Journal of Plant Diseases and Protection (2024) 131:881–890
https://doi.org/10.1007/s41348-023-00841-6

ORIGINAL ARTICLE

The significance of nematode communities in South African citrus decline orchards

M. C. Pretorius[1] · Adele McLeod[2] · Hendrika Fourie[3,4] · Mieke Daneel[3,5]

Received: 3 May 2023 / Accepted: 22 November 2023 / Published online: 11 January 2024
© The Author(s), under exclusive licence to Deutsche Phytomedizinische Gesellschaft 2023

Abstract

Citrus slow decline is a slow developing disease occurring in citrus orchards rendering the citrus orchards uneconomical in a decade or two. Causal agents have not been identified and therefore a study was initiated to identify parameters or combination of parameters that could be useful in early detection of the disease. Four citrus orchards in Mpumalanga Province, South Africa, were identified where citrus slow disease decline was present. Twenty trees of each of the three disease categories including healthy, slight sick and sick trees, were randomly selected and sampled in each orchard. Among the many parameters measured, nematode communities were evaluated as they might show differences between the three tree categories. Results showed that *Tylenchulus semipenetrans* was the most abundant nematode species present in the three tree categories making up more than 90% of the nematode communities. Nine other plant-parasitic and 21 free-living nematodes were identified among the three tree categories with *Acrobeloides* being the most abundant nematode genus followed by *Prismatolaimus*. Among the feeding groups, 73% belonged to bacterivores followed by about 10% to the fungivores. Metabolic foot prints for the three tree categories were very small and closely together in the depleted and degraded quadrat.

Keyword Bioindicators · Citrus · Nematodes · Slow decline

Introduction

Citrus is a major fruit crop worldwide and South Africa rank as the 12th largest producer of fruit with a large portion of the production being exported. Citrus is produced in many areas of South Africa and encompassed an area of around 99 600 ha in 2021 with production estimated to increase in 2022 (http://www.cga.co.za/). Among the restraints in the production of the crop are diseases and pest infestations of which plant-parasitic nematodes (PPN) represent a major constraint (Pretorius and Le Roux 2017).

In citrus, the most economically important PPN is the citrus nematode, *Tylenchulus semipenetrans* (Cobb 1913). The citrus nematode is not indigenous to South Africa, but following its introduction it has spread succefully to all citrus producing areas in the country (Pretorius and Le Roux 2017). Citrus nematode infected trees grow progressively slower as *T. semipenetrans* populations increase, eventually resulting in trees producing smaller fruit and lower yields (Pretorius and Le Roux 2017). As the South African, citrus industry is mainly focussed on fresh fruit exports, fruit size and optimum yields are of utmost importance.

Symptoms caused by *T. semipenetrans* in infected orchards include smaller leaves that may become chlorotic, often showing Zn-deficiency symptoms, and under saline conditions excessive sodium (Na) may accumulate in leaves while feeder roots appear slightly thicker than healthy roots. This eventually causes smaller and less fruit resulting in lower yields. Symptom progression is very slow since it may take years for a tree to deteriorate to an unproductive state (Le Roux 1995; Meena et al. 2018). The symptoms caused by *T. semipenetrans* may at times resemble that of

✉ M. C. Pretorius
mc@cri.co.za

1 Citrus Research International, P. O. Box 28, Nelspruit 1200, South Africa

2 Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Stellenbosch 7601, South Africa

3 Unit for Environmental Sciences and Management, Integrated Pest Management, North West University, Private Bag X 6001, Potchefstroom 2520, South Africa

4 Syngenta, 254 Hall St, Die Hoewes, Centurion 0157, South Africa

5 Agricultural Research Council - Tropical and Subtropical Crops, Private Bag X11208, Nelspruit 1200, South Africa

root related slow citrus decline, which is a very complex syndrome of citrus.

Trees within slow declining orchards exhibit different stages of decline that can be grouped into different categories based on the canopy health of the trees. No specific root pathogens have been associated with this slow decline although it is assumed that *T. semipenetrans* is likely a role player. Previous studies have found that in some, but not all, orchards with citrus slow decline significantly higher *T. semipenetrans* soil densities are associated with healthy trees versus declining trees. This is indicative of a deteriorating root system not being able to support the pathogen.

By contrast to ample knowledge being available for PPN that are parasitizing citrus, limited information has been generated for beneficial nematodes occurring in citrus orchards across the globe. Most studies have been conducted in Florida, where rainfall and irrigation regimes were shown to influence nematode community structure (Porazinska and Coleman 1995; McSorley 1997; Porazinska et al. 1998a). Porazinska et al. (1998b) reported that omnivores (Om) were strongly influenced to irrigation levels in citrus orchards although it was explained by the long term elevated water supply rather than the temporary water content. The effect of orchard ground cover management in citrus orchards on nematode communities has been investigated in two studies in Florida (Porazinska et al. 1999; Macchia et al. 2003) and one in Israel (Eshel et al. 2022). Mulching and plant ground covers were shown to frequently influence composition and abundance of feedings groups including bacterivores (Ba), fungivores (Fu), herbivores (He) and omnivores (Om) (Porazinska et al. 1999; Macchia et al. 2003; Eshel et al. 2022). Presence of free living nematodes provides a good indication of soil food webs since they contribute towards crucial processes such as the decomposition and recycling of nutrients (indicated by the ratio of Ba–Fu). The suppression of pathogenic soil organisms has also been ascribed to beneficial nematodes and hence they are indicators of community changes of such organisms. Beneficial nematodes are also indicators of polluted or disturbed soils (Neher 2001; Stirling 2014) because they are known to respond to changes caused by land management practises (Bongers 1990; Cabanillas et al. 1999), pollution (du Preez et al. 2018) and other disturbances/interventions (e.g. drought, fire, rain). It might, therefore, also be possible that beneficial communities could differ between the different categories of citrus slow decline, considering the previously reported citrus nematode associations with tree decline categories. Because it is unclear what the origin or the cause of the slow decline trees is and it is difficult to predict the disease, beneficial nematode communities might be an early indicator of changes in the rhizosphere of citrus trees.

