Sunn Hemp Cover Cropping and Organic Fertilizer Effects on the Nematode Community Under Temperate Growing Conditions

JERMAINE HINDS,1 KOOK-HUI WANG,2 SHARADCHANDRA P. MARAHATTA,3 SUSAN L. F. MEYER,4 CERRUTI R. R. HOOKS1

Abstract: Field experiments were conducted in Maryland to investigate the influence of sunn hemp cover cropping in conjunction with organic and synthetic fertilizers on the nematode community in a zucchini cropping system. Two field treatments, zucchini planted into a sunn hemp living and surface mulch (SH) and zucchini planted into bare-ground (BG) were established during three field seasons from 2009 to 2011. In 2009, although SH slightly increased nematode richness compared with BG by the first harvest (P < 0.10), it reduced nematode diversity and enrichment indices (P < 0.01 and P < 0.10, respectively) and increased the channel index (P < 0.01) compared to BG at the final harvest. This suggests a negative impact of SH on nematode community structure. The experiment was modified in 2010 and 2011 where the SH and BG main plots were further split into two subplots to investigate the added influence of an organic vs. synthetic fertilizer. In 2010, when used as a living and surface mulch in a no-till system, SH increased bacterivorous, fungivorous, and total nematodes (P < 0.05) by the final zucchini harvest, but fertilizer type did not influence nematode community structure. In 2011, when incorporated into the soil before zucchini planting, SH increased the abundance of bacterivorous and fungivorous nematodes early in the cropping season. SH increased species richness also at the end of the season (P < 0.05). Fertilizer application did not appear to influence nematodes early in the season. However, in late season, organic fertilizers increased enrichment and structure indices and decreased channel index by the end of the zucchini cropping cycle.

Key words: Crotalaria juncea, free-living nematode, living mulch, no-till, strip-till, surface mulch.

Free-living nematodes are widely recognized as nutrient recyclers that make up an important component of the soil microfauna (Ferris et al., 2001). These nematodes encompass a variety of functional groups and perform vital services within the soil ecosystem. Succession of bacterivorous and fungivorous nematodes is important for nutrient cycling of nitrogen and carbon rich organic matter (Ferris et al., 2004). In addition, free-living nematodes closely interact with other soil organisms. Therefore, nematode community structure and other soil properties are good indicators of soil health (Ferris et al., 2001, 2004).

Crop husbandry practices such as cover cropping are often investigated for their impacts on the soil microfaunal community. The use of summer and winter cover crops in combination with irrigation have been shown to increase the abundances of bacterivorous and fungivorous nematodes and resulted in increased N mineralization and tomato growth (Ferris et al., 2004). Careful selection of cover crops could also benefit crops by suppressing soilborne pathogens such as plant-parasitic nematodes. Cover crops such as marigold (Tagetes spp.), rapeseed (Brassica rapa), and sunn hemp (Crotalaria juncea) have been reported to produce allelopathic chemicals that are toxic to nematodes (Wang et al., 2002; Hooks et al., 2010). In addition, proper selection of cover crops could benefit crop production also as their residues can be incorporated into the soil to enhance nematode antagonistic soil microorganisms (Wang et al., 2001).

The current research focuses on examining the potential of sunn hemp utilized as a cover crop in a temperate region. Sunn hemp is known to be a tropical leguminous cover crop native to India with rapid biomass production, ability to fix nitrogen, and nematode suppression properties (USDA-NRCS, 1999; Marshall, 2002; Wang and McSorley, 2004; Marahatta et al., 2010). Rotor and Joy (1983) first reported sunn hemp biomass production of 6,725 kg/ha dry biomass and 165 kg N/ha in Hawaii. Additionally, sunn hemp is often cited for its positive effects on beneficial soil organisms and its ability to suppress plant-parasitic nematodes by functioning as a poor/nonhost, producing nematostatic compounds and increasing nematode-antagonistic fungi (Rich and Rahi, 1995; Wang et al., 2002, 2003, 2004; Marahatta et al., 2010).

