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Impact of No-till Cover Cropping of Italian Ryegrass on Above and Below Ground Faunal Communities Inhabiting a Soybean Field with Emphasis on Soybean Cyst Nematodes

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Abstract: Two field trials were conducted between 2008 and 2010 in Maryland to evaluate the ability of an Italian ryegrass (IR) (*Lolium multiflorum*) cover crop to reduce populations of plant-parasitic nematodes while enhancing beneficial nematodes, soil mites and arthropods in the foliage of a no-till soybean (*Glycine max*) planting. Preplant treatments were: 1) previous year soybean stubble (SBS); and 2) herbicide-killed IR cover crop + previous year soybean stubble (referred to as IR). *Heterodera glycines* population densities were very low and no significant difference in population densities of *H. glycines* or *Pratylenchus* spp. were observed between IR and SBS. Planting of IR increased abundance of bacterivorous nematodes in 2009. A reverse trend was observed in 2010 where SBS had higher abundance of bacterivorous nematodes and nematode richness at the end of the cover cropping period. Italian ryegrass also did not affect insect pests on soybean foliage. However, greater populations of spiders were found on soybean foliage in IR treatments during both field trials. Potential causes of these findings are discussed.

Key words: conservation tillage, *Heterodera glycines*, *Glycine max*, *Lolium multiflorum*, crop management, soil mite, spider, *Plathypena scabra*, nematode community.

Soybean (*Glycine max* L Merr.) is colonized by a diverse fauna of herbivores and pathogens. The soybean cyst nematode (SCN) *Heterodera glycines* Ichinoe is considered the most economically important soybean pathogen (Riggs and Niblack, 1993; Wrather and Koenning, 2006). Annual yield losses in the US alone due to SCN have been estimated at approximately $1.5 billion (Wrather and Koenning, 2006). Soybean cyst nematode can cause significant yield loss in the absence of noticeable aboveground symptoms (Wang et al., 2003). In addition to SCN, lesion nematodes, *Pratylenchus* spp., also invades soybean roots causing additional damage (Lawn and Noel, 1986).

Population levels of SCN at planting could be the primary determinant of final crop yield (Koenning et al., 1996). Current nonchemical management practices include planting resistant soybean varieties and practicing crop rotation with nonhost plants (Niblack, 2005). However, sixteen races of SCN have been identified and 12 of these have been reported in the US (Chen et al., 2001). Thus, SCN genetic variability poses limitations to the effectiveness of resistant cultivars (Niblack et al., 2002). Additionally, crop rotation may be less effective if SCN populations are moderate to high. Crop rotation of a minimum of two years may be required to reduce the population numbers below damaging levels, especially in sandy soils, but often a longer rotation period such as 5 to 6 years is recommended (Chen et al., 2001). Several nematicides are labeled for use on soybeans, but they do not provide season-long control. In addition, nematicides are expensive for soybean growers, and some nematicides can infiltrate ground water while others pose health risks to humans and the environment (Lacher et al., 1997; Abawi and Widmer, 2000). Thus, field studies are needed to develop alternative methods for SCN management.

A number of plant species have been evaluated for their suppressive effects against SCN (Jackson et al., 2005; Miller et al., 2006; Scotland and MacDonald, 1987; Warnke et al., 2006). Among these studies, leguminous non- or poor hosts reduced SCN population density most efficiently (Miller et al., 2006; Warnke et al., 2006). However, growers in some U.S. states, such as Maryland, are provided cost share funds to grow cover crops during the winter. Non-leguminous winter cover crops or grasses are recommended as they serve as good catch crops for excess nutrients that might otherwise leach from previous cash crops to sensitive waterways. Thus, a feasible practice for soybean farmers with SCN infestations is to grow a grass winter cover crop that can also help manage SCN.

A recent report indicated that the use of Italian ryegrass (IR, also known as annual ryegrass; *Lolium multiflorum*) as a cover crop can increase soybean yields if properly managed (Oregon Ryegrass Growers Seed Commission, www.ryegrasscovercrop.com). Results from several studies indicated that IR can also significantly reduce SCN populations. For example, Creech et al. (2008) found that IR was the only non-leguminous plant among several tested winter cover crops that reduced SCN population densities after one year. Three mechanisms proposed by Riga et al. (2001) by which IR suppresses SCN include: 1) increasing egg hatch in the absence of a host; 2) depleting lipid reserves of juveniles (starving); and 3) inducing low nematode parasitism on the IR, thereby decreasing

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later infection on soybean. The hatched second-stage juvenile (J2) is considered the most vulnerable SCN stage because it does not survive more than a few weeks without a host plant (Ishibashi et al., 1973; Thompson and Tylka, 1997). Reductions in lesion nematode populations have also been reported in soybean fields following IR compared to fallow control (Pedersen and Rodriguez-Kabana, 1991). However, some of the SCN investigations were done in a greenhouse environment (Pedersen et al., 1988; Riga et al., 2001; Warnke et al., 2006). Thus, field studies are needed to solidify the efficacy of IR in reducing damage caused by SCN.