The overall aim of the study was to determine whether plant-associated nematode community profiling in four citrus orchards, containing trees in three different decline stages, can contribute towards a better understanding of citrus slow decline. The specific objectives of this South African study were to: (i) determine the abundance and diversity of plant-parasitic and beneficial nematode assemblages citrus orchards in the Mpumalanga Province; (ii) determine whether associations exist between plant-parasitic and beneficial nematode communities *versus* visual healthy-looking and declining (slight and advanced declining trees) citrus trees; (iii) whether the soil food web structure differ between the three tree decline categories.

Material and methods

Orchard trial sites

Four citrus trial sites in the Lowveld in the Mpumalanga Province, South Africa, representing orchards with typical slow decline symptoms within the South African citrus industry, were selected. All four sites contained 20 year-old Delta Valencia [*Citrus sinensis* (L.) Osbeck] grafted on Carrizo citrange rootstock [*Citrus sinesis x Poncirus trifoliata*] trees, and included three blocks at the Valley View farm near Karino planted on a loamy-sand soil (Alco 1 [25°27′ 27.42″S; 31°04′ 46.75″E], Alco 2 [25°27′ 27.42″S; 31°04′ 39.52″E], Alco 3 [25°27′ 30.37″S; 31°04′ 11.98″E]) and one block at the LA Visagie Estate also near Karino (25°24′ 26.22″S; 31°04′ 51.47″E) on a sandy soil. The Karino region has a subtropical climate with warm humid rainy summers and dry moderate winters. Orchard tree spacing was 6 m between the rows and 3 m within the rows. All the trees were kept at a height of 2.6–2.8 m. The tree row direction for all the orchards faced North–South. The orchards had micro irrigation systems installed with one microjet between each tree within the row. Orchard management was according to the CRI guidelines for optimum yield, fruit size and qualification to ensure the export of fruit according to internationally accepted standards (CRI 2019).

Throughout each orchard, trees exhibited a range of symptoms ranging from healthy looking to different declining stages. Trees with decline symptoms were located randomly throughout the orchards and never in a line along the irrigation system or in confined areas. Based on a visual canopy evaluation of the trees, three decline symptom categories were selected within each orchard and trees were categorised as: category 1 containing healthy trees, category 2 trees with a slight indication of decline and category 3 with an advanced state of decline. The selected trees were randomly selected in the four orchards and rated until 20 trees for each of the three tree categories were identified. In total, 60 trees per orchard were thus selected and a total of 240 trees were included in the study.

Nematode sampling and analysis

Nematode samples collected at each tree consisted of three subsamples of about 350 ml of soil each (to a total of 1050 ml of soil) at a depth of 1—20 cm in the root-zone under the tree canopy. The three subsamples per tree were placed in one bag and taken to the laboratory for further analyses. The number of *T. semipenetrans* J2 stage was analysed from 250 ml soil samples according to the method of Whitehead and Hemming (1965), a method used by the diagnostic centre of Citrus Research International. Additionally, subsamples were send to the nematology laboratory at EkoRehab at North West University, Potchefstroom, South Africa. There, the entire nematode community was extracted from 250 ml soil using the decanting and sieving method followed by adapted sugar-flotation method (Marais et al. 2017). A Peter's 1 ml-counting slide (Hooper et al. 2005) and a Nikon (Eclipse 50*i*) light microscope (40 × magnification) were used for nematode counting and identification (Coyne et al. 2007). Beneficial nematodes were identified to genus/family level while PPN were identified to genus level.

Statistical analyses

For each of the nematode genera prominence values (PV = mean population density *x* frequency of occurrence/10) were calculated (Fourie et al. 2001) with frequence of occurrence (FO) = (number of location where a nematode genus is present * 100)/total numer of locations and Mean Population Density (MPD) = Total number of nematode per genus/number of samples.

In addition, soil nematode data were subjected to the online Nematode Indicator Joint Analysis (NINJA) programme (Sieriebriennikov et al. 2014) for calculation of nematode-based indices. The Enrichment Index (EI: measure of resource availability) and Structure Index (SI: measure of the complexity of the soil ecosystem) were used to conduct a food web analysis and create a metabolic footprint. The Basal Index (BI) was used to provide an indication of the main decomposition pathway; lower and higher values represent bacterial and fungal dominated decomposition pathways, respectively (Ferris and Bongers 2009; Sánchez-Moreno and Ferris 2018).

Results

Abundance and diversity of plant-parasitic and beneficial nematode assemblages

In total, 34 genera/families were recovered from the plant-associated soil samples collected in the four citrus orchards. The most abundant nematode genus recovered from the samples was *Tylenchulus* with a PV value of 719.8. It was found as the highest number in all sites and within the three tree categories.

