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Survey of nematodes in the Herbert region to develop a nematode-hazard index and evaluate soil health

Shamsul A Bhuiyan[1], Kylie Garlick[1], Lawrence Di Bella[2], Ellie McVeigh[2] and Michael Sefton[2]

[1]*Sugar Research Australia Limited, Woodford, Qld 4514*; sbhuiyan@sugarresearch.com.au
[2]*Herbert Cane Productivity Service Limited (HCPSL), Ingham, Qld 4850*

Abstract Soil nematodes are microscopic worm-like organisms and are important members of the soil ecosystem. Some nematodes are either plant or animal parasites, and some are 'free-living' feeding on bacteria, fungi or on other nematodes. Plant-parasitic nematodes can cause significant yield loss in sugarcane. Free-living nematodes are vital contributors to soil health through their involvement in mobilisation of nitrogen and carbon and are important indicators of soil health. The objectives of this study were to determine the extent of plant-parasitic nematodes and their potential impact on sugarcane production in the Herbert region, and the use of nematode community analysis to determine the soil health of the surveyed sugarcane farms. Soil samples were collected from 56 locations from six sugarcane productivity zones, Central Herbert, Lower Herbert, Ingham Line, Stone River, Abergowrie and Wet Belt from one-year plant crops. Most of the sugarcane fields surveyed had medium (484 nematodes/200g soil) to high (1137 nematodes/200 g soil) numbers of root-lesion nematodes. Other nematodes, including root-knot nematodes, were relatively low in number. A nematode-hazard index (HI) was developed by adding the weighted number of parasitic nematodes determined from a hazard factor derived from the damage caused relative to damage caused by root-knot nematodes. Approximately 73% of surveyed farms had medium to high HI values, an indication of moderate to high potential impact on yield. As an indicator of soil health, soil food-web maturity indices (MI) were determined on the basis of nematode community. Analysis of nematode community showed low maturity indices (MI and MI2-5) (<3.5) in all farms, an indication of low soil food-web maturity and persistent use of chemical fertilisers. Soil food-web indices and metabolic footprints indices also indicated perturbation of sugarcane soil and a farming system dominated by herbivorous nematodes. Our study demonstrated that nematodes community analysis can be used as indicators of soil health for the sugar industry as well as a monitoring tool to determine the effects of management practices.

Key words Sugarcane nematodes, nematode community analysis, soil health, soil food web, soil health indicator

INTRODUCTION

Nematodes are microscopic worm-like organisms present in all agricultural soil including sugarcane. They are an important part of the soil ecosystem, play an important role in the movement of soil carbon and nitrogen. Several nematode species are important pathogens of plants. Nematode pests of sugarcane cause in excess of 15% yield loss in Australia costing more than $80 million across the industry in productivity (Blair and Stirling 2007). Although, the impacts of plant parasitic nematodes are well-documented (Blair and Stirling 2007; Blair *et al.* 1999, Bull 1981), no targeted survey has been conducted in the last 15 years. A change in management practices, adoption of new varieties and climate change can influence the prevalence and impact of pests and diseases. A n ongoing monitoring and survey of nematodes in Australian sugarcane fields is long overdue.

Over the last decades, our knowledge of nematode communities and their response to soil health has improved significantly. Nematodes are vital contributors to soil health through their involvement in mobilisation of nitrogen and carbon and are essential part of soil ecosystem. They occur almost everywhere and in high numbers, represent a trophically heterogeneous group. They are taxonomically and functionally diverse, and differ in their physiological, developmental, behavioural and food preferences. They are differentially sensitive to soil environmental conditions. Some groups are highly sensitive to soil disturbance – such as pollutants, chemicals, high input organic matters. Some are tolerant to pollutants and can survive in resource depleted environments, and some of them are opportunists, able to survive in a wide range to soil environmental conditions. As soil recovers after a disturbance, a successional change occurs in the nematode community. It is possible to give an increasing value to each of the taxa that subsequently colonises the disturbed soil. Nematode community analysis can be used to (a) determine physical and chemical condition of soil, including toxicity, salinity, sodicity, impact of chemical fertiliser application, (b) monitor farm management practices, (c) monitor water quality in agricultural soil and rivers, and (d) monitor land degradation and revegetation (Sanchez-Moreno and Howard 2018; Ferris *et al.* 2001; Neher *et al.* 2001; De Goede *et al.* 1993).

