

Management Strategies for Control of Soybean Cyst Nematode and Their Effect on
Nematode Community

A Thesis
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

Zane Grabau

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Dr. Senyu Chen

June 2013

Acknowledgements

I would like to acknowledge my committee members John Lamb, Robert Blanchette, and advisor Senyu Chen for their helpful feedback and input on my research and thesis. Additionally, I would like to thank my advisor Senyu Chen for giving me the opportunity to conduct research on nematodes and, in many ways, for making the research possible. Additionally, technicians Cathy Johnson and Wayne Gottschalk at the Southern Research and Outreach Center (SROC) at Waseca deserve much credit for the hours of technical work they devoted to these experiments without which they would not be possible. I thank Yong Bao for his patient in initially helping to train me to identify free-living nematodes and his assistance during the first year of the field project. Similarly, I thank Eyob Kidane, who, along with Senyu Chen, trained me in the methods for identification of fungal parasites of nematodes. Jeff Vetsch from SROC deserves credit for helping set up the field project and advising on all things dealing with fertilizers and soil nutrients. I want to acknowledge a number of people for helping acquire the amendments for the greenhouse study: Russ Gesch of ARS in Morris, MN; SROC swine unit; and Don Wyse of the University of Minnesota. Thanks to the University of Minnesota Plant Disease Clinic for contributing information for the literature review. Many others from SROC spent significant time assisting with this study, especially Jeff Ballman, as well as Nick Hoverstad, Shun Xiao, Ray Johnson, and the SROC soil crew.

Abstract

Soybean cyst nematode (SCN), *Heterodera glycines*, is the major yield-limiting pathogen on soybean and various plant-parasitic nematodes can damage corn. Additionally, the nematode community is a useful bioindicator for soil health. In chapter 1, relevant research is reviewed. Chapter 2 describes experiments testing ten organic soil amendments at various rates for SCN control in the greenhouse. Some amendments—particularly canola meal, pennycress seed powder and condensed distiller’s solubles—effectively reduced SCN populations at 40 days after planting soybeans. By 70 days after planting, SCN control by amendments was diminished. Additionally, phytotoxicity was a concern, particularly at 40 days after planting. Based on these experiments, organic soil amendments have value for SCN management, but more work is needed to optimize amendment efficacy particularly at the field scale. Chapter 3 describes the impact of tillage, granular nematicide (aldicarb or terbufos), synthetic fertilizers (NPKS combinations), and organic fertilizer (swine manure) on plant-parasitic nematodes, the nematode community, and plant yield as assessed in a corn-soybean cropping system. *H. glycines*, *Helicotylenchus* spp, *Xiphinema* spp, and *Pratylenchus* spp were the major plant-parasitic nematodes present at the sites. Tillage had only minor impacts on populations of major plant-parasitic nematode genera. While aldicarb reduced *H. glycines* and *Helicotylenchus* populations, albeit inconsistently, terbufos did not affect major plant-parasitic nematode populations. Nematicides increased soybean and corn yields under some conditions suggesting plant-parasitic nematodes impacted corn and soybean, although this impact was inconsistent. Tillage, fertilizer, and nematicide impacts on the nematode community were often site- and season-specific. Manure application compellingly shifted the nematode community to one of increased enrichment and decreased community structure. The inorganic fertilizers had minimal impact on the nematode community. Conventional tillage decreased nematode community structure based on some measures, but increased bacterivore and fungivore population densities. In contrast, aldicarb nematicide decreased bacterivore and fungivore population densities. Effects of terbufos nematicide on nematode populations and community composition were inconsistent.

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Chapter 1: Literature Review

1. Introduction

The objectives of the research described in this thesis are three-fold. (i) Evaluate current and potential agronomic practices for their impact on soybean cyst nematode, *Heterodera glycines*. In particular, tillage, organic soil amendments, organic fertilizers, inorganic fertilizers, and nematicides were evaluated. (ii) Evaluate the impact of various plant-parasitic nematodes on corn and soybean growth in Minnesota and evaluate how the aforementioned agronomic practices affect this relationship. (iii) Evaluate the impact of these agronomic practices on soil and plant health by chemical measures of the soil, biological measures using the nematode community, and direct measures of plant yield. To complete these objectives, one greenhouse and one field study were conducted. In the greenhouse study, described in chapter 2, various organic soil amendments were screened for efficacy against SCN populations. In the field study, described in chapter 3, the effect of tillage, inorganic and organic fertilizers, and nematicide on SCN, plant-parasitic nematodes of corn and soybean, crop yield, and soil health were investigated. Chapter one gives background information on relevant plant-parasitic nematodes and reviews research related to the work for this thesis.

