

# Effect of *Crotalaria juncea* Amendment on Nematode Communities in Soil with Different Agricultural Histories<sup>1</sup>

K.-H. WANG,<sup>2</sup> R. MCSORLEY,<sup>3</sup> R. N. GALLAHER<sup>4</sup>

**Abstract:** Effect of sunn hemp (*Crotalaria juncea*) hay amendment on nematode community structure in the soil surrounding roots of yellow squash (*Cucurbita pepo*) infected with root-knot nematodes was examined in two greenhouse experiments. Soils were from field plots treated long-term (LT) with yard-waste compost or no yard-waste compost in LT experiment, and from a short-term (ST) agricultural site in ST experiment. Soils collected were either amended or not amended with *C. juncea* hay. Nematode communities were examined 2 months after squash was inoculated with *Meloidogyne incognita*. Amendment increased ( $P < 0.05$ ) omnivorous nematodes in both experiments but increased only bacterivorous nematodes in ST experiment ( $P < 0.05$ ), where the soil had relatively low organic matter (<2%). This effect of *C. juncea* amendment did not occur in LT experiment, in which bacterivores were already abundant. Fungivorous nematodes were not increased by *C. juncea* amendment in either experiment, but predatory nematodes were increased when present. Although most nematode faunal indices, including enrichment index, structure index, and channel index, were not affected by *C. juncea* amendment, structure index values were affected by previous soil organic matter content. Results illustrate the importance of considering soil history (organic matter, nutrient level, free-living nematode number) in anticipating changes following amendment with *C. juncea* hay.

**Key words:** community structure indices, *Cucurbita pepo*, *Meloidogyne incognita*, organic amendments, squash, sunn hemp.

Sunn hemp, *Crotalaria juncea* L., is a legume that is receiving increased attention due to its efficient green manure properties, including its ability to fix nitrogen, rapidly produce biomass, increase soil organic matter, sequester carbon (Rotar and Joy, 1983), and suppress many plant-parasitic nematodes (McSorley et al., 1999; Robinson et al., 1998; Santos and Ruano, 1987; Wang et al., 2001). *Crotalaria juncea* also has been documented to increase nematode-antagonistic fungi (Quiroga-Madrigal et al., 1999; Rodríguez-Kábana and Kloepper, 1998; Wang et al., 2001) and bacterivorous nematodes involved in soil nutrient cycling (Venette et al., 1997; Wang et al., 2002) and improve soil nutrient levels (Reeves et al., 1996). Therefore, it is considered a good rotation crop for organic and other sustainable agricultural systems.

Meeting crop nitrogen (N) demand through organic N sources may be one of the most critical issues in developing sustainable agricultural systems (Powers and McSorley, 2000). Using leguminous cover crops such as *C. juncea* has been proven to meet N demand for various crops (Marshall, 2002; Reeves et al., 1996), but often N needs must be supplemented by other N sources or hays (Powers and McSorley, 2000). Dried hays or residues of cover crops are particularly versatile for use as amendments in agricultural production. There is increasing interest in using amendments of *C.*

*juncea* hay as a means of achieving fertilizer needs in organic agricultural production (Marshall, 2002). Availability of nutrients from a hay or other organic matter to the crop plant depends on the mineralization of inorganic nutrients from the organic matter by soil microflora and microfauna (Clarholm, 1984). However, microbes also can immobilize inorganic phosphorous and nitrogen in the soil (Ingham et al., 1985). Free-living nematodes, especially bacterivorous nematodes, graze on the microbes and release these nutrients (Ingham et al., 1985). Bacterivorous nematodes play an important role in nutrient cycling because of their abundance, high turnover rate, and strong interactions with other soil microbes (Ferris et al., 1996, 1997; Freckman, 1988; Ingham et al., 1985), and with predators (Laakso and Setälä, 1999; Yeates and Wardle, 1996).

Previously, *C. juncea* amendment was shown to stimulate population growth of free-living nematodes in soils infested with *Rotylenchulus reniformis* Linford and Oliveira (Wang et al., 2001, 2002) or *Meloidogyne incognita* (Kofoid and White) Chitwood (Wang et al., unpubl.). Elevated numbers of free-living nematodes, specifically bacterivores and fungivores, may improve plant tolerance to plant-parasitic nematodes by increasing plant vigor through more efficient nutrient cycling, or increase numbers of predatory and omnivorous nematodes that can then feed on plant-parasitic nematodes. Due to their relatively low numbers, inconsistent occurrence, and non-specific food habits, the capability of predatory nematodes for providing consistent control of specific pest species is generally limited (Kerry, 1987). However, predatory nematodes represent only one component of the soil ecosystem, whose small individual contribution may become significant only in concert with other nematode-antagonistic flora and fauna present in most soils. For example, increasing free-living nematode abundance also may increase the abundance of tardigrades (Hallas and Yeates, 1972)

Received for publication 8 Nov 2002.

<sup>1</sup> This research was supported in part by U.S. Department of Agriculture, CSREES grant no. 00-51102-9571, "Integrating Pest Management Alternatives to Methyl Bromide with Sustainable Crop Production," and in part by the Florida Agricultural Experiment Station, and approved as Journal Series No. R-09183.

<sup>2</sup> Postdoctoral Research Associate, <sup>3</sup> Professor, Department of Entomology and Nematology, University of Florida, P.O. Box 110620, Gainesville, FL 32611.

<sup>4</sup> Professor, Department of Agronomy, University of Florida, P.O. Box 110730, Gainesville, FL 32611.

The authors thank J. J. Frederick, H. Palmer, and J. Chichester for their technical assistance, and D. Porazinska and J. J. Frederick for reviewing the paper.