The second most abundant genus found during the study was a bacterivore, *Acrobeloides* with a PV value of 175.7. Other genera observed at the four sites during the study had much lower PV values with their PV ranging from 11.1 to 2.5. These genera included *Prismatolaimus, Eucephalobus, Aphelenchus, Criconema, Acrobeles* and *Panagrolaimus* (Table 1).

The other genera/families seemed to be linked to specific orchards and often to only a few trees in those orchards (Table 1).

Plant-parasitic nematode communities and their association with the tree declining categories

No significant differences for the total PPN community were observed between the three tree categories nor for the *Tylenchulus* populations for the combined data set.

Significant differences ($P < 0.05$) were observed between the abundance of *Tylenchulus* between the three tree categories for Alco 1 and Alco 2. In Alco 1, significantly lower *Tylenchulus* numbers were observed in the category 1 trees whereas in Alco 2, the category 3 trees had significantly lower numbers compared to category 1 and 2 trees. When percentage *Tylenchulus* was determined for each tree category in Alco 1, *Tylenchulus* numbers made up 91–93% in category 1 and 2 trees respectively while *Tylenchulus* numbers made up 99.5% of the plant-parasitic nematode community in category 3 trees. In Alco 2, *Tylenchulus* numbers constituted 97.5% in Category 3 trees compared to 99.5% in category 1 and 2 trees.

Concerning other PPN genera, some significant differences were observed but this was mostly due to genera being present in one tree category and not in the other two categories such as *Criconema* and *Ogma* in LA Visagie and *Hemicriconemoides* in Alco 1 and 2 (Table 2).

Beneficial nematode community complex associated with the tree decline categories

No difference could be observed between the free living nematode communities associated with the disease categories for the entire dataset (Table 3). Bacterivores made up more than 85% of the free living nematode community whereas fungivores made up less than 10%, followed by omnivores and a very small percentage carnivores (Table 3). When the orchards were evaluated individually, only in Alco 3, a significant difference in percentage bacterivores was observed. The percentages bacterivore community in category 1 trees were significantly higher than the bacterivore community in the category 3 trees. In the other orchards, no

Table 1 Nematode genera in citrus rhizosphere soils (250 ml) and their associated feeding group and trophic value, prominence value (PV), frequency of occurrence (FO), mean population density (MPD) in four orchards (Alco 1, Alco 2, Alco 3 and LA Visagie)

Genus or family	Guild[a]	PV[b]	FO[c]	MPD[d]	Alco 1	Alco 2	Alco 3	LA Visagie
Tylenchulus	He3	719.80	100	719.80	√	√	√	√
Criconema	He3	9.60	100	9.60	√	√	√	√
Ogma	He3	5.40	50	7.70	–	–	√	√
Hemicrionemoides	He3	3.30	25	6.60	–	–	–	√
Meloidogyne	He3	0.60	25	1.20	√	–	–	–
Hemicycliophora	He3	0.20	25	0.50	–	√	–	–
Tylenchorhynchus	He3	0.20	25	0.30	–	–	–	√
Scutellonema	He3	0.30	50	0.40	√	–	–	√
Nanidorus	He4	0.30	50	0.50	√	–	–	√
Xiphinema	He5	0.30	50	0.40	√	√	–	–
Acrobeloides	Ba2	175.70	100	175.70	√	√	√	√
Anaplectus	Ba2	7.50	50	10.70	–	√	√	–
Plectus	Ba2	8.50	75	9.80	√	√	√	–
Prismatolaimus	Ba3	11.10	100	11.10	√	√	√	√
Eucephalobus	Ba2	10.70	100	10.70	√	√	√	√
Chiloplacus	Ba2	4.30	50	6.00	√	√	–	–
Cephalobus	Ba2	3.90	75	4.50	√	–	√	√
Rhabditis	Ba1	5.00	75	5.80	√	–	√	√
Acrobeles	Ba2	5.90	100	5.90	√	√	√	√
Panagrolaimus	Ba1	2.50	100	2.50	√	√	√	√
Cervidellus	Ba2	0.70	50	1.00	–	–	√	√
Pseudacrobeles	Ba2	0.10	25	0.20	–	–	√	–
Diplogastridae	Ba2	0.04	25	0.08	–	–	√	–
Enoplidae	Ba3	0.04	25	0.08	–	–	√	–
Aphelenchus	Fu2	9.70	100	9.70	√	√	√	√
Ditylenchus	Fu2	2.10	75	2.40	√	–	√	√
Aphelenchoides	Fu2	1.10	50	1.60	√	√	–	–
Diphtherophora	Fu3	0.04	25	0.08	–	–	√	–
Dorylaimidae	O5	4.30	75	4.90	√	√	√	–
Aporcelaimus	O5	0.50	25	0.90	–	–	–	√
Mylonchulus	Ca4	0.30	50	0.40	√	–	√	–
Discolaimus	Ca4	0.04	25	0.08	√	–	–	–
Mononchoides	Ca4	0.02	25	0.04	√	–	–	–
Clarkus	Ca5	0.02	25	0.04	√	–	–	–

[a]Feeding group and trophic value (1 to 5). He = herbivores (plant-parasitic), Ba = bacterivores, Fu = fungivores, O = omnivores and Ca = carnivores

[b]PV = mean population density x frequency of occurrence/10

[c]FO = (number of location where a nematode genus is present * 100) / total number of locations

[d]MPD = mean population density = Total number of nematodes per genus / number of samples

√Nematode genera presence

significant differences were observed between disease tree categories.

