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Influence of cover crops and soil amendments on okra (*Abelmoschus esculentus* L.) production and soil nematodes

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Abstract

A pot experiment to determine the effects of summer cover crops and soil amendments on okra yields and population densities of various soil nematode taxa was conducted in two consecutive growing seasons in a subtropical region. Two cover crops, sunn hemp (*Crotalaria juncea*) and sorghum sudangrass (*Sorghum bicolor* × *S. bicolor* var. sudanense), were grown and returned to the soil with fallow as a control. As soon as these cover crops were harvested, they were soil-incorporated together with one of several organic amendments. These organic amendments were biosolids, N-Viro soil (a 1 : 1 mixture of coal ash and biosolids), coal ash, co-compost (a 3 : 7 mixture of biosolids and yard wastes), and yard waste compost compared with a control (no additional amendment). Other treatments were fumigation with MC-33 (a mixture of 33% of methyl bromide and 67% of chloropicrin) and cover crop removal (harvested and removed cover crops and their residues from the soil). A nematode-susceptible vegetable crop, okra (*Abelmoschus esculentus* L.), was grown under these treatments. Among organic amendments, the application of biosolids produced the highest okra yield and biomass, and greatly suppressed root-knot nematodes, *Meloidogyne incognita*, in the soil. Between these two cover crops, sunn hemp was superior to sorghum sudangrass in improving okra production and in suppressing root-knot nematodes. The result indicates that growing sunn hemp as a cover crop and applying certain organic amendments can improve okra production and suppress root-knot nematodes, which are very damaging to okra plants. Such combined practices show a significant potential for application in organic farming and sustainable agriculture systems in a tropical or subtropical region.

Key words: amendments, biosolids, composts, coal ash, cover crop, nematode, okra

Introduction

Soil organic amendments, e.g. biosolids, composts, etc., are a valuable source of plant nutrients, particularly N and some micronutrients, which can provide essential nutrition to crops through decomposition. However, the plant available nutrients supplied by these amendments vary considerably depending on their components and mineralization rate[1]. Cover crops, commonly used as green manures, are also important soil organic amendments for sandy or gravelly soils in Florida, USA[2]. The C : N ratio in green manure or compost amendments usually plays a vital role in controlling the decomposition and nutrient release rates[3]. Biosolids resulting from the treatment of waste water or sewage sludge contain higher amounts of N and of some other nutrients, which had a lower C : N ratio than regular composts. Biosolids usually combine with other organic materials, such as green and woody wastes, and act much like slow-release organic fertilizers[4]. Other composted municipal solid wastes (paper, cardboard, food waste, yard waste, textiles, etc.), including yard waste compost (mainly from leaves and branches of trees and shrubs, and grass

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clippings) and coal ash or fly ash (a byproduct from power plants) when used as soil amendments have shown a high potential to improve soil fertility and crop production[5,6]. These materials may serve as a partial substitute for inorganic fertilizer for tomatoes[7] and bell peppers[8], and as general soil conditioners[4]. Vegetable crop yield responses to such amendments have been evaluated for many crops, including tomato, bell pepper, broccoli, sweet corn, cucumber, eggplant, okra, snap bean, squash and watermelon[9].

Compost production has become a promising industry. In the State of Florida alone, over 27 million $t\,yr^{-1}$ of solid waste is produced, which is about 4.5 kg per capita per day[10]. The generation of municipal solid wastes is increasing rapidly in step with the rapid rate of population growth in this state. However, since Florida is a major vegetable-producing state, with 418,000 acres under cultivation each year[10], there is a great potential to utilize composts. According to USEPA, 30–60% of a community's municipal solid wastes can be processed as municipal solid waste composts[11]. The agricultural industry is the largest consumer of those composts and has the potential to use up to 800 million m^3 annually, which is more than 10 times the US production of solid waste composts[12,13].

Numerous studies have demonstrated that the application of composts can increase soil organic matter[14,15], cation exchange capacity[16], soil water-holding capacity[17,18], pH of acidic soils[15] and soil microbial[19] and enzymatic activities[18] and decrease soil bulk density[17]. The application of these composts usually does not contaminate soils, edible crops or groundwater with pathogens or heavy metals[6,25]. Crop responses to different composts vary widely: from induced N-deficiency in pepper[20] to yield increases of 22% in snap bean and 38% in tomato[15]. On the other hand, applications of certain composts—because of immaturity or a high C:N ratio—may lead to the immobilization of soil mineral N[21,22] and thereby induce N-deficiencies in plants and depress crop yields[20].

Soil nematodes, especially root-knot nematodes (*Meloidogyne* spp.), are major soil-borne pests that damage crops and significantly diminish yields. Indeed plant-parasitic nematodes cause annual crop losses estimated at approximately \$78 billion worldwide and \$8–\$12 billion in the United States[23]. However, certain cover crops as green manure have been shown to improve soil fertility and crop yields[3,24,25], as well as suppress plant-parasitic nematodes[26–29]. Certain compost amendments have also been found to suppress soil-borne nematodes and plant pathogens[30–33], and some researchers have suggested that it may be possible to develop their use as reliable alternatives to methyl bromide for soil fumigation in vegetable production systems[2,34]. Therefore, the objective of this experiment was to elucidate the effect of growing certain green manure crops and the application of various organic soil amendments on okra yields and on the population densities of various soil nematode taxa.

