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# Can using a strip-tilled cover cropping system followed by surface mulch practice enhance organisms higher up in the soil food web hierarchy?

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## ABSTRACT

Field trials were conducted in 2008 and 2009 to evaluate the potential of using sunn hemp (SH), *Crotalaria juncea*, and marigold (MG), *Tagetes patula*, in a strip till cover cropping system (STCC) followed by clipping SH and MG to provide surface mulch (SM). The overall objective was to examine if the STCC + SM could improve the structure of the soil food web compared to bare ground (BG) system where weeds were maintained at minimum level prior to planting. Cucumber (*Cucumis sativus*) and winter gourd (*Benincasa hispida*) were planted as cash crops in 2008 and 2009, respectively. Both the SH and MG in STCC + SM system suppressed herbivorous nematodes through the end of 2008 and up to mid-term crop cycle in 2009. The abundance of bacterivorous and fungivorous nematodes were consistently greater in SH plots during both trials. The structure index was significantly greater in SH treatment plots in 2009, indicating a more structured soil food web than BG treatment. SH and MG plots resulted in higher ( $P < 0.05$ ) abundances of collembolans and predatory mites, respectively. Although crop yields were similar among treatments in 2008, winter gourd yield was significantly higher in SH during 2009. Possible mechanisms of why using the SH STCC + SM system resulted in improved soil food web structure in a relatively short time frame is discussed.

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## 1. Introduction

Cover crops may be regarded as a backbone component in helping annual cropping systems attain ecosystem sustainability (Sullivan, 2010). Ecosystem services provided by cover crops include reducing soil erosion and compaction, increasing soil organic matter, enhancing beneficial soil organisms (Snapp et al., 2005) and improving soil health (Wang et al., 2008). A healthy soil is a soil that is capable of supporting essential life properties such as plant anchorage and nutrient cycling, chemical and physical soil processes, soil biodiversity, and pest suppression (Doran and Zeiss, 2000). Each of these processes may be influenced by cover cropping. In terms of nutrient cycling, although bacteria and fungi release nitrogen (N) to soils as their populations turn over, these organisms also render N unavailable as immobilized microbial biomass. However, the mesofauna that feed on bacteria and fungi can excrete nitrogen-rich waste, contributing up to 30% of mineralized N (De Ruiter et al., 1993; Griffiths, 1994) and increasing plant uptake of N as much as 50% (Laakso et al., 2000). Plants rely on all trophic groups within the soil food web for nutrient mineralization. Thus,

maintaining the integrity of a soil food web is a key component of sustaining soil and associated plant health.

Nematodes are proven to be good soil health bioindicators (Ferris, 2010; Ferris et al., 2001; Neher, 2001; Sánchez-Moreno et al., 2009) and thus can be used to examine how various land management practices impact soil health. A healthy soil food web should sustain nematodes with different life strategies and feeding behaviors ranging from fast growing and breeding bacteria-feeding nematodes at the bottom of the food chain, to slow growing, slow reproducing predaceous nematodes at the top. In addition, nematodes have diverse life strategies, ranging from colonizers (short life and high reproduction rate) to persisters (long life and low reproduction rate) (Bongers and Bongers, 1998). Most importantly, nematodes have numerous interactions with other soil organisms and play important roles in soil nutrient cycling (Ingham et al., 1985). Therefore, nematode faunal analysis provides a reliable insight into soil food web hierarchy and associated soil health (Wang and Mcsorley, 2005).

Several attempts have been made to use cover crops in combination with conservation tillage practices to reduce soil disturbances and increase the abundance of soil organisms higher in the soil food web hierarchy (DuPont et al., 2009; Sánchez-Moreno and Ferris, 2007; Marahatta et al., 2010). However, these studies generally found that agricultural management practices caused a disturbance in the soil food web, and that long term conservation tillage

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(more than 2 years) is required before enhancement of omnivorous or predaceous nematodes can be observed. DuPont et al. (2009) found that although the abundance of bacterivorous and fungivorous nematodes was consistently higher in cover crop treatments, SI (which is mainly a weight abundance of the omnivorous and predaceous nematodes) was not different between cover cropping practice and fallow treatment during the two-year of no-till farming. This suggests that cover cropping and no-till practices are capable of enriching the soil nutrients without increasing the stability of the soil food web structure. They speculated that the initial population densities of omnivorous and predaceous nematodes were too low from previous agricultural disturbances to be influenced by the cover cropping. Other studies have also failed to show increases in soil food web structure following two years of no-tillage (Hanel, 2003; Minoshima et al., 2007) or strip-tillage (Marahatta et al., 2010). However, incorporating sunn hemp, *Crotalaria juncea*, at 1% of soil weight (w/w) in a greenhouse pot experiment within 8 weeks of yellow squash (*Cucurbita pepo*) growth significantly increased the abundance of predaceous and omnivorous nematodes (Wang et al., 2003). Thus, in addition to reducing soil disturbances, adding resources such as organic matter into the soil can also enhance nematodes that are of higher hierarchy in the soil food web.

Soil mites also contribute to the maintenance of soil structure and fertility. Mites influence decomposition by grazing on fungi and other soil organisms, thus promoting the formation of humus in the soil (Coleman and Crossley, 1996). It has been suggested that any management practice that influences soil mite abundance may impact organic matter decomposition and nutrient availability (Bedano et al., 2006). Soils in agricultural fields are often disturbed and plant residues are not allowed to accumulate on the surface especially when polyethylene mulch is used as surface mulch. These practices are detrimental to enhancing the number and diversity of free-living mites (Minor and Norton, 2004).

The current research focused on examining two cover crops known to have nematode antagonistic properties: sunn hemp variety 'Tropic Sun' and French marigold (*Tagetes patula*) variety 'Single Gold'. Sunn hemp is a leguminous cover crop that creates much biomass (7 t/ha of air-dried organic matter at 2 months of growth), and produces 150–165 kg/ha of N under favorable conditions (Rotar and Joy, 1983). Sunn hemp supported low reniform nematode (*Rotylenchulus reniformis*) numbers during growth, but its leachate was lethal to *R. reniformis* (Wang et al., 2001). However, French marigold var. 'Single Gold' produces 1390 kg/ha of dry biomass and 31 kg N/ha at 2 months of growth (Wang, K.-H., unpublished data) and releases nematicidal compounds during growth (Hooks et al., 2010). Sunn hemp and marigold have been shown to enhance total free-living nematodes, but failed to enhance omnivorous and predaceous nematodes over a 2-year strip-till cropping cycle (Marahatta et al., 2010; McSorley et al., 2009).

The aim of this study was to examine a cover cropping system in which the cover crop is used as living mulch in a strip-till system. It was hypothesized that greater ecosystem benefits could be obtained by planting a vegetable crop into a strip-tilled cover crop system (STCC) and periodically trimming the cover crop (living mulch) to provide surface mulch (SM) (i.e. STCC + SM). Strip tilling is cultivating only the portion of the soil that is to contain the cash crop row. Specific benefits anticipated with respect to using this strategy include: (1) greater suppression of herbivorous nematodes by partially tilling the cover crop residue into the planting strip, (2) a reduction in soil disturbances compared to conventional tillage practice (i.e., tilling the entire plot), (3) continuous enrichment of the soil food web through longer provision of organic surface mulch, and (4) the establishment of a more structured and stable soil food web. Thus, the objective of this study was to examine if

using a STCC + SM system could suppress herbivorous nematodes and enhance populations of organisms higher up in the soil food web hierarchy within two cropping cycles.