Among the free living nematodes, *Acrobeloides* was consistently the genus with the highest abundance. Its percentage abundance ranged between 48 and 81% of the free living nematode communities between the 4 orchards. However, within each tree category, no consistency was observed, as in Alco 1 and 2, *Acrobeloides* numbers in category 1 were close to 45% whereas in Alco 3 and LA Visagie, percentage *Acrobeloides* was 60% and 80% respectively in category 2 (data not shown).

Biodiversity parameters for the nematode communities in different tree decline categories

Because there was a significant interaction between orchards and categories, combined orchard analysis was

Table 2 Number of plant-parasitic nematode genera and the total of all genera in soil sampled from three citrus tree categories (1 = healthy; 2 = slight indication of decline; 3 = advanced state of decline)

Nematode numbers	Category 1		Category 2		Category 3		*P* value	*F* ratio
Alco 1								
Tylenchulus	2.60 (940)	b	3.05 (1670)	a	3.25 (2010)	a	0.001	7.80
Criconema	1.03 (56)	a	0.49 (16)	b	0.07 (1)	b	< 0.01	8.89
Ogma	0.21 (4)	a	0.48 (83)	a	0.15 (3)	a	0.248	1.43
Hemicriconemoides	0.28 (5)	b	0.91 (74)	a	0	b	< 0.001	9.77
Total PPN	2.74 (1012)	b	3.08 (1845)	a	3.25 (2021)	a	< 0.001	7.99
Alco 2								
Tylenchulus	3.34 (2435)	a	3.40 (2810)	a	2.79 (965)	b	< 0.001	23.32
Criconema	0.50 (5.5)	a	0.35 (6)	a	0.21 (4)	a	0.273	1.33
Hemicriconemoides	0	a	0	a	0.02 (0.1)	a	0.374	1.00
Total PPN	3.40 (2449)	a	3.35 (2824)	a	2.79 (990)	a	< 0.001	22.62
Alco 3								
Tylenchulus	3.26 (2055)	a	3.21 (1980)	a	3.21 (1850)	a	0.762	0.27
Criconema	0.47 (9)	a	0.46 (7)	a	0.16 (4)	a	0.190	1.72
Ogma	0.06 (1)	a	0	a	0	a	0.374	1.00
Total PPN	3.27 (2065)	a	3.21 (1987)	a	3.22 (1862)	a	0.754	0.28
LA Visagie								
Tylenchulus	3.34 (2555)	a	3.48 (3405)	a	3.31 (2340)	a	0.076	2.70
Criconema	0.42 (8)	a	0	b	0	b	< 0.001	7.92
Ogma	0.07 (1)	a	0	a	0	a	0.374	1.00
Total PPN	3.35 (2564)	a	3.48 (3405)	a	3.31 (2340)	a	0.073	2.74
Combined orchards								
Tylenchulus	3.30 (1996)	a	3.39 (2466)	a	3.25 (1791)	a	0.750	0.50
Criconema	3.28 (20)	a	0.29 (7)	a	0.14 (2)	a	0.128	2.61
Total PPN	3.28 (2022)	a	3.39 (2515)	a	3.24 (1802)	a	0.427	0.94

Values are the Log10 $(x+1)$ data with the actual nematode numbers in brackets. Row values with the same letter do not differ significantly from one another ($P < 0.05$) according to Fisher's protected least significant difference test

not conducted, instead each orchard was analysed separately (Table 4).

No significant differences were observed between diversity indices for the different disease categories in most of the orchards except for Alco 1 where category 2 trees had a significantly lower value than category 1 and 3 trees (Table 4).

When nematode based indices were compared between the different tree decline categories, some significant differences were observed. In Alco 1, structure index (SI) showed a significantly lower value for category 3 trees compared to the other two categories.

Two other NBIs that showed significant differences were Basal (BI) and Enrichment Indices (EI). BI was significantly higher in category 1 trees compared to the other 2 categories in Alco 3 and EI was significantly higher in category 1 trees compared to the other 2 categories in LA Visagie (Table 4).

Another parameter showing significant differences between the tree categories was F + B/H. In Alco 2, category 3 trees had a significantly higher value whereas in LA Visagie it was category 2 trees that had a significantly higher value.

The metabolic footprint of the nematode communities for the three tree categories was situated in quadrat D representing a degraded and depleted soil. Analyses of the four orchards independently also exhibited the same metabolic footprint as the combined orchard dataset. The footprint, showing enrichment and structural dimensions, was very small for each of the categories since the three tree categories were positioned very close together indicating no difference between the three tree categories concerning structure or enrichment (Fig. 1A). The basal triangle positions the nematode community of trees based on stability, enrichment and stress. The majority of trees, irrespective of decline category was therefore positioned close to 100% stress (Fig. 1B).