E-mail: koonhui@ufl.edu

This paper was edited by Deborah A. Neher.

and nematophagous fungi (Jaffee *et al.*, 1993), which may be more efficient predators than predatory nematodes.

The effect of *C. juncea* amendment on free-living nematodes in different nematode trophic groups is not clear. Ferris *et al.* (2001) suggested that indices derived through faunal analysis of key nematode trophic groups and guilds may provide insight into the condition of the soil food web. The objective of the current research was to determine the effects of *C. juncea* hay amendment on nematode community structure in the soil surrounding plants infected with root-knot nematodes. It is important that organic amendments and other methods used to improve plant and soil ecosystem condition be examined in the context of root-knot nematodes because they are key pests in many agricultural crops grown in the southeastern United States. In particular, we examined hypotheses that amendment of *C. juncea* will: (i) increase numbers of bacterivores and fungivores involved in nutrient cycling, (ii) increase omnivores and predatory nematodes that have a potential to feed on plant-parasitic nematodes, and (iii) change nematode community structure (as evaluated by nematological community indices). To determine if performance of *C. juncea* amendment varies under different soil conditions, these hypotheses are tested in the context of several very different agricultural histories. We hypothesize that these amendment effects of *C. juncea* would have more effect in a soil with low organic matter than in a soil that already has a high level of organic matter.

#### MATERIALS AND METHODS

Two greenhouse experiments were conducted on the University of Florida campus in Gainesville, Florida, in 2001 to examine the differences in nematode communities between *C. juncea*-amended or unamended soils. These effects of *C. juncea* were tested in soils from sites with long-term (LT) experiment and short-term (ST) experiment agricultural histories.

*LT Experiment:* The first experiment was conducted in spring 2001. Soils were from two field sites previously used for LT yard-waste compost studies at the former University of Florida Green Acres Agronomy Farm in Alachua County, Florida. Soils were Arredondo loamy sand (loamy, siliceous, hyperthermic, Grossarenic Paleudult) (Thomas and Wittstruck, 1985), with 94% sand, 2% silt, and 4% clay. Based on previous agricultural and experimental history (McSorley and Gallaher, 1996), these soils are characterized as having a previous history of yard-waste compost (YWC+) or no yard-waste compost (YWC-). The YWC+ soil was collected from field plots amended with 269 mt/ha/yr of composted yard-waste plant materials, including sticks, clippings, and wood fragments, each year from 1993 to 1998. The composting process and application are described in

detail elsewhere (McSorley and Gallaher, 1996); by 2001, when significant decomposition had occurred, the main impact from these past treatments was an accumulation of a great amount of soil organic matter. The site was planted with two successive cycles of maize (*Zea mays* L.) with cowpea (*Vigna unguiculata* [L.] Walp.) as an intercycle cover crop during 1998 and 1999, fallowed with weeds, and remained undisturbed until soil collection in March 2001. Soil organic matter content of this site was 8.44%. The YWC- soil was collected from the same experimental sites as the YWC+ (McSorley and Gallaher, 1996) but from field plots not amended with yard-waste compost. Organic matter content at this soil was 2.42%. Collected soil (approximated 25 to 30 kg from each site) was sieved through a mesh (2-mm-pore) to remove coarse plant debris but still allow smaller flora and fauna to pass through, and the soil was then homogenized for use in a greenhouse experiment.

*Soil amendment:* On 7 March 2001, these two soils were either amended or not amended with *C. juncea* hay at 1% (w/w) rate based on soil dry weight. *Crotalaria juncea* hay was harvested from a crop grown at another site during fall 2000. The hay was air-dried and stored over the winter for more than 3 months. Soil with dry weight of 454 g (adjusted according to soil moisture of each soil) was placed into a 12.7-cm-diam. × 7.5-cm-deep plastic pot. The experiment was a 2 × 2 factorial (compost × *C. juncea* amendment), arranged in a randomized complete block design with four replications.

On 18 March 2001, one germinating squash (*Cucurbita pepo* L., 'Yellow Crookneck') seed was planted per pot. Five days after planting, the soil was infested with 200 *M. incognita* second-stage juveniles (J2) in 3 ml of water by pipeting into three holes made around the seedling. These nematodes had been cultured previously in a greenhouse on pepper (*Capsicum annuum* L., 'California Wonder'). Eggs were extracted from root systems in 0.35% NaOCl (Hussey and Barker, 1973) and incubated on Baermann trays for 7 days to obtain hatched J2 (Barker, 1985).

Plants were watered daily and fertilized weekly with 50 ml/plant of a solution of 0.54 g/L of 15-30-15 (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O) of Miracle-Gro (Scotts Miracle-Gro Product Inc., Marysville, OH) fertilizer, using an equal amount of water or fertilizer for each plant. Squash flowers were picked as soon as they appeared to prevent fruiting and uneven growth among the pots. Safer Brand Insecticidal Soap (Safer Inc., Bloomington, MN) was sprayed on the foliage to manage populations of silverleaf whiteflies (*Bemisia tabaci*), but all plants showed some silver leaf symptoms by the end of the experiment.

The experiment was terminated on 15 May 2001, 8 weeks after *M. incognita* inoculation. Soil from each pot was placed in a plastic bag and mixed well, and 100 cm<sup>3</sup> soil was subsampled to extract nematodes by a sieving

and centrifugal flotation method (Jenkins, 1964). The extracted nematodes were heat-killed (2.5 minutes at 60 °C), preserved with 1% formalin and 0.5% streptomycin sulfate, and stored at 4 °C until counted. All nematodes from the subsample were identified to genus level, or to family or order level if genus was not clear, and counted using an inverted microscope.