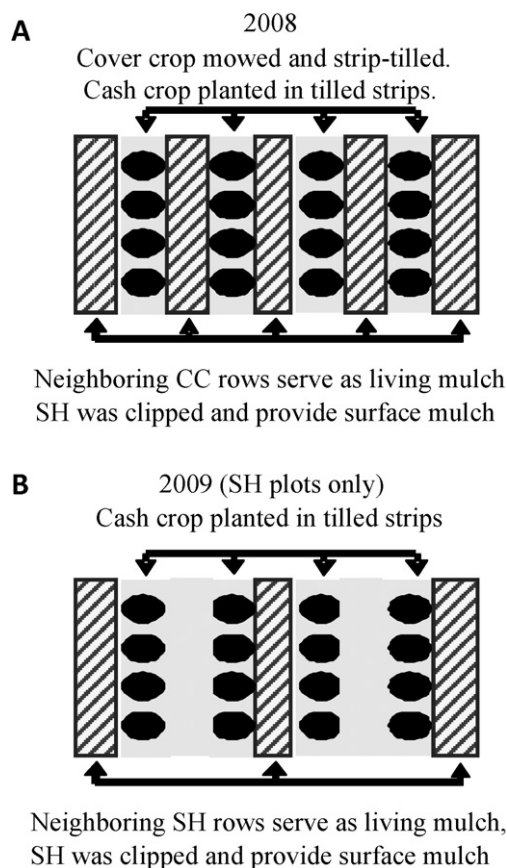
## 2. Materials and methods

A field experiment was conducted at the University of Hawaii at Manoa Poamoho Experiment Station on Oahu, Hawaii in 2008 and 2009. The soil type at the site is Wahiawa silty clay with Tropeptic Eutrustox, clayey, kaolinitic, isohyperthermic soil, containing 18.6% sand, 37.7% silt, and 43.7% clay in the top 25-cm soil. Soil organic matter was approximately 2% with pH of 6.5. This site was cultivated with a broccoli crop before, but had been fallow with weeds for two years prior to the initiation of this experiment. Field was plowed and rototilled. Soils at the experimental site resulted in natural populations of several herbivorous nematode species including *R. reniformis* and root-knot (*Meloidogyne incognita* and *M. javanica*) nematodes. The experiment composed of three pre-plant treatments: sunn hemp (SH) variety 'Tropic Sun', marigold (MG) variety 'Single Gold', or bare ground (BG). Each treatment was replicated 4 times and arranged in a randomized complete block design. Individual plots were  $\sim 11 \times 11 \text{ m}^2$  and separated by at least 6 m of bare soil.

### 2.1. 2008 Trial

In 2008, cover crops were planted between May 2 and 7. Seeds of SH and MG were planted at 29 and 2 g/row (equivalent to 44 kg/ha and 2.95 kg/ha), respectively at 60-cm row spacing and drip-irrigated. Weeds found in BG treatment plots were managed by spot spraying them with the herbicide glyphosate. Weeds in SH and MG plots were managed by hand weeding or spot treatment with glyphosate. No fertilizer was applied during this cover cropping period. At 10 weeks after cover crop planting, alternate rows of cover crops were cut using weed whacker and tilled into the soil at approximately 15–20 cm deep using hand held tiller (Tiller FRC800, Honda Motor Co., Ltd., Japan) between 18 and 23 July 2008 (Fig. 1A). Planting rows in BG plots were also tilled. SH and MG biomass was estimated on 23 July 2008 by clipping the above ground vegetation from three randomly selected 0.19 m<sup>2</sup> quadrants in each plot. After collection, cover crop samples were oven dried at 70 °C to a constant weight and a sub-sample was collected and submitted to A & L (Great Lakes Laboratories, Inc., Fort Wayne, IN) for carbon and nitrogen content analysis.

Two-week old cucumber (*Cucumis sativus*) variety 'sweet slice' (Harris Seeds, Rochester, NY) seedlings were transplanted into the cover crop tilled rows and bare-ground plots with an intra- and inter-row spacing of 1.8- and 1.2-m, respectively, on August 1, 2008. A total of 6 cucumber plants per row, 9 rows per plot were planted, i.e. a total of 54 plants/plot. The remaining cover crop (non-tilled strips) surrounding the cucumber rows remained as an interplant living mulch with the cucumber until completion of the cropping cycle (Fig. 1). The sunn hemp was trimmed to a height of 0.5 m and residues were remained on the soil under the cucumber crop canopy as surface mulch, covering the entire cucumber crop planting rows. The sunn hemp was clipped again on 4 September and 2 October 2008 to reduce shading of cucumber plants. MG was clipped on one occasion (18 September 2008). Cucumber plants were fertilized with urea at 15 g/plant (equivalent to 66 kg/ha) on 13 August 2008. To help manage heavy insect infestations, GF-120 Fruit Fly bait (Dow Agrosciences, Indianapolis, IN) was sprayed on rows of sorghum  $\times$  sudangrass (*Sorghum bicolor*  $\times$  *S. bicolor* var. *sudanense*) neighboring the field site and *Basillus thuringiensis* (Crymax® WDG, Certis, LLC., U.S.A.) was sprayed on cucumber foliage for melon fly (*Bactrocera cucurbitae*) and pickleworm (*Diaphania nitidalis*), respectively.



**Fig. 1.** Illustration of strip-till cover cropping (CC) practice followed by living mulch and surface mulch practice in 2008 (A) and a slight modification for sun hemp (SH) plots in 2009 (B).

Soil samples were taken at cover crop planting (27 June 2008), at cucumber planting (1 August 2008), 3 weeks after strip-tilling (12 August 2008), 6 weeks after cucumber planting (25 September 2008) and after termination of cucumber crop (20 November 2008). Six shovel of soil of 20 cm-deep were collected systematically in a diagonal pattern throughout a plot and composited. Soil was taken from the rhizosphere of cover crop or from bare-ground in BG plot prior to cucumber planting, but was taken from cucumber root zone after cucumber planting. Soil samples collected were transported to the laboratory and nematodes were extracted as described below. Soil sample collected after cucumber planting were also subjected to soil mesoarthropod extraction. Another group of soil fauna monitored were macroarthropods above the soil surface. To monitor isopods and other arthropods on the soil surface, pitfall traps were set up from 26 August to 21 October 2008 using a 500-cm<sup>3</sup> tri-corner beaker filled with 300-ml of dilute detergent solution. Traps were embedded in the ground so that the lip of the trap remained level with the soil surface. Five traps were placed in each experimental plot and the contents were examined, counted, and recorded; traps were replaced weekly.

Cucumber plants were harvested weekly beginning 6 September and ending on 2 October 2008. Fruits damaged by pickleworms and/or fruit flies were categorized as unmarketable. Marketable and unmarketable fruits were counted and weighted.

## 2.2. 2009 Trial

To examine the longer-term impact of STCC+SM practice on the soil fauna, the second trial was super-imposed on treatment plots from 2008 Trial (i.e., similar treatments were repeated in the

same plots). At termination of the 2008 Trial, the SH was close to senescence and the MG reached senescence but had reseeded itself. The previous cucumber rows were tilled under between 1 and 8 December 2008. Weeds were sprayed with glyphosate in February 2009 prior to planting the next crop. Due to the severe cucumber damage caused by pickleworms and fruit flies in 2008, a less susceptible cucurbit, winter gourd (*Benincasa hispida*) 'Ballon' (Kitazawa Seed Company, Oakland, CA) was planted on 2 April in 2009. Cover crops were reseeded on 23 April 2009 only in the non-tilled strips (Fig. 1B). To prevent the SH from shading out the low growing winter gourd, the treatment was modified by planting sunn hemp between two winter gourd rows (Fig. 1B). A lower SH seeding rate (22 g/row or 33 kg/ha) was used in 2009 because of the excessive sunn hemp biomass noted in 2008. The sunn hemp was clipped on 23 June 2009 to provide surface mulch. Early in the season, winter gourd seedlings became infected with a seed-borne fungal disease (charcoal rot) caused by *Macrophomina phaseolina*. Thus, charcoal rot incidence was recorded. Stem diameter at the base of 10 plants randomly selected from each plot were measured at mid-term (29 July 2009) of the winter gourd crop as a measurement of plant growth.

Soil samples were taken prior to initiation of the 2009 Trial (5 January 2009), and at 2 months (18 June 2009) and 4 months (20 August 2009) after winter gourd planting. Soil was subjected to nematodes extraction as described in Section 2.3. Pitfall traps were set up from 17 April to 13 August 2009 similar as described in 2008 to mainly monitor isopods and amphipods.

