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# Survey of plant-parasitic and free-living nematodes in New South Wales cane-growing areas

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**Abstract** Plant-parasitic nematodes are important pests of sugarcane worldwide, causing more than $80 million loss in productivity per year in Australia. A second group of nematodes are known as 'free-living' and feed on bacteria, fungi or on other nematodes. 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 three mill areas in New South Wales, and the use of nematode community analysis to determine the soil health of the surveyed sugarcane farms. Soil samples were collected from 43 locations from three mill areas, Broadwater, Condong, and Harwood. Sixteen farms (37%) surveyed had medium (>300 nematodes/200 g soil) and 19 farms (44%) had high (>800 nematodes/200 g soil) numbers of root-lesion nematodes. Spiral nematodes were the only other abundant species and occurred in all farms surveyed. A nematode hazard index (HI) was developed by adding the weighted number of plant-parasitic nematodes. Approximately 93% of surveyed farms had medium (>300) to high (>800) HI values, an indication of moderate (5% to 20%) to high (>20%) potential yield loss. Soil food-web indices and metabolic-footprint indices also indicated perturbation of sugarcane soil and a farming system dominated by herbivorous nematodes. This survey demonstrated that plant-parasitic nematodes are prevalent in New South Wales cane-growing areas and are probably causing yield loss.

**Key words** Sugarcane nematodes, nematode community analysis, soil health, root-lesion nematodes, nematode hazard index, soil health indicator

## INTRODUCTION

Nematodes are worm-like organisms, mostly microscopic, present in all agricultural soil including sugarcane production environments. Several species of nematodes are important pests of agricultural crops, including sugarcane. They are also an important part of the soil ecosystem and play an important role in the movement of soil carbon and nitrogen. In sugarcane, more than 317 species of 48 genera of plant-parasitic nematodes have been recorded from roots and rhizospheres (Ramouthar and Bhuiyan 2018). Some genera, such as *Pratylenchus* (lesion nematodes), *Meloidogyne* (root-knot nematodes) and *Helicotylenchus* (spiral nematodes), are more common and widespread in cane fields in Australia. Among them, lesion and root-knot nematodes are considered the most destructive nematodes in sugarcane. Nematode pests of sugarcane cause in excess of 15% yield loss in Australia, costing more than $80 million 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), the last targeted survey for the Queensland sugarcane industry was conducted 20 years ago and no survey was conducted for the New South Wales (NSW) sugar industry. As a result, no information is available on the prevalence and types of nematodes in NSW cane-growing areas.

Change of management practices, adoption of new varieties and climate change can influence the prevalence and impact of pests and diseases. An ongoing monitoring and survey of plant-parasitic nematodes in Australian sugarcane fields is long overdue.

Over the decades, our knowledge of nematode communities and their relationship to soil health has been enriched significantly. 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 Ferris 2018; Ferris *et al.* 2001; Neher 2001; De Goede *et al*. 1993).

Given this, we surveyed NSW cane-growing areas to determine the extent of plant-parasitic nematodes and their potential impact on sugarcane production, and explored the use of nematode community analysis to determine the soil health of each surveyed sugarcane farm.

## MATERIALS AND METHODS

Soil samples were collected in February and March 2019 from 43 locations covering three mill areas in the NSW cane-growing region (Table 1) using standard protocols (https://sugarresearch.com.au/sugar_files/2017/02/Pachymetra-and-Nematode-sampling-procedure.pdf), and sent to SRA Woodford for assessment. The soil types varied from clay, peat, loam to sandy soil. Surveyed farms were under either second ratoon of one-year crops, or first ratoon of two-year crops planted with variety Q208.