*ST experiment:* The amendment experiment was repeated in fall 2001. However, only one soil was tested. Due to the closing of University of Florida Green Acres Agronomy Farm, another field soil was used in the second experiment. This soil, collected from the University of Florida, Experimental Designs Field Teaching Laboratory, Gainesville, Florida, was Millhopper sand (loamy, siliceous, hyperthermic, Grossarenic Paleudult) (Thomas and Wittstruck, 1985) with 92% sand, 3% silt, and 5% clay, and 1.95% organic matter. In contrast to the sites described previously (LT experiment), this site is considered to have an ST agricultural history because it was first planted to vegetable crops rather recently (1997). The field site was cropped with short-term rotations of various vegetable crops, and is continuously disrupted by rototilling after each short-lived vegetable crop. A cover crop of rye (*Secale cereale* L.) was intercropped with lupine (*Lupinus angustifolius* L.) in the season prior to soil sampling. Soil for the greenhouse test was either amended or not amended with *C. juncea* hay as described in LT experiment. Therefore, the experimental design was a randomized complete block design with four replications. Squash seeds were planted on 24 September 2001 and inoculated 1 week after germination. Due to the low recovery of *M. incognita* at the termination of LT experiment, a greater number of *M. incognita* (800 J2/pot) was used for inoculation in ST experiment. The experiment was terminated on 26 November 2001, 8 weeks after nematode inoculation. Methods for data collection were identical to those described for LT experiment.

*Nematode community analysis:* Nematodes were assigned to five trophic groups: bacterivores, fungivores, herbivores, omnivores, and predators (Yeates et al., 1993a). Although feeding habits of Tylenchidae (mostly *Filenchus* and *Tylenchus*) and *Eiphyadophora* are considered unclear (Yeates et al., 1993a), they were classified as fungivores (McSorley and Frederick, 1999) in this experiment. *Monhystera* was grouped as a bacterivore rather than a substrate ingestor (Yeates et al., 1993a). The total number of nematodes in every trophic group and the percentage of every trophic group in the nematode community were calculated.

On the basis of nematode data, several indices of the nematode community were computed. Nematode richness was determined as the total number of different taxa recorded per sample. Simpson's index of dominance (Simpson, 1949) was calculated as  $\lambda = \sum (p_i)^2$ , where  $p_i$  is the proportion of each genus  $i$  present (those identified to the family or order level were ex-

cluded). Simpson's index of diversity was calculated as  $1/\lambda$ . Fungivore to bacterivore (F/B) ratios were calculated to characterize decomposition and mineralization pathways, using the F/B ratio of Freckman and Ettema (1993) and the F/(F+B) ratio of Neher (1999). Total maturity index (MI) as defined by Yeates and Bird (1994) was calculated as  $\sum (p_i c_i)$ , a weighted mean of the colonizer-persister (c-p) values of nematodes in all trophic groups including herbivores, where  $c_i$  is the c-p rating for taxon  $i$  according to the 1-to-5 c-p scale (Bongers and Bongers, 1998). Nematode fauna were further analyzed by a weighting system for the nematode functional guilds in relation to enrichment and structure of the food web as suggested by Ferris et al. (2001). These indices include the enrichment index (EI), structure index (SI), and channel index (CI). The EI and SI are proposed to describe the enrichment and the structure condition of the soil food web, respectively (Ferris et al., 2001). They are calculated as  $EI = 100 \times [e/(e+b)]$  and  $SI = 100 \times [s/(s+b)]$ , where  $e$ ,  $s$ , and  $b$  are the abundance of nematodes in guilds representing enrichment (guilds Ba<sub>1</sub>, Fu<sub>2</sub>), structure (Ba<sub>3</sub>-Ba<sub>5</sub>, Fu<sub>3</sub>-Fu<sub>5</sub>, Om<sub>3</sub>-Om<sub>5</sub>, Ca<sub>2</sub>-Ca<sub>5</sub>), and basal (guilds Ba<sub>2</sub>, Fu<sub>2</sub>) food web components, respectively (Ferris et al., 2001). The CI represents the predominant decomposition pathway in the soil food web, and is calculated as  $CI = 100 \times [0.8Fu_2/(3.2Ba_1 + 0.8Fu_2)]$ , where Fu<sub>2</sub> and Ba<sub>1</sub> are the abundance of fungivorous nematodes in the guild with a c-p value of 2 and bacterivorous nematodes in the guild with c-p value of 1, respectively.

*Statistical analysis:* Nematode community data collected from LT experiment were analyzed as a two-way (compost × amendment) analysis of variance (ANOVA), whereas data from ST experiment were analyzed as a one-way ANOVA. Both analyses were performed using SAS (SAS Institute, Cary, NC) to determine significant effects at  $P \leq 0.05$ , or  $P \leq 0.01$ . Nematode count data were log-transformed prior to ANOVA, and untransformed means are presented.

## RESULTS

*Effect of C. juncea on nematode numbers:* Amendment with *C. juncea* (Cj) resulted in different responses on nematode communities in the two experiments. Abundance of several nematode genera and of total nematodes were affected ( $P \leq 0.05$ ) by Cj in ST experiment but not in LT experiment (Tables 1, 2). However, in the LT experiment, history of yard-waste compost had significant impact on the abundance of several genera of nematodes in the bacterivorous and herbivorous groups ( $P \leq 0.05$ ).

*Bacterivores.* Generally, abundance of bacterivores was greater in LT experiment than ST experiment. However, none of the bacterivorous taxa were affected by Cj amendment in LT experiment (Table 1) except *Acrobelles*, which was increased significantly by Cj amend-

TABLE 1. Nematode genera in soils with or without history of yard-waste compost (YWC+ or YWC-), with or without *Crotalaria juncea* (Cj+ or Cj-) amendment 2 months after squash was planted in a greenhouse experiment using soil from a long-term agricultural history site, summer 2001.