Winter gourd fruits were harvested weekly beginning 25 June until 20 August 2009. Fruits damaged by pickleworms and fruit flies were categorized as unmarketable. Marketable and unmarketable fruits were counted and weighed. Due to heavy infestation of *Meloidogyne* spp. in 2009, 10 winter gourd plants per plot were removed from the soil at the end of the experiment to examine for root-galls. Roots were rated for nematode galling on a 0–10 scale, where 0 = 0 galls; 1 = 1–2 galls; 2 = 3–10 galls; 3 = 11–30 galls; 4 = 31–100 galls; 5 = >100 galls per root system or less than 25% of root system galled; 6 = 50% of root system galled, 7 = 75% root system galled; 8 = no healthy roots, 9 = completely galled, and 10 = plant and roots are dead (Taylor and Sasser, 1978; Netscher and Sikora, 1990). Roots collected from each plot were brought back to the laboratory and 50 g of roots were subsampled for nematode egg extraction using 0.35% NaOCl (Hussey and Barker, 1973) and incubated on Baermann trays for 10 days to obtain vermiform stage nematodes (Rodriguez-Kabana and Pope, 1981).

## 2.3. Nematode assays

Nematodes were extracted from a 250-cm<sup>3</sup> subsample of soil by elutriation followed by centrifugal flotation (Jenkins, 1964). Nematodes were identified to the lowest taxonomic group possible, counted, and assigned to one of six trophic groups: algivores, bacterivores, fungivores, herbivores, omnivores, or predators (Yeates et al., 1993). The feeding habit of Tylenchidae (mainly *Filenchus* and *Tylenchus*) was classified as fungivore (McSorley and Frederick, 1999; Okada and Kadota, 2003). *Prismatolaimus* was grouped as a bacterivore rather than a substrate ingester claimed by Yeates et al. (1993). The total numbers of every trophic group in the community were calculated. Nematode richness was the total number of 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 of the  $i$  genera present (specimens identified only to the order level were excluded). Simpson's reciprocal index of diversity was calculated as  $1/\lambda$ . The fungivore to bacterivore (F/B) ratio was calculated to characterize decomposition and mineralization pathways (Twinn, 1974 in Freckman and Ettema, 1993). Total maturity index (MI) as defined by Yeates (1994) was cal-



culated as  $\sum(p_i c_i)$ , where  $c_i$  is the colonizer-persister (c-p) rating of taxon  $i$  according to the 1–5 c-p scale of Bongers and Bongers (1998). The nematode fauna was also analyzed by a weighting system for nematode functional guilds in relation to enrichment and structure of the soil food web. The enrichment index (EI) assesses food web responses to available resources, and structure index (SI) reflects the degree of trophic connection in food webs of increasing complexity as the system matures (Ferris et al., 2001). These indices were calculated as  $EI = 100 \times [e/(e+b)]$  and  $SI = 100 \times [s/(s+b)]$  where  $e$ ,  $s$ , and  $b$  are enrichment, structure, and basal food web components calculated as proposed by Ferris et al. (2001). They are sum of weighted abundance of nematodes in guilds representing those components. Whereas the channel index (CI) represents the decomposition pathway in the soil food web, calculated as  $CI = 100 \times [0.8Fu_2/(3.2Ba_1 + 0.8Fu_2)]$ . In general, higher EI and lower CI suggest greater bacterial activity.

#### 2.4. Mesarthropods assay

One hundred g of soil subsampled from each sample was incubated in a Berlese trap (Kim and Jung, 2008) for 3 days using 25 W bulbs (soft white, GE lightening, General Electric Company, OH). Soil mesarthropods were collected in glass jars containing 70% ethyl alcohol, identified to order under a stereo microscope (Nikon, Serco Technical Services, CA), and categorized into trophic groups (Coleman and Crossley, 1996).

#### 2.5. Statistical analysis

All data were checked for normality using Proc Univariate (SAS Institute, Cary, NC). Parameters that were not normally distributed were subjected to appropriate transformation as suggested in Steel and Torrie (1980). Nematode abundance data were log-transformed ( $\log_{10}[x+1]$ ) before analysis of variance (ANOVA), other transformations are listed in Section 3. Only untransformed means are presented. Means were separated using the Waller–Duncan  $k$  ratio ( $k = 100$ )  $t$ -test when the treatment effects were significant ( $P \leq 0.05$ ). Mesarthropods data collected from pit-fall traps at weekly interval were subjected to repeated measure analysis using Proc Mix (SAS Institute, Cary, NC).

### 3. Results

#### 3.1. Cover crop biomass and nutrient content

At termination of cover cropping period in 2008, SH produced 7.5 times more dry biomass than MG (Table 1). Although SH had a higher C:N than MG, when SH tissues were compartmentalized into stem, flower and leaf, C:N ratios of SH were 37.4:1, 11.2:1, and 9.26:1, respectively (data not shown in table). Most importantly, the majority of SH biomass was contributed from leaf tissue. SH also produced 8.2, 5.7, 4.0 and 3.7 times more organic matter, total

**Table 1**  
Biomass and nutrient content from sunn hemp (SH) and marigold (MG) residues harvested on 23 July 2008.

	SH	MG
Dry biomass (t/ha)	11.49	1.53
C:N	29.3:1	20.4:1
Total organic C (kg/ha)	5239.43	636.03
Organic matter (kg/ha)	9036.16	1099.11
Total N (kg/ha)	178.82	31.18
NH <sub>4</sub> -N (kg/ha)	5.83	1.04
Organic N (kg/ha)	172.99	30.14
P (P <sub>2</sub> O <sub>5</sub> ) (kg/ha)	26.39	6.60
K (K <sub>2</sub> O) (kg/ha)	234.57	63.96

**Table 2**

Numbers of key herbivorous nematodes in sunn hemp (SH), marigold (MG), and bare ground (BG) treatment plots throughout the cropping cycle in 2008.

Treatment	P <sub>0</sub> <sup>a</sup>	P <sub>1</sub>	P <sub>2</sub>	P <sub>3</sub>	P <sub>4</sub>
Reniform nematodes/250 cm <sup>3</sup> soil					
SH	267 <sup>b</sup>	202	112	180 b	230 b
MG	132	292	98	85 b	970 ab
BG	230	228	142	968 a	2162 a

<sup>a</sup> Population densities of nematodes at P<sub>0</sub> = 6/27/08, P<sub>1</sub> = 8/1/08, P<sub>2</sub> = 8/12/08, P<sub>3</sub> = 9/25/08, P<sub>4</sub> = 11/20/08.

<sup>b</sup> Means are average of 4 replications. Means in a column for each nematode in each trial followed by the same letter(s) are not different according to Waller–Duncan  $k$ -ratio ( $k = 100$ )  $t$ -test at  $P < 0.05$  based on  $\log(x+1)$  transformation.

N, P in P<sub>2</sub>O<sub>5</sub> form, K in K<sub>2</sub>O form, than MG respectively (Table 1). The majority of the N in SH and MG was in organic form rather than the N–NH<sub>4</sub> form which would be more available for the plant to uptake.

#### 3.2. Effects of STCC + SM on herbivorous nematodes

Among the herbivorous nematodes present, *R. reniformis* was the most abundant nematode at the experimental site in 2008. *Meloidogyne* spp. was not detected during the first 3 sampling dates in 2008, and its population densities was relatively low with no difference among treatments at the last sampling date (P<sub>4</sub>). Thus, data on *Meloidogyne* was not shown for 2008. During the initial 3 sampling dates (P<sub>0–2</sub>), no difference existed in *R. reniformis* population densities among treatments (Table 2). However, it was significantly lower ( $P < 0.05$ ) in SH than BG at P<sub>4</sub>. In 2009, SH reduced ( $P < 0.05$ ) population densities of *R. reniformis* at 2 months after winter gourd planting (P<sub>1</sub>) compared to BG, but not at termination of the winter gourd crop (P<sub>2</sub>, Table 3). Numbers of *R. reniformis* hatched from 50 g of winter gourd roots at 4 months after planting were lower ( $P < 0.05$ ) in MG plots followed by SH plots. Population densities of *Meloidogyne* spp. increased at the experimental site from 2008 to 2009. Although there was no difference among treatments in the soil populations from P<sub>0</sub> to P<sub>2</sub>, MG plots resulted in the lowest *Meloidogyne*. Highest numbers of hatched *Meloidogyne* spp. ( $P > 0.05$ ) and root gall rating ( $P < 0.05$ ) were found in the winter gourd roots from the BG plot as compared to those in SH and MG plots ( $P < 0.05$ ; Table 3).

