Soybean Yield and *Heterodera glycines* Responses to Liquid Swine Manure in Nematode Suppressive Soil and Conducive Soil

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Abstract: The soybean cyst nematode (SCN), *Heterodera glycines*, is a major factor limiting soybean yield. Experiments were conducted in 2009 and 2010 to determine the effects of liquid swine manure and chemical fertilizer PK on soybean and corn yields, and on SCN population in an SCN-suppressive field (S-Site) and an SCN-conducive field (C-Site) in Minnesota. The experiment was a split-plot design with crop sequences as main plots and fertilizer treatments as subplots. The 2-yr crop sequences were Sus-Sus, Res-Sus, and Corn-Sus, where Sus was SCN-susceptible soybean, and Res was SCN-resistant soybean. The fertilizer treatments were manure, PK, and a nonfertilizer as control. Manure did not reduce SCN egg population density but resulted in 31% lower SCN second-stage juvenile (J2) population density at the S-Site at 45 d after planting (DAP) in 2009. Manure also reduced spiral nematode (*Helicotylenchus* spp.) population density by 52% compared with PK and nonfertilizer treatments at S-Site at 45 DAP in 2009. The crop sequence of Corn-Sus and Res-Sus reduced the SCN egg and J2 but increased spiral nematode population density at both sites. An increase of 1.4 Mg/ha and 0.5 Mg/ha in yield of susceptible soybean was observed in manure and PK treatments, respectively, at the C-Site in 2009. Corn yield was 2.8 Mg/ha and 5.0 Mg/ha greater when treated with manure than nonfertilizer at the S-Site and C-Site, respectively. This study suggests that soil fertility management may be a useful strategy to alleviate the SCN damage to soybean.

Key words: Helicotylenchus, Heterodera glycines, management, nematode suppressive soil, swine manure, soybean, soybean cyst nematode.

The soybean cyst nematode (SCN), *Heterodera glycines*, has become a significant problem in soybean production in the United States (Wrather et al., 2003; Monson and Schmitt, 2004; Wrather and Koenning, 2006). Despite the presence of SCN-resistant soybean (*Glycine max*) cultivars and common annual rotation with corn (*Zea mays*), SCN management has proven difficult. Successful long-term SCN management strategies should have multiple components and include various means to suppress SCN population density and improve soybean yield. One potential component of SCN management program is to use fertilizer application to improve soil health, which may suppress plant-parasitic nematodes and provide sufficient nutrients for plant growth.

Animal manure is one of most popular organic amendments for soils, because incorporation of animal manure adds nutrients into the soil and can positively affect soil quality by increasing organic matter and improving soil structure (Oka, 2010; Thoden et al., 2011). For example, a 7-yr study showed that the treatment with beef cattle (*Bos primigenias*) manure increased the average yields of potato tubers by 27% (Kimpinski et al., 2003). In addition, nematicidal compounds generated from decomposition of animal manure may help suppress various soil-borne pathogens, such as the wilt fungus *Verticillium dahliae* (Lazarovits et al., 2000; Tenuta et al., 2002; Conn et al., 2005). Beef, poultry, and swine (*Sus domesticus*) manures have been reported as effective in reducing the population densities of lesion (*Pratylenchus* spp.), root-knot (*Meloidogyne* spp.), cyst (*Heterodera*

spp.), and other economically important plant-parasitic nematodes in various cropping systems (Kimpinski et al., 2003; Everts et al., 2006; Nahar et al., 2006; Briar et al., 2007; Min et al., 2007; Oka et al., 2007; Mahran et al., 2008a, 2009; Liang et al., 2009). A number of researchers have reported that liquid swine manure had the potential for reducing plant-parasitic nematodes, such as lesion nematodes and SCN (Min et al., 2007; Xiao et al., 2007; Mahran et al., 2008a, 2008b). Xiao et al. (2007) suggested that liquid swine manure controlled SCN effectively within 35 d after application in a greenhouse study, and the mechanism involved volatile fatty acids. The suppressive effect on lesion nematodes increased in liquid swine manure with lower pH levels (Mahran et al., 2008a). However, the effect of liquid swine manure on nematodes was inconsistent in field experiments. A single application of liquid swine manure reduced the population density of plant-parasitic nematodes for up to 3 yr in one field but had little or no effect in another field (Conn and Lazarovits, 2000). Liang et al. (2009) reported significantly greater numbers of cyst nematodes in plots treated with composted swine manure in a long-term field study. In a study by Reynolds et al. (1999), swine manure resulted in greater SCN egg population density at harvest.

The present study represents the first investigation of liquid swine manure's influence on SCN population dynamics in SCN-suppressive soil (S-Site) as compared with SCN-conducive soil (C-Site). Suppressiveness to SCN at the two field sites used in this study had been demonstrated in greenhouse assays (Chen, 2007; Bao et al., 2011). Nematode populations in the S-Site on susceptible soybean were relatively low compared to the average infestation level in the fields in the region, but SCN population densities were high in the C-Site. Greenhouse studies further demonstrated that this nematode suppressiveness was at least partially attributed to soil microbial activities (Bao et al., 2011).

