

Amendments of sugarcane trash induce suppressiveness to plant-parasitic nematodes in a sugarcane soil

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Abstract. In a field experiment at Bundaberg, Queensland, sugarcane trash was incorporated into soil with, or without, additional nitrogen supplied as either soybean residue or ammonium nitrate. The carbon inputs from plant material (10 t C/ha) were the same in all treatments, while both plus-nitrogen treatments received the same amount of nitrogen (210 kg N/ha). Sugarcane was planted 23 weeks after amendments were incorporated, and 24 weeks later there were 95% fewer lesion nematodes (*Pratylenchus zae*) in roots growing in amended soil than in roots from the unamended control. Populations of *P. zae* and *Tylenchorhynchus annulatus* in amended soil were reduced by 85% and 71%, respectively. Bioassays in which forage sorghum was planted in soil collected during the experiment and inoculated with *P. zae* indicated that amended soils were suppressive to the nematode at 20 weeks but not after 47 weeks. All amendments increased readily oxidisable carbon, microbial biomass, microbial activity and numbers of free-living nematodes, but had no effect on known predators of nematodes (i.e. dorylaimid and mononchid nematodes and three naturally occurring species of nematode-trapping fungi, *Arthrobotrys conoides*, *A. thaumasium* and *Drechlerella dactyloides*). However, an unidentified predatory fungus was found only in soil amended with sugarcane trash and was possibly involved in the suppression of plant-parasitic nematodes.

Additional keywords: soil nitrogen, nematophagous fungi, soil food webs, biological control.

Introduction

Lesion nematode (*Pratylenchus zae*) is an important pest of sugarcane in Australia and, together with a suite of other plant-parasitic nematodes (Blair *et al.* 1999a, 1999b), is one of many physical, chemical and biological constraints reducing the productivity of soil with a long history of sugarcane monoculture (Garside *et al.* 1997; Stirling *et al.* 2001). Since previous studies have shown that some organic amendments enhance the suppressiveness of sugarcane-growing soil to *P. zae* (Stirling *et al.* 2003), we explored the possibility of using plant residues that are readily available in the sugar industry for nematode control. One such material is sugarcane trash (the tops and leaves remaining in the field after sugarcane is mechanically harvested), as 25 t/ha of dry sugarcane residue is left on the soil surface when crops yielding ~125 t/ha are harvested (Mitchell and Larsen 2000).

Soybean residue was also used because ~7.5 t/ha of dry matter is produced when a soybean crop is grown between sugarcane cycles (Garside *et al.* 1998).

Previous work on organic amendments for nematode control (reviewed by Stirling 1991; Akhtar and Malik 2000) has mainly been done with nitrogen-rich amendments such as animal manure, meat and bone meal, soy meal, oil cakes and chitin. When these amendments are applied to soil at application rates of 5–100 t/ha, nematicidal compounds such as ammonia and nitrous acid are produced at concentrations that are sufficient to kill plant-parasitic nematodes (Rodriguez-Kabana 1986; Lazarovits *et al.* 1999; Oka and Yermiyahu 2002). However, nematicidal effects are relatively short-lived because ammonia concentrations remain high for a limited time. The high inputs of nitrogen associated with these amendments are also potentially

damaging to human health and the environment (Cowling *et al.* 2001; Oka and Pivonia 2002). An alternative approach is to use organic amendments to enhance naturally occurring mechanisms of biological control. The pioneering work of Linford *et al.* (1938) showed that free-living nematodes multiply during the decomposition of organic matter and that these nematodes support large populations of nematode-capturing fungi, non-trapping fungal parasites, predacious nematodes and predacious mites. Collectively, these predators kill many nematodes, including plant-parasitic nematodes, and are one of the reasons that nematode control is consistently obtained with organic amendments (Muller and Gooch 1982; Stirling 1991).

In our initial work with organic amendments in the sugar industry (Stirling *et al.* 2003; Pankhurst *et al.* 2005), eight different organic materials were added to a sugarcane-growing soil and changes in selected soil chemical and biological properties were monitored over the following 12 months. Results from laboratory bioassays showed that amendments with a high carbon to nitrogen ratio (e.g. sawdust, grass hay and sugarcane trash) induced suppressiveness to *Meloidogyne javanica* and *Pratylenchus zaei* 4 and 7 months after they were added to the soil. In contrast, nitrogen-rich amendments (e.g. lucerne hay, feedlot manure, poultry manure, chitin and mill mud) did not suppress plant-parasitic nematodes. Multiple regression analyses indicated that suppression of nematodes was associated with low levels of nitrate-nitrogen in soil, a fungal-dominant soil biology and high numbers of omnivorous nematodes (Stirling *et al.* 2003). Since some predatory hyphomycetes and several genera of wood-decaying basidiomycetes are known to be most active in nitrogen-limited habitats (Barron 1992; Tzean and Liou 1993), predation by fungi and possibly nematodes was suspected to have been partly responsible for the nematode control obtained.