#### 3.3. Effects of STCC + SM on nematode communities

All nematode taxa in each trophic group were listed in Table 4. Throughout the sampling period during 2008, SH plots supported higher abundance of bacterivorous and fungivorous nematodes compared to BG and to a lesser extent to MG (Fig. 2A, B). SH suppressed herbivorous nematodes throughout 2008 whereas MG suppressed their population up to the third sampling date ( $P < 0.05$ ; Fig. 2C). Abundance of omnivorous nematodes was low compared to other trophic groups (Fig. 2D). Although no significant differences were detected among treatments, the abundance of omnivorous nematodes increased to a higher level in SH plots compared to BG and a similar trend was observed in MG plots toward the end of the cucumber crop (Fig. 2D). Abundance of algivores and predacious nematodes were very low, and no differences in their abundance were detected among treatments. Thus, their abundances were not shown.

Soil health condition was more clearly depicted by nematode richness (Fig. 3A) as SH consistently maintained higher number of nematode genera (richness) compared to BG ( $P < 0.05$ ). MG had higher richness and diversity than BG on one sampling date ( $P < 0.05$ ; Fig. 3A, B). SH and MG had lower MI than BG (Fig. 3C),

**Table 3**

Numbers of key herbivorous nematodes in sunn hemp (SH), marigold (MG), and bare ground (BG) treatment plots throughout the cropping cycle in 2009.

Treatment	P <sub>0</sub> <sup>a</sup>	P <sub>1</sub>	P <sub>2</sub>	Winter gourd roots <sup>b</sup> (50 g roots)	Root-gall index <sup>c</sup>
Reniform nematodes/250 cm <sup>3</sup> soil					
SH	435	362 b	1210	1162 ab	–
MG	252	625 ab	1350	227 b	–
BG	48	1830 a	953	2000 a	–
Root-knot nematodes/250 cm <sup>3</sup> soil					
SH	20	0	252	15,975	2.10 b
MG	0	2	75	16,115	2.25 b
BG	10	57	297	63,750	4.70 a

<sup>a</sup> Population densities of nematodes at P<sub>0</sub> = 1/5/09, P<sub>1</sub> = 6/18/09, and P<sub>2</sub> = 8/20/09.<sup>b</sup> Nematodes were extracted from 50 g of winter gourd roots at termination of winter gourd.<sup>c</sup> Root-gall index in a scale of on a 0–10 scale, where 0 = 0 galls; 1 = 1–2 galls; 2 = 3–10 galls; 3 = 11–30 galls; 4 = 31–100 galls; 5 = >100 galls per root system or less than 25% of root system galled; 6 = 50% of root system galled, 7 = 75% root system galled; 8 = no healthy roots, 9 = completely galled, and 10 = plant and roots are dead.

but no difference in SI was detected among treatments after the initiation of 2008 (Fig. 3D). SH resulted in a higher EI than BG for the first 3 sampling dates after cucumber planting but not at the final sampling date ( $P < 0.05$ ). The BG treatment resulted in a higher CI value than SH and MG treatments until the last sampling date (Fig. 3F).

Similar to 2008, the SH in 2009 Trial resulted in higher populations of bacterivorous and fungivorous nematodes than BG (Fig. 4A, B). SH and MG were able to suppress herbivorous nematodes up to 2 months after winter gourd planting (Fig. 4C), but no differences existed among treatments at 4 months after planting. Number of omnivorous nematodes was highest in SH treatment although not significantly higher than the BG treatment, and over all its abundance was 4.5 times higher in 2009 than 2008 (Fig. 4D). SH resulted in the highest richness among treatments toward the end of the winter gourd trial ( $P < 0.05$ ; Fig. 5A). SH also resulted in the highest nematode diversity by the end of 2009 Trial ( $P < 0.05$ ; Fig. 5B). No difference existed in richness and diversity between MG and BG. Although MI in SH was significantly lower than MG and BG at 2 months after planting winter gourd ( $P < 0.05$ , Fig. 5C), SI was higher ( $P < 0.05$ ) in SH and MG as compared to BG at 4 months

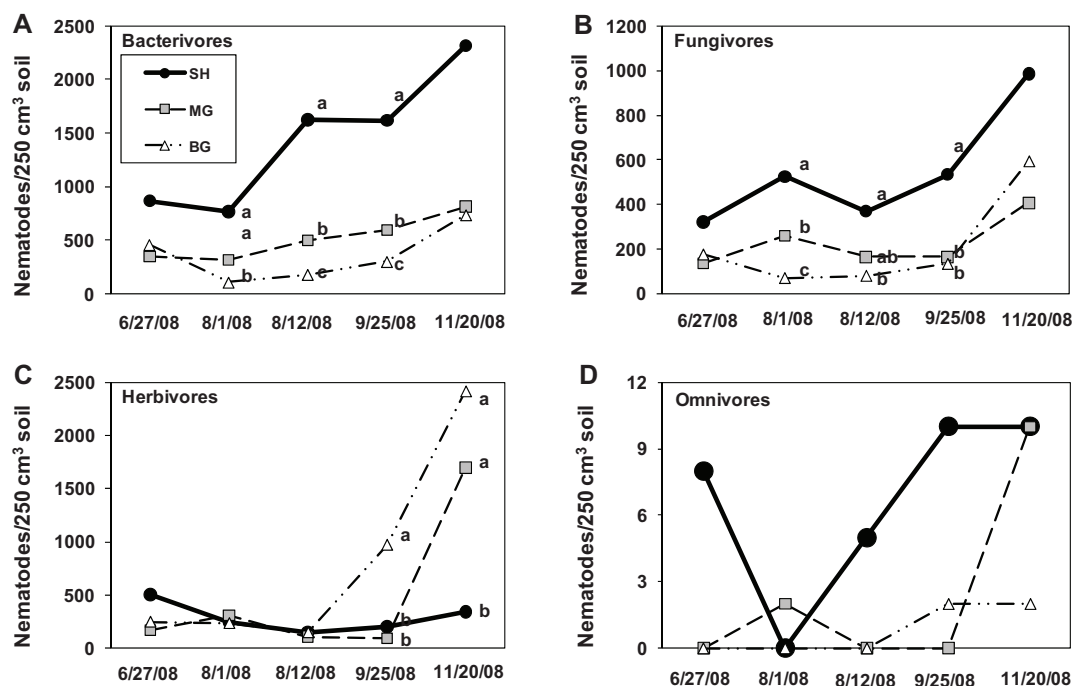
after planting (Fig. 5D). Unexpectedly, EI was higher ( $P < 0.05$ ) in BG than SH at the end of this trial (Fig. 5E) and no difference in CI was observed between SH and BG (Fig. 5F).