The long-term goal of this study was to develop sustainable management of SCN by integrating organic amendments into the management program to suppress SCN population densities and improve soybean yield. The specific objectives of this study were (i) to quantify the effects of liquid swine manure and chemical fertilizer (P and K) on SCN population densities, other plant-parasitic nematodes, and crop yield; (ii) to determine the interactive effects of fertilizer treatments and crop rotations on SCN and soybean yield; and (iii) to compare soil chemical properties among fertilizer treatments and crop rotations.

Materials and Methods

Experimental design: Field experiments were conducted in the SCN-suppressive (S-Site, 44° 04′ 21″ N, 93° 31′ 24″ W) and -conducive (C-Site, 44° 05′ 30″ N, 93° 32′ 47″ W) fields at Waseca, MN, in 2009 and 2010. The soil at the S-Site was a Nicollet clay loam (fine loamy, mixed, mesic Aquic Hapludoll), and the soil at C-Site was a Webster clay loam (fine loamy, mixed, mesic Endoaquoll). Both sites were planted to SCN-susceptible soybean in 2007 and 2008. For each site, the experiment was a split-plot design with three crop sequences as main plots and three fertilizer treatments as subplots. Each subplot was 6 m long and 3 m wide, and consisted of four rows. Each treatment had four replicates. The crop sequences included Sus-Sus, Res-Sus, and Corn-Sus, where Sus was SCN-susceptible soybean 'DeKalb 22-52', Res was SCN-resistant soybean 'Pioneer Brand R92Y20', and Corn was 'DeKalb 50-44'; and the first and second abbreviations represent crops in 2009 and 2010, respectively. All of the soybean and corn cultivars were resistant to glyphosate (2-phosphonomethylamino acetic acid). The fertilizer treatments were manure (liquid swine manure at 37.4 m^3/ha or 239 kg total N/ha + 26 kg P/ha + 112 kg K/ha), PK (49 kg P/ha + 93 kg K/ha), and nonfertilizer as control (no fertilizer). The manure was injected into the soil 10 cm under each intended row with 25-cm-wide sweep injector on 22 April 2009, and the corn and soybean were planted on 4 May 2009. The PK fertilizer was broadcast on the soil surface and then incorporated into the soil before planting in 2009. In 2010, all plots were planted to Dekalb 22-52 soybean on 6 May, and no fertilizer was applied. Glyphosate was used for preemergence and postemergence weed control. No insecticide and fungicide were used.

Soil sampling: A composite soil sample consisting of 20 soil cores (2 cm in diam. and 15 to 20 cm deep) was collected from the two central rows of each four-row plot at four different times: prior (to fertilizer application in 2009 or planting in 2010), 45 d after planting (DAP) in 2009 (no sampling at 45 DAP in 2010), mid-season (around 2 mon after planting), and harvest. The soil was placed in a plastic bag, stored in a cool room (approximately 10°C), and processed within 2 d.

Soil chemical analysis: Soil samples taken prior to fertilizer application and harvest in 2009, and at harvest in 2010 were analyzed in the University of Minnesota Soil Test Laboratory for pH, organic matter, and concentrations of P, K, Zn, Fe, Cu, and Mn according to Recommended Chemical Soil Test Procedures for the North Central Region (Brown, 1998). In addition, NO_3^--N (Willis and Gentry, 1987) and NH_4^+-N (Keeney and Nelson, 1982) levels were determined at 45 DAP in 2009.

Nematode extraction and counting: The soil in each sample was thoroughly mixed before nematode extraction. The SCN egg population density was determined from a subsample of 100 cm^3 of soil. Cysts were extracted from the soil using an elutriator (Byrd et al., 1976) and centrifugation in 63% (w/v) sucrose solution for 5 min at 1,500 *g*. Eggs were released from the cysts by breaking the cysts with a mechanical device (Faghihi and Ferris, 2000) and then collected in a 50-ml tube. They were stored at 4°C before being counted within 2 wk. Another subsample of 100-cm^3 soil was used to extract vermiform nematodes using the hand-decanting and sucrose centrifugal-flotation technique (Jenkins, 1964) to determine the densities of SCN second-stage juveniles (J2) and other plant-parasitic nematodes.

Yield measurement: Soybean and corn were harvested in October each year from 6.1 m of the two central rows of each plot. Soybean seed yield was computed at 13.0% water, and corn grain yield was computed at 15.5% water.

Data analysis: The general linear model (GLM) procedures in Statistical Analysis System (SAS) Version 9.2 (SAS Institute, Cary, NC) were used to perform the split-plot analysis of variance (ANOVA). Dependent variables were evaluated for normality and transformed as necessary before performing the ANOVA. The nematode population densities were square-root transformed, while yield data were not transformed. Crop sequences (three levels), fertilizer treatments (three levels), and replicates (four) were treated as whole plots, subplots, and blocks, respectively. Across the two sites, a mixed model ANOVA with site, crop, and fertilizer as fixed factors was used in GLM procedure. The interactions of site with crop and/or fertilizer treatments were significant in most dependent variables, so the data from each site were analyzed separately without the site factor. Means of the treatment of crop sequences and fertilizers within each site were compared with least significant difference (LSD) test at $P < 0.05$ in GLM procedure.