Discussion

The overall aim of the study was to determine whether plant-associated nematode community profiling in four citrus orchards, containing trees in three different decline stages, could contribute towards a better understanding of

Table 3 Percentage beneficial nematodes per feeding group associated with soil collected from three citrus tree categories (1 = healthy; 2 = slight indication of decline; 3 = advanced state of decline) in four orchards (Alco 1, Alco 2, Alco 3 and LA Visagie)

Beneficial nematode feeding groups	Category 1		Category 2		Category 3		*P* value	*F* ratio
Alco 1								
Bacterivores	89.3	a	87.8	a	82.0	a	0.460	0.79
Fungivores	5.7	a	6.8	a	16.3	a	0.109	2.31
Omnivores	4.4	a	3.5	a	0.6	a	0.180	1.77
Carnivores	0.6	a	1.9	a	1.1	a	0.763	0.27
Alco 2								
Bacterivores	80.3	a	90.4	a	90.9	a	0.554	0.60
Fungivores	5.5	a	5.6	a	5.3	a	0.997	0.00
Omnivores	7.6	a	3.9	a	3.8	a	0.469	0.77
Carnivores	0.5	a	0.1	a	0.0	a	0.415	0.89
Alco 3								
Bacterivores	95.5	a	78.8	ab	73.3	b	0.047	3.24
Fungivores	3.3	a	14.6	a	13.2	a	0.154	1.94
Omnivores	0.6	a	6.5	a	12.2	a	0.287	1.28
Carnivores	0.7	a	0.1	a	0.2	a	0.504	0.69
LA Visagie								
Bacterivores	96.7	a	97.6	a	96.8	a	0.929	0.07
Fungivores	1.7	a	1.2	a	3.0	a	0.746	0.29
Omnivores	1.6	a	1.2	a	0.1	a	0.492	0.72
Carnivores	0.0	a	0.03	a	0.01	a	0.438	0.84
Percentage per feeding group								
Bacterivores	91.9	a	88.6	a	85.5	a	0.131	2.05
Fungivores	4.1	a	7.1	a	9.7	a	0.063	2.80
Omnivores	3.6	a	3.8	a	4.4	a	0.927	0.08
Carnivores	0.5	a	0.5	a	0.3	a	0.945	0.06

Values are the Log10 $(x+1)$ data with the actual nematode numbers in brackets. Row values with the same letter do not differ significantly from each another ($P<0.05$) according to Fisher's protected least significant difference test

citrus slow decline. The communities were evaluated per tree category for possible changes related to the degree of root decline status. The study was conducted in the Lowveld region of South Africa.

No significant association was found between nematode numbers and citrus slow decline, indicating that nematode abundance is not a reliable early indicator for this disease. Significant differences between *T. semipenetrans* abundance in the disease categories were observed in two orchards, however, these differences were not consistently linked with a specific disease category in those orchards as it was found in the category 1 trees in one orchard and in category 3 trees in the other orchard. Abd-Elgawad (2020) touches on this phenomenon by stating that *T. semipenetrans* numbers are influenced by a wide variety of factors including soil pH, moisture content and tree nutrition, which could explain some of the variation among the numbers linked to the disease categories.

Significant differences in nematode abundance were also observed for *Criconema* and *Hemicriconemoides* in one and two orchards, respectively. However, the difficulty with these results concerning our objective of identifying indicators is the very low nematodes numbers for both *Criconema* and *Hemicriconemoides,* ranging from 56 to 0, and 73–0, respectively. The inconsistent presence of both the nematodes disqualifies them from the possible utilisation as a good indicator of decline in citrus orchards. In addition, these low nematode numbers are probably not causing any damage to roots of the citrus trees. Currently, it is not known at which level the above-mentioned nematode genera would contribute to the decline syndrome in citrus orchards. Many other nematode genera have been found on citrus but besides the citrus nematode, very little is known about the damage potential of *Radopholus similis, Hemicycliophora, Pratylenchus* and *Meloido*gyne (Pretorius and Le Roux 2017; Shokooi and Duncan 2018; Abd-Elgawad 2020).

For *Acrobeloides*, the second most abundant genus found during this study, significant differences were only observed between the decline categories in the Alco 3 orchard, with the highest abundance found in the healthy tree category. Unfortunately, this tendency was not observed in the other orchards.

Table 4 Average nematode indices obtained from four citrus orchards where soil was sampled at three citrus tree categories (1 = healthy; 2 = slightly declined; 3 = advanced state of decline)