Though many studies have investigated the potential use of sunn hemp as a cover crop, most of these studies were conducted in tropical and subtropical climates such as Hawaii and Florida (Sipes and Arakaki, 1997; McSorley, 1999; Wang et al., 2001, 2004, 2006). Few studies have been conducted to investigate the impact of sunn hemp cover cropping on the nematode community in temperate regions (Reeves et al., 1996; Stocking-Gruver, 2007). As such, the first objective of this experiment was to evaluate the impact of sunn hemp cover cropping on nematode community structure in Maryland. Although cover crops are primarily grown during winters in Maryland, there are advantages to utilizing leguminous cover crops such as sunn hemp during the summer. Short season leguminous cover crops can potentially be integrated into a summer cash crop as living mulch to increase crop biodiversity and provide nitrogen to the cash crop. One limitation of using leguminous cover crops, however, is their relatively fast decomposition rate compared with more commonly
used cereal cover crops that may result in short-term improvement of nematode communities. Thus, the second objective of this experiment was to examine the potential for an organic fertilizer utilized in conjunction with a sunn hemp cover crop to provide a synergistic effect in improving nematode community structure in the zucchini-sunn hemp interplanted agroecosystem.

MATERIALS AND METHODS

Experimental design: Field experiments were conducted at the University of Maryland Central Maryland Research and Education Center in Upper Marlboro, MD, during the 2009, 2010, and 2011 growing seasons. For each experiment, eight field plots each measuring 14.6 × 14.6 m were established with 8 m of bare ground area between plots. Each year, the study site consisted of two main treatments arranged in a randomized complete block design. The two treatments, zucchini seedlings planted directly into bare-ground (BG) and zucchini seedlings planted into a sunn hemp living mulch (SH), were replicated four times. During the 2009 growing season, sunn hemp plots were prepared by sowing ‘Tropic Sun’ sunn hemp seeds on 15 June at a rate of 44.8 kg/ha in 12 rows in each plot at a row spacing of 1.2 m. The sown sunn hemp plants were allowed to grow for approximately 6 wk. One week before cash crop planting, the established sunn hemp rows were clipped to a height of approximately 45 cm using hand shears. The clipped sunn hemp foliage was spread between rows as a surface mulch. On 23 July, 2-wk-old greenhouse-grown Cucurbita pepo ‘Fortune’ seedlings (Seedway, Hall, NY) were transplanted into field plots at an inter- and intra-row spacing of 1.2 m. In sunn hemp treatment plots, zucchini seedlings were planted directly into the surface mulch between sunn hemp living mulch rows such that each row of zucchini was surrounded on either side by a row of sunn hemp. Plots were drip-irrigated to mitigate periods of low rainfall; plots and border areas were weeded via hoeing, hand weeding, and spot applications of glyphosate (Roundup, Monsanto, St. Louis, MO) applied with a backpack sprayer. Because of potential disease problems at the 2009 planting site, subsequent field trials were moved to a different field at the same research station. Similar main plot treatments were established in 2010 and 2011 trials; however, main plots were split into two subplots to compare the impact of an organic fertilizer (OF) or synthetic fertilizer (SF). In 2010, sunn hemp seeds were sown on 2 June. Due to competition with the zucchini crop during the previous year, the growth period of sunn hemp was reduced to 5 wk instead of 6 wk. Sunn hemp foliage was mowed 1 wk before zucchini planting to create surface mulch. On 24 June, 2-wk-old ‘Gold Star’ zucchini seedlings (Syngenta, Inc., Wilmington, DE) were transplanted into each plot. Organic or synthetic fertilizer was applied to their designated subplots 28 d after planting by side dressing. The synthetic fertilizer was applied at 35 g of 5-5-10 (Southern States, Upper Marlboro, MD) or 1.75 g N, 3.76 g P2O5, and 2.91 g K2O per plant, whereas organic pelletized chicken manure fertilizer was applied at 58 g (Perdue AgriRecycle, Seaford, DE) 7-2-2 equivalent to 4.06 g N, 0.49 g of P2O5, and 0.96 g K2O per plant. In general, organic fertilizers have lower mineralization rates than synthetic fertilizers (Treadwell et al., 2007). In this experiment, we included higher rates of N from organic fertilizer than synthetic fertilizer to compliment differences in N mineralization rate; and no N deficiency symptoms were observed throughout the zucchini cropping cycle.