Results

Soil chemical properties: Soil chemical properties were similar among the treatments prior to the fertilizer application within each site with the exception that K concentration was greater in the corn plots than susceptible and resistant soybean plots (data not shown). All of the soil chemical properties were different between the two

sites at harvest in 2009 (Table 1). The concentration of nitrate nitrogen at 45 DAP in the treatment that received manure was 53.3 mg/kg and 57.2 mg/kg at the S-Site and C-Site, approximately four and three times of that obtained with the PK or nonfertilizer treatment, respectively (Table 1). The ammonium nitrogen also increased in manure treatments as compared with nonfertilizer at both sites and with PK treatment at the C-Site (Table 1). Manure also increased the concentration of Cu and K in the soil at both sites, and Zn and Mn at the S-Site (Table 1). The PK treatment almost doubled P concentration in the soil at the harvest as compared with nonfertilizer treatment at both sites (Table 1). The manure also slightly increased P level at the S-Site. No treatment effects on soil pH, organic matter, and Fe concentrations were detected at harvest in 2009. There was no fertilizer treatment effect on the organic matter and soil elements at harvest in 2010, except that the P levels in the manure and PK treatments were higher than that in nonfertilizer treatment (data not shown).

SCN population density: The SCN egg and J2 population density at the C-Site was generally greater than that at the S-Site (Tables 2,3). There were no effects of fertilizer treatments on the SCN egg population density prior to planting, 45 DAP, and at harvest in 2009, and at

harvest in 2010 (Table 2). When the data from the two sites were pooled together, the midseason egg population densities in 2009 were slightly greater in the manure and PK treatments than nonfertilizer treatment, but the treatment was not significant when the data were analyzed by site (Table 2). At the S-Site, the SCN egg population density at planting in 2010 was greater in soil treated with PK than with nonfertilizer, and intermediate with manure. At the S-Site, susceptible soybean in 2009 supported greatest SCN egg population densities from midseason 2009 to midseason 2010 (Table 2). The greater SCN egg population density in SCN-susceptible soybean treatment as compared with corn and SCN-resistant soybean was observed only at harvest in 2009 and midseason in 2010 at the C-Site (Table 2). Manure and PK resulted in greater SCN egg population density at the midseason of 2010 than nonfertilizer treatment at the C-Site (Table 2).

The application of manure resulted in 31% and 46% lower SCN J2 population density at 45 DAP in 2009 as compared with nonfertilizer and PK treatment, respectively, at the S-Site (Table 3). In contrast, a greater SCN J2 population density at harvest in 2009 and at planting in 2010 was observed in C-Site with the manure treatment (Table 3). At both sites, the SCN J2 population densities

TABLE 1. Soil chemical properties as affected by treatments of fertilizers and crop sequences.†

Treatment	pH	P (mg kg^{-1})‡	K (mg kg^{-1})	OM (g kg^{-1})	Zn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	N-NO$_3^-$ (mg kg^{-1})	N-NH$_4^+$ (mg kg^{-1})
					S-Site					
Crop in 2009										
Corn	6.5§	26.7	239	59.0	3.7	57.5	1.2	16.7	21.5 b	7.1
Res	6.5	23.6	213	57.3	3.4	48.0	1.2	17.1	23.2 b	7.4
Sus	6.8	19.3	220	53.5	3.3	37.3	1.1	15.6	33.9 a	7.6
Fertilizer										
Manure	6.6	19.8 b	233 a	56.6	4.2 a	49.0	1.3 a	17.8 a	53.3 a	8.2 a
PK	6.6	32.0 a	220 b	57.0	2.9 b	48.2	1.1 b	15.6 b	13.0 b	7.2 ab
Nonfertilizer	6.6	17.8 c	219 b	56.3	3.2 b	45.5	1.1 b	16.0 b	12.4 b	6.6 b
					C-Site					
Crop in 2009										
Corn	7.6	7.4	133	66.5	2.5	12.8	0.8	7.6	31.4	9.0
Res	7.7	6.3	136	69.7	2.2	13.0	0.8	7.1	33.2	8.2
Sus	7.6	7.0	131	68.1	2.1	12.0	0.8	6.7	32.4	8.3
Fertilizer										
Manure	7.6	5.8 b	142 a	68.3	2.5 a	13.0	0.9 a	7.3	57.2 a	10.3 a
PK	7.7	10.6 a	128 ab	68.3	2.0 b	12.1	0.7 b	6.7	20.4 b	6.7 b
Nonfertilizer	7.6	4.9 b	125 b	67.7	2.3 ab	12.3	0.7 b	7.2	20.6 b	8.2 b
ANOVA (*F* values)										
Site (A)	103.9***	68.7***	106.7***	67.4***	82.7***	65.1***	67.9***	77.0***	22.8***	3.4 NS
Crop (B)	0.7 NS	0.5 NS	0.4 NS	1.3 NS	3.6 NS	1.2 NS	0.5 NS	0.5 NS	4.2*	0.0 NS
Fertilizer (F)	2.0 NS	90.8***	10.8***	0.9 NS	9.9***	3.4*	27.1***	7.2**	208.9***	14.1***
A × B	1.1 NS	0.5 NS	0.6 NS	2.2 NS	0.1 NS	0.8 NS	0.4 NS	0.0 NS	1.8 NS	0.5 NS
A × F	0.2 NS	0.1 NS	1.4 NS	0.1 NS	0.4 NS	0.5 NS	0.5 NS	3.0 NS	5.1*	3.5*
B × F	1.5 NS	1.9 NS	0.5 NS	0.6 NS	0.7 NS	0.3 NS	0.5 NS	1.3 NS	0.6 NS	1.6 NS
A × B × F	0.8 NS	0.9 NS	1.2 NS	0.3 NS	0.9 NS	0.9 NS	0.7 NS	0.9 NS	1.9 NS	1.3 NS