In addition to examining the potential of IR to suppress plant-parasitic nematodes, this study investigated its impact on soil health bioindicators, mainly free-living nematodes and soil mites. Free-living nematodes and soil mites can be used as soil health indicators because they are ubiquitous and have diverse feeding behaviors and life strategies (Ferris et al., 2001; Neher, 2001; Koehler, 1994). Soil nematode sensitivity to habitat disturbances furthers their candidacy as soil health bioindicators (Wang et al., 2006). Soil mite abundance, species richness, ability to colonize different soil habitat, occupancy of various trophic levels, and dispersal strategies make them ideal candidates for assessing the impact of farm management practices on soil health and quality (Behan-Pelletier, 1999). Most importantly, free-living nematodes and soil mites play crucial roles in soil nutrient cycling and some are predators or antagonistic to plant-parasitic nematodes. Further, soil organisms that affect plant performance could indirectly impact aboveground herbivores (Alston et al., 1991; Scheu, 2001). Examining cover crop impact on the nematode community and on aboveground herbivores will provide agricultural managers a more complete assessment of cover crop influences within an agricultural ecosystem.

Specific objectives of this study were to investigate the ability of IR grown as a winter cover crop in a no-till soybean system to: 1) reduce populations of SCN and lesion nematodes; 2) improve the community of free-living nematodes and soil mites; 3) influence insect herbivores and beneficial organisms above the soil surface (i.e., plant canopy); and 4) improve yields in a soybean agroecosystem.

Materials and Methods

Experimental site, treatment and plot layout: Field experiments were conducted in 2009 and 2010 at the University of Maryland Lower Eastern Shore Research and Education Center (LESREC) in Salisbury, MD. The soil was classified according to the USDA texture classification scheme as Fort Mott loamy sand consisting of 83.5% sand, 4.4% silt, 12.1% clay; 0.6% organic matter; and pH 6.6. The field site has a history of SCN infestation including a mixture of races 1, 3, and 5 (Kenworthy et al., 2006). The previous three-year cropping history included corn (*Zea mays*) followed by rye (*Secale cereale*) as a cover crop in 2005, soybean followed by a wheat (*Triticum aestivum)* cover crop in 2006, and corn followed by a rye cover crop in 2007. The study site had been in no-till for several years. The entire experimental area was 0.37 ha and set up in a randomized complete block design with two treatments replicated four times. Each treatment plot measured 13 m × 22 m, contained soybean residue and stubbles from the previous soybean crop, and was separated by 9 m of bare soil. The two treatments were soybeans planted into a 1) herbicide killed Italian ryegrass cover crop mulch (IR), and 2) previous year soybean stubble (SBS). Italian ryegrass was planted in the fall (29 October 2008 and 20 October 2009 in the 2009 and 2010 trials, respectively, at 22.4 kg/ha; following harvest of the previous soybean crop) and sprayed with herbicides in the spring (20 May 2009 and 13 May 2010; prior to soybean seeding). Italian ryegrass residues remained on the soil as dying mulch. Soybean cv Pioneer 94B73 and Pioneer 93M92, with no known resistance to SCN, were planted on June 08, 2009 and 2010, respectively with a 8606 White Planter featuring positive airflow seed placement for accurate no-till planting into treatment plots at 425,891 seeds per ha with a 38 cm inter-row spacing. To monitor the impact of IR over time and to enhance the residue buildup effect that may occur, treatments were maintained in the same plots for both trials. During the study, agrochemical (fertilizers and herbicides) and production practices (planting date, cover cropping techniques, etc.) used closely mimicked commercial practices of soybean managers in Maryland and neighboring Northeastern states. Yield was estimated by harvesting all rows in each plot with a small-plot combine and making adjustment for seed moisture. More details on production practices carried out during the study are listed in Table 1.

Nematode and mite sampling: Soil samples were taken from each plot prior to experiment initiation (1 November 2008), shortly after rye destruction (3 June 2009 and 8 June 2010), approximately 2 months after soybean planting (12 August 2009 and 16 August 2010), and at soybean maturity (3 October 2009 and 2 October 2010). During each sampling, 20 soil cores (2.5-cm diameter × 20-cm deep) were collected randomly from each plot with a soil auger from the intra-row area approximately 10 cm from soybean plants. All soil cores collected from each plot were composited and subsampled, bagged and nematodes were extracted in the laboratory. In 2010, soils were sampled for nematodes and soil mites. These soil samples were divided for nematode and mite faunal analyses at 100 and 250 cm^3, respectively. Eight root samples were collected per plot for SCN quantification on the final sampling dates of 2009 and 2010. An additional eight root samples were collected for mite faunal analysis in 2010. In the laboratory, roots were gently washed free of soil and weighed. To extract nematodes, soil or root samples were placed in water, massaged to remove cysts from roots, and poured

TABLE 1. Dates of key production practices during the 2009 and 2010 field trials.