With this in mind, we undertook a survey to determine the extent of plant-parasitic nematodes and their potential impact on sugarcane production in the Herbert region. We used these data in a nematode community analysis to determine the soil health of each surveyed sugarcane farm.

MATERIALS AND METHODS

Soil samples were collected in August and September 2019 using standard protocols (Bhuiyan *et al.* 2019) and sent to SRA Woodford for assessment. Soil samples were collected from 56 locations covering six sugarcane production zones in the Herbert cane growing region (Table 1). Most of the soil types were clay to sandy soil and collected from fields managed under conventional and new farming systems. All farms were under one-year plant crops, planted with one of three commercial varieties, Q208⌽, Q200⌽ and Q253⌽. Nematode samples were collected after harvesting.

Table 1. Details of soil samples (56) collected from six productivity zones in Herbert canegrowing region.

Productivity zone	No of samples	Soil types	Farming system
Central Herbert	9	Clay, terrace loam, fine sandy loam, sand ridge, river sand, coarse red sandy loam	Conventional
Lower Herbert	11	Clay, terrace silt loam, river sand, fine grey sandy loam	Conventional and New
Ingham Line	9	Clay, sandy	Conventional
Abergowrie	9	Silty clay, sandy clay red loam, fine red loam	Conventional and New
Wet Belt	9	Silty clay, sandy loam, clay, river overflow, red loam, sandy clay	Conventional and New
Stone River	9	Clay, coarse sandy loam, grey sands, sandy clay, river overflow, red loam, red sand, silty clay	Conventional and new

Nematodes were extracted from soil samples using a modified Whitehead tray method (Whitehead and Hemming 1965). Approximately 200 g of soil (with sugarcane roots) samples was placed on double-layered tissue paper on a steel mesh set in a flat tray. The soil and roots submerged in water, left for 48 hours and then the nematodes were collected using a 38-µm sieve, stored in a 20-mL vial at 5°C until counting under a light microscope at 10X and 40X magnification. Approximately 100 g of fresh soil were maintained at 105°C for one week in a drying oven to calculate soil dry matter.

Both plant-parasitic and free-living nematodes were assessed and expressed as total nematodes per 200 g of dry soil.

Plant-parasitic nematodes

To measure the collective impact of parasitic nematodes, we developed a hazard index (HI) on the basis of relative pathogenicity (hazard factor) of the particular nematodes in relation to the most pathogenic root-knot nematodes. For example, a single root-lesion nematode is deemed to cause 80% (hence hazard factor = 0.8) of the damage caused by a root-knot nematode, and a single stunt nematode causes 25% of the damage caused by a root-knot nematode (Table 2) (G Stirling, personal communication).

Table 2. Plant parasitic nematodes, pathogen status and hazard factor.

Nematodes type/species	Pathogen status	Hazard factor
Lesion (*Pratylenchus* spp.)	Highly pathogenic	0.8
Root-knot (*Meloidogyne* spp.)	Highly pathogenic	1.0
Dagger (*Xiphinema* sp.)	Moderately pathogenic	0.25
Stubby (*Paratrichodorus* spp.)	Moderately pathogenic	0.25
Stunt (*Tylenchorhynchus* sp.)	Moderately pathogenic	0.25
Ring (f. Criconematidae)	Moderately pathogenic	0.25
Spiral (*Helicotylenchus*, *Rotylenchus*)	Mildly pathogenic	0.05
Reniform (*Rotylenchulus*)	Mildly pathogenic	0.05
Pin (*Paratylenchus*)	Mildly pathogenic	0.05

The HI for a particular soil sample was calculated as HI = $\sum[Xin \times Wfn]$, where X=number of nematodes, and W= weight of the particular nematode species.

Then, *HI=Lesion*0.8+Root-knot*1+Dagger*0.25+Stubb*0.25+Stunt*0.25+Ring*0.25+Spiral*0.05+Reniform*0.05+ Pin*0.05.*

Estimated yield loss was determined on the basis of HI (Table 3).

Table 3. Estimated reduction of yield in relation to HI threshold.

Hazard index (HI)*	Risk category	Estimated yield loss
0 to 300	Low	<5%
300 to 800	Medium	5% to 20%
800>	High	>20%

*The HI is the values are the equivalent root-knot (*Meloidogyne* spp.) damage threshold. (Modified from Blair 2005, and G Stirling pers. comm.)