† The values are means of four replicates of the split-plot experiment including three crop sequences as main plots and the fertilizers as subplots in SCN-suppressive (S-Site) and -conducive (C-Site) sites, respectively, of the soil samples taken at harvest in 2009. Res is SCN-resistant soybean, and Sus is SCN-susceptible soybean.

‡ P levels were measured as Bray-P at S-Site, and Olsen-P at C-Site.

§ The values followed by the different letters in the same column are significantly different according to least significance test at $P < 0.05$. *, **, and *** denote the *F* values significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. NS = not significant at $P \geq 0.05$.

TABLE 2. Effects of fertilizer and crop sequence on *Heterodera glycines* egg population density.†

Treatment	2009				2010		
	Pi09	P45d-09	Pm09	Pf09	Pi10	Pm10	Pf10
				S-Site			
Crop sequence‡							
Corn-Sus	4,390§	2,922	2,502 b§	1,808 b	1,355 b	1,443 b	3,758
Res-Sus	4,706	2,513	3,017 b	2,196 b	2,167 b	1,837 b	5,667
Sus-Sus	6,829	5,896	6,554 a	9,600 a	5,567 a	4,324 a	3,363
Fertilizer							
Manure	4,833	3,362	4,296	5,008	2,908 ab	2,270	3,971
PK	5,702	4,102	4,319	4,781	3,546 a	2,631	4,775
Nonfertilizer	5,390	3,867	3,458	3,815	2,634 b	2,702	4,042
				C-Site			
Crop sequence							
Corn-Sus	16,813	9,908	11,094	7,246 b	7,042	4,660 b	9,371
Res-Sus	16,463	10,146	9,760	7,113 b	7,733	5,408 b	9,821
Sus-Sus	13,871	9,923	11,200	12,121 a	10,152	8,090 a	10,471
Fertilizer							
Manure	16,617	11,063	11,142	9,871	8,617	6,473 a	8,746
PK	16,942	10,650	11,090	8,667	8,817	6,560 a	11,488
Nonfertilizer	13,588	8,265	9,823	7,942	7,494	5,125 b	9,429
ANOVA (*F* values)							
Site (A)	146.8***	61.3***	55.1***	48.9***	98.7***	60.9***	62.3***
Crop sequence (B)	0.0 NS	1.9 NS	3.8 NS	35.9***	17.2***	16.6***	1.2 NS
Fertilizer (F)	2.0 NS	1.7 NS	4.1*	3.1 NS	6.0**	1.8 NS	2.4 NS
A × B	3.4 NS	2.5 NS	2.8 NS	5.0*	2.6 NS	0.3 NS	1.6 NS
A × F	2.2 NS	3.1 NS	0.1 NS	0.0 NS	0.3 NS	2.2 NS	0.8 NS
B × F	1.4 NS	0.8 NS	1.3 NS	0.3 NS	0.8 NS	1.2 NS	0.7 NS
A × B × F	0.1 NS	0.1 NS	1.0 NS	0.6 NS	1.5 NS	3.1*	1.7 NS

† Pi09, P45d-09, Pm09, Pf09, Pi10, Pm10, and Pf10 are population densities (number of nematode eggs per 100-cm³ soil) prior to applying fertilizer, 45 d after planting, at midseason, and at harvest in 2009, and at planting, midseason, and harvest in 2010, respectively.

‡ The 2-yr crop sequences were Sus-Sus, Res-Sus, and Corn-Sus, where Sus is SCN-susceptible soybean and Res is SCN-resistant soybean; the first and second abbreviations represent the crops in 2009 and 2010, respectively.