Materials and Methods

Soil properties

The soil used in this experiment was Krome, very gravelly loam (loamy-skeletal, carbonatic, hyperthermic Lithic Udorthents) collected from a field in the Tropical Research and Education Center, University of Florida, Homestead, FL, USA. The soil contained 58.8% gravel (>2 mm) and was composed of 48.4% sand, 30.3% silt and 21.3% clay in the fine earth (gravel-free) fraction. Also the soil contained 60% $CaCO_3$, pH 7.8 (water), soil organic C 28 $g\,kg^{-1}$, total N 1.1 $g\,kg^{-1}$, and ammonium bicarbonate-diethylene triamine pentaacetic acid (AB-DTPA) extractable P 22.7 $g\,kg^{-1}$ and K 129 $g\,kg^{-1}$, respectively. Since the populations of root-knot nematodes (*Meloidogyne incognita*) in the field were known to be quite low[29,31], plant roots of tomatoes with root galls caused by root-knot nematodes were collected from several farms in Miami-Dade County of Florida, and pieces of these roots were uniformly distributed to the pot soil to increase the nematode population. The experiment was conducted with 8.3-liter black plastic pots 23 cm in diameter and 20 cm high with a capacity of 8 kg of soil per pot.

Experimental design

A split plot design was conducted with 24 pots for each of two cover crops and a fallow (control) treatment as main plots. Eight soil amendment treatments were applied to each cover crop as subplots. Three replicates were conducted for each treatment. Summer cover crops were sunn hemp (*Crotalaria juncea* L.) and sorghum sudangrass [*Sorghum bicolor* × *S. bicolor* var. sudanense (Piper) Stapf.] compared with fallow soil (control). The amendments were biosolids, N-Viro soil (a 1:1 mixture of coal ash and biosolids), coal ash, co-compost (a 3:7 mixture of biosolids and yard wastes), yard waste compost (YW-compost), control (cover crop grown and incorporated into the soil, but without any other organic amendment), cover crop removal (the cover crop was grown and aerial parts together with roots were removed from the soil), and MC-33 (fumigated with a mixture of 33% of methyl bromide and 67% of chloropicrin after the cover crop was grown and incorporated into the soil).

Experimental management and growth conditions

These two cover crops, sunn hemp and sorghum sudangrass, were seeded on June 10, 2002 and June 17, 2003, and the okra was seeded on September 18, 2002 and September 30, 2003 for every treatment. These cover crops were harvested at the stage of sunn hemp flowering, and cut into pieces (<2 cm) before mixing into the soil together with one of the following organic amendments: biosolids, N-Viro soil, coal ash, co-compost, yard waste compost, control (cover crop only without any amendment), cover crop removed and MC-33. The amount of each organic

Table 1. Characteristics, nutrient and trace metal concentrations of soil amendments used in the experiment.

	Biosolids	N-Viro soil	Co-compost	YW-compost[4]	Coal ash
pH	6.1	9.2	6.7	7.7	11.8
EC ($mS\,cm^{-1}$)[1]	16.1	6.4	1.0	1.7	5.4
	$mg\,kg^{-1}$				
Extractable N[2]	1461.2	13.8	145.9	29.0	6.4
Extractable P	236.6	209.1	130.3	85.9	8.9
Total P[3]	29,080	5430	13,433	729	156
Total Ca	43,179	115,122	44,133	153,614	161,294
Total Mg	3774	1814	2452	2027	1781
Total Fe	8387	9094	13,210	5559	6985
Total Zn	1254	168	347	598	69
Total Mo	11.4	12.0	6.0	3.4	18.7
Total Co	2.8	16.6	2.6	4.5	41.5
Total Cd	3.6	1.7	1.8	1.8	1.6

[1] EC, electrical conductivity. The ratio of soil to water was 1 : 2.
[2] N was extracted by 2.0 *M* KCl; extractable P was extracted with ammonium bicarbonate–DTPA, and determined by means of AA3.
[3] Total content of elements was determined by means of ICP after samples were digested with the EPA 3050A method.
[4] YW, yard waste.

amendment added to the soil was 205 g pot^{-1} of dry weight basis, equivalent to 50 t ha^{-1}. After the cover crop residues and/or organic amendments were incorporated into the soil, the amended soil was allowed to equilibrate for 10 days before the okra (*Abelmoschus esculentus* L.) was seeded. The composition and properties of the various organic amendments were determined and are summarized in Table 1. More detailed information can be found in Wang et al.[25]. In the MC-33 treatment, the pots were fumigated by placing them into a chamber and using liquid N to introduce the appropriate amount of the 33% methyl bromide and 67% chloropicrin mixture after cover crops were incorporated into the soil. The chamber was kept hermetically sealed for 72 h. Thereafter the pots were exposed to the atmosphere for an entire week, and then okra was seeded. The okra planting was thinned at the three-leaf stage to six plants per pot. Drip irrigation was installed and adjusted to deliver 2 liter h^{-1} of water. A clock timer was used to control the irrigation automatically. The period and frequency of irrigation were adjusted according to needs of the successive plant growth stages. Okra fruits were harvested when they attained full development.

The experiment was carried out from June 2002 to February 2003 as the first year (Year-1), and from June 2003 to February 2004 as the second year (Year-2) in a screen house at Homestead, FL, USA, which is a subtropical region. The average annual rainfall is 1499 mm, of which 76% falls between June and October, the average annual temperature is 23.9°C with a range from a maximum of 35°C in June to a minimum of 5°C in January[24]. During these two growing seasons between June 2002 and February 2004, the air temperatures ranged from a minimum of 2.2°C to a maximum of 34.6°C with 1084.6 mm rainfall in Year-1, and from a minimum of 2.9°C to a maximum of 33.3°C with 1319 mm rainfall in Year-2. Monthly average temperatures were 35.3 and 34.1°C as maximum both in August of 2002 and August of 2003; and 28.5 and 28.6°C as minimum both in January of 2003 and January of 2004; relative humidity fluctuated between 75% (January) and 84% (June) in Year-1 and 77% (December) and 84% (August and September) in Year-2; the maximum solar radiation was 216 W m^{-2} in August 2002 and 224 W m^{-2} in July 2003; and the minima were 128 W m^{-2} in December 2002 and 129 W m^{-2} in December 2003.