2. Soybean Cyst Nematode

2.1.1 Disease caused by SCN

Soybean Cyst Nematode (SCN), *Heterodera glycines*, is the major yield-limiting pathogen in United States soybean production causing an estimated 3.05 million tonnes of soybean yield loss in 2009. This is twice as much as seedling diseases, the second most damaging soybean disease behind SCN (Koenning and Wrather, 2010). Symptoms of SCN infection include stunted plant shoots, stunted root systems, and chlorosis leading to the name Yellow Dwarf for the disease caused by SCN (Chen, 2011). Yield losses may occur without visible symptoms of SCN infection with losses of 30% in heavily infested Midwest fields (Chen et al., 2001b). Signs of the disease include SCN females or cysts visible on roots or SCN cysts, females, eggs, juveniles, or males present in the soil.

2.1.2 Life Cycle of SCN

As a nematode, SCN is a non-segmented, microscopic roundworm that lives in water-filled pore spaces in soil when not actively infecting plants. The SCN life cycle

consists of four juvenile stages and an adult stage each separated by a molt (shedding of cuticle) begins with an egg (Chen, 2011; Noel, 2004). Inside the egg, the first stage juvenile develops until the first molt into a second-stage juvenile (J2). The J2 hatches from the egg which may be stimulated by presence of host plant roots in the soil (Warnke et al., 2006). Following hatch, the J2 moves through the soil to the host plant roots which it infects by entering the plant root. Inside the plant root, the J2 contacts the plant cells in the stele or cortex inducing formation of syncytia, large multinucleated cells from which the nematode feeds with its stylet (Noel, 2004).

Once feeding is initiated, the juvenile remains at the same feeding site and molts into third-stage juvenile (J3), fourth-stage juvenile (J4), and adult stages. Females will continue to enlarge and feed through all life stages until assuming a lemon shape and color with posterior end extending outside of the root as an adult female. By J4 stage, SCN males assume a long, vermiform shape and exit roots after molting into a mature adult male. SCN males travel through the soil to inseminate sedentary females only after which they begin producing fertilized eggs (Noel, 2004). The SCN female exudes a gelatinous matrix into the soil in which some eggs are contained. Other eggs are retained in the female body which hardens and darkens into a cyst after death of the female. Inside the cyst, also the overwintering structure, eggs are protected from the environment and may remain viable for many years (Inagaki and Tsutsumi, 1971). During winter months, SCN enters and undergoes diapause, a period of reduced activity and reproduction which is induced by cool temperatures and time of year (Noel, 2004). From hatch to fertile female, the life cycle takes about 28 days to complete depending on conditions (Noel, 2004).

2.1.3 Effect of soil factors on SCN population

Soil temperature affects SCN survival and growth with different optimum temperatures for different life stages. Hatching is reported to occur at temperatures from 16 to 36 °C (Slack et al., 1961) with optimum around 24 °C (Hamblen et al., 1972). Root penetration is highest around 28 °C (Hamblen et al., 1972). Speed of nematode development increases linearly from 15 to 30 °C with optimum survival around 25 °C (Alston and Schmitt, 1988). Generally, SCN will not develop below 14 or above 35 °C (Hamblen et al., 1972). Since SCN lives in water-filled pores when in the soil, it is affected by soil moisture with studies in the southern United States suggesting nematode development is optimal when moisture is just below field capacity although

soil type also influences this (Heatherly, 1988; Heatherly and Young, 1991; Heatherly et al., 1992; Koenning and Barker, 1995; Young and Heatherly, 1988). While SCN reproduces well in a variety of soil types, reproduction may be highest in fine soils although damage may also be less severe in fine soil (Barker et al., 2004).