§ The data are main effect of the split-plot experiment with crop sequences as main plots and fertilizers as subplots, each with four replicates. The values followed by the different letters in the same column are significantly different according to least significance test at $P < 0.05$. *, **, and *** denote the *F* values significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. NS = not significant at $P \geq 0.05$.

were greater from 45 DAP in 2009 through early season (C-Site) or midseason (S-Site) in 2010 in the plots with susceptible soybean than with resistant soybean or corn in 2009 (Table 3).

Other plant-parasitic nematodes: For the plant-parasitic nematodes other than SCN, only spiral nematode (*Helicotylenchus* spp.) was detected from most plots and had enough abundance for statistical analysis. Average population density of spiral nematode was greater at the C-Site than the S-Site, with the initial population densities of 1,004, and 427 per 100 cm³ soil, respectively (data not shown). The manure treatment reduced spiral nematode population density by 52% relative to the PK and nonfertilizer treatment in S-Site at 45 DAP in 2009 (Table 4). The spiral nematode population density at harvest in 2009 was least with manure, intermediate with PK, greatest with nonfertilizer treatment in the C-Site (Table 4). Among three crop sequences, greater population density of spiral nematodes was observed on corn-soybean rotation in both sites at different sampling dates (Table 4).

Soybean yield: Soybean yield response to fertilizer application differed dramatically between the two field sites (Table 5 and Figs. 1,2). At the S-Site, average soybean yield in 2009 was 3.50 Mg/ha, but there was no difference in the soybean yield between the SCN-resistant and susceptible cultivars and among the three fertilizer treatments (Fig. 1). At the C-Site, average soybean yield in 2009 was only 2.71 Mg/ha (data not shown). An increase of 1.4 Mg/ha and 0.5 Mg/ha in yield of susceptible soybean was observed in manure and PK treatments, respectively, at the C-Site in 2009 (Fig. 1). The yield advantage of SCN-resistant cultivar over susceptible cultivar was 883 kg/ha for nonfertilizer ($P = 0.06$), 454 kg/ha for PK, and only 230 kg/ha for manure at the C-Site in 2009 (Fig. 1), although none of the differences were statistically significant ($P > 0.05$). In manure treatment, the soybean yield in 2009 was similar between the two sites, but in nonfertilizer treatment the yield of susceptible soybean was 1.56 Mg/ha less in the SCN-conducive soil than the SCN-suppressive soil (Fig. 1).

The manure and PK applied in 2009 resulted in 610 and 545 kg/ha, respectively, greater soybean yield in 2010 as compared with the nonfertilizer at the C-Site. However, there was no difference in the 2010 soybean yield among the fertilizer treatments at the S-Site (Fig. 2). The 2010 soybean yield following corn was slightly greater than following soybean at the C-Site (Fig. 2).

TABLE 3.   Effects of fertilizer and crop on the *Heterodera glycines* second-stage juvenile population density.†

Treatment	2009				2010		
	Pi09	P45d-09	Pm09	Pf09	Pi10	Pm10	Pf10
				S-Site			
Crop sequence‡							
Corn-Sus	164§	387 b§	31 b	52 b	132 b	186 b	196
Res-Sus	219	503 ab	31 b	70 b	147 b	235 b	189
Sus-Sus	290	708 a	150 a	328 a	568 a	379 a	154
Fertilizer							
Manure	203	372 b	66	161	245	227	179
PK	267	685 a	88	161	340	305	176
Nonfertilizer	203	541 a	57	127	261	268	184
				C-Site			
Crop sequence							
Corn-Sus	273	372 b	64 b	68 b	191 b	282	408
Res-Sus	305	800 a	122 b	94 b	233 b	263	347
Sus-Sus	228	725 ab	239 a	285 a	527 a	413	371
Fertilizer							
Manure	260	637	155	196 a	404 a	349	364
PK	281	613	145	109 c	269 b	289	378
Nonfertilizer	264	647	125	142 b	277 b	319	383
ANOVA (*F* values)							
Site (A)	1.74 NS	1.68 NS	23.32***	3.61 NS	10.82**	4.07 NS	36.85***
Crop sequence (B)	0.35 NS	7.11**	26.57***	61.62***	54.03***	10.13*	0.45 NS
Fertilizer (F)	0.88 NS	3.74*	0.71 NS	4.65*	2.52 NS	0.03 NS	0.01 NS
A × B	1 NS	1.21 NS	1.68 NS	1.55 NS	2.74 NS	1.11 NS	0.44 NS
A × F	0.29 NS	6.02**	0.06 NS	3.52*	2.93 NS	1.71 NS	0.06 NS
B × F	1.53 NS	0.45 NS	1.87 NS	0.09 NS	0.55 NS	1.89 NS	0.42 NS
A × B × F	0.45 NS	0.52 NS	1.51 NS	0.12 NS	0.15 NS	0.31 NS	0.65 NS

† Pi09, P45d-09, Pm09, Pf09, Pi10, Pm10, and Pf10 are population densities (number of nematodes per 100-cm^3 soil) prior to applying fertilizer, 45 d after planting, at midseason, and at harvest in 2009, and at planting, midseason, and at harvest in 2010, respectively.
‡ The 2-yr crop sequences were Sus-Sus, Res-Sus, and Corn-Sus, where Sus is SCN-susceptible soybean and Res is SCN-resistant soybean; the first and second abbreviations represent the crops in 2009 and 2010, respectively.
§ The data are main effect of the split-plot experiment with crop sequences as main plots and fertilizers as subplots, each with four replicates. The values followed by the different letters in the same column are significantly different according to least significance test at $P < 0.05$. *, **, and *** denote the *F* values significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. NS = not significant at $P \geq 0.05$.