Nematode community analysis

The community structure of soil nematodes is indicative of the condition or health of the soil in which they live, as they respond rapidly to disturbance and enrichment. We used several indices to assess the health of soil in the surveyed properties. Two maturity indices were used for free-living nematodes (MI, and MI2-5). To compute these indices, nematodes were assigned to colonizer-persister (c-p) values according to Bongers (1990).

The c-p values range from enrichment colonizer (c-p 1), disturbance colonizer (c-p 2) to persisters (c-p 4 and c-p 5). In short, c-p 1 are bacterivores, have a short life cycle and high fecundity and are only active during period of high bacterial biomass. The c-p 2 group has a relatively short life cycle, includes both bacterivores, and fungivores, and are tolerant to disturbance.

The c-p 3 and c-p 4 groups have longer life cycles and are fairly sensitive to disturbance and both groups contain bacterivores and fungivores. The c-p 5 group contains nematodes with a larger body size, slow generation time and high sensitivity to disturbance, and are predominantly carnivores and omnivores (Ferris *et al.* 2001). MI is the weighted collective values for all taxa, excluding parasitic nematodes.

To identify the nematode food web properties enrichment index (EI) and structural index (SI) were calculated according to Ferris *et al.* (2001) (Figure 1).

Nematode taxa with the same feeding habits, and inferred function (Functional guild) are shown in the food web as Ba_x, Fu_x, Ca_x, Om_x (x = 1–5 of the c-p scale), where Ba, Fu, Ca, Om are bacterivores, fungivores, carnivores and omnivores, respectively.

Nematode footprints are based on the biomass with each trophic group or functional guild were calculated as described by Ferris (2010).

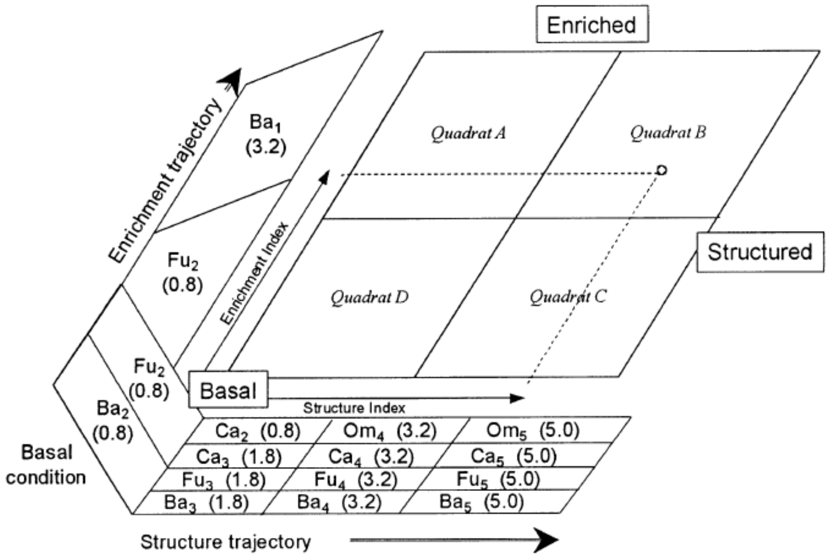

Figure 1. Representation of the nematode faunal profile indicates whether the soil community is enriched but unstructured (Quadrat A), enriched and structured (Quadrat B), resource limited and structured (Quadrat C), or resource depleted with minimal structure (Quadrat D) (from Ferris *et al.* 2001).

Statistical analysis

Nematode data were log-transformed (ln(x+1)), where x = number of nematodes per 200 g dry soil) before analysis and were analysed using linear mixed model of SAS (SAS version 9.4, SAS Institute Cary) PROC MIXED procedure. Data were back-transformed after analysis for presentation. SAS PROC CORR procedure was used to calculate correlation among nematode communities and yield.

Nematode community data were uploaded and analysed using an online program NINJA (Nematode Joint Indicator Analysis) to calculate nematode indices and metabolic footprints (Sieriebriennikov *et al.* 2014). Principal-component analysis of nematode community and cane yield (t/ha) were conducted using R.

RESULTS AND DISCUSSION

No significant differences were observed among nematode parameters regardless of soil types, variety and farming system.

Plant-parasitic nematodes

No significant differences were found among the number of plant-parasitic nematodes collected from different productivity zones or soil types. Overall lesion nematodes number were high in all surveyed field, where soil samples collected from Ingham Line zones had the highest (1,137) number of lesion nematodes followed by Abergowrie (980) (Table 4).