This paper describes experimental results which indicate that the nematode suppression previously detected in laboratory and glasshouse bioassays can be reproduced in the field. The results also confirm the suppressive effects of amending soil with sugarcane trash and show that suppression is maintained when nitrogen is added. Possible mechanisms of suppression are also discussed.

Methods

Application of organic amendments

A field near Bundaberg, Queensland (24°50'S 153°30'E) with a yellow podsollic soil [a yellow dermosol according to the classification of Isbell (1996)] was used for the experiment. The field had grown sugarcane for many years, but had been bare-fallowed for the previous 18 months. Before the experiment commenced, soil samples were collected from each plot (ten cores with a 25-mm-diameter sampling tube to a depth of 15 cm) and nematodes were extracted using methods described later. Four treatments, each replicated five times in a randomised block design, were then established at

the site: a non-amended control (NIL), sugarcane trash at 25 t dry matter/ha (SCT), sugarcane trash at 15 t dry matter/ha plus soybean trash at 10 t dry matter/ha (SCT + S) and sugarcane trash at 25 t dry matter/ha plus 210 kg N/ha applied as ammonium nitrate (SCT + N). Since both plant materials had a carbon content of ~40%, ~10 t C/ha was applied to the amended soil. The sugarcane trash contained 0.15% N, which meant that the nitrogen input from this source was either 23 or 38 kg N/ha (SCT + S and SCT, respectively). Two treatments (SCT + S and SCT + N) received an additional 210 kg N/ha from either an organic source (soybean trash, 2.1% N) or an inorganic source (ammonium nitrate, 38% N). All materials were applied on 26 March 2002 and incorporated to a depth of ~10 cm with a rotary hoe. Plots were then kept free of weeds by spraying with glyphosate as required. Each plot was 3 × 3 m, with one half used later to grow sugarcane and the other half reserved for soil sampling.

Experiment with sugarcane

On 2 September 2002 (23 weeks after amendments were incorporated), a raised bed 1 m wide was formed in each plot by using a rake to move ~10 cm of top soil from an area 25 cm wide on either side of the bed. Two trenches 40 cm apart were made in the newly formed bed and fertiliser (13% N, 14% P and 13% K) was added to each trench at a rate of 20 g/m of trench. Fourteen 3-bud billets of sugarcane (cv. Q188) were treated for pests and diseases of planting material and emerging roots [a 5-min dip in Sportak (prochloraz) at 0.2 g/L water followed by a spray with Lorsban at 1.5 L/ha] before they were planted by hand in each plot and covered with soil. Because of shortages due to drought, plots received little irrigation water and the crop was mainly sustained by 60, 60, 30 and 180 mm of rain that fell in mid-October, early November, mid-December and early February, respectively.

The number of primary shoots in each plot was counted 30 days after planting. Soon after secondary shoots had started to emerge (54 days after planting), plants growing from the billets at the end of each row (i.e. a total of four billets/plot) were removed, together with roots and associated soil. Nematodes were extracted from roots by intermittent misting (Southey 1986), and from soil by spreading it on an extraction tray for 4 days (Whitehead and Hemming 1965). In both cases, nematodes were recovered by sieving twice on a 38- μ m sieve and plant-parasitic nematodes were counted. Roots and shoots were also dried and weighed.

The remaining plants grew until 19 February 2003 (170 days after planting or ~47 weeks after organic amendments were incorporated), when the total number of stalks and the number of stalks damaged by the large moth borer (*Bathytricha truncata*) were counted. Stalks and other above-ground material were then removed and weighed, and the amount of fresh biomass was converted to dry weight by drying representative samples. Soil samples (ten cores per plot taken with a 25-mm-diameter sampling tube) were collected from around the base of plants at a depth of 0–15 cm. A composite sample of roots from five points in the same sampling area was also collected from 0–15 cm. Nematodes were extracted from roots and soil as described for the first harvest.

Schedule for monitoring the soil chemistry and biology

Soil chemical and biological changes were monitored over the course of the experiment by collecting soil samples from a 3 × 1.5 m section of each plot that was adjacent the planted area and left undisturbed throughout the experiment. Plots were sampled on 6 May, 20 June, 20 August and 13 October 2002 and 10 March 2003 (~6, 12, 20, 28 and 50 weeks after amendments were incorporated) by removing 10 L of soil from a depth of 0–15 cm. To avoid sampling the same area twice, soil was taken each time from a strip 3 × 0.3 m that had not previously been sampled. Soil was assayed for suppression to lesion nematode and

a range of chemical and biological parameters were measured using methods described below.