Corn yield: With the manure treatment, the yield of corn was 13.87 Mg/ha at the S-Site and 12.99 Mg/ha at the C-Site (Fig. 3). Corn yield was 2.8 Mg/ha and 5.0 Mg/ha greater when soil treated with manure than nonfertilizer at S-Site and C-Site, respectively (Fig. 3). There was no difference in corn yield between the PK and nonfertilizer treatments at either site (Fig. 3).

Discussion

Our study showed that soybean yield responded positively to application of liquid swine manure and chemical fertilizers, and the manure was more effective than the chemical fertilizer PK. This study agrees with the results of a previous study that liquid swine manure application increased seed yield in soybean fields in southern Minnesota (Schmitt et al., 2001). In this study, the soybean yield response to fertilizer treatments was more sensitive in SCN-susceptible than SCN-resistant cultivar and for the SCN-conducive than SCN-suppressive fields. In other words, the soybean yield advantage of using SCN-resistant cultivars in a nematode-conducive soil may at least be partially replaced by fertilizer input, especially liquid swine manure.

The lack of a reduction of SCN egg population density by manure does not agree with the results from a previous greenhouse study in which manure effectively lowered SCN egg population density (Xiao et al., 2007). However, it is similar to the results from a previous field study (Reynolds et al., 1999). In the greenhouse study, Xiao et al. (2007) planted soybean immediately following application of manure, while in the present field study the soybean was planted 2 wk after application of the manure. The concentrations of fatty acid compounds, which are the major nematicidal compounds, in the soil from the manure application declined rapidly (Mahran et al., 2009). It is possible that these fatty acids are more effective in killing SCN J2 than eggs. The SCN generally does not hatch before planting soybean, and the manure might have less effect on the unhatched SCN eggs under field conditions. In the same greenhouse study, Xiao et al. (2007) found that liquid swine manure became ineffective in lowering SCN eggs population at 61 d after application. It appears that the application time may be a reason for the different effects of manure on SCN eggs between the greenhouse and field experiments.

TABLE 4. Effects of fertilizer and crop on the spiral nematode (*Helicotylenchus* spp.) population density.†

Treatment	2009				2010		
	Pi09	P45d-09	Pm09	Pf09	Pi10	Pm10	Pf10
				S-Site			
Crop sequence‡							
Corn-Sus	399§	260	106 a	280 a	208	317	733 a
Res-Sus	428	220	69 ab	199 ab	119	172	450 ab
Sus-Sus	454	195	45 b	140 b	139	145	332 b
Fertilizer							
Manure	446	130 b	57	160	115	149	339
PK	451	273 a	54	158	172	163	423
Nonfertilizer	385	272 a	108	300	180	321	753
				C-Site			
Crop sequence							
Corn-Sus	1026	580	227	413 a	406 a	236	592 a
Res-Sus	1067	550	179	391 a	330 ab	248	469 ab
Sus-Sus	919	498	185	278 b	208 b	146	321 b
Fertilizer							
Manure	735 b	408	185	263 b	303	171	452
PK	1252 a	745	213	352 ab	305	242	413
Nonfertilizer	1025 ab	475	193	468 a	335	217	517
ANOVA (*F* values)							
Site (A)	97.78***	25.89***	82.86***	27.02***	30.81***	0.20 NS	0.00 NS
Crop sequence (B)	0.19 NS	0.70 NS	4.89*	7.67**	4.51*	2.05 NS	10.25***
Fertilizer (F)	2.47 NS	5.47**	0.75 NS	2.44 NS	0.88 NS	1.45 NS	1.73 NS
A × B	0.27 NS	0.12 NS	2.14 NSs	0.47 NS	0.85 NS	1.43 NS	0.36 NS
A × F	3.67*	1.82 NS	1.33 NS	0.68 NS	0.99 NS	0.88 NS	0.61 NS
B × F	0.42 NS	1.25 NS	1.95 NS	0.44 NS	0.58 NS	0.08 NS	1.50 NS
A × B × F	0.32 NS	0.14 NS	1.19 NS	1.00 NS	0.71 NS	0.28 NS	0.27 NS

† Pi09, P45d-09, Pm09, Pf09, Pi10, Pm10, and Pf10 are population densities (number of nematodes per 100-cm^3 soil) prior to applying fertilizer, 45 d after planting, at midseason, and at harvest in 2009, and at planting, midseason, and harvest in 2010, respectively.
‡ The 2-yr crop sequences were Sus-Sus, Res-Sus, and Corn-Sus, where Sus is SCN-susceptible soybean and Res is SCN-resistant soybean; the first and second abbreviations represent the crops in 2009 and 2010, respectively.
§ The data are main effect of the split-plot experiment with crop sequences as main plots and fertilizers as subplots, each with four replicates. The values followed by the different letters in the same column are significantly different according to least significance test at $P < 0.05$. *, **, and *** denote the *F* values significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. NS = not significant at $P \geq 0.05$.