Sampling and analysis

Each cover crop was sampled for biomass determination before the harvest and incorporation into the soil. Okra fruits were harvested to obtain the fruit yield. The okra yields and cover crop biomass weights were converted to metric tons per hectare (t ha^{-1}) equivalent based on the surface area of the pot. For determining the identities and densities of the various nematode taxa, soil samples were collected at three stages: (1) before the cover crops were seeded, (2) after cover crops were grown and (3) immediately after the okra harvest. A soil sample was taken from 5 to 15 cm below the surface of each pot. Nematodes from each sample were extracted by means of Cobb's sieving and decanting technique[35], followed by a modified Baermann funnel method[36]. Sieves used in nematode extraction were US Standard Sieve Series of 100, 200 and 325 mesh with openings of 149, 74 and 44 μm, respectively. Nematodes were fixed in hot 30 ml l^{-1} of formaldehyde solution, identified to genus level and counted using a stereoscope. Some fixed specimens were processed with anhydrous glycerin[37], and examined under a compound microscope for species identification. Nematode identifications were based on the morphology of adult and larval forms and their identities were confirmed with recent taxonomic keys[38–42]. Roots were washed free of soil and examined for galling and root-knot infection. Some roots showing lesions were cut into small pieces and left

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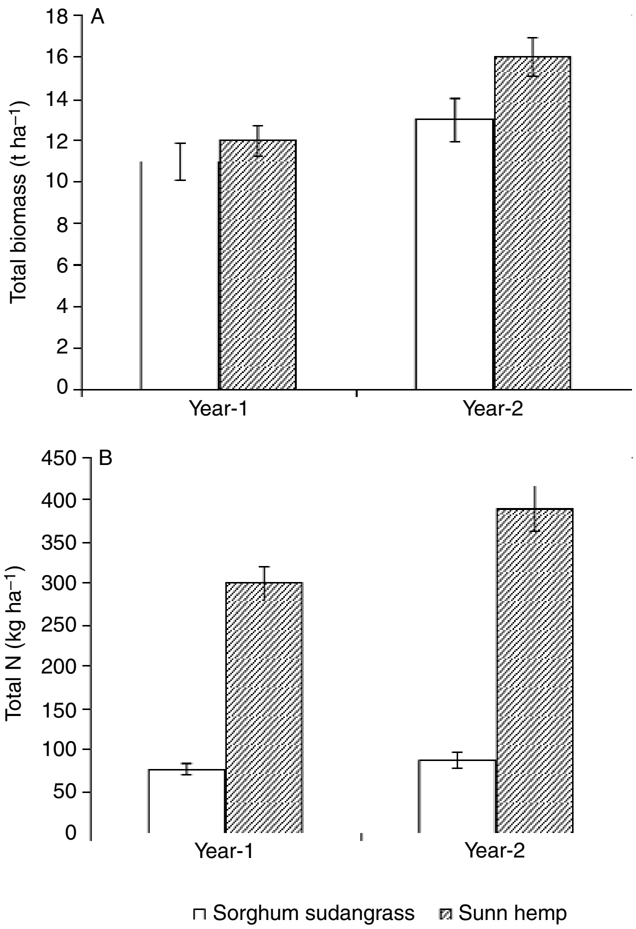

Figure 1. Total amounts of biomass (A) and N (B) accumulated by cover crops and returned to the soil in Year-1 and Year-2. The vertical bars represent standard errors of the mean values ($n = 3$).

in water for 36–48 h to reveal the presence of any lesion and other nematodes. Nematode density (number in 250 ml of soil) was determined for each species and recorded. The okra plants were individually rated for root galls and egg masses on a 0–5 scale: 0 = 0 galls, 1 = 1–2, 2 = 3–10, 3 = 11–33, 4 = 31–100, and 5>100 galls or egg masses[43].

Statistics

The data were subjected to the analysis of variance (ANOVA) and Duncan's multiple range tests for a significant difference at $P < 0.05$ by means of the SAS program[44].

Results

Cover crop biomass production and nutrient contents

There were no significant differences in biomass production between sunn hemp and sorghum sudangrass in this experiment in either of the 2 years (Fig. 1). In years 1 and 2, sunn hemp produced the equivalent of 12 and 16 t ha^{-1} of dry weight biomass, respectively, while sorghum sudangrass correspondingly produced 11 and 13 t ha^{-1}. However, the total amounts of N accumulated and returned to the soil by these two cover crops were significantly different because sunn hemp contains about 3 times the N concentration of sorghum sudangrass, i.e., 2.85 versus 0.92%[3]. Thus the total amounts of N returned to the soil in plant biomass were equivalent to 300–390 kg ha^{-1} from sunn hemp versus 77–87 kg ha^{-1} from sorghum sudangrass in both years (Fig. 1). However, the amounts of P and other elements returned in these two cover crops were similar (data not shown).

Okra yields influenced by green manure and organic amendments

Results of the ANOVA (Table 2) showed that yields of okra were significantly affected by these two cover crops, as well as by various organic amendments, but the interaction between cover crops and organic amendments was not significant.

Okra yields (Table 3) under all treatments were generally higher in the second year than in the first. Compared to the fallow, growing sunn hemp increased okra fruit yields in both years and growing sorghum sudangrass increased okra fruit yield in the second year as well.

In contrast to the control, some soil amendments, especially biosolids, increased the okra yield dramatically in both years; all the other treatments of soil amendments significantly increased okra yields in both years except those of coal ash and yard waste compost in the second year (Table 3). However, we noted that treatments of sunn hemp combined with an organic amendment that has a low N content [e.g. N-Viro (13.8 mg kg^{-1}), coal ash (6.4 mg kg^{-1}), or yard waste compost (29 mg kg^{-1})] resulted in a higher okra yield than treatments of sorghum sudangrass combined with the same organic amendment. In contrast, treatments of sunn hemp combined with an organic amendment that has a high N content [e.g. biosolids (1461 mg kg^{-1}) or co-compost (146 mg kg^{-1})] did not result in a significantly higher yield of okra than treatments of sorghum sudangrass combined with the same organic amendment (data not shown).