Bioassay for suppression of lesion nematode

Soil from each plot was bioassayed for suppressiveness to lesion nematode 20, 28 and 50 weeks after amendments were incorporated. At each of these sampling times, the number of *P. zeae* in 200 mL soil samples from each plot was determined using the tray method described previously. Pots were then filled with 400 mL of soil from each plot and inoculated with 2000 *P. zeae* from cultures maintained on sugarcane. Since the number of *P. zeae* extracted from soil prior to inoculation was never greater than 85 nematodes/200 mL soil, the initial inoculum density in the pots ranged from 2000 to 2170 *P. zeae*/pot. Five seeds of forage sorghum (cv. Jumbo) were then planted in each pot and plants were grown in a glasshouse for 6 weeks. To minimise possible nitrogen deficiency problems in soil amended with sugarcane trash, plants were watered twice with a soluble fertiliser (Aquasol, Hortico (Aust.) Pty Ltd) diluted to contain ~0.02% N. When the plants were harvested, the root ball was broken apart and nematodes were extracted from a mixture of roots and soil by spreading a 200 mL sample on a tray for 4 days (Whitehead and Hemming 1965). The number of nematodes recovered was used to estimate the final number of nematodes per pot (Pf), and Pf was divided by the number of nematodes inoculated (Pi) to determine the multiplication rate. Nematode counts were not corrected for extraction efficiency.

Soil chemistry

Nitrate nitrogen was determined at all sampling times [2M KCl extraction and colourimetric finish utilising the Griess-Ilosvay reaction (Rayment and Higginson 1992: Method 7C2)]. Twenty, 28 and 50 weeks after amendments were incorporated into soil, total soil organic carbon (C) and nitrogen (N) were determined on a Leco C-N combustion analyser (Rayment and Higginson 1992: Method 6B3). Organic carbon oxidisable by 33 mM potassium permanganate (C1) was measured following the methodology of Blair *et al.* (1995).

Soil microorganisms

Microbiological assessments were done at all sampling times. Populations of bacteria and fungi were estimated by adding 2 g soil to 18 mL of sterile phosphate-buffered saline (PBS) and shaking it at 250 r/min for 30 min. A 1 mL sample was then serially diluted and plated onto 1% tryptic soy agar plus 100 µg/mL cycloheximide (for bacteria) and 25% potato-dextrose agar plus 100 µg/mL rifampicin and 100 µg/mL novobiocin (for fungi). Microbial biomass C was determined after soil had been passed through a 2-mm sieve and adjusted to a moisture content of 70% field capacity. A 20 g soil sample was fumigated for 10 days under an atmosphere of CHCl₃ and then extracted with 0.5 M K₂SO₄. Extracts were filtered, digested with K₂Cr₂O₇ and H₂SO₄, and the carbon determined spectrophotometrically at 600 nm using sucrose as a standard (Vance *et al.* 1987). Microbial activity was estimated by measuring the rate of hydrolysis of fluorescein diacetate (Chen *et al.* 1988).

Free-living nematodes

Nematodes were extracted at all sampling times using the tray method described previously. Plant-parasitic nematodes were counted by genus and free-living nematodes by trophic group: bacterial-feeding nematodes (Rhabditida), fungal-feeding nematodes (Aphelenchina and some Tylenchidae), omnivores (Dorylaimida) and predators (Mononchida).

Nematophagous fungi

The nematode-trapping fungi present in each soil treatment were compared at 12, 20, 28 and 50 weeks using a modification of a

method described by Jaffee *et al.* (1998). A soil sample (100 g dry weight equivalent) from each plot was made up to a 200 mL volume with sterile distilled water, shaken for 20 min and then diluted 1 in 5 with water. Five 20 µL drops of the 1/5 dilution (i.e. 0.01 g soil) were pipetted onto two replicate plates of one-quarter strength corn-meal agar and then 1000 bacterial-feeding nematodes (*Caenorhabditis elegans*) were added to each plate. Plates were examined for nematode-trapping fungi after 3 weeks. Predatory hyphomycetes with teleomorphs in the Orbiliaceae (Ascomycota) were named according to the generic concept of Scholler *et al.* (1999), with *Arthrobotrys* being used for species producing adhesive networks and *Drechslerella* for species producing three-celled constricting rings.

The presence of fungi actively capturing nematodes was observed on mesh-covered agar in soil microcosms. A piece of water agar ~40 × 20 mm was placed on a glass slide at the bottom of a Petri dish and covered with 90 µm nylon mesh. The dishes were then filled with ~60 g of soil from each plot and incubated for 6 days in the laboratory at ambient temperatures of ~23°C. The soil was then discarded, the mesh was replaced with a coverslip and the agar surface was scanned under a microscope for signs of nematodes captured or parasitised by fungi.