2.2 Current SCN management

2.2.1 Resistant cultivars

Management of SCN relies heavily on resistant cultivars. Resistant cultivars will not form or maintain a functional syncytium which prevents SCN feeding on soybean roots (Koenning, 2004). Resistant cultivars have proven effective at decreasing SCN populations while increasing soybean yields in Minnesota (Chen et al., 2007; Chen et al., 2001a; Chen et al., 2001b). One southern Minnesota trial of various cultivars showed average yield increase of 28% with resistant cultivars in fields with over 5,000 SCN eggs/100 cm³ soil (Chen et al., 2001b).

However, over time SCN populations adapt and overcome resistance becoming able to reproduce on resistant cultivars, cause damage, and increase population levels (Zheng et al., 2006). This is particularly concerning because current SCN-resistant cultivars are derived from only a few sources of resistance: primarily PI 88788 and Peking, or rarely PI 437654 (Chen, 2011). As a practical measure for assessing the ability of SCN populations to overcome various sources of resistance, HG-typing was developed. An HG type is determined for an SCN population from a particular field by comparing its reproduction on seven soybean indicator lines with different sources of resistance with a susceptible control cultivar (Niblack et al., 2002). For the HG type, the SCN population is assigned the corresponding number for each of the resistant lines it can produce on. For example, an SCN population is HG type 1.2.4 if it can reproduce well (Female Index > 10) on lines 1, 2, and 4 (Peking, PI 88788 and PI437654). This a practical tool for determining what cultivars will be successful in particular fields as well as judging changes in SCN population. However, HG types do not group SCN populations into any type of meaningful taxonomic group, pathovar, or biovar as sections of a population may break different resistance sources and there is no way to detect this. For example, the population with HG type 1.2.4 may have a subpopulation that develops on line 1 only, and a subpopulation that develops on lines 2 and 4 only.

In Minnesota, there is strong evidence that SCN populations are adapting to overcome current sources of resistance (Zheng et al., 2006) while there is also evidence

this occurs in other areas of the Midwest (Kim et al., 2011b; Schmitt et al., 2004). Because of this, diversification of resistance sources is encouraged and crop rotation is used to slow resistance-breaking by SCN populations.

2.2.2 Crop rotation

A second SCN management strategy is crop rotation, which is often used in combination with resistance and other management practices. When soybean is rotated with non-host or poor host crops, SCN population is reduced minimizing soybean yield loss in subsequent years. Extensive work has been done to catalog hosts, non-hosts, and poor hosts of SCN (Chen et al., 2006; Porter et al., 2001; Warnke et al., 2006; Warnke et al., 2008). Some known non-host or poor host crops commonly grown in Minnesota include corn, perennial ryegrass, canola, red clover, oats, wheat, pea, clover, alfalfa, sugar beet, sunflower, and barley (Riggs and Hamblen, 1966; Riggs, 1987; Sortland and MacDonald, 1987; Warnke et al., 2006).

Corn is the primary rotation crop with soybean due to its economic importance, therefore most rotation studies have focused on corn-soybean rotation. While corn rotation helps reduce SCN population, in the Midwest longer periods of corn are necessary for effective SCN population reduction with five years of continuous corn needed to reduce populations below damage thresholds for SCN susceptible soybean (Chen et al., 2001a; Porter et al., 2001). However, rotation with corn combined with strategic use of resistant soybean can be effective with annual rotation of the two crops producing adequate soybean yields (Chen et al., 2001a).

Among other rotation crops, a field study at various sites in Minnesota showed barley, flax, oat, sorghum, wheat, canola, corn, potato, sunflower, alfalfa, hairy vetch, red clover, and pea can reduce in-season SCN population growth compared to susceptible soybean, although amount and consistency of the reduction varied (Miller et al., 2006b). These annual rotations did not generally affect susceptible soybean yield or SCN population in the following year suggesting longer rotations or additional strategies are needed for effective management (Miller et al., 2006b).

Greenhouse studies on rotation crops also showed potential for various crops to reduce SCN population including sunn hemp (*Crotalaria juncea*), forage pea (*Pisum sativum*), lab-lab bean (*Lablab purpureus*), Illinois bundleflower (*Desmanthus illinoensis*), and alfalfa (*Medicago sativa*) (Warnke et al., 2006). Additional greenhouse studies showed residues of sunn hemp, red clover, and perennial ryegrass incorporated

in fallow soil can reduce SCN egg population and subsequent infectivity. *In vitro* studies showed the extracts of fresh plants or plant residue from sunn hemp, red clover, soybean, and canola can reduce viability of SCN J2 (Warnke et al., 2008).