Effects on levels of root galling by cover crops and organic amendments

Significantly less root galling of okra was found with treatments of sunn hemp rather than sorghum sudangrass (Figs. 2A and 3A), which indicates that sunn hemp possesses one or more mechanisms to resist root-knot nematodes. The most important finding is that this function can last long enough to protect the subsequent nematode-susceptible cash crop.

Root galling in the biosolids treatment in both years (Figs. 2B and 3B) was quite low and statistically similar to

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Table 2. ANOVA on the effect on okra yield of cover crops, organic amendments and interactions of these two factors.

Source	DF	Sum of squares	Mean square	*F*-value	Pr>*F*
Cover crops	1	556.710708	556.710708	41.95	<0.0001
Organic amendments	7	3972.813119	567.544731	42.76	<0.0001
Cover crops × Organic amendments	7	111.093154	15.870451	1.20	0.3352
Replicates	2	3.073936	1.536968	0.12	0.8910

Table 3. Okra yields ($t\,ha^{-1}$) of various cover crop–organic amendment combinations.

	Okra fruit yield ($t\,ha^{-1}$)	
Organic amendment	**Year-1**	**Year-2**
Cover crops		
Sunn hemp	7.21 a[1]	13.25 a
Sorghum sudangrass	5.32 ab	8.21 b
Fallow	4.03 b	5.16 c
Soil amendments		
Biosolids	20.32 a	26.29 a
N-Viro soil	13.29 b	13.21 b
Coal ash	10.18 c	12.14 bc
Co-compost	12.34 b	13.21 b
Yard waste compost	12.28 b	12.19 bc
Control (cover crop only)	6.15 d	11.26 c
Cover crop removed[2]	5.02 d	9.04 d
MC-33	10.88 c	15.37 b

[1] Data with same letters in the same column for cover crops or soil amendments represent insignificant differences at $P \leqslant 0.05$.
[2] The cover crop was grown until the sunn hemp flowering stage for its harvest. At that time, both shoots and roots were removed and discarded.

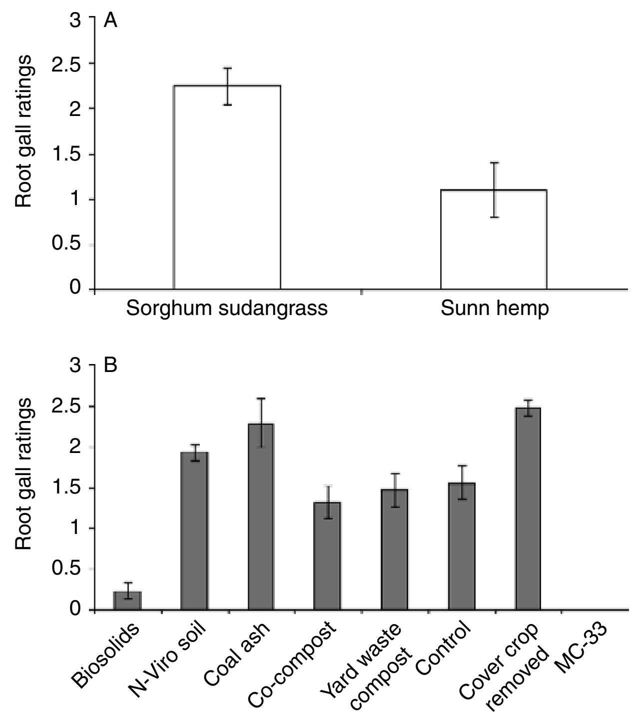

Figure 2. Root gall ratings of okra influenced by cover crops (A) and soil amendments (B) in the first year experiment. Vertical bars represent standard errors of means. Root rating scale (0–5): 0 = 0 galls, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, 5 > 100 galls or egg masses of the root-knot nematode, *Meloidogyne incognita*.

that in the MC-33 treatment. Root galling was very high in the treatment of cover crop removed in both years (Figs. 2B and 3B). In the experiment of the second year, the levels of root galling in the biosolids and coal ash treatments were not significantly different from that in the MC-33 treatment (Fig. 3B).

Effects of cover crops and organic amendments on population densities of different soil nematode taxa

The total number of nematodes decreased after sorghum sudangrass was grown (Table 4), and the total numbers of both plant parasitic and non-parasitic nematodes were suppressed. Although sorghum sudangrass did not cause a decline in the density of *M. incognita*, it did not allow a substantial build up of this or any other taxon of plant-parasitic nematode. In contrast, the sunn hemp treatment reduced the aggregate total number of nematodes slightly, but strongly suppressed the parasitic nematode taxa, and allowed the mycophagous *Aphelenchus avenae* and the bactivorous rhabditids to thrive (Table 4). The taxa of plant-parasitic nematodes that were present before sunn hemp was grown, but that could not be detected after sunn hemp had been grown, were the root-knot nematode (*M. incognita*), the stunt nematode (*Quinisulcius acutus*) and the reniform nematode (*Rotylenchulus reniformis*). After okra had been grown in soil containing sorghum sudangrass or sunn hemp residues or in fallow, the total numbers of plant-parasitic and non-parasitic nematodes had increased dramatically in all these treatments; these two cover crops did not cause a significant difference in the total number of parasitic nematodes but the population of non-parasitic nematodes in the sunn hemp treatment was significantly higher than that in the sorghum sudangrass treatment (Table 4).