### 3.4. Effects of STCC + SM on soil mesoarthropods

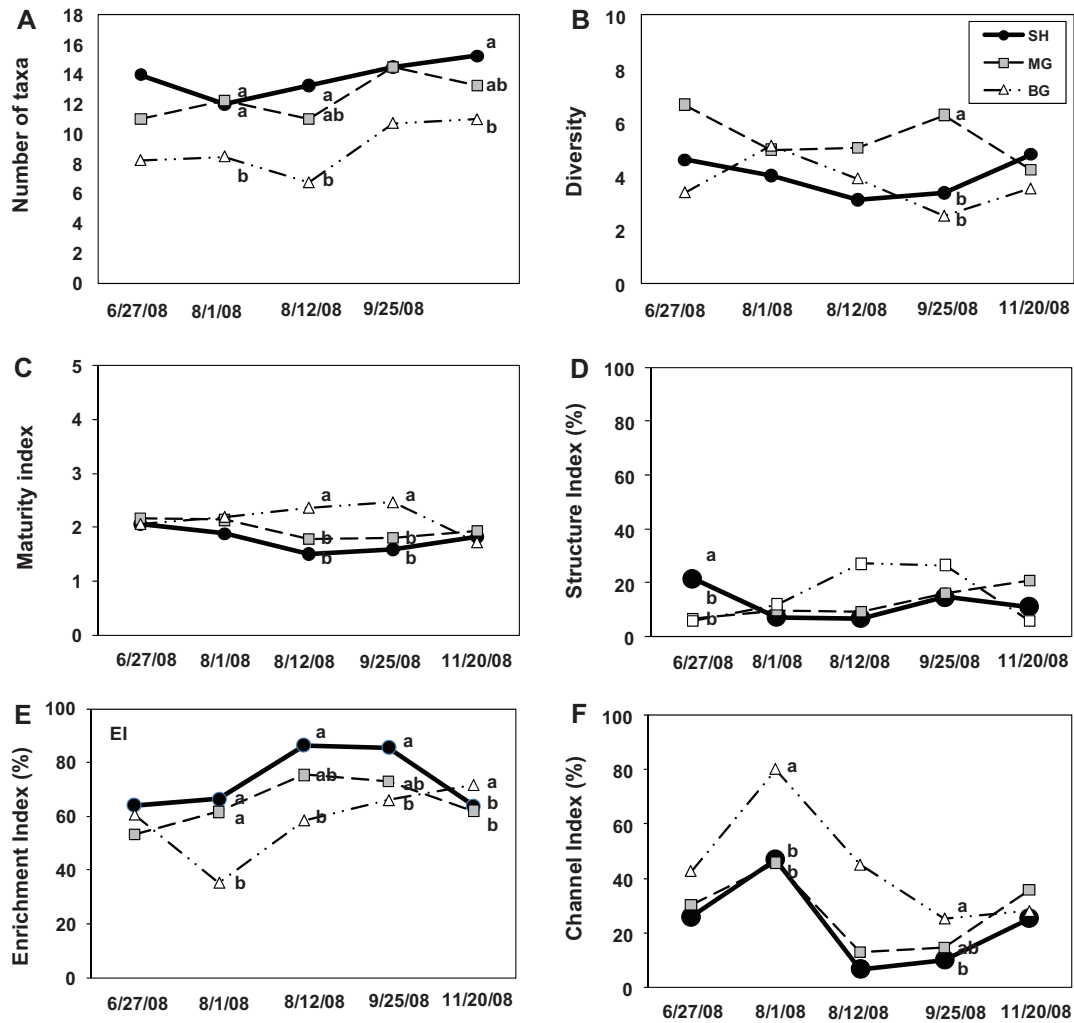
In 2008, the most abundant mesoarthropods extracted from the soil were oribatid mites, predatory mites (including Actinedida and Mesostigmata), and collembola. Although numbers of oribatid mites were not significantly different among treatments (Table 5), predatory mites and collembolans were significantly higher ( $P < 0.05$ ) in MG and SH, respectively than in the BG plots at the last sampling date (Table 5).

### 3.5. Effects of STCC + SM on soil surface arthropods

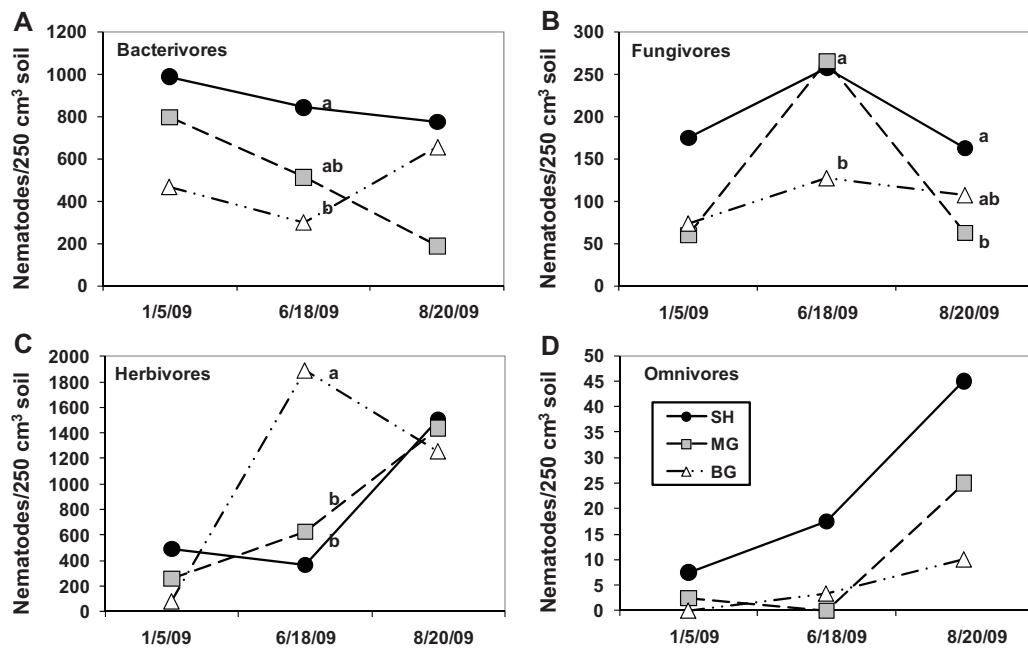
The most abundant soil surface arthropods in 2008 were isopods, whereas those in 2009 were amphipods and isopods. Repeated measures analysis of pitfall trap samples showed that SH consistently supported higher levels of isopods in 2008 and isopods and amphipods in 2009 than MG and BG ( $P < 0.05$ , Fig. 6A,



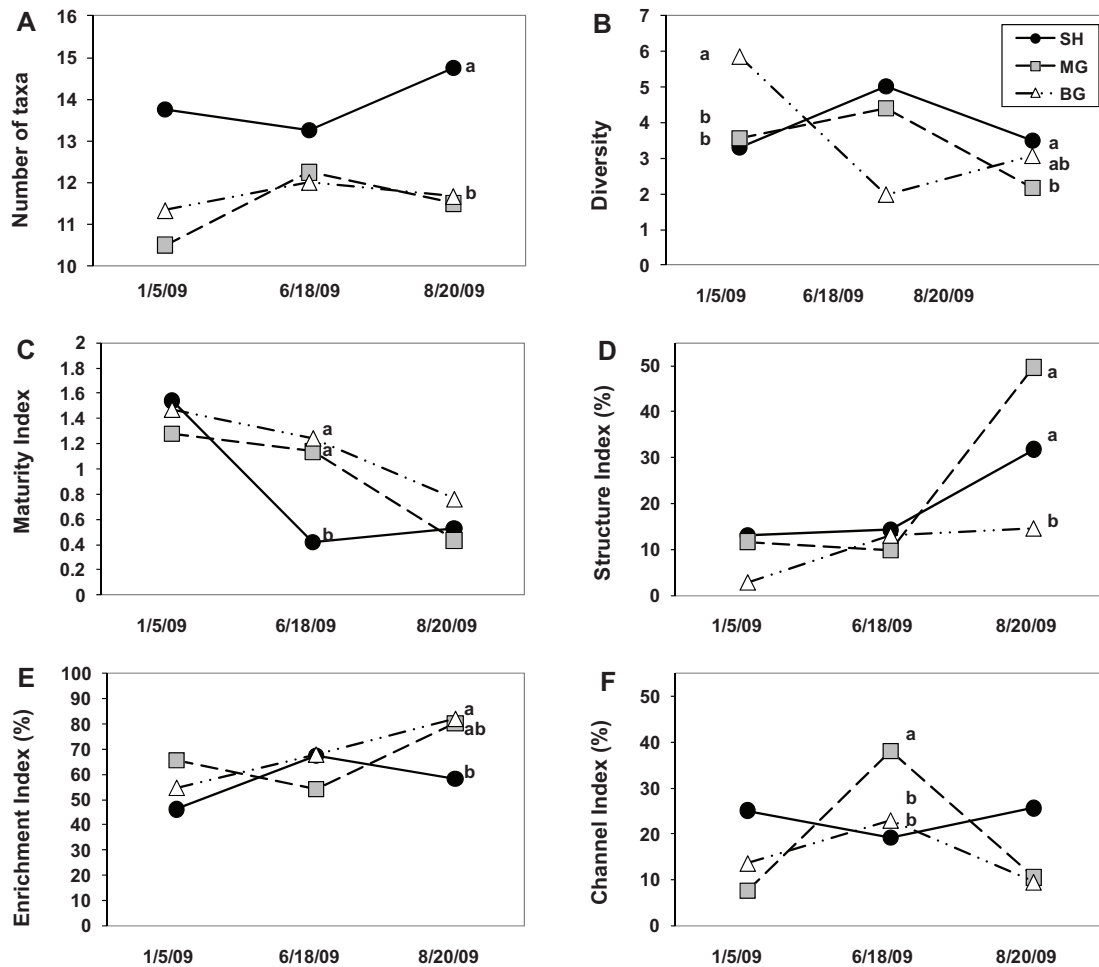
**Fig. 2.** Numbers of (A) bacterivorous, (B) fungivorous, (C) herbivorous, and (D) omnivorous nematodes in soil collected from sunn hemp (SH), marigold (MG) or bare ground (BG) treated plots in 2008. Values ( $n = 4$ ) in each sampling date followed by the same letter are not different according to Waller–Duncan ( $k$ -ratio)  $t$ -test ( $P < 0.05$ ).