A combination of potential mechanisms, rather than a single one, was involved in suppression of SCN in soil by the manure treatment. The immediate decrease in SCN J2 population density after manure application may have been due to the preexisting nematicidal compounds in manure, such as ammonia and fatty acids. It is unclear why a significant reduction of SCN J2 was detected only in SCN-suppressive field. It is possible that besides the effect of the nematicidal chemical compounds, manure might affect J2 and vermiform nematode populations through changing microbial activities, depending on soil microbial community and environmental conditions. In the nematode-suppressive soil, some nematophagous fungi including trapping fungi

TABLE 5. Analysis of variance (*F* value) for soybean and corn yield response to crop and fertilizer treatment in the soybean cyst nematode suppressive and conducive fields.

	Soybean yield		Corn yield 2009
	2009	2010	
Site (A)	31.4**	331.2***	24.9***
Crop (B)	6.5*	1.1 NS	
Fertilizer (F)	40.1***	16.1***	34.3***
A × B	1.4 NS	0.5 NS	
A × F	20.4***	9.1***	2.5 NS
B × F	7.0**	1.2 NS	
A × B × F	0.7 NS	0.2 NS	

*, **, and *** denote the *F* values significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. NS = not significant at $P \geq 0.05$.
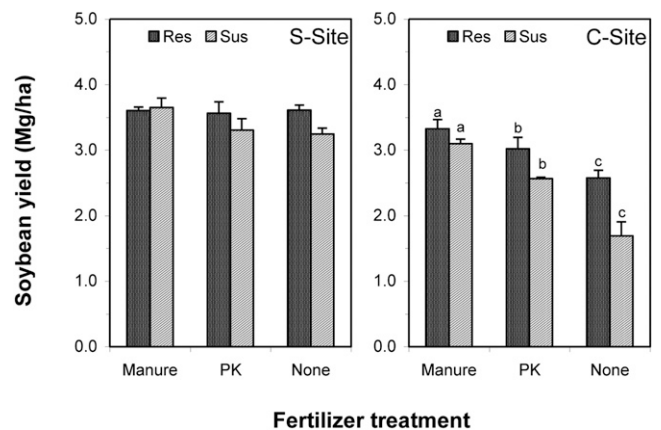

FIG. 1. Fertilizer treatment effect on yield of SCN resistant (Res), susceptible (Sus) soybean cultivars in the SCN-suppressive (S-Site) and -conducive (C-Site) sites, respectively, in 2009. The bars are mean values of four replicates, and the lines on bars indicate the standard error. Significant differences among the different fertilizer treatments within the same soybean cultivar at C-Site are indicated by different letters above the bars according to unprotected least significance difference test at $P < 0.05$. No treatment effects were detected in S-Site.
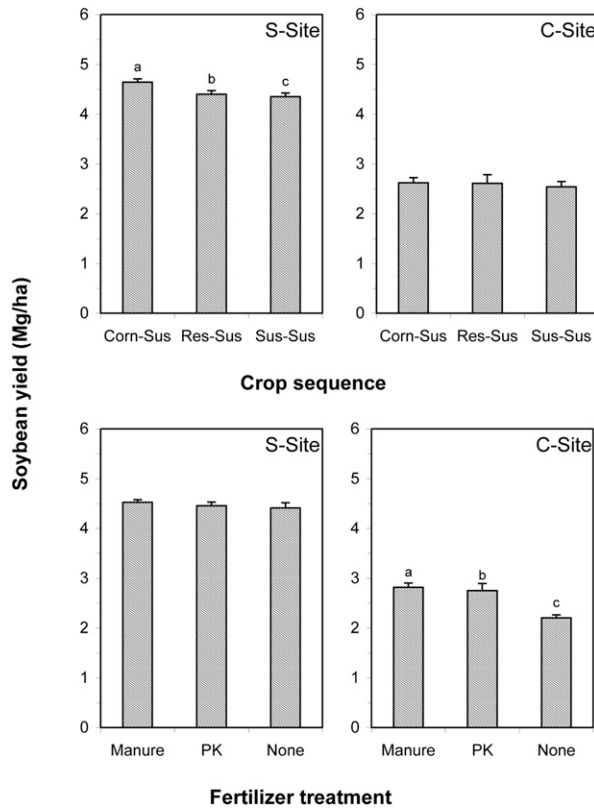
FIG. 3. Fertilizer treatment effect on corn in the SCN-suppressive (S-Site) and -conducive (C-Site) sites in 2009. The bars are mean values of four replicates, and the lines on bars indicate the standard error. Significant differences among the different fertilizer treatments within the same graph are indicated by different letters above the bars according to least significance difference test at *P* < 0.05.