***Table 1.*** List of 43 soil samples collected from three mill areas in NSW in 2019.

| Mill area | No of samples | Soil type | Crop type and class |
|---|---|---|---|
| Broadwater | 13 | Clay, Peat, Loam, Sandy loam, Peaty clay, Clay loam | 2-year, first ratoon |
| Condong | 16 | Clay, Loam, Peat, Sandy Peat, Sandy loam | 1-year, second ratoon |
| Harwood | 14 | Sandy loam, River alluvial, Clay, Silty Clay, Clay loam, Loam | 2-year, first ratoon |

Nematodes were extracted from soil samples using a modified Whitehead tray method (Whitehead and Hemming 1965). Approximately 200 g of each soil sample (with sugarcane roots) was placed on double-layered tissue paper on a steel mesh set in a flat tray. The sample was submerged in water, left for 48 hours and then the nematodes were collected using a 38 µm sieve and stored in a 20 ml vial at 5 °C until enumeration under a light microscope at 10x and 40x magnification. Approximately 100 g of fresh soil was maintained at 105 °C for 1 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.

Yield data as tonnes of cane per hectare (TCH) was recorded for each sample site (taken from subsequent harvest).

### Plant-parasitic nematodes

To estimate the collective impact of parasitic nematodes, we developed a hazard index (HI). The HI value is determined 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 causes 80% (hence, hazard factor = 0.8) of the damage caused by root-knot nematodes, and a single stunt nematode causes 25% of the damage caused by root-knot nematodes (Table 2) (Bhuiyan *et al.* 2020).

***Table 2***. Plant-parasitic nematodes, pathogen status and hazard indices.

| Nematode 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 (Criconematidae) | Moderately pathogenic | 0.25 |
| *Spiral (Helicotylenchus* spp., *Rotylenchus* spp.) | Mildly pathogenic | 0.05 |
| Reniform (*Rotylenchulus* sp.) | Mildly pathogenic | 0.05 |
| Pin (*Paratylenchus* sp.) | Mildly pathogenic | 0.05 |

The Hazard index (HI) for a particular soil sample was calculated by adding the number of nematodes after multiplying with their respective hazard factors. For this survey:

*HI = Lesion*0.8+Root-knot*1+Dagger*0.25+Stubby*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 HI threshold. (Bhuiyan *et al.* 2020).

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

## 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. To compute these indices, nematodes were assigned to colonizer-persister (c-p) values according to Bongers (1990) (Table 4). The c-p values range from enrichment colonizers (c-p 1), disturbance colonizer (c-p 2), to persisters (c-p 4 and c-p 5). C-p 1 nematodes are bacterivores, have a short life cycle and high fecundity, and are only active during high bacterial generation. The c-p 2 group have a relatively short life cycle, include 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).

***Table 4.*** Nematode families belong to c-p groups (Bongers 1990).

| Family | c-p index | Family | c-p index |
|---|---|---|---|
| Neotylenchidae | 2 | Achromadoridae | 3 |
| Anguinidae | 2 | Ethmolaimidae | 3 |
| Aphelenchidae | 2 | Cyatholaimidae | 3 |
| Aphelenchoididae | 2 | Desmodoridae | 3 |
| Rhabditidae | 1 | Microlaimidae | 3 |
| Alloionematidae | 1 | Odontolaimidae | 3 |
| Diploscapteridae | 1 | Aulolaimidae | 3 |
| Bunonematidae | 1 | Bastianiidae | 3 |
| Cephalobidae | 2 | Prismatolaimidae | 3 |
| Ostellidae | 2 | Ironidae | 4 |
| Panagrolaimidae | 1 | Tobritidae | 3 |
| Myolaimidae | 2 | Onchulidae | 3 |
| Teratocephalidae | 3 | Tripylidae | 3 |
| Diplogasteridae | 1 | Alaimidae | 4 |
| Neodiplogasteridae | 1 | Bathyodontidae | 4 |
| Diplogasteroididae | 1 | Mononchidae | 4 |
| Tylopharyngidae | 1 | Anatonchidae | 4 |
| Odontopharyngidae | 1 | Nygolaimidae | 5 |
| Monhysteridae | 1 | Dorylaimidae | 4 |
| Xyalidae | 2 | Chrysonematidae | 5 |
| Linhomoeidae | 3 | Thornenematidae | 5 |
| Plectidae | 2 | Nordiidae | 4 |
| Leptolaimidae | 3 | Qudsianematidae | 4 |
| Halaphanolaimidae | 3 | Aporcelaimidae | 5 |
| Diplopeltidae | 3 | Belondiridae | 5 |
| Rhabdolaimidae | 3 | Actinolaimidae | 5 |
| Chromadoridae | 3 | Discolaimidae | 5 |
| Hypodontolaimidae | 3 | Leptonchidae | 4 |
| Choanolaimidae | 4 | Diphtherophoridae | 3 |