Numbers of other plant-parasitic nematodes, including root-knot nematodes, were relatively low, except for a few individual farms. Nematode hazard indices (HI) ranged from approximately 71 to over 2600 (Figure 2). Nematode HI values were high in Ingham Lines and Abergowrie (HI>800), and for other productivity zones, HI values were medium. In general, 27%, 46% and 27% of farms had low, medium and high HI values, respectively.

Table 4. Mean number of plant-parasitic nematodes (/200 g of dry soil) and cane yield (t/ha) in six sugarcane productivity zones in the Herbert. Numbers in the parenthesis are standard error of means.

Productivity zone	Cane yield (t/ha)	RLN	RKN	Spiral	Stubby	Ring	Dagger	HI
Abergowrie	88.2 (±4.4)	980 (±232)	47 (±42)	19 (±13)	43 (±8)	13(±6.1)	13 (±6)	853 (±203)
Central Herbert	80.1 (±7.7)	517 (±138)	61 (±34)	38 (±25)	8 (±3)	1(±0.5)	1 (±0.5)	489 (±132)
Ingham line	87.8 (±3.5)	1137 (±345)	11 (±5)	35 (±17)	7(±3)	16(±7.6)	0	937 (±277)
Lower Herbert	79 (±5.9)	484 (±56)	109 (±82)	22 (±15)	18 (±8)	47 (±25.7)	1(±0.5)	522 (±82)
Stone River	76 (±5.2)	640 (±135)	40 (±28)	11 (±7)	5(±2)	2 (±1.3)	1(±0.5)	560 (±127)
Wet Belt	69.7 (±5.8)	690 (±222)	109 (±83)	20 (±5)	3 (±1)	46 (±21)	2(±1)	648 (±179)

RLN = root-lesion nematode, RKN = root-knot nematodes, HI = nematode hazard index.

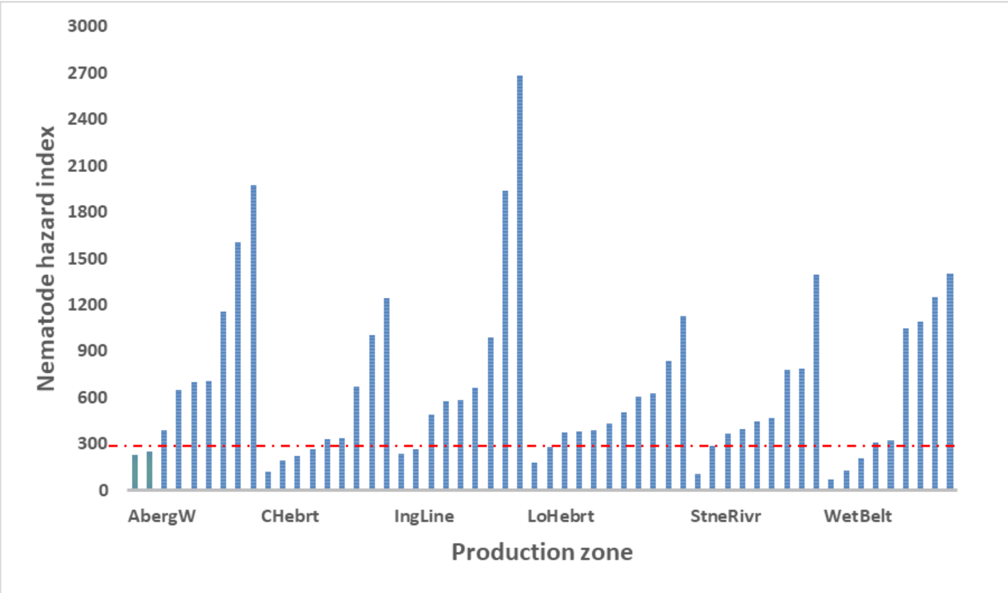

Figure 2. Nematode hazard indices (HI) for six sugarcane productivity zones, Herbert. Abergw=Aberwarie, CHebrt=Central Herbert, IngLine= Ingham Line, LoHebrt=Lower Herbert, StneRivr= Stone River, WetBelt = Wet Belt. Values above the red dotted line (HI>300) indicate medium or high HI values.