Some studies have used trap crops, these are leguminous crops that stimulate SCN hatch then are killed, in combination with corn rotation for SCN control but inconsistent reductions in SCN population when offset by corn yield losses for pea and soybean trap crops (Chen et al., 2001b). Another study used perennial ryegrass, red clover, or alfalfa as interseeded cover crops on soybean, but any reductions in SCN population were offset by yield losses (Chen et al., 2006).

2.2.3 Tillage

Minimum tillage has become a common practice in the Midwest to conserve soil and reduce fuel costs therefore its effects are considered as part of an SCN management strategy. Most studies on SCN have generally found no effects (Chen et al., 2001a; Chen, 2007b; Conley et al., 2011; Hershman and Bachi, 1995), or population decrease (Donald et al., 2009; Koenning et al., 1995; Westphal et al., 2009) under minimum tillage. Only one study found SCN increase under minimum tillage (Noel and Wax, 2003). Most literature suggests no yield benefit (Chen et al., 2001a; Conley et al., 2011; Donald et al., 2009; Hershman and Bachi, 1995) or yield reduction for soybean (Chen, 2007b; Koenning et al., 1995; Noel and Wax, 2003; Westphal et al., 2009) under minimum tillage. These results suggest tillage generally does not have a strong effect on SCN population.

2.2.4 Fertilizer

Like tillage, fertilizer application is an integral agronomic practice that may influence soil properties. For producers, fertilizer choices that impact SCN management include type (organic or synthetic), nutrients, and rate of fertilizer applied. There is evidence that SCN affects soybean nutrient concentrations particularly Ca in plant shoots (Melakeberhan, 2007; Smith et al., 2001), Mg in plant shoots (Smith et al., 2001), and K in plant roots (Smith et al., 2001). Therefore, there is speculation that fertilization could offset nutrient deficiencies induced by SCN leading to greater disease tolerance or resistance (Melakeberhan, 1999; Melakeberhan, 2007; Smith et al., 2001).

There is some evidence that synthetic fertilizers help with SCN management or tolerance. In a greenhouse experiment, SCN-susceptible soybeans inoculated with SCN had a higher photosynthetic rate when fertilized with both nitrogen and a Hoagland

solution including a suite of nutrients (phosphorus, potassium, magnesium, calcium) than Hoagland solution alone (Melakeberhan, 1999). Similarly, soybean photosynthetic rate was higher when fertilized with the Hoagland solution than without under SCN-infested conditions. More importantly, fertilization decreased SCN cyst development and egg production with Hoagland solution including nitrogen most effective followed by Hoagland solution alone then non-fertilized control (Melakeberhan, 1999). Decreased SCN development with fertilizer application could be caused by increased plant resistance.

In a Tennessee field study, phosphate-potassium (PK) fertilizers at low rates increased SCN population density compared to highest application rate or non-fertilized control (Howard et al., 1998). While SCN population decrease without fertilizer may have been due to decreased yields, yield was increased at high fertilizer rate. Therefore at the high fertilizer rate, SCN population decrease may have been due to increased plant defense.

A greenhouse study was conducted on split-root soybean seedlings grown in sand exposed to various concentrations of SCN and K (Smith et al., 2001). In this study, K fertilization did not affect SCN infection or growth on plant roots or soybean growth at 30 days. SCN infection decreased K concentration in soybean roots under medium levels of K fertilization, but high levels of K fertilization eliminated this problem. Additionally, this effect on K concentration was localized around SCN infection sites.

In a Michigan field study, soybeans showed limited responses to starter N fertilization (6.72 kg/ha) under SCN stress. In particular, yield of one SCN-susceptible cultivar was increased with nitrogen fertilizer under high SCN populations, but the other two cultivars (one resistant and one susceptible) were not affected (Melakeberhan, 2007).