Year	Date	Task
2008	October 28	Italian ryegrass was planted.
2009	April 30	Fertilized with 05-00-20 + 7%S + 0.2%B at 560 kg/ha
	May 20	Sampled Italian ryegrass biomass from four 0.32 m^2 quadrant/plot
		[a]Sprayed Roundup Weather Max (2.3 l/ha) + Dual II Magnum (3.5 l/ha)
	June 03	Sprayed Gramoxone (2.2 l/ha) + 820 Surf (0.7 1/ha)
	June 08	Planted soybean.
	June 24	Sprayed Roundup Weather Max (1.75 l/ha) + Dual II Magnum (1.2 l/ha)
	Oct. 13	Harvested soybean.
	Oct. 20	Bush-hog mowed soybean debris from harvested plots. Italian ryegrass was planted for next trial.
2010	Mar. 09	Amended soil with high-magnesium pulverized lime (2242 kg/ha)
	May 11	Fertilize with 05-00-20 + 7%S + 0.2%B at 560 kg/ha
	May 13	Spray Roundup Power Max (2.3 l/ha) + Dual II Magnum (1.2 l/ha)
	May 20	Sample Italian ryegrass biomass from four 0.32 m^2 quadrant/plot
	June 08	Planted soybean.
	June 28	Sprayed Roundup Weather Max (1.75 l/ha) + Dual II Magnum (1.2 l/ha)
	Oct. 26	Harvested soybean

[a] Herbicides when used were applied to each treatment plot.

through nested sieves: 20 mesh (850-μm-pore) over 60 mesh (250-μm-pore) over 500 mesh (25-μm-pore). Cysts were recovered and counted as described in Krusberg et al. (1994). Vermiform nematodes collected from the sieves were counted under an inverted microscope and identified to the genus level whenever possible. Developmental stages of SCN were categorized as infective J2, cysts with eggs or cysts without eggs (Melakeberhan and Dey, 2003). Nematodes were counted and converted to number of nematodes per g roots. For mite extraction, unwashed root samples were weighed and placed in a modification Berlese-funnel apparatus (Edwards, 1991) for 7 days using 25 watt light bulbs. Mites were identified to order with the exception of Mesostigmata which live in the soil as predators of various soil invertebrates and help regulate their populations (Walter and Ikonen, 1989; Moore et al., 1988); these mite's position in the soil foodweb makes them key soil health bioindicators (Beaulieu and Weeks, 2007). As such, mites in this order were identified to genus level and counted using a stereomicroscope.

Nematode community analysis: Nematodes were 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 in this study as opposed to substrate ingestor as suggested by Yeates et al. (1993). Total numbers of each trophic group in the community were calculated. Nematode richness included total number of taxa recorded per sample. Simpson's index of dominance (Simpson, 1949) was calculated as $\lambda = \Sigma\, (p_i)^2$, 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 (Freckman and Ettema, 1993). Total maturity index (MI) as defined by (Yeates, 1994) was calculated as $\Sigma\, (p_i c_i)$, where c_i is the colonizer-persister (c-p) rating of taxon i according to the 1 to 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 suggested by Ferris et al. (2001). They are the sums 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 $CI = 100 \times [0.8Fu_2/(3.2Ba_1 + 0.8Fu_2)]$.

Foliar sampling of pests and beneficial arthropods: Soybean foliage arthropods were sampled weekly with the use of a 38.1 cm diameter sweep net. A sample consisted of five sweeps down and across two rows of soybean. Two samples were taken per treatment plot. Arthropod collections were transferred into ziplock sealed plastic storage bags and temporarily stored on ice in a portable cooler while in the field, transported to the laboratory, and stored in a freezer for later species identification and counting. Sampling commenced on 7 July 2009 and 8 July 2010, and was terminated on 21 September 2009 and 14 September 2010, respectively, following soybean crop senescence.

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 and soil mite abundance data were log-transformed ($\log_{10}[x+1]$) before analysis of variance (ANOVA); other transformations are listed in the results section. Only untransformed means are presented. Means were separated using the Waller-Duncan k ratio (k=100) t-test when the treatment effects were significant ($P \leq 0.05$). Foliar arthropod counts were subjected to a repeated-measure analysis of variance (ANOVA) (SAS 9.1, SAS Institute 2002) with trial designated as a random factor (PROC Mixed). The model was constructed to examine the main effect of treatment over 12 sampling dates.

Results

Cover crop biomass: During both study years, there was good IR plant stand with the exception of an approximately 6.4 m wide row area along the border of the first plot planted which was caused by seeds being trapped in the drill during initial planting. The IR produced a biomass of 1.53 Mg/ha in 2010. Rats entered the cover crop drying facility and destroyed the IR collected for biomass data in 2009 before it was weighed, but the field establishment appeared similar to 2010.