Our results learly indicated that lesion nematodes (*Pratylenchus zeae)* were present in large number in almost all sugarcane fields surveyed. This is in-agreement with previous works that found that root-lesion nematodes are the most common parasitic nematodes associated with sugarcane and can cause significant production loss (Ramouther and Bhuiyan 2018; Stirling and Blair 2000). All plant-parasitic nematodes can cause significant damages to sugarcane crop if present above the threshold levels. The threshold that can cause significant yield loss for root-lesion nematodes for plant and first-ratoon crops is 300 nematodes/200 g of soil (Magarey 2013). Over 76% of the farm surveyed had root-lesion nematode numbers that were above this threshold. We developed a nematode-hazard index (HI) that incorporates relative values of nematode species, in terms of damage, compared to root-knot nematodes. The HI values are independent of individual species of nematodes and are easy to estimate nematode damage threshold when multiple species of plant parasitic nematodes are present.

Correlations among nematode communities and cane yield

Cane yields (t/ha) showed moderate (r=0.32) correlation with numbers of bacterivores and weak negative, but significant (r=–0.26) correlations with numbers of stunt nematodes (Table 5). Numbers of lesion nematodes showed strong (r>0.8) correlations with HI values and total numbers of plant-parasitic nematodes (PPN), and moderately correlated with bacterivores. The numbers of bacterivores had weak to moderate correlations with yield, lesion nematode and HI values. Nematode HI values showed strong correlations with numbers of lesion nematodes, total nematodes PPN and stubby nematodes. The strong correlations of numbers of lesion nematodes with total PPN numbers and HI is indicative of the major contribution of the variations in numbers of this nematode.

***Table 5*.** Correlations among plant-parasitic and free-living nematodes, yield (TCH) and HI values.

Factors	Cane yield	Stubby	Pin	Total PPN	HI	Bacterivores	Fungivores	Omnivores
Lesion		0.30*		0.83***	0.91***	0.29*		
Root-knot		0.31*					0.26*	
Spiral				0.28*				
Stunt	–0.26*						0.28*	
Total PPN		0.37*			0.97***	0.31*		
Total free living			0.28*			0.68***	0.75***	
HI		0.35**		0.97***		0.32*		
Bacterivores	0.32*	0.43*	0.36**	0.31*	0.32*		0.37**	
Predators								0.28*

* = significant ≤0.05; ** = significant ≤0.001, *** = significant ≤0.0001. Total PPN = total plant parasitic nematodes, HI= nematode hazard index.

Principal-component analysis (PCA)

The first factor (PC1) of the PCA described 53.34% of the variability and the second factor (PC2) described 27.56% of the variability (Figure 3). Along the PC1, root-lesion (RLN), HI, total PPN, and total free-living nematodes were major factors and closely located on the right side of the plot. Relative proportions of root-knot nematodes (RKN) were correlated to the negative side of the plot opposed to RLN.

The PC2 was mainly contributed to by bacterivores, stubby and stunt nematodes, where stunt nematodes were more or less opposed to stubby and bacterivores. Bacterivores and stubby nematodes were strongly correlated to pin, dagger and ring nematodes, and yield. Although, dagger, ring and spiral nematodes were located in the vicinity of the plan centre indicating that they did not differ much among treatments and had little impact on the changes.

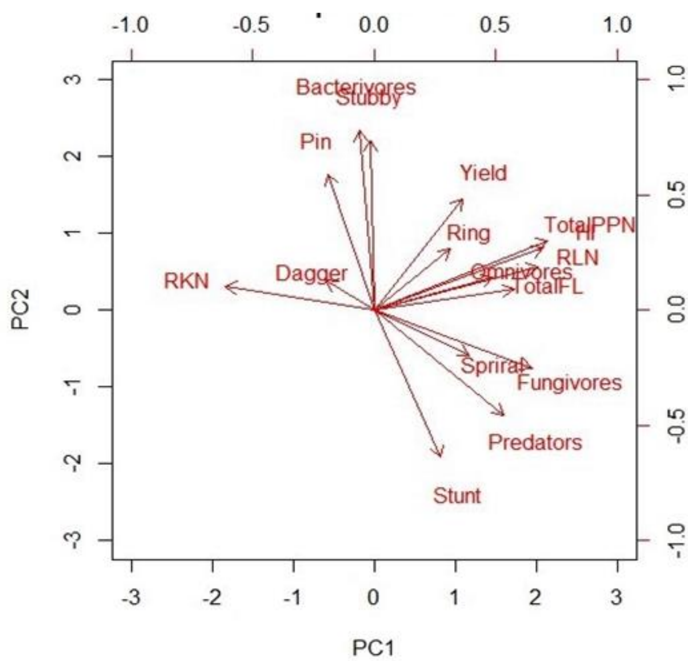

Figure 3. Principal-component analysis of nematode communities and yield. Nematode data were log-transformed before analysis. RKN= root-knot nematodes, RLN=root-lesion nematodes.