Following the 2010 field study, corners of each treatment plot were marked and similar treatments were reestablished in the same plots in 2011. The number of sunn hemp rows established during the 2011 field trial differed from the preceding years in that 24 rows of sunn hemp were planted with an inter-row spacing of 0.61 m. Sunn hemp plants were allowed to grow for 6 wk before zucchini seedlings were transplanted into the treatment plots. Before zucchini transplanting, sunn hemp plants were flail mowed to a height of ~ 20 cm. Alternating sunn hemp rows were then strip-tilled and ‘Gold Star’ zucchini seedlings (Syngenta, Inc., Wilmington, DE) were transplanted into tilled strips on 27 July. Similar to 2010, fertilizer treatments were applied 28 d after planting at 35 and 58 g for synthetic and organic fertilizers, respectively.

Soil sampling: Soil samples consisted of 10 randomly gathered samples using a 2.68-cm-diam. soil probe at a depth of 20 cm in each subplot. These samples were then combined and a subsample of 100 cm³ soil was taken. In 2009, soil samples were collected on 22 June, 27 August, and 15 September, corresponding to preplot establishment, first zucchini harvest, and final zucchini harvest, respectively. In 2010, soil samples were collected on 25 June and 19 August, corresponding to date of zucchini transplanting and date of final zucchini harvest. During the 2011 growing season, soil samples were gathered on 13 August, 1 September, and 21 September, corresponding to post-mow, first harvest, and final harvest, respectively.

Nematode community analysis: Nematodes were extracted from soil using centrifugal flotation from 100 cm³ soil (Jenkins, 1964). Nematodes were identified to the genus level and counted and categorized to seven trophic groups: algivore, bacterivore, fungivore, herbivore (plant-parasitic), omnivore, and predator-based on categorization by Yeates et al. (1993). The ratio of fungivorous to bacterivorous nematodes (F/B+F) was computed. Soil food web indices were calculated as proposed by Ferris et al. (2001). The enrichment index (EI) represents a measure of nutrient enrichment of the environment and is based on the responses of opportunistic bacterivore and fungivore colonizers. Higher numbers
are indicative of a more nutrient enriched soil. Structure index (SI) is based on the proportion of nematodes belonging to higher trophic groups. Structure index values are indicative of the overall connectedness and stability of soil food webs. Channel index (CI), which is the proportion of fungal feeding nematodes to total opportunistic bacterivores and fungivores, describes the dominant decomposition pathway. Lower channel index values suggest a bacteria-dominated decomposition pathway, whereas higher CI values suggest a fungal-dominated decomposition pathway. Nematode richness was determined by total number of taxa (nematodes identified to genus level to determine functional group). The Simpson’s Diversity Index, a measure of species richness and species evenness, was used to measure nematode diversity (Simpson, 1949).

Statistical analyses: Nematode data collected in 2009 were analyzed via one-way analysis of variance (ANOVA) (SAS Institute, Cary, NC). In 2010 and 2011, data collected on the initial sampling date at zucchini planting were analyzed by one-way ANOVA. All subsequent sampling dates were subjected to split-plot ANOVA. When necessary, nematode trophic group parameters were log(x + 1) transformed, and community indices (percentage) data were square root transformed to normalize variances (Steel and Torrie, 1984) for ANOVA. Only nontransformed means are reported.

Results

Each year, plant-parasitic, or herbivorous, nematode populations were low and sporadic at the study sites. The main genera of plant-parasitic nematodes found included Hoplolaimus, Pratylenchus, Paratrichodorus, and Xiphenema. No significant differences were observed between SH and BG, and OF and SF; and no interactions between cover crop treatments and fertilizer types were observed throughout the 3-yr study except for a slight difference detected during the first zucchini harvest period (27 August 2009) during the 2009 trial. During this sampling period, herbivorous nematode counts were higher in SH than BG plots at 307 and 182 per 100 cm$^3$ of soil, respectively ($P < 0.05$). However, this effect did not persist beyond this sampling time. Thus, this report focused on evaluating the effect of cover crop or fertilizer effect on free-living nematodes and nematode community structure.