To identify nematode food web properties, the enrichment index (EI) and structural index (SI) were calculated according to Ferris *et al.* (2001) (Figure 1). These two indices are calculated from the weighted abundance of nematode guilds that comprise of bacterivores ($Ba_x$), fungivores ($fu_x$), predators or carnivores ($Ca_x$), and omnivores ($Om_x$ (x = 1–5 of the c-p scale). The EI is based on the biomass of bacterivore and fungivoresnematodes with lower c-p values (c-p 1 and 2) that respond rapidly to the increase in bacterial and fungal populations that arise from organic matter decomposition. High values indicate high soil enrichment and high fertility. The EI value was calculated as:

$$EI = 100 \times \frac{e}{e + b}$$

where *e* represents weighted frequencies of bacterivores, and *b* represents weighted frequencies of fungivores. The SI is based the on functional guilds of nematodes with higher c-p values ranging from c-p 3 – 5, represents more stable conditions of soil, and is calculated as

$$SI = 100 \times \frac{s}{s + b}$$

where *s* represents weighted frequencies of higher functional nematode guilds (groups) (cp 3 – 5), and *b* represents weighted frequencies for lower functional nematode guilds (cp 1 – 2).

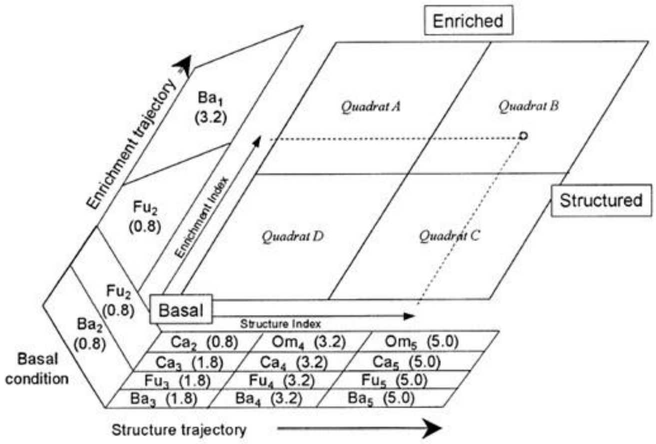


***Figure 1.*** Representation of the nematode faunal profile that indicates whether the soil community is enriched but unstructured (Quadrat A), enriched and structured (Quadrat B), resource limited and structured (Quadrat C), or resource depleted with minimal structure (Quadrat D) (from Ferris *et al.* 2001). Nematode taxa with the same feeding habits and inferred function (Functional guild) in the food web are given as $Ba_x$, $Fu_x$, $Ca_x$, $Om_x$ (x = 1–5 of the c-p scale), where Ba, Fu, Ca and Om are bacterivores, fungivores, carnivores and omnivores, respectively.

Nematode footprints are based on the biomass and metabolic activity of the assemblage with each trophic group or functional guild calculated as described elsewhere (Ferris 2010).

## 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 the 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).

## RESULTS AND DISCUSSION

### Plant-parasitic nematodes

Numbers of root-lesion nematode were high in all surveyed fields, and soil samples collected from Broadwater peat soil had the highest (1,725 nematodes/200 g soil) number of lesion nematodes followed by Condong sandy peat soil (1,348 nematodes/200 g soil) (Table 5). Among other plant-parasitic nematodes, only spiral nematodes were prevalent in relatively high number in all surveyed farm, ranging from 40 nematodes/200 g soil to >1800 nematodes/200 g soil.