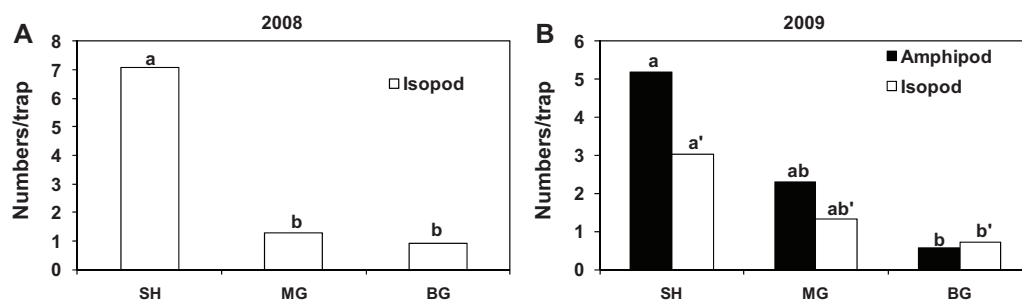
**Fig. 3.** (A) Nematode richness (number of nematode taxa), (B) diversity, (C) maturity index (MI), (D) structure index (SI), (E) enrichment index (EI), (F) channel index (CI) in soil collected from sunn hemp (SH), marigold (MG) or bare ground (BG) treated plots in 2008. Values ( $n=4$ ) in each sampling date followed by the same letter are not different according to Waller–Duncan ( $k$ -ratio)  $t$ -test ( $P<0.05$ ).



**Fig. 4.** Numbers of (A) bacterivorous, (B) fungivorous, (C) herbivorous, and (D) omnivorous nematodes in soil collected from sunn hemp (SH), marigold (MG) or bare ground (BG) treated plots in 2009. Values ( $n=4$ ) in each sampling date followed by the same letter are not different according to Waller–Duncan ( $k$ -ratio)  $t$ -test ( $P<0.05$ ).



**Fig. 5.** (A) Nematode richness (number of nematode taxa), (B) diversity, (C) enrichment index, (D) channel index and (E) structure index in soil collected from sunn hemp (SH), marigold (MG) or bare ground (BG) treated plots in 2009. Values ( $n=4$ ) in each sampling date followed by the same letter are not different according to Waller–Duncan ( $k$ -ratio)  $t$ -test ( $P<0.05$ ).



**Fig. 6.** (A) Isopods collected in 2008, and (B) isopods and amphipods collected in 2009 using pitfall traps partially buried under leaf canopy of the cash crop. Values ( $n=20$ , 5 traps in 4 replications) are repeated measure over 8 and 19 weeks throughout the cropping cycle in 2008 and 2009, respectively.

B). These isopods were also more abundant in MG than BG in both trials ( $P<0.05$ ).

### 3.6. Effects of STCC + SM on crop yield and plant growth

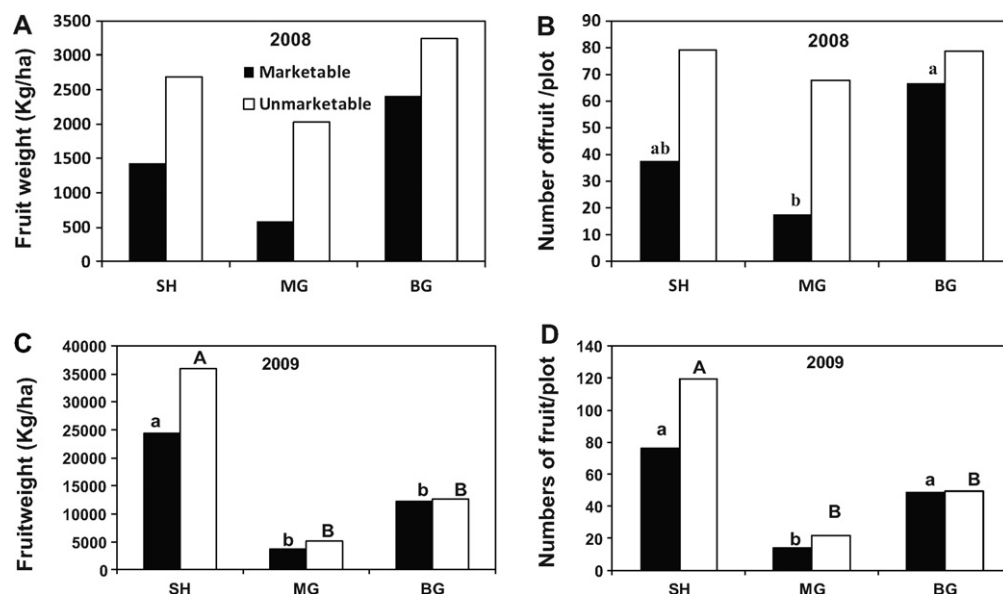
The majority of harvested cucumber fruits were damaged by fruit flies or pickleworms in 2008. No significant difference in fruit weight among treatments was detected in 2008, but BG had higher number of fruits than MG treatment ( $P<0.05$ ; Fig. 7). In 2009, although many winter gourd fruits were damaged by pickleworm, SH produced significantly higher yield of marketable and unmarketable fruits than MG and BG ( $P<0.05$ ; Fig. 7C, D). Early in the winter gourd growing season, some winter gourd transplants dis-

played symptoms of charcoal rot disease. Incidence of charcoal rot (plants with scarified stem or dead plants) was significantly lower in SH than MG and BG treatments ( $P<0.05$ ; Fig. 8A). Stem diameter of winter gourd was also greater in SH than in BG and smallest in MG plots ( $P<0.05$ ; Fig. 8B).