Soybean cyst and lesion nematodes: The four key plant-parasitic nematodes found at the experimental site were *H. glycines*, *Pratylenchus* spp., *Helicotylenchus* sp., and *Trichodorous* sp. Among these, *H. glycines* was the most abundant, followed by *Pratylenchus* spp. Initial population densities of *H. glycines* and *Pratylenchus* did not differ between IR and SBS treatment plots prior to the beginning of the experiment on November 2008 (Table 2). In general, population densities of *H. glycines* were very low. Initial population densities for both years were lower than 200 cysts/100 cm^3 soil. Numbers of *H. glycines* and of *Pratylenchus* spp. did not differ significantly between IR and SBS plots throughout 2009, but SCN J2 counts were slightly higher in IR plots than SBS plots ($P \leq 0.10$) at soybean planting (June 8) in 2010 (Table 3). In contrast to *H. glycines*, *Pratylenchus* spp. numbers were slightly lower in IR than SBS ($P \leq 0.10$) at soybean planting in 2010. Population densities of plant-parasitic nematodes did not differ between IR and SBS plots at the end of the soybean cropping cycle in 2010.

Effect of IR on nematode communities: Effects of IR on nematode communities varied between the 2009 and 2010 trials. In 2009, a higher abundance of bacterivorous nematodes was found two months after planting (on August 12) in IR treatment plots compared to SBS; numbers of these nematodes were significantly greater in IR plots by the end of the soybean crop on October 3 ($P \leq 0.01$; Table 4). Although nematode diversity was initially significantly higher in SBS ($P \leq 0.05$) prior to experiment initiation in November 2008, diversity did not differ between IR and SBS beyond that period (Table 4). Slightly higher MI was detected in SBS than in IR ($P \leq 0.10$) at termination of the IR cover crop in June 2009, but not thereafter (Table 4). In 2010, soybean planted in SBS contained a higher abundance of bacterivorous nematodes ($P \leq 0.05$) than IR at cover crop termination (May) and at midseason (August) ($P \leq 0.10$; Table 5), although the differences at June sampling were not significant. Nematode richness was also significantly higher in SBS plots than in IR plots in May 2010 ($P \leq 0.05$). In 2010, a slightly higher EI ($P \leq 0.10$) and significantly lower CI ($P \leq 0.05$) was detected in SBS plots at soybean planting in June 2010.

Soil mites: Samples for soil mites were collected only during the 2010 trial. Soil mites commonly found included Mesostigmata, Prostigmata, Oribatida, Astigmata and Endostigmata. Mesotigmatid mites sampled from the experimental plots consisted of eight genera representing the families Rhodacaridae, Laelapidae, Digamasellidae, Ascidae and Macrochelidae (Table 6). There were no differences in numbers of mites collected from soil or soybean roots between IR and SBS treatments in June and October at the end of the soybean crop cycle ($P > 0.05$; Table 6). However, much higher numbers of mesostigmatid and atigmatid mites were detected from soybean roots as opposed to the soil samples (Table 6).

Aboveground arthropods: Five main groups or species of herbivorous insect pests were found in the soybean foliage sweep net samples: 1) green stink bugs (*Acrosternum hilare* Say), 2) green cloverworm (*Plathypena scabra* Fabricius), 3) corn earworm (*Heliothis zea* Boddie), 4) three-cornered alfalfa hopper (*Spissistilus festinus* Say), and 5) Dectes stem borer (*Dectes texanus texanus* LeConte). Several other pests occurred at very low levels. Of the

Table 2. Numbers of soybean cyst and lesion nematodes in soil collected from Italian ryegrass (IR) and soybean stubble (SBS) plots in the 2009 trial.

	Nov 1, 2008		Jun 3, 2009		Aug 12, 2009		Oct 3, 2009	
Nematode	IR	SBS	IR	SBS	IR	SBS	IR	SBS
Soybean cyst	100 cm^3 soil							
Cysts (with eggs)	101[a]	74	15	12	8	6	356	529
Cysts (without eggs)	77	61	40	25	54	70	849	792
Total cysts	178	135	55	37	62	76	1205	1321
Juveniles	80	46	452	574	79	66	680	626
Lesion	72	70	12	26	12	5	294	290

[a] Means are averages of four replications. No significant difference between treatments was observed in all sampling dates.

TABLE 3. Numbers of soybean cyst and lesion nematodes in soil collected from Italian ryegrass (IR) and soybean stubble (SBS) plots in the 2010 trial.

Nematode	May 8, 2010 IR	May 8, 2010 SBS	June 8, 2010 IR	June 8, 2010 SBS	Aug 16, 2010 IR	Aug 16, 2010 SBS	Oct 2, 2010 IR	Oct 2, 2010 SBS
Soybean cyst	-----	-----	-----	100 cm^3 soil	-----	-----	-----	-----
Cyst (with eggs)	63[a]	63	46	14	19	20	140	109
Cyst (without eggs)	71	61	75	32	41	21	182	61
Total cyst	134	124	121	46	60	41	322	170
Female	0	0	0	0	0	0	9	1
Egg	88	93	84	146	274	171	404	271
Juvenile	83	162	103	68@	30	16	144	64
Lesion nematodes	7	16	2	22@	47	58	231	349

[a] Means are averages of four replications. @ indicates significant different ($P < 0.10$) between IR and SBS within a sampling date based on analysis of variance after $\log(x+1)$ transformation.

above-mentioned species, the green cloverworm, a well-known defoliator of soybean, was the only herbivore present in high numbers during both study years. Green cloverworm population levels were especially elevated in 2010 and in August reached levels that were about 4 fold higher than in the 2009 field season (Fig. 1A, B). The highest mean numbers encountered during a sampling occasion were 9.5 and 54 in 2009 and 2010, respectively; both sampled from soybean planted in IR treatment plots. However, for both study years, no significant differences were found in the green cloverworm population densities between IR and SBS treatment plots ($P > 0.05$).