Indices	Category 1		Category 2		Category 3		*P* value	*F* ratio
Alco 1								
F/B	0.21	a	0.10	a	0.07	a	0.2800	1.31
F + B/H	1.05	a	0.38	a	0.29	a	0.0700	2.74
H'	0.67	a	0.62	a	0.48	a	0.2536	1.41
Simpson	0.33	a	0.14	b	0.41	a	0.0059	5.62
MI	2.21	a	2.11	a	2.01	a	0.1428	2.02
MI2-5	2.27	a	2.27	a	2.04	b	0.0478	3.21
BI	79.10	a	65.20	a	64.10	a	0.2326	1.56
EI	19.50	a	15.10	a	7.80	a	0.3178	1.17
SI	29.60	a	27.10	a	6.60	b	0.0202	4.19
Total biomass	82.30	a	103.80	a	52.50	a	0.3119	1.19
Alco 2								
F/B	0.07	a	0.08	a	0.09	a	0.9742	0.03
F + B/H	0.16	b	0.05	b	1.21	a	0.0141	4.63
H'	0.48	a	0.22	a	0.42	a	0.0612	2.95
Simpson	0.54	a	0.67	a	0.68	a	0.6930	0.37
MI	2.20	a	2.05	a	2.03	a	0.0997	2.41
MI2-5	2.20	a	2.12	a	2.08	a	0.3016	1.21
BI	71.00	a	79.40	a	80.80	a	0.5167	0.67
EI	5.95	a	15.30	a	9.70	a	0.3837	0.98
SI	25.00	a	12.30	a	10.00	a	0.1496	1.97
Total biomass	0.31	a	0.08	a	0.41	a	0.1364	2.87
Alco 3								
F/B	0.06	a	0.39	a	1.18	a	0.4405	0.83
F + B/H	0.10	a	0.23	a	0.09	a	0.0929	2.48
H'	0.27	a	0.37	a	0.33	a	0.5309	0.64
Simpson	0.54	a	0.59	a	0.40	a	0.3997	0.93
MI	1.00	a	2.10	a	2.20	a	0.6012	0.51
MI2-5	2.03	a	2.17	a	2.24	a	0.3728	1.50
BI	89.00	a	63.20	b	54.00	b	0.0019	7.01
EI	8.90	a	24.30	a	28.70	a	0.0756	2.71
SI	3.90	a	17.30	a	22.80	a	0.0752	2.71
Total biomass	0.13	a	0.24	a	0.18	a	0.2893	1.28
LA Visagie								
F/B	0.02	a	0.01	a	0.06	a	0.5725	0.56
F + B/H	0.10	b	0.21	a	0.04	b	0.0059	5.67
H'	0.40	a	0.38	a	0.22	a	0.1432	2.03
Simpson	0.50	a	0.75	a	0.63	a	0.0736	2.74
MI	1.96	a	2.14	a	2.12	a	0.0618	2.94
MI2-5	2.11	a	2.14	a	2.12	a	0.9041	0.10
BI	59.10	a	76.50	a	79.90	a	0.0569	3.03
EI	32.30	a	1.80	b	2.00	b	< 0.0001	14.42
SI	13.50	a	22.00	a	18.10	a	0.5614	0.58
Total biomass	0.42	a	0.86	a	0.03	a	0.1787	1.78

Means with the same letter are not significantly different and the letter marked in bold show significant differences according to $P < 0.05$ between the three categories; F/B = Fungivore numbers over Bacterivore numbers; F + B/H = (Fungivore numbers + Bacterivore numbers)/Herbivore numbers; H' = Shannon Wiener diversity index; Simpson = Simpson index; MI = Maturity index; MI2-5 = Maturity index for cp2 to 5 nematodes; BI = Basal index; EI = Enrichment index; SI = Structure index

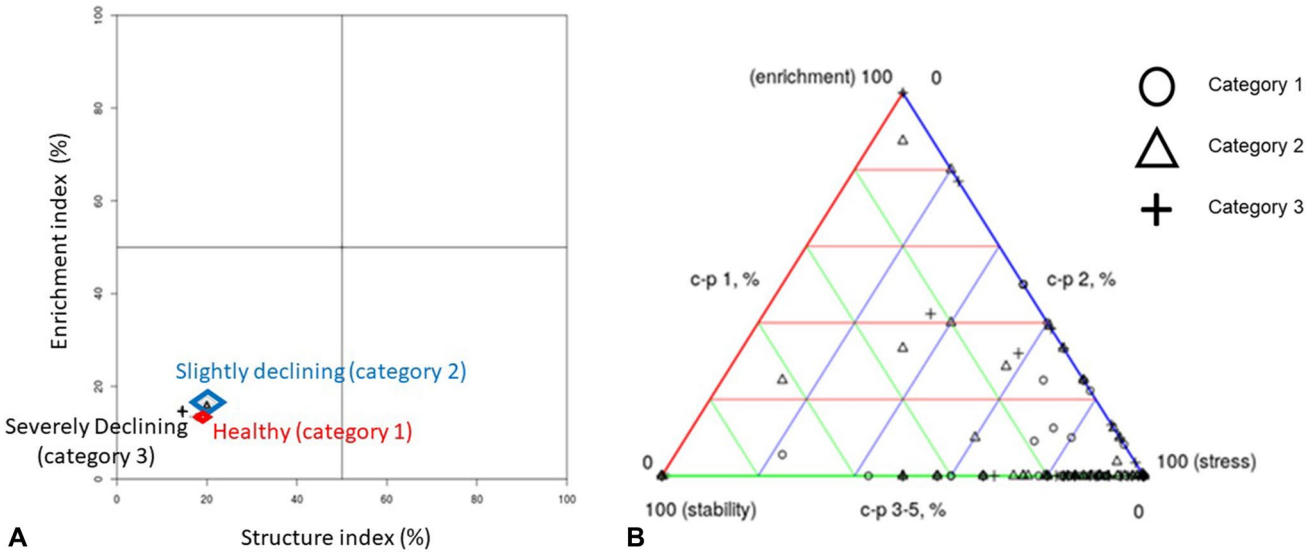


Fig. 1 **A** Metabolic footprint of beneficial trophic groups for the three categories in the four citrus orchards combined sampled in South Africa with the red square indicating the metabolic foot print of the healthy trees, blue square indicating the metabolic foot print of the slightly declining trees and black "+" indicating the metabolic foot print of the severely declining trees and **B** the basal triangle showing the position of each tree in the triangle indicating most of the nematodes being cp2 and cp3 leaning towards high stress, low stability and low enrichment

Similar as for nematode genera, investigated parameters including the beneficial feeding groups (bacteriovores, fungivores, canrivores and omnivores) obtained from the characterization of the beneficial nematodes were unable to identify an early indicator for citrus slow decline. When a combined analysis was done on the free-living nematode communities collected from all four trial sites the vast variations between the different sites as well as between the different disease categories made the interpretation of the nematode communities in the different disease categories inconclusive. In the study of Porazinska et al. (1999), mulching seemed to affect nematode populations over an extended period in contrast to other practises such as fertilization and irrigation which only affected some nematodes sporadically. In another study of Porazinska et al. (1998b) where the effect of different irrigation schemes on nematode communities was investigated, positive correlations with certain trophic groups and irrigation treatments were observed. It is therefore possible to expect differences in the nematode communities between the three disease categories. However, the impact of management practises, such as mulching, on soil health and nematode community composition is probably much more significant than the transformation between disease categories since we did not observe consistent changes in tropic groups between the disease categories.