Nematode	c-p value <sup>a</sup>	YWC+			YWC-		
		Cj+	Cj-	Means	Cj+	Cj-	Means
<b>Bacterivores</b>							
<i>Acrobeles</i>	2	124 <sup>b</sup>	25	74	80	145	112
<i>Acrobeloides</i>	2	1,938	1,435	1,686	470	630	550
<i>Eucephalobus</i>	2	55	16	36	264	129	197*
<i>Panagrolaimus</i>	1	8	3	6	40	4	22
<i>Plectus</i>	2	75	10	42	30	125	78
<i>Prismatolaimus</i>	3	6	11	8	2	0	1*
<i>Rhabditidae</i>	1	56	13	34	76	36	56
<i>Wilsonema</i>	2	5	4	4	0	0	0
<i>Zeldia</i>	2	69	59	64	67	185	126
Total		2,356	1,585	1,970	1,038	1,261	1,150
<b>Fungivores</b>							
<i>Aphelenchoides</i>	2	13	5	9	33	38	36*
<i>Filenchus</i>	2	0	4	2	10	10	10
<i>Tylenchus</i>	2	0	1	1	3	9	6
Total		24	15	20	50	59	54*
<b>Herbivores</b>							
<i>Meloidogyne</i>	3	14	12	13	14	12	13
<i>Mesocriconema</i>	3	25	61	43	122	88	105
<i>Pratylenchus</i>	3	15	15	15	5	6	6
Total		54	89 <sup>c</sup>	72	141	106	124
<b>Omnivores</b>							
<i>Eudorylaimus</i>	4	3	0	2	1	0	1
Total		5	0	2	1	0	1
Total nematodes		2,446	1,695	2,071	1,264	1,440	1,352

<sup>a</sup> See Bongers and Bongers (1998).

<sup>b</sup> Data are untransformed arithmetic means of 4 replications. \*, \*\* represent significant difference between  $\log_{10}(x+1)$  transformed means of YWC+ and YWC- treatments at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively. No significant ( $P \leq 0.05$ ) YWC  $\times$  Cj interactions occurred, except for *Acrobeles*.

<sup>c</sup> Difference between Cj+ and Cj- was significant at  $P \leq 0.05$ .

ment only in the YWC+ soil ( $P \leq 0.05$ ). The abundances of many of bacterivorous taxa were increased by Cj amendment in ST experiment ( $P \leq 0.05$ , Table 2)

except for the abundance of *Cephalobus* and *Zeldia*, which were decreased by Cj amendment ( $P \leq 0.05$ , Table 2). Total number of bacterivores was significantly

TABLE 2. Nematode genera in squash planted pots 2 months after incorporation of *Crotalaria juncea* (Cj) amendment in a greenhouse experiment using soil from a short-term agricultural history site, fall 2001.

Nematode	Nematodes/100 cm <sup>3</sup> soil		Nematode	Nematodes/100 cm <sup>3</sup> soil	
	Cj+	Cj-		Cj+	Cj-
<b>Bacterivores</b>			<b>Herbivores</b>		
<i>Acrobeles</i>	20	2*	<i>Meloidogyne</i>	170	43*
<i>Acrobeloides</i>	206	26*	<i>Mesocriconema</i>	0	1
<i>Cephalobus</i>	4	12*	<i>Paratrichodorus</i>	0	8
<i>Eucephalobus</i>	33	5**	Total	170	52**
<i>Panagrolaimus</i>	86	5	<b>Omnivores</b>		
<i>Plectus</i>	13	9	<i>Aporcelaimellus</i>	0	1
<i>Rhabditidae</i>	86	18*	<i>Eudorylaimus</i>	8	2*
<i>Wilsonema</i>	6	0*	Total	8	3*
<i>Zeldia</i>	0	3*	<b>Predators</b>		
Total	461	88**	<i>Ironus</i>	1	0
<b>Fungivores</b>			<i>Nygolaimus</i>	4	0
<i>Aphelenchoides</i>	24	9	<i>Tobrilus</i>	1	0
<i>Filenchus</i>	6	1	Total	6	1**
<i>Nothotylenchus</i>	24	3	<b>Total Nematode</b>		
<i>Tylenchus</i>	2	1		724	162**
<i>Tylencholaimus</i>	0	1			
Total	56	14			

Data are untransformed arithmetic means of 4 replications. \*, and \*\* represent significant difference between  $\log_{10}(x+1)$  transformed means of Cj+ and Cj- treatments at  $P \leq 0.05$ , and 0.01, respectively, according to analysis of variances.

increased by Cj amendment in ST experiment ( $P \leq 0.01$ , Table 2) but not in LT experiment (Table 1).

**Fungivores.** Total number and the abundance of each nematode genera of fungivores were not affected by Cj in either experiment ( $P > 0.05$ , Tables 1, 2). Yard-waste compost history decreased the total number of fungivorous nematodes in LT experiment (Table 1), mainly due to the decrease of *Aphelenchoides* in the YWC+ soil ( $P < 0.05$ , Table 1).

**Herbivores.** Amendment with Cj increased total number of herbivores, especially *M. incognita*, in ST but not in LT experiment ( $P < 0.01$ , Table 2). Yard-waste compost history had no impact on the abundance of herbivores (Table 1).

**Omnivores and predators.** *Crotalaria juncea* amendment increased abundance of omnivorous nematodes (mainly *Eudorylaimus*) in ST experiment ( $P < 0.05$ , Table 2) but had no significant effect in LT experiment. In ST experiment, Cj increased the total number of predatory nematodes as well ( $P < 0.01$ , Table 2), but no predatory nematodes were detected in LT experiment.