Soil food-web maturity index

Soil maturity index, MI was calculated by including enrichment opportunistic taxa (c-p 1), and MI2-5 was calculated by excluding the c-p 1 group in order to determine the long-lasting environmental effects (Figure 4). No significant differences were found among the maturity indices among productivity zones. Both maturity indices were less than 3, indicating low soil-food web maturity, persistence of chemical fertilisers, and high sensitivity to soil-borne pests and diseases (Sanchez-Moreno and Howard 2018). Due to the continuous monoculture of sugarcane soil, the low soil web maturity indices were not unexpected. Growers can use these indices as a guide to improve soil health by increasing complex source of organic matter, reduce chemical fertiliser and reduce soil physical perturbation (Sanchez-Moreno and Howard 2018).

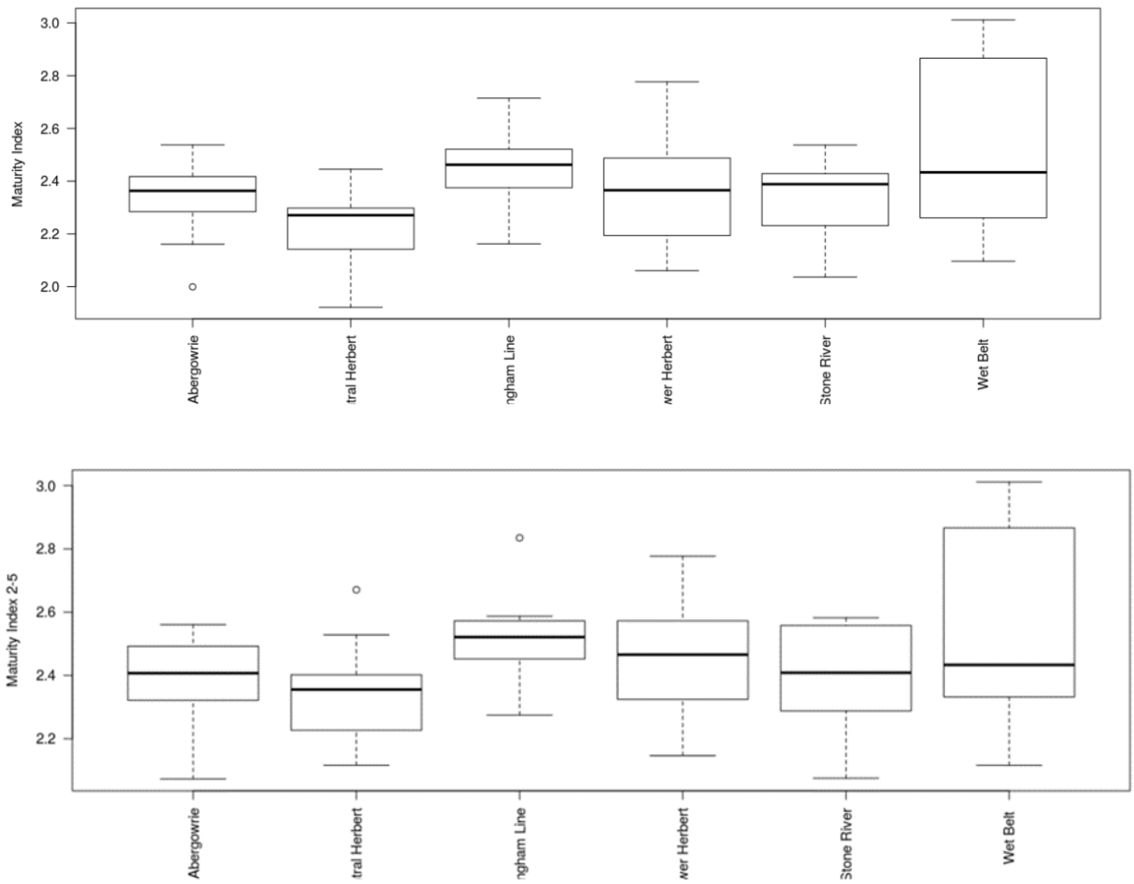

Figure 4. Nematode maturity indices for six sugarcane productivity zones in Herbert.