***Table 5.*** Number and range of nematodes (/200 g of dry soil) and yield (TCH), in the three mill areas.

| Mill area | Soil type | [1]TCH | RLN | Spiral | Other PPN | Total PPN | Total FL |
|---|---|---|---|---|---|---|---|
| Broadwater | Clay | 164(±35) | 879(±178) | 322(±75) | 60(±16) | 1260(±217) | 1995(±207) |
| Broadwater | Clay loam | 118 | 761 | 119 | 14 | 894 | 1487 |
| Broadwater | Loam | 180 | 2149 | 227 | 168 | 2545 | 1162 |
| Broadwater | Peat | 181(±37) | 1725(±848) | 1820(±965) | 143(±75) | 3692(±196) | 4003(±320) |
| Broadwater | Peaty caly | 102 | 439 | 141 | 59 | 638 | 1821 |
| Broadwater | Sandy loam | 90(±5) | 395(±44) | 247(±174) | 83(±55) | 727(±188) | 1655(±312) |
| Condong | Clay | 104(±11) | 768(±235) | 206(±87) | 25(±7) | 1007(±245) | 2700(±544) |
| Condong | Loam | 145 | 330 | 287 | 43 | 667 | 2674 |
| Condong | Peat | 122±12 | 1015(±104) | 556(±214) | 113(±65) | 1690(±322) | 2278(±287) |
| Condong | Sandy loam | 60 | 852 | 357 | 34 | 1248 | 3197 |
| Condong | Sandy peat | 89(±11) | 1348(±383) | 288(±87) | 25(±2) | 1661(±460) | 1969(±230) |
| Harwood | Clay | 207 | 618 | 40 | 11 | 669 | 800 |
| Harwood | Clay loam | 226 | 1242 | 545 | 32 | 1819 | 1236 |
| Harwood | Loam | 137 | 733 | 1140 | 55 | 1928 | 2169 |
| Harwood | River aluvial | 292 | 111 | 55 | 31 | 197 | 1281 |
| Harwood | Sandy loam | 182 (±19) | 649(±229) | 467(±220) | 59(±22) | 1177(±372) | 2586(±868) |
| Harwood | Silty clay | 169(±19) | 842(±151) | 151(±65) | 44(±16) | 1038(±187) | 1401(±154) |

[1]TCH = tonnes of cane/ha, RLN = root-lesion nematodes, Spiral = spiral nematodes, PPN = plant-parasitic nematodes, FL = free-living nematodes. Values with ± standard error) of mean for each soil type.

Nematode hazard indices (HI) ranged from approximately 33 to over 1300 (Figure 2). Only three of 43 farms (7%) surveyed possessed a low HI value, compared to 23 (53%) and 17 (40%) of 43 farms that had medium and high HI values respectively (Figure 2).

The results from the survey clearly indicated that lesion nematodes (*Pratylenchus* spp*.)* were the dominant plant-parasitic nematodes in the NSW cane-farming system, present in large numbers in almost all sugarcane fields surveyed. This is consistent with a recent survey in the Herbert cane-growing region, and previous work that found that root-lesion nematodes are the most common parasitic nematodes associated with sugarcane and can cause significant production loss (Bhuiyan *et al.* 2020; Stirling and Blair 2000). All plant-parasitic nematodes can cause significant damage to sugarcane crops if present above the threshold levels. The threshold for root-lesion nematodes for plant and first ratoon crop is 300 nematodes/200g of soil (Magarey 2013), and over 95% of the farms that we surveyed had root-lesion nematode numbers above the threshold.