FIG. 2. Soybean yield in response to the fertilizer treatment and crop sequence in the SCN-suppressive (S-Site) and -conducive (C-Site) sites, respectively, in 2010. The 2-yr crop sequences were Sus-Sus, Res-Sus, and Corn-Sus, where Sus is SCN-susceptible soybean and Res is SCN-resistant soybean; the first and second abbreviations represent the crops in 2009 and 2010, respectively. The bars are means and the lines on the bars are the standard error of main effect with four replicates. The different letters within a graph denote the significant differences according to unprotected least significance difference test at *P* < 0.05. No letter in the graph indicates no difference.

and endoparasites of nematodes have been detected (Chen, 2007). The swine manure might enhance the activities of the nematophagous fungi especially the trapping fungi. Wachira et al. (2009) reported that cow (*Bos primigenius*) manure and chicken (*Gallus gallus domesticus*) manure stimulated the occurrence of nematode-destroying fungi in the soil and also reduced plant-parasitic nematodes. When alfalfa leaves (*Medicago sativa*) were added to soil, both microbivorous nematodes and the nematode-trapping fungus *Dactylellina candidum* increased (Jaffee, 2006). Further studies are needed to determine whether or not the effect of the swine manure on nematophagous fungi is a major mechanism in suppressing plant-parasitic nematodes.

A number of plant-parasitic nematodes were observed at the two sites, but only spiral nematodes were observed in most samples in addition to SCN. Spiral nematodes can parasitize both corn and soybean, but the pathogenicity was low. At the population density we observed at the two sites, the nematode should cause limited damage, if any, to corn and soybean. Thus, the manure
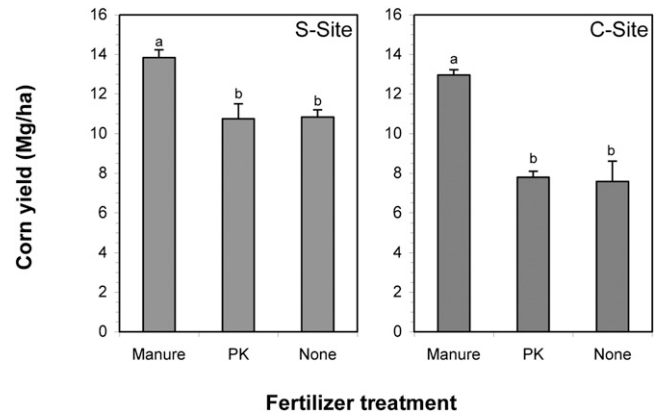
treatment effect on corn yield was probably due to the nutrient effect. The manure provided nitrogen as well as other nutrients for corn growth and resulted in higher yield than both PK fertilizer and nonfertilizer treatments. We realize that N is generally needed for corn production, and the increase of corn yield by manure was mainly because of the nitrogen effect. Considering our emphasis on SCN and soybean yield, we used PK fertilizer treatment without nitrogen for both soybean and corn crops for a balanced experimental design, because chemical nitrogen fertilizer is generally not used for soybean.

Differences in soil chemical properties among fertilizer treatments might have a direct or indirect influence on nematode population dynamics, including free-living nematodes via plant growth or microbial activity (Bulluck et al., 2002; Okada and Harada, 2007). Manure application has been shown to significantly increase the concentrations of nitrate and ammonium in the soil (Jokela, 1992; Mikha and Rice, 2004). The concentration of nitrate was tripled with the manure treatment. More abundant plant nutrition from manure amendment might not only increase crop tolerance to SCN infestation but also provide soil microbes with a new energy source that resulted in the increased diversity and activities of soil microorganism communities. Effectiveness of liquid swine manure in suppressing SCN may be affected by specific soil conditions, such as soil type and pH. For example, the results from Mahran et al. (2008a) indicated that liquid swine manure may be an effective means to kill *P. penetrans* in slightly acid and neutral soil when application is combined with low rates of acid. In acidic soils, swine manure killed *Verticillium dahliae* within a day after application but had no effect in neutral or alkaline soils (Lazarovits et al., 2001). The soil pH was near neutral at the S-Site and slightly higher in the C-Site. Whether the soil pH affected control of nematodes by the manure is unclear in the present study.

In conclusion, our results suggest that soil fertility management plays an important role in developing sustainable strategies to manage SCN and improve crop yield in soybean-corn cropping system. The population density of SCN J2 was significantly lower in soil treated with manure in the SCN-suppressive soil, but no reduction of SCN egg population densities by manure was observed in either site. The liquid swine manure treatment, a source of N, P, K, and other macro- and micro-nutrients, and the chemical fertilizer PK treatment, a source of P and K, increased soybean yield in the SCN-conducive soil. Manure also increased corn yield in both sites. The soil fertility management, especially the application of swine manure, can partially replace the yield advantages of SCN-resistant cultivars in the SCN-conducive soil.

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