During the 2009 growing season, no significant differences were detected between SH and BG treatments in terms of nematode abundance in each trophic group examined except for fungivorous nematodes. Fungivorous nematodes were slightly higher in SH than BG before plot establishment and during the first zucchini harvest period (27 August 2009). These effects, however, did not persist beyond these sampling periods. Nematode genera richness was greater in SH (23) than BG (20) during first zucchini harvest ($P < 0.10$), but this effect did not persist to final harvest (data not shown). Contradictorily, at final harvest, diversity and EI were lower ($P < 0.05$ and $P < 0.10$, respectively) and CI higher ($P < 0.01$) in SH than BG treatment (Table 1). No significant interactions between main- and subplot treatment factors were observed throughout the 2009 trial.

During the 2010 trial, there were no significant differences among SH and BG with respect to nematode trophic groups or indices at zucchini transplanting (25 June 10). However, by the final harvest (19 August 2010), SH had higher total nematode abundance than BG ($P < 0.01$, Table 2). This was mainly attributed to higher numbers of bacterivores and fungivores in SH than BG at final harvest ($P < 0.01$). Abundances of omnivorous and algivorous nematodes were also slightly higher in SH than in BG at final harvest ($P < 0.10$, Table 2). There were few differences between fertilizer types (OF and SF) with respect to the nematode community. Only at final zucchini harvest were differences between OF and SF detected. At this time, abundance of bacterivorous nematode was higher in SF than OF plots ($P < 0.10$, Table 2). Fertilizer type did not affect nematode community indices ($P ≥ 0.10$). No significant interaction between main- and subplot treatment factors was observed in 2010 trial.

In the 2011 trial, just after mowing and strip-till (13 August 2011), SH increased total nematodes compared with BG ($P < 0.01$, Table 3), mainly because of higher bacterivorous and fungivorous nematodes in SH than BG ($P < 0.05$). The effect of SH on these trophic groups was not significant on the first harvest ($P ≥ 0.1$, data not shown), but fungivorous and omnivorous nematodes were higher ($P < 0.05$) in SH than BG plots at final harvest (9-21-11). Although SF subplots contained more bacterivorous and total nematodes than OF subplots just after SH mowing and strip tilling ($P < 0.10$, Table 3), numbers of bacterivorous ($P < 0.10$), omnivorous ($P < 0.01$), predatory ($P < 0.10$), and total ($P < 0.10$) nematode numbers were greater in OF than SF plots by final harvest (Table 3).

In terms of nematode community indices in 2011, SI was lower in SH compared with BG plots just after mowing and strip-tilling. No significant differences were detected between SH and BG treatments in terms of nematode abundance in each trophic group examined except for fungivorous nematodes. Fungivorous nematodes were slightly higher in SH than BG before plot establishment and during the first zucchini harvest period (27 August 2009). These effects, however, did not persist beyond these sampling periods. Nematode genera richness was greater in SH (23) than BG (20) during first zucchini harvest ($P < 0.10$), but this effect did not persist to final harvest (data not shown). Contradictorily, at final harvest, diversity and EI were lower ($P < 0.05$ and $P < 0.10$, respectively) and CI higher ($P < 0.01$) in SH than BG treatment (Table 1). No significant interactions between main- and subplot treatment factors were observed throughout the 2009 trial.

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mowing and strip tilling the sunn hemp ($P < 0.05$). By first harvest, nematode diversity was greater ($P < 0.10$) and channel indices were lower ($P < 0.05$) in SH (Table 3). With regards to subplot treatments, EI and SI were greater, whereas CI was lower in OF compared with SF plots by final harvest, ($P < 0.05$, Table 3). No significant interaction between main- and subplot factors was observed except for species richness by final harvest. The addition of OF only increased nematode richness in BG treatment plots ($P < 0.05$).