**Effect of *C. juncea* on indices of nematode community structure:** Most indices of community structure were not affected by Cj-amendment (Tables 3, 4) except for the maturity index in LT experiment. The majority of nematodes present in these communities were bacterivores, with an especially large percentage of bacterivores present in LT experiment. The F/B ratios and F/(F+B) tended to be less and the (F+B)/PP ratios greater in LT experiment than ST experiment (Tables 3, 4). In LT experiment, communities in soils with a history of compost application (YWC+) had a greater ( $P \leq 0.01$ ) structure index than YWC- soils (Table 3). Averaged over both soils, amendment with Cj resulted

TABLE 4. Nematode community indices in squash planted pots 2 months after incorporation of *Crotalaria juncea* amendment in a greenhouse experiment using soil from a short-term agricultural history site, fall 2001.

Indices	Cj+	Cj-
% bacterivores	59.65	55.12
% fungivores	7.49	8.70
% herbivores	27.40	31.66
% omnivores	1.47	1.89
% predators	1.21	0.55
F/B	0.13	0.16
F/(F+B)	0.13	0.11
(F+B)/PP	3.06	2.16
Richness	18	17
Dominance	0.19	0.15
Diversity	5.31	8.18
Maturity index	1.97	2.06
Enrichment index	69.68	60.64
Structure index	21.53	19.29
Channel index	7.94	11.48

Data are means of 4 replications. No differences ( $P \leq 0.05$ ) between Cj+ and Cj- for any index measured.

in a lower ( $P \leq 0.05$ ) value of maturity index in LT experiment.

## DISCUSSION

**Effect of *C. juncea* amendment on nematode numbers:** Effect of *C. juncea* amendment on nematode communities differed between compost histories and between experiments in which soils representing LT (LT experiment) and ST (ST experiment) agricultural sites were used. In general, *C. juncea* amendment increased total numbers of bacterivores in ST experiment, but not in LT experiment. This amendment did not alter abundance of fungivores during the experiments, but it in-

TABLE 3. Nematode communities indices in soil with or without yard-waste compost (YWC+, YWC-), treated or not treated with *Crotalaria juncea* (Cj) amendment 2 months after squash was planted in a greenhouse experiment using soil from a long-term agricultural history site, summer 2001.

	YWC+			YWC-			ANOVA	
	Cj+	Cj-	Means	Cj+	Cj-	Means	Compost	Cj
% Fungivores	2.31 <sup>c</sup>	1.74	2.02	3.73	4.95	4.34	NS <sup>d</sup>	NS
% Bacterivores	87.40	75.08	81.24	77.85	73.61	75.73	NS	NS
% Omnivores	0.34	0	0.17	0.12	0	0.06	NS	NS
% Herbivores	4.23	13.05	8.64	10.74	7.90	9.32	NS	NS
F/B <sup>a</sup>	0.03	0.03	0.03	0.05	0.07	0.06	NS	NS
F/(F+B)	0.03	0.03	0.03	0.05	0.06	0.05	NS	NS
(F+B)/PP <sup>b</sup>	19.13	58.78	38.96	14.02	11.12	12.57	NS	NS
Richness	11.00	12.50	11.75	10.75	10.75	10.75	NS	NS
Diversity	2.67	2.65	2.66	4.93	4.48	4.71	NS	NS
Dominance	0.46	0.52	0.49	0.34	0.24	0.29	NS	NS
Maturity index	2.00	2.12	2.06	1.97	2.02	2.00	NS	*
Enrichment index	18.81	9.85	14.33	31.41	15.76	23.58	NS	NS
Structure index	5.29	5.88	5.59	1.11	0.00	0.56	**	NS
Channel index	6.92	20.00	13.46	16.32	46.21	31.26	NS	NS

<sup>a</sup> F/B = fungivorous/bacterivorous nematode abundance.

<sup>b</sup> PP = plant-parasitic nematode abundance.

<sup>c</sup> Data are means of 4 replications. \*, \*\* represent significant difference between corresponding main effect means at  $P \leq 0.05$ , and  $P \leq 0.01$ , respectively.

<sup>d</sup> ANOVA effect not significant at  $P \leq 0.05$ .

creased the number of omnivorous and predatory nematodes, provided that they were at a detectable level (ST experiment).

These results only partially support our hypothesis that *C. juncea* amendment could alter the nematode community structure in favor of bacterivores and fungivores. We expected that *C. juncea* amendment would increase numbers of bacterivorous nematodes more than fungivorous nematodes in these ST experiments because dried residue of *C. juncea* a legume, has a relatively low C:N ratio (C:N ratio = 18.92; Marshall, 2002). Increased abundance of fungal-feeding nematodes during the period of organic decomposition would be more likely in crop residues with a greater C:N ratio (Beare *et al.*, 1992; Ferris *et al.*, 1996). However, McSorley and Frederick (1999) found that bacterivore and fungivore numbers were greater following legume and non-legume amendment application, compared to unamended soil throughout 10 months of study.