The main predators collected from soybean foliage were various species of spiders. Spider families found in the soybean foliage included Salticidae, Agelendidae, Oxyopidae, Thomisidae, and Araneidae. The family Oxyopidae was the most dominant group of spiders present. For both study years, total spider numbers were significantly greater ($P \leq 0.01$ and $P \leq 0.05$ in 2009 and 2010, respectively) on soybean plants in IR than in SBS treatment plots (Fig. 2A, B). Other generalist predators occurring in low numbers included the minute pirate bug, *Orius* sp., damsel bug, *Nabis* spp., big-eyed bug, *Geocoris* spp., green and brown lacewings (family Hemerobiidae and Chrysopidae) and various lady beetles (family Coccinellidae). However, weekly densities were too low to be subjected to an analysis and accumulative numbers for the entire season did not differ significantly between treatments ($P > 0.05$).

Soybean yield: Similar to other parameters measured, no significant differences were detected in soybean yield between IR and SBS treatments (Table 7). However, in both years, SBS tended to have slightly higher soybean yields than IR. There was a significant drop in yield during the 2010 growing season, possibly due to the low rainfall.

DISCUSSION

Despite the extensive sampling of numerous above and below ground organisms during two growing seasons,

TABLE 4. Effects of Italian ryegrass on nematode communities in the 2009 trial.

	Nov 1, 2008 IR	Nov 1, 2008 SBS	Jun 3, 2009 IR	Jun 3, 2009 SBS	Aug 12, 2009 IR	Aug 12, 2009 SBS	Oct 3, 2009 IR	Oct 3, 2009 SBS
	-----	-----	-----	Nematodes/100 cm^3 soil	-----	-----	-----	-----
Bacterivores	103[a]	79	26	35	61	35 *	5625	2970 *
Fungivores	76	46	12	31	14	15	505	205
Herbivores	176	93 *	415	653	71	77	1205	610
Omnivores	82	99	8	17	6	12	40	30
Predators	24	24	0	0	3	2	10	5
% Bacterivores	21.26	23.50	7.04	4.96	39.60	25.09	77.56	75.34 *
% Fungivores	15.77	13.57	3.38	4.60	9.65	9.86	6.34	5.52
% Herbivores	37.64	26.54 *	86.64	86.90	41.79	52.87	13.61	15.87
% Omnivores	18.54	27.62	2.40	2.81	4.07	7.80	0.49	0.62
% Predators	5.38	7.14	0	0	1.76	1.28	0.18	0.12
Fungivores/ Bacterivores	0.80	0.61	1.20	1.38	0.24	0.42	0.08	0.08
Richness	17	14	7	10	10	10	13.50	11.25
Diversity	6.80	8.18*	1.38	1.36	4.23	3.48	3.12	3.30
MI	2.36	2.70	1.54	2.00*	1.35	1.64	1.03	1.04
EI	48.52	43.50	51.22	48.03	40.82	33.43	88.39	87.67
SI	76.97	83.53	50.95	69.77	33.40	41.04	10.56	13.65
CI	51.33	57.78	58.01	70.00	35.79	61.36	2.92	2.86

[a] Means are averages of four replications. * indicates significant differences ($P \leq 0.05$) between Italian ryegrass (IR) and soybean stubs (SBS) at each sampling date based on analysis of variance. MI=Maturity index, EI=Enrichment index, SI=Structure index, CI=Channel index.

TABLE 5. Effects of Italian ryegrass on nematode communities in the 2010 trial.

	May 18, 2010		Jun 18, 2010		Aug 16, 2010	
	IR	SBS	IR	SBS	IR	SBS
	Nematodes/100 cm^3 soil					
Bacterivores	12[a]	40*	132	118	472	519*
Fungivores	54	66	125	134	199	194
Herbivores	22	167*	92	68	58	76
Omnivores	5	22	17	12	16	15
Predators	8	15	7	4	6	10
% Bacterivores	11.79	13.44	33.54	31.82	60.71	61.99
% Fungivores	43.48	23.78	37.99	31.08	26.68	24.24
% Herbivores	24.04	45.25*	23.90	21.23	7.08	8.48
% Omnivores	4.58	5.81	4.57	3.79	1.89	1.64
% Predators	7.41	4.40	4.55	2.84	0.82	1.30
Fungivores/Bacterivores	5.34	1.72	0.99	1.48	0.44	0.40
Richness	11	18.25*	21.75	19	21.75	20.25
Diversity	4.16	5.16	6.26	4.77	4.32	3.80
MI	2.53	2.70	2.29	2.24	2.06	2.05
EI	55.76	54.72	35.31	41.82*	39.30	37.83
SI	62.17	71.26	43.00	38.24	23.68	22.06
CI	71.64	58.52	91.04	73.91*	54.98	50.85