Nematode c-p triangle

By plotting the community groups in a c-p triangle plot, most groups of the nematodes belong to c-p2 and c-p3-5 groups, where the former group was dominant (Figure 5). The majority of the nematodes were from c-p2 group, which indicated physical or chemical stress. De Goede *et al.* (1993) described the nematodes of c-p2 group as most abundant in the soil and are resistant to pollution or stress.

The main advantage of the c-p triangle is that it can be employed for short-term monitoring, such as impact of application of fertiliser or chemical or tillage, or for long-term monitoring of soil health, such as monitoring of nematode succession over time under a particular farming system (De Goede *et al.* 1993).

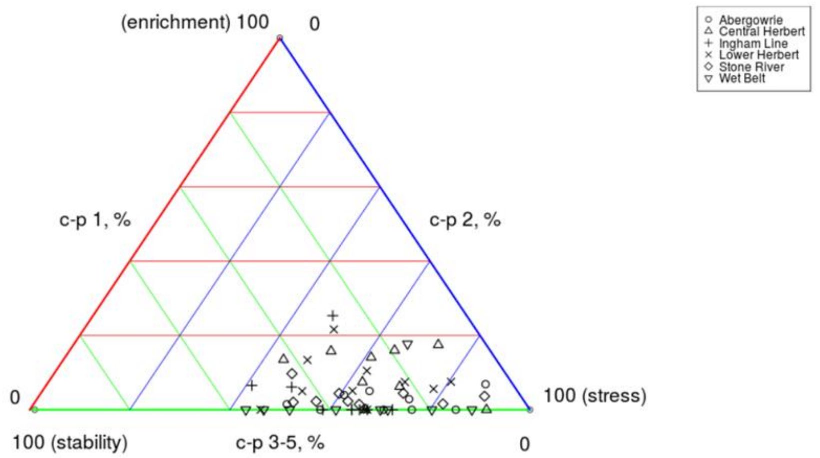

Figure 5. Representation of the enrichment profile (c-p triangle), unweighted proportional representation of cp-1, cp-2 and cp-3–5 groups of the nematode fauna in sugarcane field.

Soil food-web condition

Out of 56 sample locations, 15 (27%) indicated an extremely depleted and stressed condition (Figure 6, lower-left quadrant), four (7%) were highly enriched and disturbed with a bacterial dominated system (Figure 6, upper-left quadrant), seven (12.5%) were low to moderately disturbed and in a N-enriched system (Figure 6, upper-right quadrant), and 30 (54%) sample locations were relatively undisturbed but stressed system with moderate or highly structured system (Figure 6, lower-right quadrant).

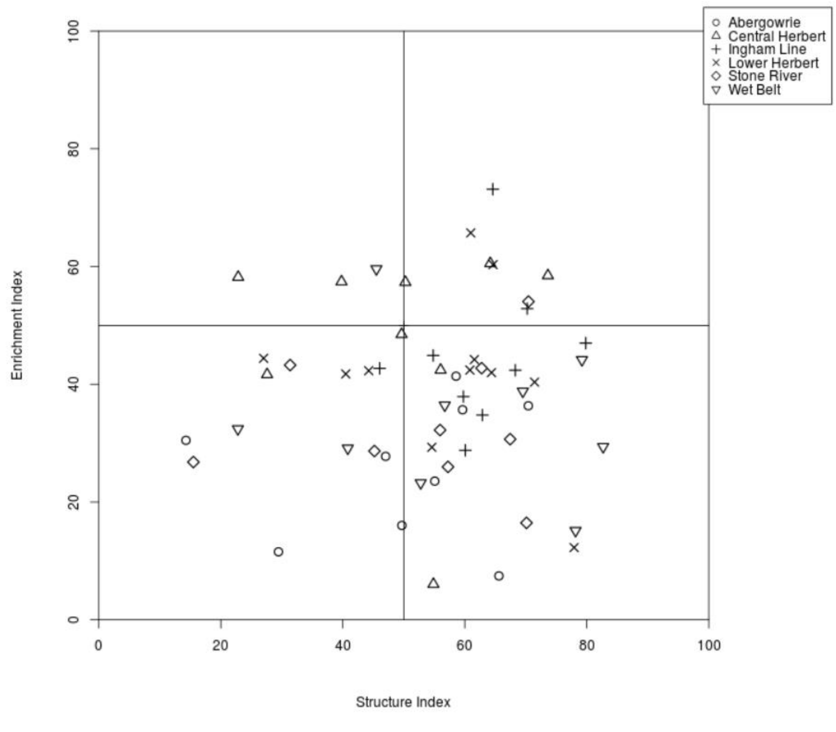

Figure 6. Soil food web conditions of six sugarcane productivity zones in the Herbert region. Higher enrichment index (EI) values are associated with higher soil fertility, and higher structure index (SI) values are indication of higher soil suppressiveness to opportunistic or invasive species.