None of the ecological indices provided a reliable indication of the soil quality status of the three disease categories because significant differences between the tree categories were only observed in one of the orchards with examples being EI, BI, F + B/H, MI2-5, H' and Simpson. Another observation in our study was that the above-mentioned indices did not gradually increase or decrease from category 1–3. Even if only two tree categories namely sick (representing category 3) and healthy (representing category 1) were analysed, none of the calculated indices could predict the soil health status between the disease categories. Two studies (Porazinska et al. 1998a, 1999) found MI to be a reliable indicator for soil health but both studies compared different management practises which probably had a higher impact on soil health than disease development.

The soil food web indicated that the three tree decline categories were positioned in the depleted and degraded quadrat and had a very low footprint. This is an indication of the harsh conditions in citrus orchards in South Africa with management practises that include frequent applications of chemicals and fertilizers as well as a weed free under tree canopy area. Unfortunately, these practises seem to be much more detrimental to the soil habitat than changes in the root zone caused by citrus slow decline. This was also observed by Eshel et al. (2022) who noticed that nematode communities and trophic diversity was seriously impacted by floor management and that a strong association existed between the two resulting in a shift in the soil food web structure and functionality.

In our study, *T. semipenetrans* was the most abundant species as it occupied more than 90% of nematode communities found in the soil samples collected at the different decline categories and could therefore not be used as an indicator

of disease development. It was probably responsible for the nematode damage caused to the root systems of the citrus trees which agrees with studies conducted world-wide (Le Roux 1995; Duncan 2005, 2009; Abd-Elgawad et al. 2016; Pretorius and Le Roux 2017; Shokoohi and Duncan 2018; Verdejo-Lucas 2022).

The influence of PPN in citrus slow decline has previously been investigated but could not be confirmed as a causal agent. Although the citrus nematode can be associated as one of the factors of the citrus root disease complex in which root-rot fungi, salinity, poor management practises such as soil preparation and irrigation management and other predisposing factors (tristeza and Huanglongbing (HLB), citrus greening disease) are involved, it is important to determine which factor is the limiting factor in the complex and should therefore be addressed first (Pretorius and Le Roux 2017; Verdejo-Lucas 2022).

Although our results were not able to identify indicators for citrus slow decline, it should stimulate citrus growers to implement more environmentally friendly methods to control pest and diseases and consider cover crops, mulches and organic amendments underneath tree canopies. It is anticipated that these changes could positively contribute to nematode community composition and consequently influence soil health and hopefully contribute to lower incidence of citrus tree decline. Further, long term studies are needed to support this observation. However, other studies have shown that soil health can be improved with green mulching for example (Macchia et al. 2003; Tu et al. 2021; Eshel et al. 2022; Van Dung et al. 2022; Wang et al. 2022).

Also the studied orchards were 20 year old and citrus nematode numbers were high. It would however be interesting to investigate the potential of citrus nematodes as indicator when sampling young trees from 3 years old trees over time and investigate how they influence the initial development of citrus slow decline and whether nematode control at this early stage could slow down the development of the citrus slow decline syndrome in orchards, and when orchard floor management by means of green- or other mulching methods will benefit beneficial nematode communities.

Author contributions MCP conceived and designed the study; MCP collected the data, DF counted the nematode data while MCP and MSD authors contributed to the data analysis and manuscript preparation. All authors read and approved the manuscripy.

Declarations

Conflict of interest All authors declare no conflict of interest.

References

Abd-Elgawad, Mahfouz MM (2020) Managing nematodes in Egyptian citrus orchards. Bull Natl Res Cent 44:41. https://doi.org/10.1186/s42269-020-00298-9

Abd-Elgawad MMM, Koura FFH, Montasser SA, Hammam MMA (2016) Distribution and losses of Tylenchulus semipenetrans in citrus orchards on reclaimed land in Egypt. Nematology 18:1141–1150

Bongers T (1990) The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecolog 83:14–19

Cabanillas HE, Bradford JM, Smart JR (1999) Effect of tillage system, soil type, crop stand, and crop sequence on reniform nematodes after harvest. Nematropica 29(2):137–146

Citrus Research International (2019) CRI Integr Prod guidelines, 2, 1–142

Coyne D, Nicol JM, Claudius-Cole B (2007) Practical plant nematology: a field guide and laboratory guide SP-IPM Secretariat. International Institute of Tropical Agriculture (IITA), Cotonou

Du Preez GC, Daneel MS, Wepener V, Fourie H (2018) Beneficial nematodes as bioindicators of ecosystem health in irrigated soils. Appl Soil Ecol 132:155–168

Duncan LW (2005) Nematode parasites of citrus. In: Luc M, Sikora RA, Bridge J (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, Wallingford, pp 437–466