Conversely, we expected the increase in abundance of bacterivorous nematodes by *C. juncea* amendment to be more consistent. Previous research reported that amendment increased the abundance of bacterivorous nematodes in organic compared to conventional farming systems without amendments (Ferris *et al.*, 1996; Freckman, 1988; Griffiths *et al.*, 1994; Neher, 1999). In the current study, total number of bacterivorous nematodes were increased by *C. juncea* amendment in a soil with initial organic matter <2% (ST experiment) but not in a soil with organic matter of 8% (YWC+ in LT experiment). Low organic matter (<2%) is typical of agricultural soils in north-central Florida; the high level of organic amendment achieved through history of repeated yard-waste compost application is unusual and extreme (McSorley and Gallaher, 1996). The most abundant bacterivore in the YWC+ soil in LT experiment was *Acrobeloides*, reaching abundance of >1,400/100 cm<sup>3</sup> soil in both amended and unamended treatments. It is possible that the abundance of *Acrobeloides* reached a carrying capacity in the soil contained in these greenhouse pots with their high organic matter, so that further amendment of *C. juncea* might not stimulate a flush of the bacteria sufficient to support further growth of the already large *Acrobeloides* population. However, bacterivores in the YWC- soil that had less organic matter content than the YWC+ soil did not respond to the *C. juncea* amendment either. But when comparing results of LT experiment (greater bacterivore abundances) with those of ST experiment (less abundance of bacterivores), it appears that *C. juncea* amendment was more effective in increasing bacterivores when their populations were low in the unamended soil rather than high. This suggests that bacterivore population density may be more critical than level of organic matter in determining the effect of *C. juncea* amendment on the abundance of bacterivores.

Amendment of *C. juncea* did not affect abundance of

herbivorous nematodes (mainly *M. incognita*) in LT experiment but increased them in ST experiment, possibly because a greater abundance of *M. incognita* was used as inoculum in ST experiment. Yeates *et al.* (1997) also found that plant-parasitic nematodes were more abundant under an organic management system. Squash shoot weight was actually greater in *C. juncea*-amended than in unamended soil in ST experiment (Wang *et al.*, unpubl. data). Therefore, the improved plant growth in amended soil may have provided more root growth and feeding sites for *M. incognita*, resulting in greater abundance.

Increase of omnivorous and predatory nematodes by the *C. juncea* amendment supported our hypothesis that amendment would stimulate these beneficial nematodes. Wardle *et al.* (1995) reported that abundance of omnivores and predators was increased when their bacterivore prey had increased 3 months earlier. However, a significant contribution of predatory nematodes toward suppression of plant-parasitic nematodes might not be obvious (Kerry, 1987). Yeates and Wardle (1996) provided another perspective of the beneficial effect of predatory nematodes in an agroecosystem: the increase in predatory nematodes may result in increased cycling of plant nutrients rather than a reduction in plant-parasitic nematodes. This was because predatory nematodes were often feeding on bacterivorous or fungivorous nematodes that apparently served as conduits through which nutrient resources passed from microbes to predacious trophic levels (Yeates and Wardle, 1996). This is an important process in maintaining soil ecosystem health because availability of nutrients from the soil organic matter to plants relies on the mineralization of nutrients from their immobilized forms (Clarholm, 1984).

*Effect of C. juncea amendment on nematode community indices:* *Crotalaria juncea* amendment did not change the richness, diversity, and dominance values of the nematode communities nor the percentages of most nematode trophic groups in both experiments. Yeates *et al.* (1997) also observed similar proportions of free-living nematodes in the cp = 1, 4, and 5 groups in soils whether managed organically or conventionally. Generally, nematode communities in ST experiment contained more species and were more diverse than those in LT experiment, allowing a more diverse community of nematodes to respond to disturbance. In contrast, the nematode fauna of LT experiment was clearly dominated by bacterivores.

Among the faunal indices (MI, EI, SI, and CI), only MI was able to detect any differences due to *C. juncea* amendment, and only in LT experiment, where the maturity index was less in *C. juncea*-amended soil. Although *C. juncea* increased omnivorous nematode numbers, their numbers were extremely low (average of 0.23% of total nematode community in *C. juncea* amended soil, Table 3) and therefore had little impact

on MI compared to the abundant bacterivores (average of 83% of total nematode community in *C. juncea* amended soil, Table 3). In ST experiment, an increase in bacterivores (mostly with  $c-p \leq 2$ ) and omnivores or predators (mostly with  $c-p \geq 3$ ) in the amendment treatment balanced out the overall  $c-p$  value and resulted in a similar MI value to the unamended control. McSorley and Frederick (2002) also suggested that if a treatment similarly affected nematode taxa in different trophic or  $c-p$  groups, no effects could be detected with commonly used indices of nematode community structure, such as MI and F/B. Although some researchers (Neher, 1999; Yeates et al., 1993b) have recommended the use of F/(F+B) rather than F/B, the high abundance of bacterivores resulted in similar values of F/B and F/(F+B) in the current experiments. Examination of additional indices such as EI and SI revealed a similar dilemma. The overabundance of the Ba<sub>2</sub> guild in these experiments reduced the sensitivity of these indices. Despite this dilemma, MI and SI values were greater in the YWC+ than YWC- soil, indicating that the multiple years of compost treatment improved the structure of the nematode communities. Neher (1999) did not detect differences in MI between soil managed conventionally and organically, and suggested that cultivation in both management systems created a greater disturbance on nematode communities that overcame any effect of management system on the communities (Neher and Campbell, 1994).

In contrast, when we compare SI and EI between the two experiments, important information regarding the food web condition of these soils is revealed. When the SI and EI of each soil at the termination of these experiments were placed on the enrichment and structure trajectories (Ferris et al., 2001), soil from LT experiment mapped in Quadrat D (representing a stressed and degraded condition) whereas soil from ST experiment mapped in Quadrat A (representing a highly disturbed but N-enriched condition). The empirical data also support the classification of the soil condition from LT experiment as stressed. In LT experiment, nematode diversity, predator and omnivore numbers, and richness were less than in ST experiment. Richness in soils from LT experiment was only 64% of that in ST experiment, an average difference of six taxa per sample. Furthermore, the soil nematode community from LT experiment was highly skewed toward bacterivores (>73% of the nematode community), especially Cephalobids like *Acrobeloides* and *Eucephalobus*. Wasilewska (1997) suggested that Cephalobidae, bacterivores in guild Ba<sub>2</sub>, become abundant in the early successional stage after enrichment, under a food web that has been diminished due to limitation of resources, adverse environmental conditions, or recent contamination. It is unclear why the nematode community structure of soils in LT experiment had reached this stressed condition with high cephalobid numbers.