**DISCUSSION**

**Effects of sunn hemp cover cropping on nematode communities:** The overall goal of the current research was to investigate the impact of sunn hemp as a living and surface mulch on nematode abundances and community indices in a temperate region. Previous work has demonstrated that sunn hemp, when used as a soil-incorporated green manure, can have beneficial effects on nematode communities and its associated soil health parameters (Wang et al., 2001, 2003, 2011; Wang and McSorley, 2004). These impacts include improved soil nutrient cycling and soil food web structure as indicated by various nematode community indices (Wang et al., 2006, 2011), reduction in plant-parasitic nematode populations and increased abundances of nematode-antagonistic organisms (Sipes and Arakaki, 1997; Wang et al., 2002, 2011). Results from the current study suggest that effects of sunn hemp cover cropping on nematode communities vary depending on the associated cultural practice.

When used manually clipped as surface mulch in 2009, sunn hemp had a negative impact on nematode community composition. Community indices calculated in 2009 suggest lower diversity and EI, and higher CI in SH than BG at final harvest. These results are indicative of a relatively simple, nutrient-depleted, and fungal-dominated decomposition pathway in SH plots. This finding could be explained by slower decomposition rate of sunn hemp when used as surface mulch as opposed to using as soil incorporated green manure (Reeves, 1996). The relatively high C:N ratio in sunn hemp stem tissue (> 40:1) (Marshall et al., 2002) could initially rob N from fertilizer, resulting in a more stressed, fungal-dominated decomposition pathway (Wang et al., 2011).

In 2010, when the sunn hemp cover crop was mowed to finer pieces instead of hand clipped and spread over the soil as a surface mulch, beneficial effects on nematode communities were apparent toward the end of the zucchini crop. Sunn hemp enhanced bacterivorous, fungivorous, algivorous, and omnivorous nematodes at final harvest, but decreased diversity and SI as compared with BG. This suggests an enriched but disturbed soil food web in SH plots in 2010. Thus, results obtained using SH solely as organic mulch as in 2010 provides credence to the belief that this cover cropping practice alone is not a viable approach to improve nematode communities and soil food web structure.

In 2011, sunn hemp rows were flail mowed and alternating sunn hemp rows were strip-tilled. When sunn hemp foliage was strip-tilled, or incorporated into the soil, leaf residues underwent rapid bacterial decomposition as suggested by higher numbers of bacterivorous nematodes in SH plots by mid-August. Wang et al. (2004) found that nutrient mineralization of sunn hemp foliage peaked at 2 wk after incorporation. In addition, incorporation of the high C:N sunn hemp stem tissue resulted in an increase of fungivorous nematodes compared with BG. This practice that enhanced different decomposition pathways resulted in
increased nematode diversity at zucchini harvest. Increased in both bacterivorous and fungivorous nematodes also provide food source for omnivorous nematodes, thus omnivorous and SI (which is a weight abundance of omnivorous or predatory nematodes) also increased in SH plots. This result suggests the combined protocol of incorporating sunn hemp residues into the soil initially for soil nutrient enrichment and leaving sunn hemp on the soil surface as a surface mulch to allow nutrients to be released slowly over a longer period of time will help sustain nematodes at higher trophic levels (omnivorous nematodes) toward the end of a zucchini crop. When Wang et al. (2011) practiced strip-till cover cropping of sunn hemp followed by continuous clipping of sunn hemp living mulch periodically to supply fresh sunn hemp organic mulch throughout a cucurbit crop, they observed a significant increase in SI after two consecutive cropping cycles. Enhancement of omnivorous nematodes by SH toward the end of zucchini harvest in 2011 could be attributed partly to consecutive plantings of SH in the same field plots.

**Effect of organic fertilizer on nematode communities**: Synergistic effects of sunn hemp cover cropping and organic fertilizer applications were not detected in this study. This was evident by the fact that no significant interactions between cover cropping and fertilizer treatments were found in 2010 or 2011. The exception was nematode richness at final harvest in 2011 where the addition of OF increased nematode richness in BG treatment plots but not in SH plots. This occurred possibly because the strip-tilled SH plots had already sustained a high level of nematode richness. Therefore, further addition of OF to these plots would not have caused an additional increase in nematode richness.