[a] Means are averages of four replications. * indicates significant differences between IR and SBS for the particular parameter at that sampling dates based on analysis of variance ($P \leq 0.05$).
MI=Maturity index, EI=Enrichment index, SI=Structure index, CI=Channel index.

spiders were the only fauna consistently affected by the IR. Spiders were significantly greater on soybean foliage grown in the IR compared to SBS habitat during both study years. This difference in spider populations was not unexpected as a review by Sunderland and Samu (2000) showed that spider abundance was increased by diversification in 63% of the studies and in 80% of the studies by 'interspersed diversification' e.g., undersowing, partial weediness, mulching and reduced tillage. However, there was no apparent impact of growing IR as a winter cover crop on arthropod pests, soil mites, plant-parasitic nematodes or free-living nematodes monitored during the study.

The main purpose of conducting this research was to test the hypothesis that IR could stimulate hatching of SCN prior to soybean planting and subsequently reduce the SCN population levels in the succeeding soybean crop. In a greenhouse study, Riga et al. (2001) showed that incorporation of plant residues from IR was effective in reducing SCN in both the soil and roots of soybean plants. They also discovered that root exudates of IR increased egg hatch of SCN compared to root exudates of soybeans. Both findings suggest that IR may be useful in a soybean rotation to manage SCN. However, results of the current field investigation did not agree with the findings of Riga et al. (2001) which were performed in a greenhouse. Only during the early period of the 2010 growing season was hatching of SCN eggs slightly greater in IR than in SBS. However, this stimulation of hatching by IR during the early season did not result in greater suppression of SCN by the end of the soybean cropping cycle. These results are also at variance with the results of Creech (2001), who found that SCN density on soybean in Indiana was reduced to 3,480 eggs/100 cm^3 soil in IR plots compared to 5,940 eggs/100 cm^3 in no IR soybean plots. However, it is probable that SCN population densities were too low to observe differences in the current study.

Among populations of soil health bioindicator organisms e.g., free-living nematodes and soil mites, IR only enhanced bacterivorous nematodes in 2009 but not in 2010. Having IR cover crop residues in addition to

TABLE 6. Mean mite densities sampled from the soil and soybean roots in June and at the end of the soybean crop cycle in the 2010.

	Soil June 8, 2010		Soil Oct 2, 2010		Roots Oct 9, 2010	
	IR	SBS	IR	SBS	IR	SBS
	Numbers/250 cm^3 soil				Numbers/26 g roots	
Mesostigmata[a]	6[b]	6	2	0	89	113
Prostigmata	0	0	1	0	2	3
Oribatida	13	17	4	1	11	12
Astigmata	2	2	2	0	203	136
Endostigmata	2	2	0	0	0	1

[a] Mesostigmata consisted of following genera: *Protogamasellus, Rhodacarus, Macrocheles, Cosmolaelaps, Geolaelaps, Hypoaspis* s.l. and *Dendrolaelaps*
[b] Means are averages of four replications. No significant differences were detected between treatments for either mite in soil or in root samples.