However, the site from which the YWC+ and YWC- soils were collected was fallow (except for sparse weeds) and free of crops for more than a year, while soil for ST experiment was collected from a recently cropped (within 2 months) site. The lack of agricultural crops may have had an impact on the soils used in LT experiment, suggesting that regular plant and root growth may be needed to maintain optimum food web condition and nutrient cycling.

One limitation to studies of nematode communities is in the difficult choice of an arena (field, microplot, greenhouse, microcosm) in which to conduct such studies. Studies in greenhouse pots are relatively easy to control but may not always reflect field conditions. The collection, sieving, and mixing of field soil were critical steps in these experiments that have affected some members of the nematode communities or other members of the soil flora and fauna. For instance, some Dorylaimida such as trichodorids are affected by disturbance such as dropping samples, although mixing of soil did not have much effect on these nematodes (Bor and Kuiper, 1966). An alternative would be to fill pots with field soil without mixing, collected directly from points in the field. The disadvantage of not mixing soil is that the high spatial variability of nematode field populations and nutrients would then be included in the greenhouse test, causing extreme variability in data and making statistical analysis problematic. The mixing helps to ensure more uniformity among all pots, allowing for fewer numbers of replications for samples that are especially time consuming to examine.

In conclusion, effect of *C. juncea* on nematode communities varies among agricultural histories. The *C. juncea* amendment increased numbers of bacterivorous, omnivorous, and predatory nematodes during the enrichment stage of decomposition in a recently cultivated Florida agricultural soil, with a typical low level of organic matter (<2%). This effect of *C. juncea* amendment on bacterivores did not occur when numbers of bacterivores were already abundant and dominated by a stressed soil food web. Increased abundance of omnivorous and predatory nematodes by *C. juncea* amendment did not reach a sufficient level to suppress *M. incognita* but may play an indirect role in nutrient mineralization and recycling. Stimulation of nematodes by amendment occurred mainly in the soil that had a short-term agricultural history and was originally low in organic matter content. Thus, farmers need to take field history into consideration when using *C. juncea* hay as an amendment because a greater response is anticipated on soils that are low in nutrients, free-living nematodes, and organic matter.

#### LITERATURE CITED

- Barker, K. R. 1985. Nematode extraction and bioassay. Pp. 19–35 in K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An advanced treatise