The effect of fertilizer type alone on nematode communities was highly significant in some cases. However, contradictory results were obtained in 2010 and 2011. Numbers of bacterivorous nematodes were lower in

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### Table 3. Effects of sunn hemp living mulch on nematode communities in a zucchini agroecosystem at Upper Marlboro, MD, during the 2011 growing season. OF and SF indicates samples collected from organic fertilizer and synthetic fertilizer subplots, respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bare-ground (BG)</th>
<th>Sunn Hemp (SH)</th>
<th>P</th>
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<tr>
<td></td>
<td>OF</td>
<td>SF</td>
<td>OF</td>
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<tr>
<td></td>
<td>Post-mow and strip-till, 13 August 2011</td>
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<tr>
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<tr>
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<tr>
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<tr>
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<td>Omnivores</td>
<td>94</td>
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</tr>
<tr>
<td>Predators</td>
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</tr>
<tr>
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<tr>
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<td>First zucchini harvest, 1 September 2011</td>
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<td>Flower queen</td>
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<td>Trophic groups</td>
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<tr>
<td>Bacterivores</td>
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<td>400</td>
<td>1,092</td>
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<tr>
<td>Fungivores</td>
<td>282</td>
<td>176</td>
<td>340</td>
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<tr>
<td>Herbivores</td>
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<td>30</td>
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<tr>
<td>Omnivores</td>
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<tr>
<td>Total</td>
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<td>Final zucchini harvest, 21 September 2011</td>
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<td>Flower queen</td>
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</table>

Nematodes were extracted from 100 cm$^3$ of soil. Means followed by an “m” or “s” indicate significant main plot effect and subplot effects, respectively based on $2 \times 2$ split-plot analysis of variance * $P < 0.10$, ** $P < 0.05$, *** $P < 0.01$. NS = nonsignificant.
OF than SF in 2010 and early sampling dates in 2011. However bacterivorous nematode abundance was higher in OF than SF at final zucchini harvest in 2011. It is anticipated that the benefits associated with organic fertilizer is the additional organic matter. Previous studies have indicated that application of organic matter can sustain higher abundances of bacterivorous and fungivorous nematodes than synthetic fertilizer (Ferris et al., 1996; Wasilewska, 1998; Wang et al., 2006; Okada and Harada, 2007; Sanchez-Moreno et al., 2009). Results obtained at the end of the 2011 cropping cycle are similar to those found by Wang and McSorley (2005) where OF subplots were more nutrient-enriched (higher EI), less disturbed (higher SI), and less stressed (lower CI) than their SF counterparts. Reverse trends observed in OF subplots in 2010 could be because of the use of sunn hemp strictly as surface mulch. Slow decomposition rate of sunn hemp organic mulch might have robbed N available from the chicken pellet fertilizer, and thus resulted in lower bacterivores in OF than SF. The implication of this study is that nematode communities would benefit better from application of organic fertilizer if it is used in conjunction with strip-tillage of sunn hemp cover crop. Modification of cultural practices of sunn hemp cover cropping between trials allow dynamic assessment of the impact of cover cropping and organic fertilizer use on nematode community structure.

In conclusion, sunn hemp cover cropping was not beneficial for nematode community structure when used solely as a living and surface mulch. Conversely, sunn hemp provided a short-term increase in bacterivorous and fungivorous nematodes after being strip-tilled in 2011 and increased omnivorous nematodes toward the end of a zucchini crop. Applications of organic fertilizer may extend elevated beneficial nematode abundances toward the end of a zucchini cropping cycle when applied as part of a SH strip-till system. Findings from this study suggest that there are benefits to integrating a SH strip-till practice with organic fertilizer for the maintenance of beneficial nutrient cycling nematodes in temperate environments. In addition, when interplanted with zucchini, sunn hemp has been found to reduce insect pest numbers and their associated plant impairments (Manandhar et al., 2009; Manandhar and Hooks, 2011; Hinds and Hooks, 2013). As such, future studies should be directed toward examining the long-term impacts of using sunn hemp concurrently as a green manure and surface mulch in combination with organic fertilizer on nematode abundances, community indices and other associated soil health parameters, insect pest levels and crop productivity concomitantly.

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