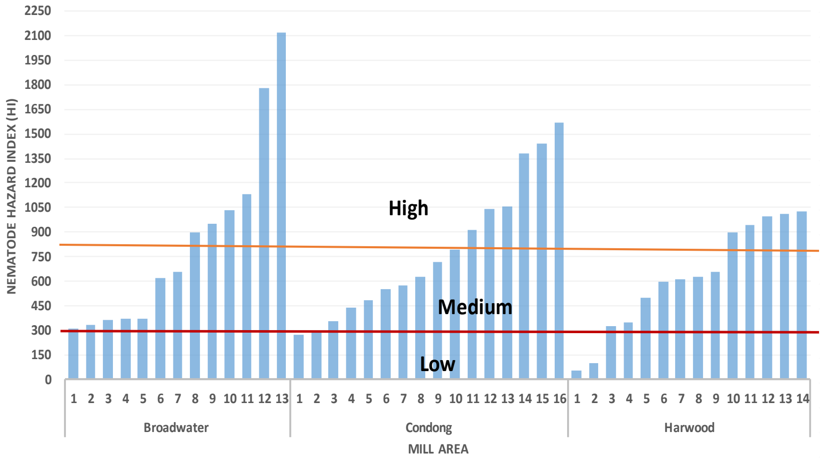


***Figure 2.*** Nematode hazard indices (HI) for farms in the three mill areas. Values between the red and orange lines indicate medium HI and above the orange line indicate high HI (Table 3).

Our nematode hazard index (HI), which incorporates relative numbers of nematode species with the level of crop damage they cause, is independent of individual species of nematodes and simplifies the interpretation of the production risk when multiple species of plant-parasitic nematodes are present. In our survey, plant-parasitic nematodes, and root-lesion nematodes in particular were present in high numbers at most locations, which presumably results in significant regional production losses.

## Correlations among nematode communities and yield (TCH) component

Sugarcane yield (TCH) showed weak or weak negative correlation with all parasitic and free living (*Dorylim*, *Monochid*, *Rhabit* and *Tylenchid*) nematode genera (Figure 3). Lesion nematodes showed strong (r=0.99) correlations with total plant-parasitic (PPN) and other parasitic nematodes, and were moderately correlated with bacterivores. The strong correlations of lesion and spiral nematodes with total PPN indicates that the major source of variation was due to these nematodes.

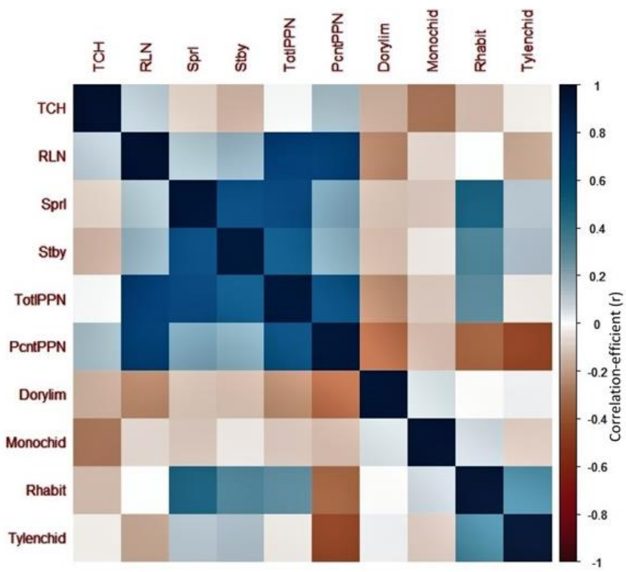


**Figure 3.** Correlations among plant-parasitic and free-living nematodes, and yield. TCH= tonnes of cane/ha, RLN = root-lesion nematodes, Sprl= spiral nematodes, Stby = stubby nematodes, TotlPPN = total plant-parasitic nematodes, PcntPPN = percent of PPN.

## Nematode c-p triangle

By plotting the community groups as a c-p triangle plot, most nematodes belonged to the c-p 2 and c-p 3-5 groups, (Figure 4). The majority of the nematodes were from the c-p 2 group, which indicated physical or chemical stress. De Goede *et al.* (1993) described the nematodes of c-p 2 group as most abundant in the soil and resistant to pollution or stress. The main advantage of the c-p triangle is that it can be employed for monitoring soil health in the short term, such as the impact of application of fertiliser or chemical or tillage, or in the long-term, such as monitoring of nematode succession over time under a particular farming system (De Goede *et al.* 1993).