However, other studies suggest synthetic fertilizer application does not affect SCN populations or soybean tolerance of SCN. In particular, there has been some interest in zinc fertilizer as a control for SCN because zinc solutions are used for *in vitro* research studies to stimulate SCN egg hatch and zinc is also a common corn fertilizer (Martin-Ortiz et al., 2009; Soleimani, 2012). In one study, zinc sulfate increased and zinc chelate decreased SCN egg hatch *in vitro*, but did not affect SCN egg hatch in greenhouse trials at rates up to 112 kg Zn/ha. Similarly, in the field, zinc fertilizers did not affect SCN egg populations at rates up to 22.4 kg Zn/ha despite increasing corn yield

(Behm et al., 1995). This suggests zinc fertilizers do not have practical value for SCN management.

Similarly, inorganic NPK fertilizers are not always beneficial for SCN management. In an Arkansas field study under SCN-infested conditions, NPK (13-54-54 kg/ha) fertilization did not provide consistent benefits for soybean tolerance or resistance to SCN populations (Riggs et al., 1989). In a north Alabama field study, neither NPK together or individual nutrients applied singly affected SCN populations (Pacumbaba et al., 1997). A study in Waseca, Minnesota showed no effect of synthetic PK treatment on SCN populations (Bao et al., 2010).

Among organic fertilizers, swine manure has received interest as an agent for SCN management. Anaerobically digested swine manure has also been shown to affect SCN hatch and survival *in vitro* (Xiao et al., 2007) and SCN population density in greenhouse studies (Xiao et al., 2008) with volatile fatty acids apparently driving SCN reductions (Xiao et al., 2007; Xiao et al., 2008). However, in a field trial effects of swine manure application were inconsistent as it increased and decreased SCN population in SCN-conducive and suppressive soils respectively (Bao et al., 2010).

In summary, there is some evidence that fertilization may help manage SCN populations although more research is needed to verify this and determine which types of fertilizers are most beneficial for nematode management.

2.2.5 Nematicide

Generally, nematicide application is not economically viable for SCN management in the Midwest. It also carries environmental and human health risks (Chen, 2011; Matthiessen and Kirkegaard, 2006; Oka, 2010; Rich et al., 2004). However, nematicide application is a primary way to assess yield loss caused by nematodes including SCN and is an economically viable management technique for other nematodes and in other regions. Aldicarb, a granular carbamate labeled for a variety of pests, is the main nematicide tested for SCN management, particularly in the Midwest (Niblack et al., 1992; Noel, 1987; Rotundo et al., 2010; Smith et al., 1991).

In all of these Midwest studies aldicarb had some benefits for soybean growth or SCN population reduction, but efficacy was also inconsistent to varying degrees (Niblack et al., 1992; Noel, 1987; Rotundo et al., 2010; Smith et al., 1991). In a two-year field study in northern Iowa, aldicarb at 2.5 kg active ingredient (a.i.)/hectare (ha) applied in furrow increased early stage soybean biomass, but did not affect soybean yield or SCN

population (Rotundo et al., 2010). In another study in north central Iowa, aldicarb at 2.24 kg a.i./ha applied in a 15 cm band increased soybean yield by 33% in SCN infested fields but only in one of two years (Niblack et al., 1992). In an Illinois field study, aldicarb (5.6 to 27.2 g/100 m row in 18 cm band) decreased SCN female population and increased yield 72% compared to non-amended control, but only in 1 of 3 years (Noel, 1987). In that study, nematicides were not effective and SCN did not affect yield in years with excess rainfall, so water availability may have contributed to nematicide efficacy (Noel, 1987). In a Missouri study that included 16 fields, aldicarb at 5.43 kg a.i./ha applied in furrow increased yields in 7 fields (25% highest increase), but decreased yields 5.7% in one field compared to non-treated plots. Similarly, aldicarb decreased SCN egg population in 2 fields (up to 49% decrease), but increased it in 3 fields (up to 110% increase) (Smith et al., 1991).

Aldicarb has also been effective against SCN in the Southeastern United States (Koening et al., 1998; Schmitt et al., 1983; Schmitt et al., 1987) although not under all conditions (Koening et al., 1998; Young, 1998). In the deep south, Aldicarb has generally not been effective against SCN in mixed populations with RKN (Dickson and McSorley, 1991; Gourd et al., 1993; Herbert et al., 1987; Weaver et al., 1988). Aldicarb is also reported to have some benefits for soybean growth in the absence of disease pressure depending on the application rate (Barker et al., 1988; Schmitt et al., 1987).