The MC-33 treatment significantly suppressed the total number of parasitic nematodes but increased the population of *R. reniformis* after okra had been grown. However, the suppression of root-knot nematode (*M. incognita*) by

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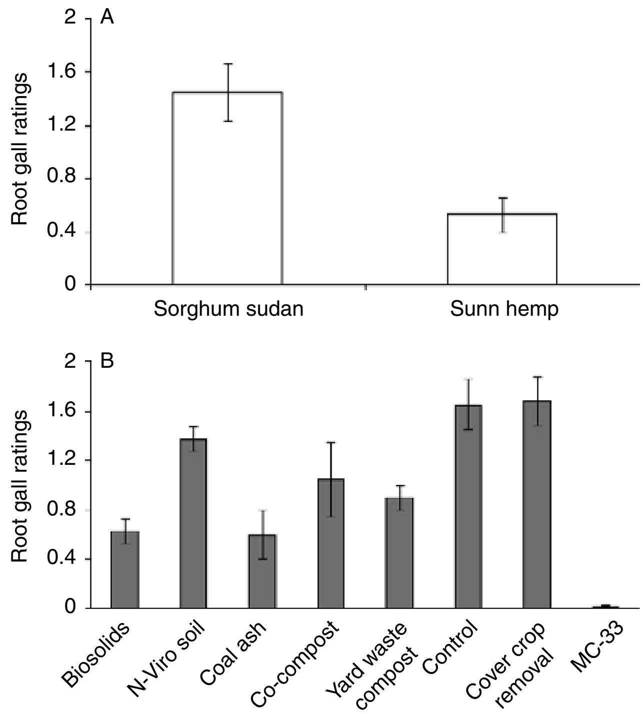

Figure 3. Root gall ratings of okra influenced by cover crops (A) and soil amendments (B) in the second year experiment. Vertical bars represent standard errors of means. Root rating scale (0–5): 0 = 0 galls, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, 5 > 100 galls or egg masses of the root-knot nematode, *Meloidogyne incognita*.

the MC-33 treatment was not significantly different from the treatment with biosolids (Table 4).

In the first year experiment, under treatments of sorghum sudangrass combined with various organic amendments (Table 5), the treatment of MC-33 significantly suppressed the total and parasitic nematode population compared to all other treatments, and especially reduced the population of root-knot nematode (*M. incognita*). In contrast, under treatments of sunn hemp combined with various organic amendments, the application of biosolids showed a superior suppression on root-knot nematodes, *M. incognita*, which was as effective as the treatment fumigated with MC-33 but the population of the stunt nematode (*Quinisulcius acutus*) was significantly increased (Table 5). The highest populations of root-knot nematode were found in the cover crop removed treatment although the difference between this treatment and others, except biosolids, coal ash and MC-33 were insignificant. The treatment of coal ash combined with sunn hemp showed a significant reduction in root-knot nematode (*M. incognita*) population versus the treatment of cover crop removed (Table 5).

In the second year experiment, although combinations of cover crops with various organic amendments (Table 6) produced some inconsistent effects compared with the first year, the treatment of MC-33 with either of those two cover crops had the lowest population of root-knot nematodes, *M. incognita*, and the total numbers of parasitic nematodes were significantly lower than those with some other treatments except the treatment of biosolids combined with sunn hemp. The combination of sunn hemp with biosolids reduced the population of root-knot nematodes (*M. incognita*) and the total number of parasitic nematodes, which produced the same result as the treatment of sunn hemp combined with MC-33 (Table 6). Also, besides the treatments of MC-33 and biosolids, the application of coal ash or co-compost showed a significant reduction of the total number of parasitic nematodes compared to treatments of yard waste compost, control or cover crop removed.

In both years of the experiment, the number of *Aphelenchus avenae* nematodes was generally improved by growing sunn hemp compared to sorghum sudangrass regardless of treatments with various organic amendments (Tables 4–6).

Discussion

Sunn hemp usually produces significantly higher amounts of biomass than sorghum sudangrass when grown in the field. For example, Wang et al.[24] obtained 12 t ha^{-1} of sunn hemp biomass versus 5 t ha^{-1} of sorghum sudangrass from a field experiment. The difference in biomass production in pots and in the field might be related to respective differences in growing conditions. Since sunn hemp is a tropical crop, its vegetative growth and development are favored by hot and humid summers and relatively long day lengths as occur from June through September in south Florida. Sunn hemp appears to grow less well in pots than sorghum sudangrass.

Yield responses of vegetable or field crops to the application of soil organic amendments have been reported by a number of researchers. For instance, Warman[45] reported that with compost amendments in field plots, marketable carrot yields were increased from 67 to 76%, compared to those from fields amended with the conventional fertilizers. Maynard[15] reported that over a 3-year period, the average tomato fruit yields from compost-amended plots ranged from 4.89 to 8.85 kg per plant compared with 3.54 to 7.67 kg per plant in the control, and the average yield increase in compost-amended plots was 18%. In Washington State, barley and wheat yields increased by 35% when prior to planting each year a form of sewage sludge was ploughed into the field at a rate of 4.5 t ha^{-1} dry weight equivalent[46]. Many trials have been performed using municipal solid waste and various composts as soil amendments[9,47–49]. However, most of these studies focused on improvements in soil quality rather than on differences in crop performance[4].

The significant improvement of okra fruit yield achieved by growing sunn hemp in both years indicates that the high N contribution from sunn hemp residues can be a main factor to improve okra yields. Also the dramatic increase in okra fruit yields under the treatment of biosolids implies that a high N requirement of okra can be met by application

Table 4. Population densities (number per 250 ml) of different soil nematode taxa before and after various treatments with cover crops and organic amendments.