## 4. Discussion

The most significant findings of this study were that STCC system (1) allowed MG to release its allelopathic compounds,  $\alpha$ -terthienyl (Gommers and Bakker, 1988) on the cash crop planting strips prior to crop planting; (2) allowed SH residues to be incorporated into the cash crop planting strips where its





**Fig. 7.** (A) Marketable and unmarketable cucumber fruits weight, and (B) numbers of marketable and unmarketable cucumber fruits accumulated throughout the season in 2008; (C) marketable and unmarketable winter gourd fruits weight, and (D) numbers of marketable and unmarketable winter gourd fruits accumulated throughout the season in 2009. Values ( $n=4$ ) for each fruit category in each trial followed by the same letter are not different according to Waller–Duncan ( $k$ -ratio)  $t$ -test ( $P<0.05$ ).

allelopathic compound, monocrotaline, could be released into the cash crop planting zone (Wang et al., 2002); thus leading to suppression of herbivorous nematodes on the subsequent cash crops planted at least toward mid-term of the cropping season; (3) reduced soil disturbance by only tilling half of the cover cropping area, resulted in enhancement of SI in the SH plots in the second cropping cycle; and (4) continued to enrich soil food web through clipping of cover crop residues and provision of organic

surface mulch directly under the cash crop canopy throughout the cropping season.

The amount of biomass (11.49 t/ha) generated by SH during the cover cropping period in 2008 was much higher than the 7 t/ha previously reported for SH growth in Hawaii at a similar seeding rate of 44 kg/ha (Rotar and Joy, 1983). The amount of SH biomass also far exceeded that of MG produced in this experiment. The abundant SH biomass, organic matter, and N, P, and K contents compared to that of MG provided a good basis of why SH out performed MG in this STCC + SM system.

**Table 4**  
Nematode taxa in each trophic group reported in 2008 and 2009 Trials.

	2008	2009		2008	2009
Algivore			Fungivore		
<i>Achromadora</i>	+	+	<i>Aphelenchoides</i>	+	+
			<i>Aphlenchus</i>	+	+
Bacterivore			<i>Diphtherophora</i>	+	+
<i>Acrobeles</i>	+	+	<i>Ditylenchus</i>	+	+
<i>Acroboides</i>	+	+	<i>Filenchus</i>	+	+
<i>Alaimus</i>	+	+	<i>Leptonchus</i>	+	+
<i>Alirhabditis</i>	+	+	<i>Nothotylenchus</i>	+	+
<i>Cephalobus</i>	+	+	<i>Psilenchus</i>	+	+
<i>Cervidillus</i>	+	+	<i>Tylenchus</i>	+	+
<i>Drilocephalobus</i>	+	+	<i>Tylencholaimus</i>	+	+
<i>Eucephalobus</i>	+	+			
<i>Heterocephalobus</i>	+	+	Herbivore		
<i>Panagrellus</i>	+	+	<i>Helicotylenchus</i>	+	+
<i>Panagrobellus</i>	+	+	<i>Meloidogyne</i>	+	+
<i>Panagrocephalus</i>	+	+	<i>Mesocriconema</i>	+	+
<i>Panagrolaimus</i>	+	+	<i>Paratrichodorus</i>	+	+
<i>Paracrobeles</i>	+	+	<i>Pratylenchus</i>	+	+
<i>Pladorida</i>	+	+	<i>Rotylenchulus</i>	+	+
<i>Plectus</i>	+	+			
<i>Prismatolaimus</i>	+	+	Omnivores		
<i>Pseudoacrobeles</i>	+	+	<i>Aporcelaimellus</i>	+	+
Rhabditidae	+	+	<i>Ecuminicus</i>	+	+
<i>Teratocephalus</i>	+	+	<i>Eudorylaimus</i>	+	+
<i>Turbatrix</i>	+	+	<i>Mesodorylaimus</i>	+	+
<i>Tylocephalobus</i>	+	+	<i>Paraxonchium</i>	+	+
<i>Wilsonema</i>	+	+	<i>Pungentus</i>	+	+
<i>Zeldia</i>	+	+			
			Predator		
			<i>Nygolaimus</i>	+	+
			<i>Tobrilus</i>	+	+

+ indicates genus/taxa is present.

#### 4.1. Effects of STCC + SM on herbivorous nematodes

Planting nematode-antagonistic cover crops such as SH and MG in this STCC system suppressed herbivorous nematodes efficiently in 2008 and up to midseason during 2009. It is well known that SH released monocrotaline that is suppressive to herbivorous nematodes when incorporated into the soil (Wang et al., 2002, 2008). This STCC practice allowed SH to suppress *R. reniformis* efficiently during 2008 but the greater abundance of *R. reniformis* found in the root system of winter gourd grown in SH plots compared to the BG

**Table 5**  
Oribatid and predatory mites (include Actinedida and Mesostigmata) in soil collected from sunn hemp (SH), marigold (MG) or bare ground (BG) treated plots in 2008.

Treatment	6/27/2008	8/1/2008	9/25/2008
Oribatid mite/100 g soil			
SH	0 <sup>a</sup> a	0 a	5.25 a
MG	0 a	0 a	7.75 a
BG	0.50 a	0.50 a	1.00 a
Predatory mite/100 g soil			
SH	0 a	8.50 a	16.00 ab
MG	0.25 a	5.00 ab	22.25 a
BG	0 a	1.50 b	7.75 b
Collembola/100 g soil			
SH	0 a	0.75 a	1.50 a
MG	0.25 a	0.50 a	1.00 ab
BG	0 a	0 a	0 b

<sup>a</sup> Means are average of 4 replications. Means for each arthropod group in each sampling date followed by the same letter are not different according to Waller–Duncan ( $k$ -ratio)  $t$ -test ( $P<0.05$ ).

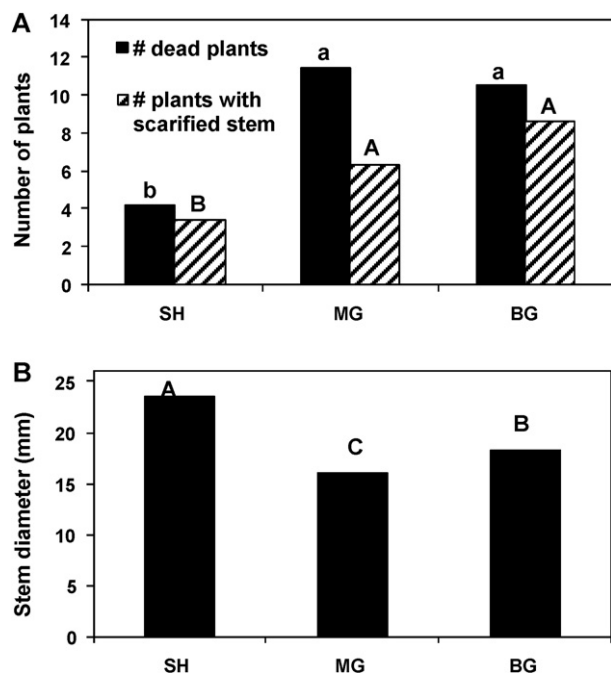


Fig. 8. (A) Numbers of plant die or with scarified stem caused by charcoal rot, and (B) stem diameter of winter gourd measured on 29 July 2009.

treatment at the end of 2009 could be due to more vigorous winter gourd plant growth in SH plots. However, winter gourd plants in SH plots had lower root-galling caused by *Meloidogyne* spp. Thus, STCC + SM practice allowed suppression of herbivorous nematodes by SH as anticipated, unlike the no-till SH cover cropping strategy reported by Wang et al. (2008) in which SH failed to suppress *Meloidogyne* when its residues were not soil incorporated.

Growing MG as a cover crop for 10 weeks also resulted in suppression of *R. reniformis* by the third cucumber sampling period in 2008, and suppression of *R. reniformis* and root-gall index by the final sampling of winter gourd in 2009. Marigold is more effective as a cover crop than as a soil amendment for nematode suppression because it is reported that nematodes that do not penetrate the root or ingest the activated  $\alpha$ -terthienyl will not be killed (Bakker et al., 1979; Gommers, 1972). This is supported later by findings showing that  $\alpha$ -terthienyl compounds are completely devoid of nematocidal activity when mixed into soil (Kagan et al., 1987; Marles et al., 1992). Contradictory results on growing MG as intercrop to suppress herbivorous nematodes on the cash crop had been reported (Govindaiah et al., 1991; Powers et al., 1993) and reviewed (Hooks et al., 2010). However, poorer growth of winter gourd in the MG plots due to competition in growth could be a drawback of using MG in a STCC practice.