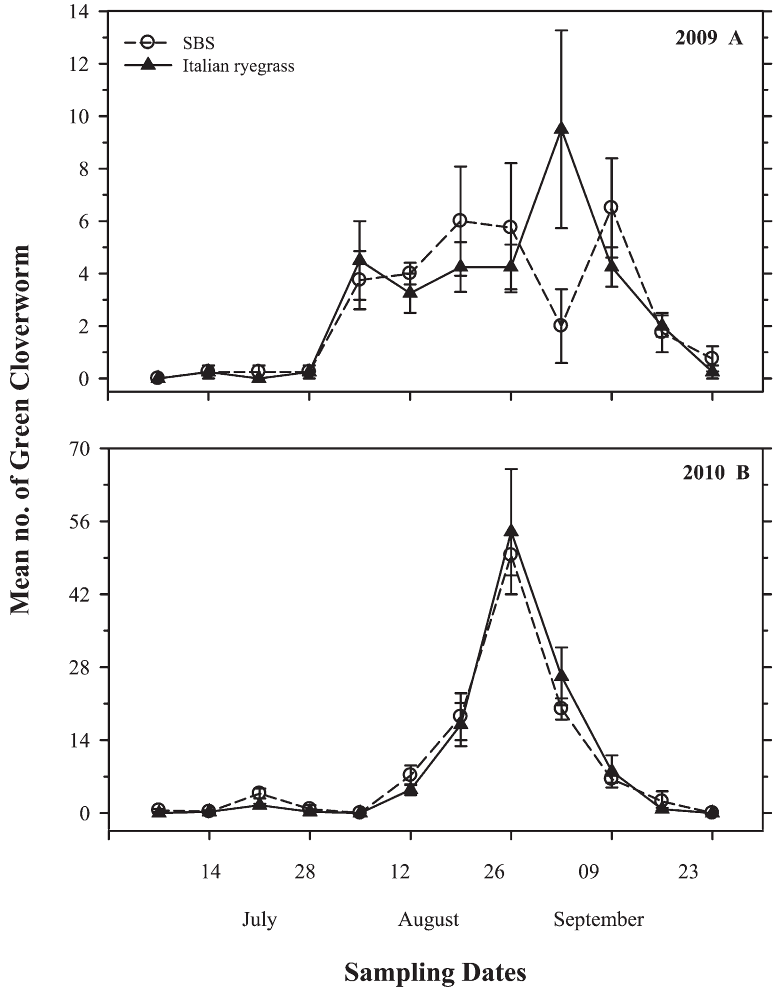

FIG. 1. Total mean number of green cloverworm, *Plathypena scabra* (F.) sampled on soybean planted into herbicide-killed Italian ryegrass cover crop or soybean stubble only in A) 2009 and B) 2010.

soybean stubble from the previous cropping cycle in IR plots should have resulted in greater microbial activities in IR plots and an associated increase in the abundance of bacterivorous and fungivorous nematodes in IR plots. Lack of differences in the abundance of free-living nematodes and soil mites between IR and SBS treatments could be attributable to the fact that the IR did not produce enough biomass i.e. 1.53 Mg/ha, to offset the impact of the soybean residues and stubble that remained on the soil surface in both treatments following soybean harvest. Rahman et al. (2007) evaluated the impact of stubble retention on nematodes in a wheat, *Triticum aestivum,* –wheat and wheat–lupin, *Lupinus angustifolius,* rotation and found that retention of stubble contributed greatly to enhancing population densities of free-living nematodes as compared to burning of stubble. Bacterivorous nematode populations were greater in IR plots than in SBS plots in October 2009, but significant differences were not observed earlier in the season. Numbers of bacterivorous and fungivorous nematodes remained very low in IR and in SBS plots until the final sampling date in 2009. Though it cannot be concluded unequivocally that nematode community structures increase more favorably in no-till as opposed to conventional till soybean fields, a study conducted by Okada and Harada (2007) in soybean showed that tillage had a definite effect on nematode diversity and community indices. In their study, diversity indices, maturity index (MI) and related indices, structure index, and channel index were higher in no-till whereas enrichment index (EI) was lower in no-till. Thus, it is conceivable that planting IR for one or two winters is not enough to override the influences on free-living nematodes in a field that has been subjected to no-till practices for several years. Although, the adverse effect of tillage on soil mite numbers has been well documented (Petersen and Luxton, 1982; Wallwork, 1976; Werner and Dindal, 1990; Sanches-Moreno et al., 2009; Gupta, 1994; Peachey et al., 2002), unlike free-living nematodes, it is less likely that their populations were more affected by the no-till field condition as opposed to the IR residues. Studies have shown that soil mite populations recover quickly to pre-till numbers within months after a tillage operation (Minor et at., 2004; Moore et al., 1984; Mallow et al., 1985).

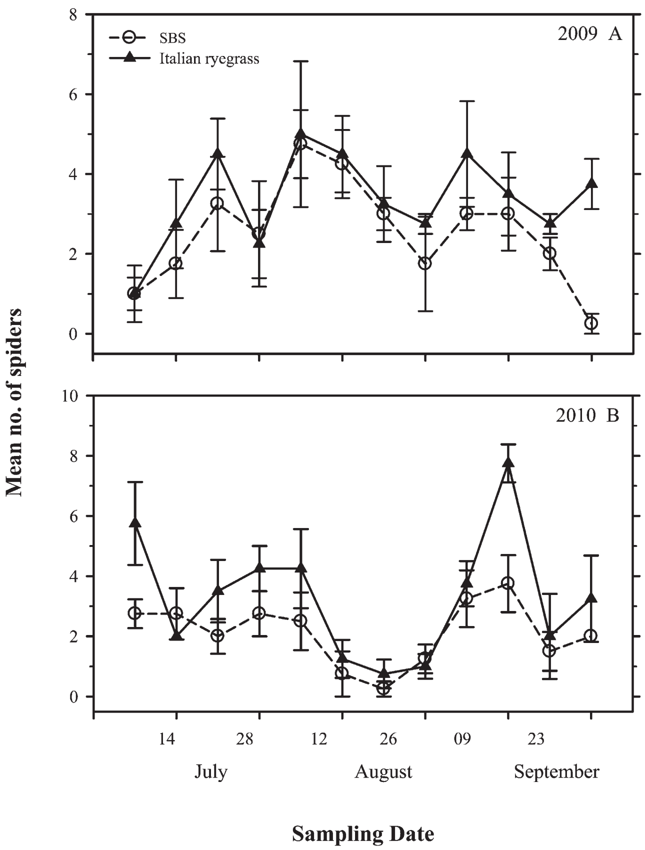

FIG. 2. Total mean number of spiders sampled on soybean foliage planted into herbicide-killed Italian ryegrass cover crop or soybean stubble only in 2009 and 2010.