	Aphelenchus avenae	***Helicotylenchus dihystera***	***Meloidogyne incognita***	***Quinisulcius acutus***	***Rotylenchulus reniformis***	***Heterodera* sp.**	***Tylenchus sandneri***	**Dorylaimids**	**Rhabditids**	**Total**	**Subtotal parasitic[1]**	**Subtotal non-parasitic**
Before cover crops were grown												
	22.0	24.3	7.0	16.0	4.3	–	6.3	9.7	14.3	104.4	51.7	52.3
When the cover crops were terminated												
Sorghum sudangrass	0.3 b[2]	0.0	8.8 a	0.3 a	0.0	–	0.0	0.0 a	4.6 b	13.9 b	9.1 a	4.8 b
Sunn hemp	29.5 a	0.0	0.0 b	0.0 a	0.0	–	0.0	2.0 a	60.5 a	92.0 a	0.0 b	92.0 a
Fallow (control)	0.0 b	0.0	0.0 b	0.0 a	0.0	–	0.0	0.0 a	3.0 b	3.0 c	0.0 b	3.0 b
After okra had been grown in soil containing cover crop residues or on fallow												
Sorghum sudangrass	0.0 b	33.7 b	423.3 a	50.4 b	–	–	1.5 b	59.0 a	63.4 a	575.1 ab	470.8 a	104.3 b
Sunn hemp	10.0 a	50.0 b	193.3 b	157.2 b	–	–	7.5 a	38.3 a	129.0 a	736.0 a	543.9 a	192.1 a
Fallow (control)	3.3 b	151.7 a	35.0 b	265.0 a	–	–	0.0 b	60.0 a	77.6 a	396.5 b	301.3 a	95.2 b
After okra had been grown in soil containing both cover crop residues and various organic materials[3]												
Biosolids	13.3 ab	75.8 ab	46.7 b	325.0 a	0.0 b	0.0 a	10.0 a	17.0 a	87.5 a	575.3 ab	447.5 a	127.8 a
N-Viro soil	6.7 ab	93.3 a	265.0 ab	165.0 b	0.0 b	160.0 a	2.5 a	45.8 a	92.2 a	830.5 a	683.3 a	147.2 a
Coal ash	30.0 a	94.2 a	190.8 ab	73.3 b	0.0 b	275.0 a	10.0 a	40.8 a	94.4 a	808.5 a	633.3 a	175.2 a
Co-compost	13.3 ab	93.3 a	200.5 ab	80.0 b	0.0 b	385.0 a	11.7 a	52.5 a	85.6 a	921.9 a	758.8 a	163.1 a
YW-compost	20.0 ab	56.7 ab	234.2 ab	118.0 b	0.0 b	433.0 a	0.0 a	52.5 a	110.3 a	1025.0 a	842.2 a	182.8 a
Cover crop only (control)	13.4 ab	68.9 ab	186.8 ab	93.1 b	0.0 b	262.3 a	3.3 a	35.7 a	83.0 a	746.5 ab	611.1 a	135.4 a
Cover crop removed	14.1 ab	53.3 ab	374.6 a	46.2 b	0.0 b	259.0 a	2.5 a	44.7 a	59.9 a	854.3 a	733.1 a	121.2 a
Cover crop + MC-33	1.2 b	10.8 b	66.8 b	45.9 b	6.3 a	0.0 a	0.0 a	19.7 a	150.1 a	300.8 b	129.8 b	171.0 a

[1] Plant parasitic nematodes include *Helicotylenchus dihystera*, *Heterodera* sp., *Meloidogyne incognita*, *Pratylenchus neglectus*, *Quinisulcius acutus*, *Rotylenchulus reniformis* and *Tylenchus sandneri*, and non-plant parasitic nematodes include Dorylaimids, *Mononchus* and Rhabditids, respectively, if any identified.

[2] Data followed by same letters within a column represent insignificant differences at $P \leqslant 0.05$.

[3] Pooled data from three replicates involving either sorghum sudangrass or sunn hemp as green manure to which an organic amendment was added.

Table 5. Effects of cover crops combined with various organic amendments on the population densities (number per 250 ml) of different soil nematode taxa in the first year experiment.

	Aphelenchus avenae	*Helicotylenchus dihystera*	*Meloidogyne incognita*	*Quinisulcius acutus*	*Heterodera* sp.	*Tylenchus sandneri*	Dorylaimids	Rhabditids	Total	Subtotal parasitic[1]	Subtotal non-parasitic
With sorghum sudangrass											
Biosolids	–	51.7 a	93.3 a	125.0 a	0.0 a	0.0 a	28.3 a	36.7 a	335.0 ab	270.0 ab	65.0 a
N-Viro soil	–	58.3 a	80.0 a	55.0 ab	160.0 a	5.0 a	31.7 a	26.0 a	416.0 ab	358.3 ab	57.7 a
Coal ash	–	98.3 a	136.7 a	30.0 b	275.0 a	0.0 a	31.7 a	73.5 a	645.2 ab	540.0 a	105.2 a
Co-compost	–	45.0 a	100.0 a	51.7 ab	385.0 a	–	31.7 a	48.0 a	562.3 ab	482.7 ab	79.7 a
YW-compost	0.0 b[2]	28.3 ab	135.0 a	66.7 ab	433.3 a	–	30.0 a	88.3 a	880.7 a	762.4 a	118.3 a
Control	0.0 b	33.7 ab	193.3 a	30.7 b	262.3 a	–	59.0 a	51.7 a	630.7 ab	520.0 a	110.7 a
Cover crop removed	12.3 a	28.3 ab	231.7 a	42.3 b	259.0 a	–	44.0 a	63.3 a	680.9 a	561.3 a	119.7 a
MC-33	1.7 b	20.0 b	1.0 b	1.7 b	–	0.0 a	25.0 a	43.4 a	92.7 c	22.7 c	70.0 a
With sunn hemp											
Biosolids	13.3 a	100.0 ab	0.0 c	525.0 a	–	20.0 a	5.7 b	86.7 b	750.7 ab	645.0 ab	105.7 a
N-Viro soil	6.7 a	128.3 a	450.0 ab	275.0 b	–	0.0 a	60.0 ab	101.7 b	1021.7 a	853.3 a	168.4 a
Coal ash	30.0 a	90.0 ab	245.0 bc	116.7 bc	–	13.3 a	50.0 ab	103.3 b	648.3 ab	465.0 b	183.3 a
Co-compost	13.3 a	141.7 a	400.0 ab	108.3 bc	–	11.7 a	73.3 a	123.3 b	871.7 a	661.7 ab	209.9 a
YW-compost	26.7 a	85.0 ab	333.3 abc	170.0 bc	–	0.0 a	75.0 a	128.3 b	818.3 ab	588.3 ab	230.0 a
Control	10.0 a	50.0 bc	423.3 ab	51.7 c	–	3.3 a	38.3 ab	85.0 b	661.6 ab	528.3 ab	133.3 a
Cover crop removed	5.0 a	26.7 bc	616.7 a	10.7 c	–	2.5 a	30.0 ab	63.4 b	755.0 ab	656.6 ab	98.4 a
MC-33	0.0 a	0.0 c	3.3 c	0.0 c	–	–	3.3 b	340.3 a	347.0 b	3.3 c	343.6 a