- on *Meloidogyne*, vol. 2. Raleigh, NC: North Carolina State University Graphics.
- Beare, M. H., R. H. Parmelee, P. F. Hendrix, W. Cheng, D. C. Coleman, and D. A. Crossley, Jr. 1992. Microbial and fauna interactions and effects on litter nitrogen and decomposition in agroecosystems. *Ecology Monograph* 62:569–591.
- Bongers, T., and M. Bongers. 1998. Functional diversity of nematodes. *Applied Soil Ecology* 10:239–251.
- Bor, N. A., and K. Kuiper. 1996. Gevoeligheid van *Trichodorus teres* en *T. pachydermus* voor uitwendige invloeden. *Mededelingen Rijksfaculteit Landbouwwetenschappen Gent* 31:609–616.
- Clarholm, M. 1984. Heterotrophic, free-living protozoa: Neglected microorganisms with an important task in regulating bacterial populations. Pp. 321–326 in M. J. Klug and C. A. Reddy, eds. *Current perspectives in microbial ecology*. Washington, DC: American Society of Microbiology.
- Ferris, H., T. Bongers, and R. G. M. deGoede. 2001. A framework for soil food web diagnostics: Extension of the nematode faunal analysis concept. *Applied Soil Ecology* 18:13–29.
- Ferris, H., R. C. Venette, and S. S. Lau. 1996. Dynamics of nematode communities in tomatoes grown in conventional and organic farming systems, and their impact on soil fertility. *Applied Soil Ecology* 3:161–175.
- Ferris, H., R. C. Venette, and S. S. Lau. 1997. Population energetics of bacterial-feeding nematodes: Carbon and nitrogen budgets. *Soil Biology and Biochemistry* 29:1183–1194.
- Freckman, D. W. 1988. Bacterivorous nematodes and organic-matter decomposition. *Agriculture, Ecosystems and Environment* 24:195–217.
- Freckman, D. W., and C. H. Ettema. 1993. Accessing nematode communities in agroecosystems of varying human intervention. *Agriculture, Ecosystems and Environment* 45:239–261.
- Griffiths, B. S., K. Ritz, and R. E. Wheatley. 1994. Nematodes as indicators of enhanced microbiological activity in a Scottish organic farming system. *Soil Use and Management* 10:20–24.
- Hallas, T. W., and G. W. Yeates. 1972. Tardigrada of the soil and litter of a Danish beech forest. *Pedobiologia* 12:287–304.
- Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Disease Reporter* 57:1025–1028.
- Ingham, R. E., J. A. Trofymow, E. R. Ingham, and D. C. Coleman. 1985. Interactions of bacteria, fungi, and their nematode grazers: Effects on nutrient cycling and plant growth. *Ecological Monograph* 55:119–140.
- Jaffee, B. A., E. C. Tedford, and A. E. Muldoon. 1993. Tests for density-dependent parasitism of nematodes by nematode-trapping and endoparasitic fungi. *Biological Control* 3:329–336.
- Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter* 48:692.
- Kerry, B. R. 1987. Biological control. Pp. 233–263 in R. H. Brown, and B. R. Kerry, eds. *Principles and practice of nematode control in crops*. Sydney, Australia: Academic Press.
- Laakso, J., and H. Setälä. 1999. Population- and ecosystem-level effects of predation on microbial-feeding nematodes. *Oecologia* 120:279–286.
- Marshall, A. J. 2002. Sunn hemp (*Crotalaria juncea*) as an organic amendment in crop production. M.S. thesis, University of Florida, Gainesville, FL.
- McSorley, R., and J. J. Frederick. 1999. Nematode population fluctuations during decomposition of specific organic amendments. *Journal of Nematology* 31:37–44.
- McSorley, R., and J. J. Frederick. 2002. Effect of subsurface clay on nematode communities in a sandy soil. *Applied Soil Ecology* 19:1–11.
- McSorley, R., and R. N. Gallaher. 1996. Effect of yard-waste compost on nematode densities and maize yield. Supplement to the *Journal of Nematology* 28:655–660.
- McSorley, R., M. Ozores-Hampton, P. A. Stansly, and J. M. Conner. 1999. Nematode management, soil fertility, and yield in organic vegetable production. *Nematropica* 29:205–213.
- Neher, D. 1999. Nematode communities in organically and conventionally managed agricultural soils. *Journal of Nematology* 31:142–154.
- Neher, D. A., and C. L. Campbell. 1994. Nematode communities and microbial biomass in soils with annual and perennial crops. *Applied Soil Ecology* 1:17–28.
- Powers, L. E., and R. McSorley. 2000. *Ecological principles of agriculture*. Albany, NY: Delmar Thomson Learning.
- Quiroga-Madrigal, R., R. Rodríguez-Kábana, D. G. Robertson, C. F. Weaver, and P. S. King. 1999. Nematode populations and enzymatic activity in rhizospheres of tropical legumes in Auburn, Alabama. *Nematropica* 29:129 (Abstr.).
- Reeves, D. W., Z. Mansoer, and C. W. Wood. 1996. Suitability of sunn hemp as an alternative legume cover crop. Pp. 125–130 in *Proceedings of the new technology and conservation tillage*, vol. 96-07. Jackson, TN: University of Tennessee Agricultural Experiment Station.
- Robinson, A. F., C. G. Cook, and A. C. Bridges. 1998. Comparative reproduction of *Rotylenchulus reniformis* and *Meloidogyne incognita* race 3 on kenaf and sunn hemp grown in rotation with cotton. *Nematropica* 28:143 (Abstr.).
- Rodríguez-Kábana, R., and J. W. Kloepper. 1998. Cropping systems and the enhancement of microbial activities antagonistic to nematodes. *Nematropica* 28:144 (Abstr.).
- Rotar, P. P., and R. J. Joy. 1983. 'Tropic Sun' sunn hemp, *Crotalaria juncea* L. HITAGR Research Extension Series 036. University of Hawaii, Honolulu, HI.
- Santos, M. A., and O. Ruano. 1987. Reação de plantas usadas como adubos verdes a *Meloidogyne incognita* raça 3 e *M. javanica*. *Nematologia Brasileira* 11:184–197.
- Simpson, E. H. 1949. Measurement of diversity. *Nature* 163:668.
- Thomas, E. C., and W. H. Wittstruck. 1985. Soil survey of Alachua County, Florida. USDA Soil Conservation Service, Washington, DC.
- Venette, R. C., F. A. M. Monstafa, and H. Ferris. 1997. Trophic interactions between bacterial-feeding nematodes in plant rhizospheres and the nematophagous fungus *Hirsutiella rhossiliensis* to suppress *Heterodera schachtii*. *Plant and Soil* 191:213–223.
- Wang, K.-H., B. S. Sipes, and D. P. Schmitt. 2001. Suppression of *Rotylenchulus reniformis* by *Crotalaria juncea*, *Brassica napus*, and *Target erecta*. *Nematropica* 31:237–251.
- Wang, K.-H., B. S. Sipes, and D. P. Schmitt. 2002. Management of *Rotylenchulus reniformis* in pineapple, *Ananas comosus*, by intercycle cover crops. *Journal of Nematology* 34:106–114.
- Wardle, D. A., G. W. Yeates, R. N. Watson, and K. S. Nicholson. 1995. Development of the decomposer food-web trophic relationships, and ecosystem properties during a 3-year primary succession in sawdust. *Oikos* 73:155–166.
- Wasilewska, L. 1997. Soil invertebrates as bioindicators, with special reference to soil-inhabiting nematodes. *Russian Journal of Nematology* 5:113–126.
- Yeates, G. W., R. D. Bardgett, R. Cook, P. J. Hobbs, P. J. Bowling, and J. F. Potter. 1997. Faunal and microbial diversity in three Welsh grassland soils under conventional and organic management regimes. *Journal of Applied Ecology* 34:453–470.
- Yeates, G. W., and A. F. Bird. 1994. Some observations on the influence of agricultural practices on the nematode faunae of some South Australian soils. *Fundamental and Applied Nematology* 17:133–145.
- Yeates, G. W., T. Bongers, R. G. M. DeGoede, D. W. Freckman, and S. S. Georgieva. 1993a. Feeding habits in soil nematode families and genera—an outline for soil ecologists. *Journal of Nematology* 25:101–313.
- Yeates, G. W., and D. A. Wardle. 1996. Nematodes as predators and prey: Relationships to biological control and soil processes. *Pedobiologia* 40:43–50.
- Yeates, G. W., D. A. Wardle, and R. N. Watson. 1993b. Relationships between nematodes, soil microbial biomass, and weed-management strategies in maize and asparagus cropping systems. *Soil Biology and Biochemistry* 25:869–876.