Another production practice that could have impacted free-living nematode and mite populations in both treatment habitats is the application of herbicide sprays. It was previously reported that SI is a good indicator for herbicide disturbance from glyphosate (Wang et al., 2006). During that study, Wang et al. (2006) showed that fallow control that received glyphosate had a very low SI and that plots that did not receive a glyphosate treatment had higher SI. However, the disturbance from glyphosate was transient and disappeared three months after a pepper crop was planted (Wang et al., 2006). The current research partially supports the supposition that predatory or omnivorous nematodes, also reflected by SI, were more sensitive to herbicide treatment as opposed to bacterivorous and fungivorous nematodes (as reflected by EI or CI) in 2009 when the weather pattern was normal. In 2009, SI continued to be low towards the end of the cycle indicating disturbed conditions, whereas EI decreased and CI increased at the end of the cycle in 2009 indicating the soil health condition had improved. Although previous literature claims that this disturbance is transient (Griffiths et al., 2008; Wang et al., 2006), the impact of herbicide sprays on SI appeared irreversible during the duration of this soybean cropping season. In addition to nematodes, mites may also be influenced by herbicide. In a multi-year study evaluating the impact of soil cultivation and herbicides on soil mites, Minor et al. (2004) observed that negative effects of tillage on soil mite abundance during year one of the study were superseded by herbicide effect the following years. Similarly, other studies have shown herbicides to negatively impact soil mite abundance and species richness (Moore et al., 1984; Koehler, 1994; Kay et al., 1999). However, similar to tillage, herbicide effect may be short-lived if herbicides are not applied regularly (Moore et al., 1984; Tsonev and Furnadzhieva, 1984, Doles et al., 2001; Minor et at., 2004; Minor and Norton 2008). An interesting finding with respect to soil mites is that extracting soil mites directly from soybean roots provided far more totals for each mite order as oppose to the standard procedure of extracting them from the soil. Further, the diversity of Mesostigmata mites was at least three times higher in root than in soil samples. This may suggest that root and soil samples are needed to provide a more adequate assessment of soil mite abundance and species richness.

TABLE 7. Soybean yields and root fresh weight at the end of the 2009 and 2010 trials.

	Root weight (g)		Yield (kg/ha)	
	2009	2010	2009	2010
IR	8.30[a]	6.01**	2123.58	1403.52
SBS	7.70	4.08	2369.38	1405.54

[a] Means are average of four replications. ** indicates root weight significantly greater in IR than SBS treatment plots ($P \leq 0.01$). No significant differences in yield were detected between treatments in either trial.

The most abundant herbivorous insect found in soybean foliage during both years of the study was the green cloverworm whose population varied during the two seasons and was the highest in 2010. Similar to belowground pests, there was no impact of treatment on their population density. In a similar study conducted by Smith et al. (1988) using rye as a cover crop, green cloverworm numbers were the highest in the rye no-till (soybean planted no-till into standing rye) during the first year, but were the lowest the second year in rye no-till compared to soybean planted in plots that did not have a rye cover crop, plots that had rye plowed and disked into the soil, and plots that had rye disked only.

The current 2-year study did not demonstrate any yield benefits to soybean planted in IR plots. However, Pedersen and Rodriguez-Kabana (1991) reported low soybean yields following IR and high soybean yields following the fallow control in both study years. Creech et al. (2008) found greater soybean yields during the initial year of another study where IR was compared to no IR treatment plots. It is possible that a longer term study would be required to see a yield increase. For example, no-till planting of corn after two years of IR resulted in yields 1.8 × higher than no IR, whereas six years of IR yielded 3.5 × more than no IR (ORGSC, 2011). Improvement of soybean yields with IR might take longer than with corn, as no-till planting of soybean without IR still benefits from the nitrogen fixing by the previous soybean crop.

In conclusion, growing cover crops in a no-till cropping system should increase residue levels on surface soils, thus enhancing soil mite and free-living nematode populations. However, this 2-year field investigation did not demonstrate any benefit of planting IR on plant parasitic or free-living nematode and soil mite populations, or on crop yield. However, it may take several years of growing Italian ryegrass for it to influence both below-ground organisms and crop yield in soybeans that have been in no-till for several years prior to IR planting. The potential influence of IR on below-ground soil organisms is further complicated by the fact that soybean growers in the Northeast typically use pre- and/or post- emergent herbicides. These practices may temporarily derail some of the positive influences that IR and other winter cover crops have on free-living nematodes, soil mites, and other soil organisms. Thus, continuous evaluation of this no-till IR system may be necessary to demonstrate if IR will lead to reduction of SCN and increase free-living nematode and soil mite populations and yields in soybean fields.

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