[1] Plant parasitic nematodes include *Helicotylenchus dihystera*, *Heterodera* sp., *Meloidogyne incognita*, *Pratylenchus neglectus*, *Quinisulcius acutus*, *Rotylenchulus reniformis* and *Tylenchus sandneri*, and non-plant parasitic nematodes include Dorylaimids, *Mononchus* and Rhabditids, respectively, if any identified.
[2] Data followed by same letters within a column represent insignificant differences at $P \leqslant 0.05$.

Table 6. Effects of cover crops combined with various organic amendments on the population densities (number per 250 ml) of different soil nematode taxa in the second year experiment.

	Aphelenchus avenae	***Helicotylenchus dihystera***	***Meloidogyne incognita***	***Rotylenchulus reniformis***	**Dorylaimids**	**Rhabditids**	**Total**	**Subtotal parasitic**[1]	**Subtotal non-parasitic**
With sorghum sudangrass									
Biosolids	1.7 a[2]	7.3 a	35.0 bc	88.3 abc	68.3 a	40.0 b	200.6 ab	130.6 ab	70.0 b
N-Viro soil	0.7 a	3.0 ab	46.7 b	78.3 abc	64.3 a	35.0 b	193.0 ab	128.0 ab	65.0 b
Coal ash	1.3 a	0.7 b	125.0 a	100.0 abc	100.0 a	58.3 b	327.0 a	225.7 a	101.3 a
Co-compost	0.0 a	1.0 b	65.0 ab	126.7 ab	103.0 a	34.7 b	295.7 a	192.7 a	103.0 a
YW-compost	0.0 a	0.7 b	44.0 b	40.3 bc	53.0 a	19.0 b	138.0 b	85.0 b	53.0 b
Control	0.0 a	1.3 b	46.0 b	81.0 abc	80.7 a	35.7 b	209.0 ab	128.3 ab	80.7 a
Cover crop removed	0.0 a	2.7 ab	85.0 ab	156.7 a	99.4 a	46.7 b	343.8 a	244.4 a	99.4 a
MC-33	1.0 a	0.0 b	1.0 c	1.3 c	221.4 a	131.7 a	224.7 ab	2.3 c	222.4 a
With sunn hemp									
Biosolids	13.3 b	17.3 a	23.7 bc	25.0 a	276.7 b	160.0 b	338.0 ab	48.0 bc	290.0 b
N-Viro soil	20.7 b	0.0 b	106.7 ab	10.0 a	426.3 a	138.3 b	564.0 a	116.7 ab	447.3 ab
Coal ash	4.7 b	1.7 b	53.3 ab	16.0 a	125.6 b	93.3 b	201.3 b	71.0 b	130.3 c
Co-compost	17.7 b	0.0 b	52.0 ab	16.7 a	265.7 b	181.7 b	352.1 ab	68.7 b	283.4 b
Yard waste compost	8.0 b	5.0 ab	105.0 ab	48.3 a	237.3 b	180.0 b	403.6 ab	158.3 a	245.3 b
Control	10.7 b	4.0 b	111.7 a	49.0 a	296.7 b	216.7 b	472.1 ab	164.7 a	307.4 a
Cover crop removed	5.7 b	1.7 b	93.3 ab	43.3 a	210.0 b	161.7 b	354.0 ab	138.3 a	215.7 b
MC-33	52.0 a	0.0 b	5.0 c	0.0 a	545.7 b	490.0 a	602.7 a	5.0 c	597.7 a

[1] Plant-parasitic nematodes include *Helicotylenchus dihystera*, *Meloidogyne incognita*, *Pratylenchus neglectus*, *Quinisulcius acutus*, *Rotylenchulus reniformis* and *Tylenchus sandneri*, and non-plant-parasitic nematodes include Dorylaimids, *Mononchus* and Rhabditids.

[2] Data followed by same letters within a column represent insignificant differences at $P \leqslant 0.05$.

of high N content amendments. This might be the main reason that application of some low N content amendments, such as coal ash and yard waste compost, did not improve okra yields consistently. In addition, the significant increase in okra yield in treatments of sunn hemp combined with low N content soil amendments, such as coal ash, and yard waste compost, indicates that the N requirement by okra plants and that of the soil N balance can be adequately met by the organic amendments with high N contents, but not by those with low N contents because sunn hemp contains much more N than sorghum sudangrass, which can be contributed to okra plants. Therefore, to achieve high crop yields, soils amended with N-Viro soil, coal ash and yard waste compost must also be amended with additional N from another source. Furthermore, okra yields in the second year in most treatments were substantially higher than in the first year, which might be also related to the amount of N input into the soil because about 25% more cover crop biomass and N were produced in the second year than in the first (Fig. 1).