Statistical analyses

Data were analysed by analysis of variance using Genstat 5 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK), with nematode counts in soil transformed [\log_{10} (no. nematodes/200 mL soil + 1)] for analysis. Chemical and biological parameters that were measured at several sampling times were analysed using a repeated measures procedure, in which treatment and time were included as factors. Means were compared using Fisher's protected LSD test. The variance ratios and LSDs for the time and interaction terms were adjusted for the degree of auto-correlation between times by the Greenhouse-Geisser epsilon (Greenhouse and Geisser 1959).

Results

Experiment with sugarcane

P. zeae and *Tylenchorhynchus annulatus*, the predominant plant-parasitic nematodes at the site, were present at low population densities before the amendments were incorporated (Table 1). However, 20 weeks after incorporation, populations of both species had declined significantly in amended plots but had not changed in non-amended plots (Table 1).

Sugarcane emerged well in all treatments and 30 days after planting (27 weeks after amendments were incorporated), the number of primary shoots was similar in all treatments (Table 2). Primary and secondary shoots were present 54 days after planting, and there were 14% fewer shoots in amended plots than in non-amended plots. However, numbers of shoots and weight of above-ground biomass did not differ significantly between treatments (Table 2). Numbers of plant-parasitic nematodes were low, but population densities of *P. zeae* and *T. annulatus* in soil and *P. zeae* in roots were generally lower in amended soil than in the non-amended control (Table 2).

When the crop was harvested 170 days after planting, stalks were generally more than 1.5 m long, but there were some stalks less than 1 m in length that had been killed by moth borer. The level of moth borer damage varied across the

Table 1. Populations of plant-parasitic nematodes in non-amended soil (NIL) and in soil amended with sugarcane trash (SCT) with or without soybean residue (S) or nitrogen (N), before amendments were applied, and 20 weeks later

	Prior to incorporating amendments		20 weeks after incorporation	
	<i>P. zoeae</i>	<i>T. annulatus</i>	<i>P. zoeae</i>	<i>T. annulatus</i>
NIL	1.95 ^A (88)	0.47 (2)	1.93 (84.1)	0.85 (6.1)
SCT	1.84 (68)	0.45 (2)	0.59 (2.9)	0.16 (0.4)
SCT + S	1.81 (64)	0.44 (2)	0.35 (1.2)	0.00 (0)
SCT + N	1.81 (64)	0.45 (2)	0.49 (0.2)	0.08 (0.2)
LSD ($P=0.05$)	n.s.	n.s.	0.68	0.43

^ATransformed means [$\log(\text{no. nematodes}/200 \text{ mL soil} + 1)$], with back-transformed means in parentheses.

Table 2. Effect of amending soil with sugarcane trash (SCT) with or without soybean residue (S) or nitrogen (N) on growth of sugarcane 30 and 54 days after planting (DAP), and numbers of plant-parasitic nematodes in soil and roots 54 DAP

	No. shoots/plot		DW tops (g)	Log (no. nematodes/200 mL soil + 1)		Log <i>P. zoeae</i> /g roots
	30 DAP	54 DAP		<i>P. zoeae</i>	<i>T. annulatus</i>	
NIL	29.6	51.2	48.1	1.43	1.29	1.80
SCT	25.6	43.8	42.5	0.73	0.39	1.51
SCT + S	25.6	42.8	39.5	0.68	0.42	1.32
SCT + N	28.4	44.8	40.6	0.89	0.86	1.06
LSD ($P=0.05$)	n.s.	n.s.	n.s.	0.353	0.361	0.451

site, largely because weeds harbouring the pest were unevenly distributed around the plots. Since there was a significant negative relationship ($P=0.008$, $R^2=0.33$) between per cent moth borer and biomass yield, the increased yield in plots amended with sugarcane trash (Table 3) was probably due to a lower incidence of moth borer, rather than an effect of the amendment.

All amendments reduced numbers of *P. zoeae* and *T. annulatus* 170 days after planting (Table 3). The effects on *P. zoeae* were particularly significant, as average nematode counts (back-transformed treatment means) were reduced from 3482 *P. zoeae*/g root in non-amended soil to 162 *P. zoeae*/g root in the amended soil, a reduction of 95%. Populations of *P. zoeae* and *T. annulatus* in soil were reduced by 85% and 71%, respectively. Two amended treatments (SCT and SCT + N) had more free-living nematodes than the non-amended control (Table 3).

Bioassay for suppression of lesion nematode

When *P. zoeae* was added to soil collected 20 weeks after amendments were incorporated, the nematode did not multiply as readily in amended soil as it did in the non-amended soil (Table 4). At 28 weeks, sugarcane trash was the only amendment to reduce nematode multiplication, while at 50 weeks there were similar numbers of lesion nematodes in soil and roots from amended plots as there were from the control (Table 4).