Among other nematicides tested in the Midwest, Telone C-35 (Dow Agrosciences, Indianapolis, IN) is a fumigant with 1,3 dichloroprene and chloropicrin as active ingredients with effects on both nematodes and fungi. In a study conducted at three field sites in Iowa, Telone increased soybean yield around 10% and decreased SCN egg populations 42% on average (De Bruin and Pedersen, 2008).

2.2.6 Biocontrol

A final strategy for SCN management is biocontrol or use of live organisms to manage a disease. For SCN, biocontrol is based on fungi and bacteria that are endemic in the soil and parasitize SCN at various life stages. Five groups of fungi have been observed to parasitize SCN: (i) trapping or predacious fungi, (ii) endoparasites of vermiform nematodes, (iii) egg parasites, (iv) antibiotic-producing fungi, and (v) vesicular-arbuscular mycorrhizae (VAM) fungi (Chen, 2004).

As their name suggests, trapping fungi capture motile vermiform nematodes using specialized structures including adhesive hyphae, branches, nets or knobs;

constricting or non-constricting hyphal rings, or stephanocysts (Chen, 2004; Liou and Tzean, 1992). Once captured, nematodes are colonized by fungal hyphae which consume resources from the nematode. *Monacrosporium drechsleri* has been observed at low levels in Minnesota soils (Liu and Chen, 2000). While many species of trapping fungi are present in the soil (Jaffee and Muldoon, 1995; Jaffee et al., 1998; Liu and Chen, 2000; Ribeiro et al., 1999), some species are host specific (Jaffee and Muldoon, 1995; Ribeiro et al., 1999). For example, some *Monacrosporium* species exhibit greater ability to capture RKN than cyst nematode juveniles (Jaffee and Muldoon, 1995; Ribeiro et al., 1999).

Among species known to parasitize SCN, endoparasitic fungi attack by encysting nematodes or producing adhesive conidia (Chen, 2004; Liu and Chen, 2000). As SCN J2 move through the soil, they contact endoparasitic fungi conidia which attach to the nematode and germinate, forming hyphae which penetrate and digest the nematode. Two species of endoparasitic fungi, *Hirsutella minnesotensis* and *Hirsutella rhossiliensis* are common in Minnesota and the Midwest in general (Chen and Liu, 2007; Chen and Reese, 1999; Chen et al., 2000a; Liu and Chen, 2000). In Minnesota, *Hirsutella* may parasitize up to 60% of SCN juveniles in a field (Chen and Reese, 1999) and is one factor that contributes to SCN-suppressive soil (Bao et al., 2011; Chen and Liu, 2005; Chen, 2007a). In a greenhouse trial, inoculation of field soil with *H. rhossiliensis* or *H. minnesotensis* isolates successfully controlled SCN populations with *H. rhossiliensis* generally more effective (Chen and Liu, 2005).

Many species of fungi are present in SCN cysts or eggs, although some may be saprophytic rather than parasitic (Chen and Chen, 2002; Chen et al., 1996; Chen and Chen, 2003). Egg-parasitic fungi penetrate living SCN eggs with their hyphae and consume resources killing the eggs. In a survey of SCN-infested soils across Minnesota, 55% of cysts and 3.4% of females were parasitized with only 1% of eggs parasitized suggesting natural suppression of SCN by egg-parasitic fungi is low (Chen and Chen, 2002). However, some isolates of egg-parasitic fungi are highly parasitic to SCN eggs (Chen et al., 1996; Kim and Riggs, 1991) and have exhibited control of SCN populations (Chen et al., 1996; Kim and Riggs, 1991; Timper and Riggs, 1998) suggesting they have potential as biocontrol agents.

Antibiotic-producing fungi produce compounds that are toxic to nematodes or affect egg hatch (Chen, 2004). In one study, filtrates from *Purpureocillium lilacinum*,

Stagonospora heteroderae, *Necosmospora vasinfecta*, and *Fusarium solani* were toxic to SCN J2 and filtrates from *Purpureocillium lilacinum*, *Stagonospora heteroderae*, and *Neocosmospora vasinfecta* inhibited SCN hatch (Chen et al., 2000b).