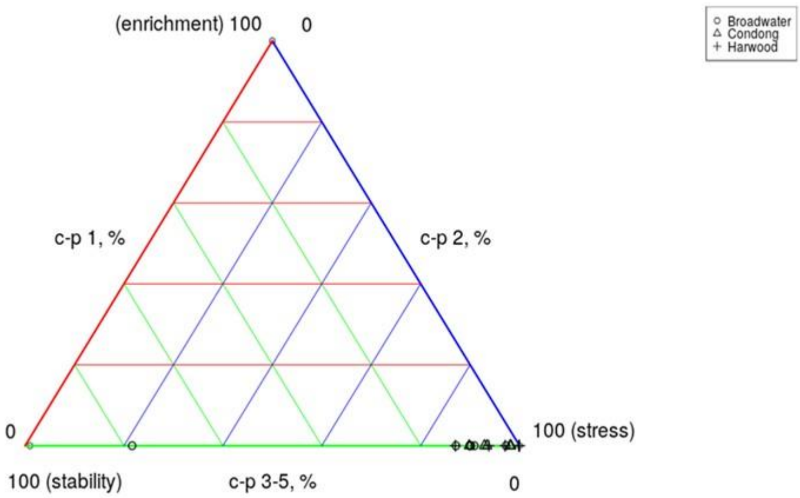


***Figure 4***. Representation of the enrichment profile (c-p triangle), the unweighted proportional representation of c-p 1, c-p 2 and c-p 3–5 groups of the nematode fauna in sugarcane fields in the three sugarcane mill areas.

## Soil food-web condition

Of the 43 sample locations, 42 (98%) had extremely depleted and stressed conditions (Figure 5, lower left quadrant), and only one (2%) location was a relatively undisturbed but stressed system with a moderate or high structured system.

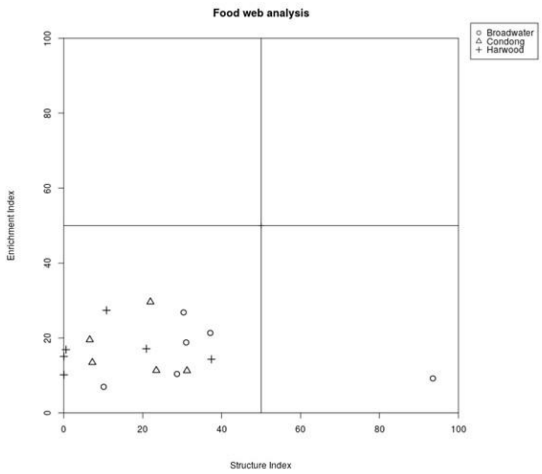


***Figure 5***. Soil food-web conditions of the three mill areas in the NSW region. Higher enrichment index (EI) values are associated with higher soil fertility, and higher structure index (SI) values are indicative of greater soil suppressiveness to opportunistic or invasive species.

## CONCLUSIONS

Although plant-parasitic nematodes are one of the major constraints to sugarcane productivity in Australia, this is the first report on the prevalence of plant-parasitic nematodes in NSW cane-growing areas. We developed a nematode hazard index (HI) that is independent of nematode types or species. Our study indicated that over 93% of the farms surveyed had medium to high hazard index values and are likely subject to significant yield loss. Among the plant-parasitic nematodes, lesion nematodes were found in all sugarcane fields in moderate to high numbers, while spiral nematodes were commonly identified but are expected to be a minor driver of yield losses. Root-knot nematodes were not prevalent, except on a few farms. As expected, there was weak relationship between productivity and nematode numbers for the crops in the survey, indicating that there are issues other than nematodes driving the range in Q208 cane yield from 60 t/ha to 226 t/ha. The farms identified with very high root lesion nematode numbers will be potential options for validating the value of clones with nematode resistance currently under development by Sugar Research Australia.

A comprehensive survey to quantify the extent of plant-parasitic nematodes would confirm the scale of economic losses across the Australian sugar industry. Analyses of the Herbert and NSW production areas have demonstrated that root-lesion nematodes are common and may be a major driver of significant yield limitations. Our findings from NSW and Herbert suggests that yield loss due to plant-parasitic nematodes due to plant-parasitic nematodes are underestimated.

Soil food-web indices and metabolic footprints indices indicated perturbation of sugarcane soils and a farming system dominated by herbivorous nematodes. We 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.

## ACKNOWLEDGMENTS

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