Soil chemistry

Treatments had marked effects on soil carbon and nitrogen. Concentrations of total organic C and the most labile C fraction (C1) were initially higher in amended than non-amended soil, but the differences declined with time (Fig. 1a and c). At 20 and 28 weeks, soil which had been

Table 3. Effect of sugarcane trash (SCT) with or without soybean residue (S) or nitrogen (N) on sugarcane biomass production 170 days after planting, and on populations of plant-parasitic and free-living nematodes

	Biomass (kg/plot)	Log <i>P. zoeae</i> /g root	Log (no. nematodes/200 mL soil + 1)		
			<i>P. zoeae</i>	<i>T. annulatus</i>	Free-living
NIL	7.45	3.54	3.25	2.83	2.93
SCT	9.51	2.17	2.29	2.31	3.38
SCT + S	6.11	2.13	2.25	2.32	3.10
SCT + N	6.46	2.34	2.73	2.27	3.19
LSD ($P=0.05$)	1.55	0.414	0.384	0.425	0.246

Table 4. Rate of multiplication of *Pratylenchus zeae* [final nematode population (Pf)/initial nematode population (Pi)] when pots of organically amended and non-amended soil collected at three sampling times were inoculated with 2000 *P. zeae*, planted with forage sorghum and grown for 6 weeks

	20 weeks Pf/Pi	28 weeks Pf/Pi	50 weeks Pf/Pi
NIL	1.07	1.12	2.16
SCT	0.28	0.39	2.75
SCT + S	0.52	2.13	1.41
SCT + N	0.47	1.14	3.38
LSD ($P = 0.05$)	0.262	0.844	1.040

amended with sugarcane trash and either soybean residue or ammonium nitrate had more total N than non-amended soil (Fig. 1b). Concentrations of N as NO_3^- were variable, but were higher in the two treatments that had received additional nitrogen (Fig. 1d).

Soil microorganisms

The three organic amendments affected most of the microbial parameters that were measured. Populations of culturable bacteria increased in some amended treatments at 20 and 28 weeks (Fig. 2a), whereas fungal populations increased significantly in all treatments at all sampling times (Fig. 2b). Microbial activity declined with time, and all amendments increased microbial activity compared with the

non-amended control until 28 weeks (Fig. 2c). Amendments also increased microbial biomass carbon, but the effect was not as consistent as for microbial activity (Fig. 2d).

Free-living nematodes

Populations of free-living nematodes increased markedly in amended plots and reached population densities around 10 000 nematodes/200 mL soil in some treatments after 20 weeks (Fig. 3d). All components of the nematode community (fungivores, bacterivores and omnivores) responded to the addition of organic matter and except for the omnivores, population densities were still higher than in the non-amended control after 52 weeks (Fig. 3a–c). At the first four sampling times (6, 12, 20 and 28 weeks), the ratio of fungal-feeding nematodes to fungal- and bacterial-feeding nematodes ranged from 0.55 to 0.80 and did not differ significantly between treatments (data not shown). However, after 50 weeks, this ratio was 0.59 in soil amended with sugarcane trash and ~ 0.35 in all other treatments, indicating that bacterial-feeding nematodes were predominant in the latter.

Numbers of omnivorous nematodes (Dorylaimida) increased in amended soil (Fig. 3c), but this group comprised only 1–3% of the total nematode population. There were relatively few large dorylaims, the most common being *Eudorylaimus* sp. More than 50% of the dorylaims were relatively small nematodes (~ 0.6 mm long) in the genus *Dorylaimellus*. Predatory nematodes in the family Mononchidae were rarely observed.