#### 4.2. Effects of STCC + SM on nematode communities

As reviewed in the introduction, the biggest challenge of improving soil health condition by cover cropping is to suppress herbivorous nematodes while simultaneously enhancing the soil fauna higher in the soil food web hierarchy. Strip-tilling the cover crop into the cash crop planting rows ensured a more resource enriched soil than no-till. This was further indicated by the fact that SH and MG plots had higher abundance of bacterivorous and fungivorous nematodes than the BG during both trials. According to Ingham et al. (1985), higher abundance of these two nematode trophic groups implies that both bacterial and fungal decomposition processes are active in the soil ecosystem. When leguminous cover crop was used in conventional cover cropping practices, cover

crop residues were fully incorporated into the soil prior to crop planting, but this practice only enhanced bacterivorous nematodes and associated EI early in the cropping season without enhancement toward the end of the vegetable crop cycle (Wang et al., 2006). However, when leguminous cover crop residues were left on the soil as surface mulch, bacterivorous nematodes only increased toward the end of the vegetable crop cycle without the early season enhancement (Wang et al., 2008). On the other hand, if cover crops were mowed and strip-tilled prior to planting the vegetable crop, bacterivorous and fungivorous nematodes were again enhanced only toward the end of the cash crop season (DuPont et al., 2009; Marahatta et al., 2010). In another study, where the cover crop was grown as living mulch and mowed without strip-tilling, no enhancement of bacterivorous or fungivorous nematodes occurred (McSorley et al., 2009). The current study provided some variation to the cover cropping practices researched above. The SH was strip-tilled just prior to planting the vegetable and clipped periodically to provide fresh surface mulch throughout the crop cycle. This strategy resulted in enhanced populations of bacterivorous and fungivorous nematodes compared to BG treatment throughout the vegetable growth cycle. While the increment in the abundance of omnivores was not statistically significant, the increase in SI appeared significant toward the end of the 2009 Trial. This can indicate more structured and less disturbed soil food web in SH and MG treatments compared to BG treatment in 2009, although MI rather declined in all treatments.

This finding is encouraging with respect to developing a strategy that increases the soil food web structure (as indicated by SI) in a short time period. Minoshima et al. (2007) found that after 2 years of conversion to no-till practice, no significant increases in soil food web structure measured by structure index were observed. Hanel (2003) observed increases in omnivorous and predaceous nematodes 2 years after agricultural fields were abandoned. However, in an active agriculture field, little change in nematode community structure was observed even 5 years after initiation of no-till practices (Parmelee and Alston, 1986). Okada and Harada (2007) found differences in nematode community structure between no-till and conventional till plots after 6 years of consecutive no-till practices. Using nematodes as soil health bioindicators, this study demonstrated the potential use of STCC + SM practice to (1) enrich the soil early during the crop growth cycle, (2) support a continuous enrichment of the soil food web; and (3) reduce soil disturbances associated with conventional tillage practices in which the entire plot is tilled. It is believed that STCC + SM practice outperformed STCC practice in terms of enhancing nematodes higher in the soil food web hierarchy because the continuous enrichment of soil food web with organic matter provided a constant food source that sustained organisms that in turn was used as a food source for omnivorous nematodes. The higher nematode richness encounter in the SH STCC + SM plots provided further evidence of improved soil health condition in SH plot than BG treatment. SH planting rows and thus its associated biomass were reduced in 2009 (sunn hemp living mulch was kept between every cash crop row in 2008 whereas it was kept between every two rows of cash crop rows in 2009) as compared to 2008. In addition, SH was clipped once as opposed to three occasions after cash crop planting during the 2008 Trial. It is believed that these changes contributed to the lack of difference between SH and BG in EI and CI in 2009 Trial.

Despite the enhancement of SI, predaceous nematodes were barely detectable. Predaceous nematodes are most sensitive to soil disturbance (Yeates et al., 1993). Agricultural systems often have low omnivorous and predaceous nematodes in comparison to natural areas (Ferris et al., 2001; McSorley et al., 2007). This is due to continuous disturbance from soil cultivation. Further, attempts to introduce omnivorous and predaceous nematodes into a no-till farming system failed to establish these groups of nematodes

(DuPont et al., 2009). Thus, more work is needed in agricultural systems to determine how the nematode fauna higher in the soil food web hierarchy can be raised following a disturbance.

#### 4.3. Effects of STCC + SM on soil mesoarthropods and soil surface arthropods

Soil mites have also been shown to be sensitive to disturbances such as tillage (Coleman and Crossley, 1996; Wardle et al., 1995; Sánchez-Moreno et al., 2009). The current study showed that the abundance of mesoarthropods was almost undetectable prior to initiation of the experiment. However, STCC + SM of MG significantly increased predatory mites in the soil, whereas STCC + SM of SH significantly increased collembolans compared to BG plots at the end of 2008. Previously, Sánchez-Moreno et al. (2009) demonstrated that predatory mites were associated with high values of nematode EI and SI, and thus were also abundant in a no-till organic cropping system. Similar findings between this study and Sánchez-Moreno et al. (2009) helps confirm the reliability of using these two groups of organisms to predict soil health.

Another group of soil fauna, isopods and amphipods, were added to this soil health monitoring system. Isopods and amphipods are relatively small crustaceans that act mainly as detritivores, feeding on decaying plant and animal material, and help in decomposition of organic matter such as phenolic compounds that cannot be digested by other decomposers (Zimmer et al., 2002). These organisms play important roles in recycling nutrients from organic matter that is harder to be decomposed by bacteria and fungi. Enhancement of this group of organisms is adding another benefit to STCC + SM practice in our system.

#### 4.4. Effects of STCC + SM on crop yield and plant growth

Ultimately, soil health should be consistent with plant health. The current study showed an improvement in crop growth only during 2009. This may be attributed to multiple reasons. Cucumber plants were more susceptible to pickleworm injury than winter gourd (personal observation from this study). In 2008, shading from SH and MG may have created a more favorable environment for fruit flies compared to the BG treatment and directly reduced plant growth through competition. Competition in space between MG and cash crop was more critical due to similar plant height between the living mulch and the cash crop. High levels of organic matter added to the soil surface in 2008 may have eventually resulted in a period of nutrient starvation, whereas synthetic fertilizer added to BG plots was more available for crop uptake. In 2009, vigorous winter gourd growth in SH plots was observed early in the season as shown by the stem diameter data. This tentatively helped the winter gourd seedlings better survive the seed-borne charcoal rot disease. Although STCC of SH improved soil health condition and ultimately resulted in significantly higher marketable yield of winter gourd than MG and BG in 2009, it significantly increased unmarketable cash crop yield. Thus, cover crops can improve soil food web condition (as indicated by greater SI values and greater abundance of soil arthropods) but can also increase the incidence of some plant pests such as fruit fly.

In conclusion, the current study demonstrated that STCC + SM practice especially that with SH not only enriched soil food web as indicated by EI, but also enhanced numbers of nematodes and mites higher in the soil food web hierarchy within two cropping cycles and resulted in higher SI in the second crop. The soil enrichment effect not only lasted to the end of a shorter-term vegetable crop (2 months) such as cucumber, but also to the end of a longer-term vegetable crop (4.5 months) such as winter gourd. These effects might have been attributed to less soil disturbance from STCC and a continuous supply of surface organic mulch which helped sustain a

higher hierarchy soil fauna. This allowed the development of a more structured soil food web. However, numbers of predaceous nematode remained low and would need further improvement. Besides the benefits of improving soil health conditions, STCC + SM of SH also suppressed herbivorous nematodes efficiently at least consistently to the mid-term of both cropping seasons. The healthier soil food web conditions in SH plots resulted in improved crop growth, allowed winter gourd seedlings to overcome charcoal rot, and thus increased marketable yield during year 2 of the STCC + SM study. Further research is needed to reduce fruit fly damage on cucurbit crops in this STCC system.

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