VAM fungi form symbiosis with plants with the fungi providing phosphorus to the plant while the plant provides carbohydrates to the fungi (Verbruggen and Kiers, 2010). However, VAM may also affect populations of various plant-parasitic nematodes by competing for root space, altering plant physiology, altering nematode feeding sites, or releasing nematotoxins (Bagyaraj et al., 1979; Habte et al., 1999; Ingham, 1988; Surech et al., 1985). However, VAM does not seem to affect SCN population (Francl and Dropkin, 1985; Tylka et al., 1991).

Among bacteria, *Pasteuria* spp. are the primary interest for biocontrol of SCN (Chen, 2004). *Pasteuria* are common soil dwellers that are obligate parasites which form endospores and mycelium and attack vermiform stages (Atibalentja et al., 2004; Chen and Dickson, 1998; Noel and Stanger, 1994; Noel et al., 2010). One species of *Pasteuria*, *P. nishiwae* reduced SCN populations when introduced to an Illinois field (Noel et al., 2010) and when occurring naturally in Japan (Nishizawa, 1986). An unclassified *Pasteuria* species has also been found on SCN in Illinois (Noel and Stanger, 1994) and demonstrates host-specificity to *Heterodera* species (Atibalentja et al., 2004). In a field study, endemic populations of this unclassified *Pasteuria* isolate limited endemic SCN populations (Atibalentja et al., 1998). In addition, some plant-growth-regulating bacteria including *Bacillus* and *Pseudomonas* have decreased SCN infection or population (Kloepper et al., 1992; Tian and Riggs, 2000; Tian et al., 2000). However results are variable with rhizobacteria exhibiting no effect on SCN populations in some cases (Tian and Riggs, 2000).

While some biocontrol of SCN is observed naturally, the challenge is to find ways to enhance or transfer biocontrol organisms or their properties such that meaningful management of SCN is possible with these organisms. One strategy is inoculating fields with the biocontrol agent, generally in the form of spores. Field inoculation with some biocontrol agents has successfully reduced SCN populations (Kim and Riggs, 1991; Noel et al., 2010; Tian et al., 2000) although this strategy is generally inconsistent (Chen, 2004). For this strategy to be worthwhile and feasible, agents must be highly damaging to SCN with host specificity and limited saprophytic ability. Additionally, there must be an economically feasible way to produce enough viable spores or inoculum of

the biocontrol agent to apply on a field scale. An alternative strategy is to enhance natural biocontrol, generally through application of soil amendments that increase the population of antagonist organisms. While this strategy can be successful, efficacy is quite variable (Oka, 2010). Fields have been identified in Illinois, Arkansas, Minnesota, and Florida that naturally maintain low populations of SCN and are called suppressive soils (Atibalentja et al., 1998; Carris et al., 1989; Chen, 2004; Chen et al., 1996; Kim and Riggs, 1991). These soils tend to have high populations of SCN-antagonistic organisms created by long periods of soybean monoculture (Carris et al., 1989; Chen, 2007a; Kim and Riggs, 1991). However, monoculture is not a viable strategy for enhancing biocontrol since yields would be low until and possibly after biocontrol was enhanced. Thus, there is much interest in finding other strategies to create suppressive soil.

3. Plant-parasitic nematodes of corn

In the Midwest, there are questions and concerns among producers and agronomists about plant-parasitic nematodes in corn due to changes in agronomic practices, namely reduction of insecticide application and reduction of tillage (Jackson, 2006; Tylka, 2007). Additionally, the genera of plant-parasitic nematodes that are important on corn generally have wide host ranges which often include soybean, so nematodes other than SCN also damage soybeans in the Midwest (Kinloch, 1998; Windham, 1998).

As organisms that reside in the soil in mixed populations, there are many challenges to recognizing and quantifying yield loss caused by specific genera of plant-parasitic nematodes. Therefore, in some instances specific genera are only associated with disease or yield loss and not proven as the cause of disease. The main tool for assessing yield loss due to nematodes is nematicide trials where crop yield and nematode populations are compared between nematicide treated and untreated plots. Other sources of information on plant-parasitic nematodes include formal surveys of nematode populations (rare), data from samples sent to diagnostic labs by producers (readily available but biased and small sample size), and other studies on plant-parasitic nematodes (uncommon).