Duncan LW (2009) Managing nematodes in citrus orchards. In: Ciancio A, Mukerji KG (eds) Integrated management of fruit crops and forest nematodes. Springer, Berlin, pp 135–173. https://doi.org/10.1007/978-1-4020-9858-1

Eshel G, Unc A, Egozi R, Shkartchy E, Doniger T, Steinberger Y (2022) Orchard floor management effect on soil free-living nematode communities. Soil Res 60:310–319

Ferris H, Bongers T (2009) Indices developed specifically for analysis of nematode assemblages. In: Wilson MJ, Kakouli-Duarte T (eds) Nematodes as environmental indicators. CABI Publishing, Wallingford, pp 124–145

Fourie H, Mc Donald AH, Loots GC (2001) Plant-parasitic nematodes in field crops in South Africa. Nematol 3(5):447–454

Hooper JH, Hallmann J, Subbotin S (2005) Methods for extraction, processing and detection of plant and soil nematodes. In: Luc M, Sikora MA, Bridge J (eds) Plant parasitic nematodes in subtropical and tropical agriculture, 2nd edn. CAB International, Wallingford, pp 53–86

Macchia ET, McSorley R, Duncan LW, Syvertsen JS (2003) Effects of perennial peanut (*Arachis glabrata*) ground cover on nematode communities in citrus. J Nematol 35:450–457

Marais M, Swart A, Fourie H, Berry SD, Knoetze R, Malan A (2017) Techniques and procedures. In: Fourie H, Spaull VW, Jones RK, Daneel MS, De Waele D (eds) Nematology in South Africa: a view from the 21st Century. Springer, Berlin, pp 73–118. https://doi.org/10.1007/978-3-319-44210-5

McSorley R (1997) Relationship of crop and rainfall to soil nematode community structure in perennial agroecosystems. Appl Soil Ecol 6(2):147–159. https://doi.org/10.1016/S0929-1393(97)00001-2

Meena AK, Dutta F, Marak MC, Meena RK (2018) Citrus decline. Int J of Curr Microbiol Appl Sci 7:2807–2815

Neher DA (2001) Role of nematodes in soil health and their use as indicators. J Nematol 33:161–168

Porazinska DL, Coleman DC (1995) Ecology of nematodes under influence of Cucurbita spp. and different fertilizer types. J Nematol 27:617

Porazinska L, McSorley R, Duncan LW, Gallaher RN, Wheaton TA, Parsons L (1998a) Relationships between soil chemical status,

soil nematode community, and sustainability indices. Nematropica 28:249–262
Porazinska L, McSorley R, Duncan LW, Graham JH, Wheaton TA, Parsons LR (1998b) Nematode community composition under various irrigation schemes in a citrus soil ecosystem. J Nematol 30:170–178
Porazinska DL, Duncan LW, McSorley R, Graham JH (1999) Nematode communities as indicators of status and processes of a soil ecosystem influenced by agricultural management practices. Appl Soil Ecol 13(1):69–86. https://doi.org/10.1016/S0929-1393(99)00018-9
Pretorius MC, Le Roux HF (2017) Nematode pests of citrus. In: Fourie H, Spaull VW, Jones RK, Daneel MS, De Waele D (eds) Nematology in South Africa: a view from the 21st century. Springer, Berlin, pp 311–324. https://doi.org/10.1007/978-3-319-44210-5
Le Roux HF (1995) Control of the citrus nematode in South Africa. PhD dissertation, Pretoria University, Pretoria
Sánchez-Moreno S, Ferris H (2018) Nematode ecology and soil health. In: Sikora R, Coyne D, Hallmann J, Timper P (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, Wallingford, United Kingdom, pp 62–86
Shokoohi E, Duncan LW (2018) Nematode parasites of citrus. In: Sikora R, Coyne D, Hallman J, Timper P (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, Wallingford, pp 446–476
Sieriebriennikov B, Ferris H, de Goede RG (2014) NINJA: an automated calculation system for nematode-based biological monitoring. Europ J Soil Biol 61:90–93
Stirling GR (2014) Biological control of plant-parasitic nematodes: soil ecosystem management in sustainable agriculture. CAB International, Wallingford
Tu A, Songhua Xie S, Zheng H, Li H, Li Y, Mo M (2021) Long-term effects of living grass mulching on soil and water conservation and fruit yield of citrus orchard in south China. Agric Water Manag 252:106897. https://doi.org/10.1016/j.agwat.2021.106897
Van Dung T, Ngoc NP, Van Dang L, Hung NN (2022) Impact of cover crop and mulching on soil physical properties and soil nutrients in a citrus orchard. Peer J. https://doi.org/10.7717/peerj.14170
Verdejo-Lucas S (2022) Pre-Planting solutions for the slow decline of citrus caused by *Tylenchulus semipenetrans*. In: Sikora RA, Desaeger J, Molendijk L (eds) Integrated nematode management: state-of-the-art and visions for the future. CAB International, Wallingford, pp 174–181
Wang N, Li L, Gou M, Jian Z, Hu J, Chen H, Xiao W, Liu C (2022) Living grass mulching improves soil enzyme activities through enhanced available nutrients in citrus orchards in subtropical China. Front Front Plant Sci Sec Plant Nutrition. https://doi.org/10.3389/fpls.2022.1053009
Whitehead AG, Hemming JR (1965) A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Ann of Appl Biol 55(1):25–38

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.