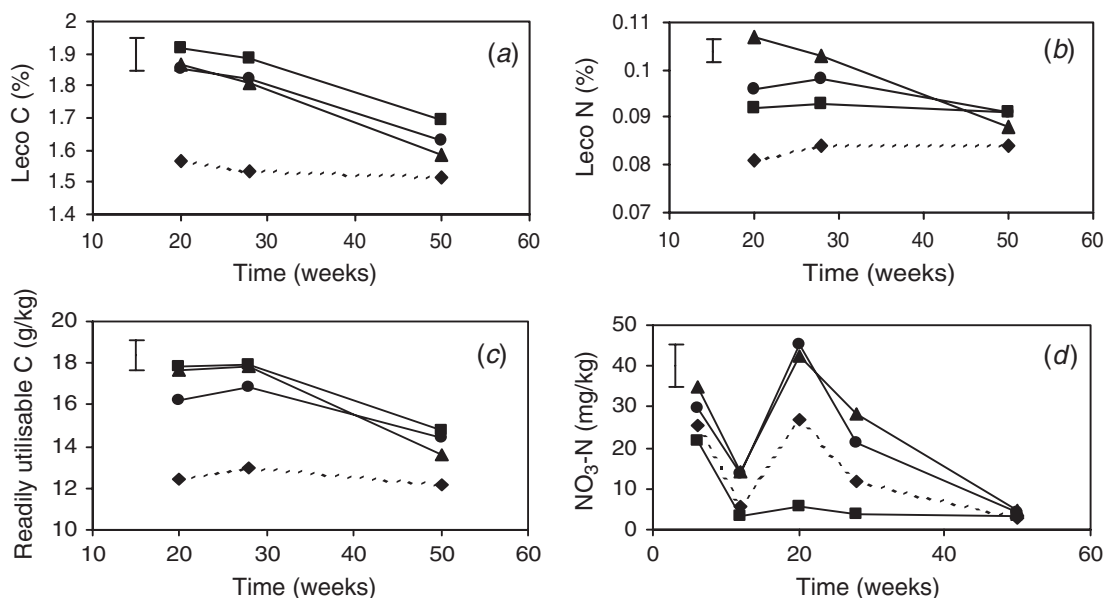


Fig. 1. Temporal changes in (a) total carbon, (b) total nitrogen, (c) readily utilisable carbon and (d) nitrate nitrogen in non-amended soil (\blacklozenge) and in soil amended with sugarcane trash (\blacksquare), sugarcane trash + soybean residue (\blacktriangle) and sugarcane trash + nitrogen (\bullet). Bars indicate LSD ($P = 0.05$).

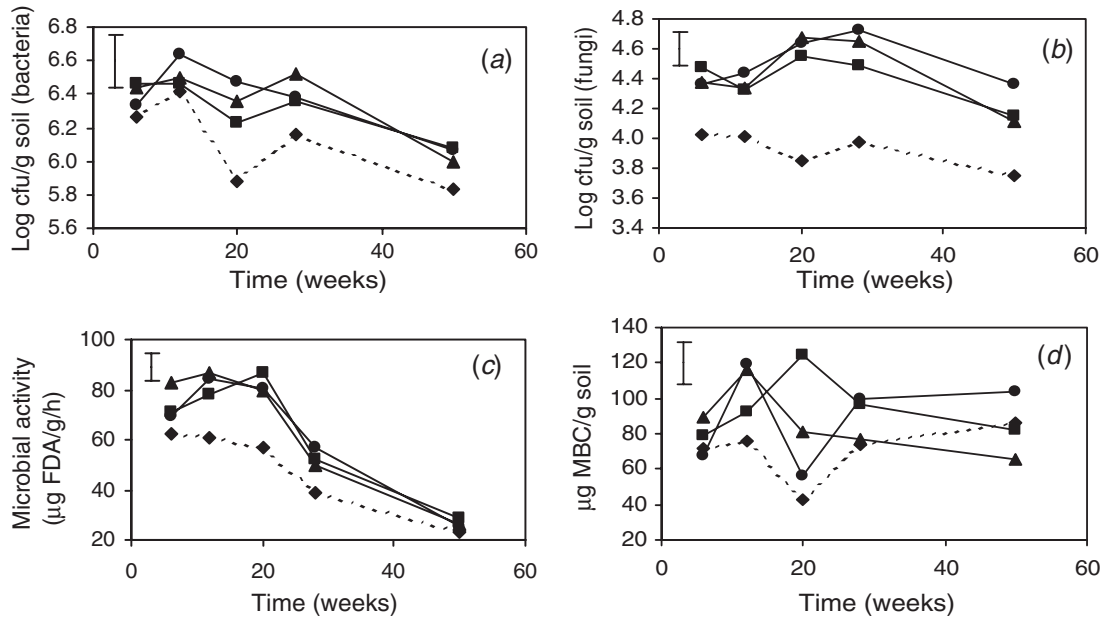


Fig. 2. Temporal changes in (a) numbers of culturable bacteria, (b) numbers of culturable fungi (Log no. colony forming units/g soil), (c) microbial activity (μg FDA/g/h) and (d) microbial biomass carbon (μg MBC/g soil) in non-amended soil (\blacklozenge) and in soil amended with sugarcane trash (\blacksquare), sugarcane trash + soybean residue (\blacktriangle) and sugarcane trash + nitrogen (\bullet). Bars indicate LSD ($P=0.05$).

Nematophagous fungi

Three species of predatory hyphomycetes (*Arthrobotrys conoides*, *A. thaumasium* and *Drechlerella dactyloides*) were isolated from the site by adding soil and bait nematodes

to agar plates. However, none of these fungi were seen frequently, as predatory hyphomycetes were observed on only 13% of plates processed from all treatments at 12, 20, 28 and 50 weeks. Also, trapping structures or nematodes captured