The results of root galling on okra plants from both years indicate that root-knot nematodes can be effectively suppressed by sunn hemp and biosolids. The coal ash treatment failed to suppress root-knot nematodes in the first year, but did so in the second. As a matter of fact, a number of researchers[3,29,50–52] have reported that *Crotalaria* spp., which include sunn hemp, suppress sedentary plant parasitic nematodes including root-knot nematodes, soybean cyst nematode (*Heterodera glycines*) and the reniform nematode as well as migratory nematodes such as sting (*Belonolaimus longicaudatus*), stubby root (*Paratrichodorus minor*), dagger (*Xiphinema americanum*) and burrowing (*Radopholus similis*) nematodes. The current experiment showed that sunn hemp combined with biosolids can suppress root-knot nematode populations almost as effectively as MC-33. The nematicidal action of MC-33 is nearly instantaneous, but that of the cover crop plus biosolids treatment is prolonged by the decomposition process, and growers might be able to schedule farm operations to take practical advantage of this approach in crop protection.

There have been several mechanisms reported on cover crops to suppress nematodes, such as acting as traps, which allow nematodes, especially the sedentary endoparasitic nematodes, to invade their roots but prevent completion of development within their roots[52], and favoring rhizosphere microflora that are strongly antagonistic to plant-parasitic nematodes[29,53]. However, nematicidal compounds in sunn hemp root exudates have been postulated to suppress root-knot nematodes[50,52,54]. The fact that removal of cover crop roots and shoots from the soil seemed to result in a higher population of root-knot nematodes, *M. incognita*, especially with and without sunn hemp, even though the difference was not significant (Tables 4 and 5), indicates that the decomposition of sunn hemp roots and foliar tissues after they were incorporated into the soil produces some impact on root-knot nematodes to protect the following crop. Of special importance is the suppression by sunn hemp of root-knot nematodes (*M. incognita*), since they are highly damaging to okra roots. Also it is important to note that sunn hemp either favored or at least did not suppress the build up in density of *Aphelenchus avenae*, Dorylaimids and Rhabditids, which are non-parasitic nematodes.

Table 7. Soil electrical conductivity (EC) of 1 : 2 soil to water mixtures with cover crops and soil amendments in the first year experiment after okra harvest.

	Soil EC (mS cm^{-1})[1]
Cover crops	
Sorghum sudangrass	1.399 a[2]
Sunn hemp	0.973 b
Fallow	0.885 b
Soil amendments	
Biosolids	1.021 a
N-Viro soil	1.376 a
Coal ash	1.171 a
Co-composts	1.204 a
YW-composts[3]	1.359 a
Control	1.280 a
Cover crop removed	1.051 a
MC-33	1.026 a

[1] The ratio of soil to water was 1 : 2.
[2] Data followed by same letters either for cover crops or for soil amendments represent insignificant differences at $P \leqslant 0.05$.
[3] YW, yard waste.

After the fallow treatment, all of the nematode taxa had decreased to non-detectable levels with the exception of the Rhabditids, which also had declined probably because in the fallow there was no plant to provide nutrition for the reproduction of those nematodes. Thus, growing sunn hemp or keeping the land fallow can effectively suppress root-knot nematodes, which shows the importance of this in the vegetable production of okra, one of the very nematode-susceptible crops in the tropical and subtropical regions. The same effect of treatments with MC-33 and biosolids on the suppression of root-knot nematode shows that both MC-33 and biosolids can effectively control the population of root-knot nematodes, *M. incognita*. The reduction of root-knot nematode population with sunn hemp rather than with sorghum sudangrass implies that the parasitic nematode-resistant cover crop, sunn hemp, may release some nematicidal compounds during the decomposition process to impact the reproduction of root-knot nematodes.

The increase in *Aphelenchus avenae* nematode by growing sunn hemp rather than sorghum sudangrass indicates that sunn hemp may facilitate the development and reproduction of the mycophagous nematode, *Aphelenchus avenae*, which helps to control some *Rhizoctonia* and *Fusarium* root-rot fungi[29,55].

The mechanism whereby biosolids combined with the incorporation of the root-knot nematode-resistant cover crop, sunn hemp, suppress root-knot nematodes is not quite clear, but it might be related to the plant growth and development. Biosolids application induced okra plants to grow more vigorously than other treatments, which resulted in a great biomass and higher yields. Vigorous plants are often less vulnerable to attack by pests and pathogens than poorly developed or weakened plants[56,57]. A high content of ammonia in biosolids might result in the plasmolysis of nematodes and the proliferation of nematophagous fungi[58,59]. In addition, a high electrical conductivity (EC), i.e., 16.1 mS cm^{-1} (Table 1), in biosolids might play a vital role to control those nematodes. However, after okra harvest, there was no significant difference observed in soil EC under treatments with all soil amendments, but, for cover crops, soil EC in the treatment of sorghum sudangrass was significantly higher than that of either sunn hemp or fallow (Table 7). This result implies that rather complex mechanisms might be involved for cover crops or soil amendments in controlling plant-parasitic nematodes, which definitely need further investigation. Coal ash suppressed plant-parasitic nematodes somewhat, possibly because it has a high pH, i.e. about 12 in this experiment (Table 1), and the high pH may result in increasing the mortality of some parasitic nematodes[60,61]. Javed et al.[58] also found that the frequency of spore attachment of *Pasteuria penetrans* to *Meloidogyne javanica* increased with increasing the soil pH, e.g., the number of spores attached was significantly higher at pH 9 versus pH 7 or 5, i.e., 6.28, 5.44 and 1.00, respectively. However, we can conclude that growing and incorporating the nematode-resistant cover crop, sunn hemp, versus the conventional one, sorghum sudangrass, in the tropical or subtropical region, combined with some organic amendments, e.g., biosolids, can effectively improve okra production and suppress the root-knot nematode, *M. incognita*, which shows a promising potential to improve the organic farming and sustainable agriculture systems in the tropical and subtropical regions.

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