Nematode metabolic footprint

Most of the sugarcane fields surveyed were dominated by herbivores (Figure 7). This is an indication of greater carbon and energy possessed predominantly by plant parasitic and other herbivores nematodes. This is also an indication of an intensive agricultural system with high soil perturbation and high herbivore pressure. In an ideal agricultural system of low herbivore pressure, high mineralisation service and high pest suppression would be presented by a balanced, cup-shaped figure (Sanchez-Moreno and Howard 2018).

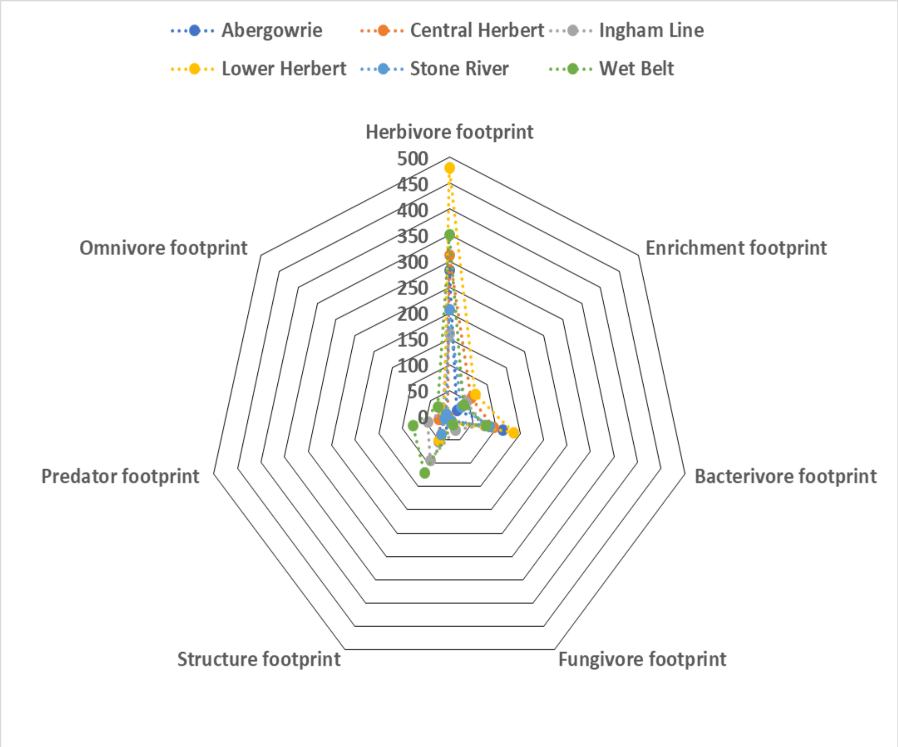

Figure 7. Radar chart of nematode metabolic footprints of 56 sugarcane fields from six sugarcane productivity zones in the Herbert.

CONCLUSIONS

Plant-parasitic nematodes are one of the major constraints of sugarcane productivity in Australia. We developed a nematode hazard index (HI) that is independent of nematode types or species. Our study indicated that over 70% of the farms surveyed had medium to high hazard index values and are subject to potential significant yield loss. Among plant-parasitic nematodes, lesion nematodes were found in all sugarcane fields in moderate to high numbers, indicating a potential contributor of yield loss. Root-knot nematodes were not prevalent, except for a few farms. A comprehensive survey is needed to quantify the extent of plant-parasitic nematodes across the Australian sugar industry.

Our study is the first to demonstrate that soil health of sugarcane farms can be measured by nematode community analysis. Analyses revealed low maturity indices (MI and MI2-5) (<3.5) in all farms surveyed, which is indicative of low soil food web maturity and persistent use of chemical fertiliser. Soil food-web indices and metabolic footprints indices also indicated perturbation of sugarcane soil and a farming system dominated by herbivorous nematodes.

Our study demonstrated that a nematode community analysis can be used as one of the indicators of soil health for the sugar industry as well as a monitoring tool for determining the impact of management practices. However, a set of comprehensive and independent measurements are needed for industry application.

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