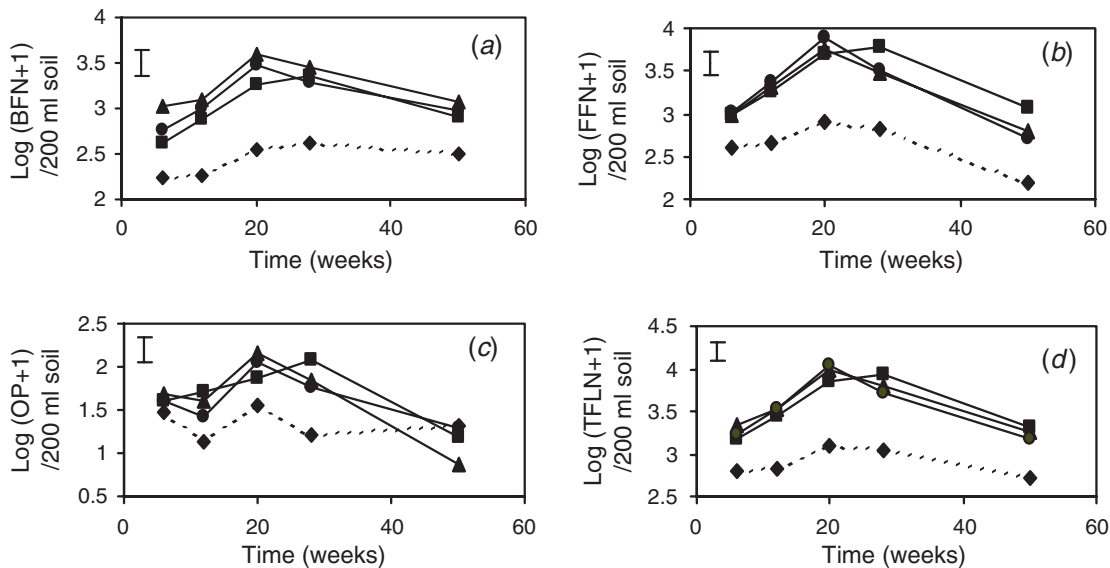


Fig. 3. Numbers [Log (no. nematodes/200 ml soil + 1)] of (a) bacterial-feeding nematodes, (b) fungal-feeding nematodes, (c) omnivore-predator nematodes and (d) total free-living nematodes in non-amended soil (\blacklozenge) and in soil amended with sugarcane trash (\blacksquare), sugarcane trash + soybean residue (\blacktriangle) and sugarcane trash + nitrogen (\bullet) at various times after amendments were incorporated into soil. Bars indicate LSD ($P=0.05$).

by these fungi were never observed on the surface of water agar in soil microcosms. The frequency of occurrence of all species was similar in amended and non-amended soil.

The most common nematophagous fungus did not sporulate and, therefore, was not a predatory hyphomycete. At 12, 20 and 28 weeks, it was seen capturing nematodes on agar surfaces that had been buried under amended soil for 6 days (Table 5). It was never seen in non-amended soil. On agar plates, the fungus produced distinct hyphal filaments with relatively few septa. These hyphae, which are $\sim 3 \mu\text{m}$ wide and exhibit protoplasmic streaming, grew across the agar surface and captured nematodes on bulbous hyphal extensions. Individual nematodes were captured and then the hypha grew on, so that several captured nematodes were often seen along the length of a single hypha. Most of the free-living nematodes on an agar plate were killed whenever this fungus was observed. Attempts to isolate it in pure culture were unsuccessful, but the fungus has been maintained in the laboratory for more than 18 months by transferring pieces of agar containing the fungus to new plates and adding bacterial-feeding nematodes. When *P. zae* and juveniles of *M. javanica* were added to these plates, they were captured in the same way as bacterial-feeding nematodes.

Discussion

Our results showed that amending soil with sugarcane trash (with or without soybean residue or ammonium nitrate) reduced populations of *P. zae* and *T. annulatus* on a crop of sugarcane that was planted 23 weeks after the amendments were incorporated into soil. Since the sugarcane grew satisfactorily in amended soil, this control of plant-parasitic nematodes appeared to be a direct effect of the amendments rather than an indirect effect caused by poor plant growth. The amendments had their main effect during the first 20 weeks of the decomposition process, as populations of *P. zae* and *T. annulatus* were $\sim 97\%$ lower in amended than non-amended soil at the time sugarcane was planted. These differences then diminished with time, either because multiplication rates increased when initial nematode densities

were low (McSorley and Phillips 1993), or because amended soil became less suppressive (Table 4).

Measurements taken during the experiment showed that chemical and microbiological changes occurred while suppressiveness was developing. Total C increased when amendments were added but, perhaps more importantly, levels of the readily oxidisable C fraction were much higher than the non-amended control at 20 and 28 weeks. Since this fraction comprises organic compounds that are readily utilisable by soil microorganisms (Conteh *et al.* 1997), it was not surprising that populations of bacteria and fungi, and enzyme activity (measured as the rate of hydrolysis of FDA) also increased in amended soil. Free-living nematodes responded to this increase in their food source, their population densities reaching a maximum 20–28 weeks after decomposition commenced.

Since our objective was to use organic matter to conserve or enhance potentially useful biological control agents, we minimised the detrimental effects of nitrogen on omnivorous and predatory nematodes (Kimpinski and Welch 1971; Yeates and King 1997; Sarathchandra *et al.* 2001; Stirling *et al.* 2003; Tenuta and Ferris 2004) by limiting nitrogen inputs and by using sugarcane trash to immobilise added nitrogen. This strategy was successful, as concentrations of N as NO_3^- in amended soil ranged from 3 to 45 mg/kg, the concentration of N as NH_4^+ was never greater than 5 mg/kg and, 12–28 weeks after amendments were incorporated, there was a 3–8 fold increase in numbers of omnivorous and predatory nematodes in amended soil compared with the non-amended control. The fact that soil amended with sugarcane trash was suppressive to lesion nematode confirms previous results (Stirling *et al.* 2003) and shows that limited quantities of N can be added to materials with a high C/N ratio without affecting their suppressiveness. The results also suggest that suppression is occurring through biological mechanisms rather than through the effects of ammonia and other nitrogenous compounds.

It has been suggested that enhanced predation on microbivorous and plant-parasitic nematodes occurs when soil is amended with organic matter (Linford *et al.* 1938;

Table 5. Incidence of an unidentified nematophagous fungus on water-agar plates containing bacterial-feeding nematodes and 0.01 g of soil, or in soil microcosms. Samples were collected 12, 20, 28 and 50 weeks after soil was amended with sugarcane trash (SCT) with, or without, soybean residue (S) or nitrogen (N)

	Water-agar plates				Soil microcosms			
	12 wk	20 wk	28 wk	50 wk	12 wk	20 wk	28 wk	50 wk
NIL	0 ^A	0	0	0	0	0	0	0
SCT	3	7	4	0	0	4	1	1
SCT + S	6	7	4	0	4	3	3	0
SCT + N	2	6	4	0	4	3	0	0

^ANumber of occurrences in two water-agar plates from each of five replicate plots, or one microcosm from each of the five replicate plots.

Stirling 1991; Wang *et al.* 2002), but there was little evidence to suggest that known predators of nematodes were highly active in our amended soil. Predatory hyphomycetes were isolated infrequently and their traps were never observed in microcosms containing amended soil, while the most common species (*A. conoides*) is considered a relatively poor predator in field soil (Jaffee 2003) and organically amended soil (Jaffee 2002, 2004). Predatory mononchids were rarely observed in amended soil, while the predominant dorylaimid was *Dorylaimellus*, which is thought to feed on plants or fungi rather than nematodes (Yeates *et al.* 1993).

An unidentified nematophagous fungus was the only predator observed more commonly in amended than non-amended soil. This fungus had the capacity to capture large numbers of nematodes, including plant-parasitic nematodes, and its predatory activities may have been one of the reasons that amended soil was suppressive to lesion nematode. Recent studies have shown that some species of nematophagous fungi can have a major impact on nematode population densities (Bouwman *et al.* 1996; Jaffee 2003).

The fact that the unidentified nematophagous fungus was found only in soil to which large quantities of sugarcane trash had been added may be significant. Amended soil was carbon rich and nitrogen poor, which means that the environment was somewhat similar to situations where predatory fungi are often found. These fungi are thought to have evolved the capacity to obtain additional nitrogen by capturing nematodes (Barron 1992; Tzean and Liou 1993), and may be most active in situations where nitrogen is immobilised by the microbial community. This situation probably occurred in our experiment, as the C/N ratio of the amended soil ranged from 17 to 20, and nitrogen is usually immobilised at a C/N ratio of ~20 (Myrold 2005). We, therefore, hypothesise that two conditions must be met before an organic amendment will enhance fungal predation on nematodes: 1) inputs of carbon must be high enough to increase populations of free-living nematodes and 2) the C/N ratio must be high enough to immobilise most of the nitrogen.

The problem with being definitive about the mode of action of organic amendments is that when organic matter is added to soil, it has a cascading effect on all trophic levels in the soil food web. Bacteria and fungi (trophic level 1) are fed on by consumers such as protozoa and free-living nematodes in trophic level 2, and they in turn are consumed by predators in trophic level 3 (e.g. nematophagous fungi, predatory nematodes and predatory arthropods). The food web in amended soil, therefore, consists of many diverse and interacting elements and its biological buffering capacity tends to prevent any component (e.g. a nematode pest) from becoming dominant. In this situation, it is never completely clear which organisms occupy the highest trophic level, as turnover is rapid and unknown predators may be involved. We, therefore, suggest that instead of focusing on the role of individual organisms in suppressing nematode pests, future

research should concentrate on defining the level of activity and complexity within the food web that is needed to achieve suppression.

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