3.1 Plant-parasitic nematodes in Minnesota

In Minnesota, a number of plant-parasitic nematodes have been reported in association with various plants (Table 1.1).

Table 1.1. Plant-parasitic nematodes reported in Minnesota	
Nematode	Reference
<i>Aphelenchoides</i> spp.	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Aphelenchus avenae</i>	(Taylor and Schleder, 1959)
<i>Aphelenchus</i> spp.	(Taylor et al., 1958)
<i>Boleodorus</i>	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Cactodera cacti</i>	(Spears, 1956)
<i>Cactodera cacti</i> group	(Taylor et al., 1958)
<i>Criconemalla inusitata</i>	(Pinochet and Raski, 1976)
<i>Criconemalla rusium</i>	(Anonymous, 1984)
<i>Criconemoides</i> sp.	(Taylor and Schleder, 1959)
<i>Croconemoides rusticum</i>	(Taylor et al., 1958)
<i>Ditylenchus</i> spp	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Gotholdsteineria</i> sp.	(Taylor and Schleder, 1959)
<i>Gracilacus straelani</i>	(Anonymous, 1984)
<i>Gracilacus</i> sp. (as <i>Gracilicus</i> sp. <i>G. marylandicus</i> like)	(MacDonald, 1979)
<i>Helicotylenchus dihystera</i>	(Anonymous, 1984)
<i>Helicotylenchus erythrinae</i>	(Taylor and Schleder, 1959)
<i>Helicotylenchus galeatus</i>	(Anonymous, 1984)
<i>Helicotylenchus mannus</i>	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Helicotylenchus pseudorobustus</i>	(MacDonald, 1979)
<i>Helicotylenchus</i> spp	(Taylor et al., 1958)
<i>Heterodera glycines</i>	(MacDonald et al., 1980)
<i>Heterodera schachtii</i>	(Anonymous, 1984)
<i>Heterodera</i> spp.	(Taylor and Schleder, 1959)
<i>Heterodera trifolii</i>	(Taylor et al., 1958; Wallace et al., 1993)
<i>Hoplolaimus coronatus</i>	(Taylor et al., 1958)
<i>Hoplolaimus tylenchiformis</i>	(Taylor and Schleder, 1959)
<i>Hoplolaimus galeatus</i>	(MacDonald, 1979; Wallace and MacDonald, 1979)
<i>Meloidogyne hapla</i>	(Crow and MacDonald, 1978)
<i>Meloidogyne</i> spp.	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Merlineus brevidens</i>	(Taylor et al., 1958)
<i>Neotylenchus</i> spp.	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Nothocriconema sphagni</i> (<i>Criconemoides sphagni</i>)	(Hoffman, 1974)
<i>Nothoglylenchus</i> spp.	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Paratylenchus projectus</i>	(Crow and MacDonald, 1978; MacDonald, 1979)
<i>Paratylenchus</i> spp.	(Taylor et al., 1958)
<i>Pratylenchus coffeae</i>	(Anonymous, 1984)
<i>Pratylenchus crenatus</i>	(Anonymous, 1984)

Table 1.1 continued. Plant-parasitic nematodes reported in Minnesota	
Nematode	Reference
<i>Pratylenchus hexincisus</i>	(MacDonald, 1979; Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Pratylenchus minyus</i>	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Pratylenchus neglectus</i>	(Taylor and Schleder, 1959)
<i>Pratylenchus penetrans</i>	(Taylor et al., 1958; Taylor and Schleder, 1959; Wallace and MacDonald, 1979)
<i>Pratylenchus pratensis</i>	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Pratylenchus scribneri</i>	(Crow and MacDonald, 1978; Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Pratylenchus tenuis</i>	(Crow and MacDonald, 1978)
<i>Pratylenchus vulnus</i>	(Anonymous, 1984)
<i>Psilenchus</i> spp.	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Punctodera punctata</i>	(Spears, 1956)
<i>Quinisulcius acutus</i>	(Anonymous, 1984)
<i>Rotylenchus robustus</i>	(Taylor et al., 1958)
<i>Rotylenchus</i> spp.	(Taylor et al., 1958)
<i>Subanguina graminophila</i>	(Goto and Gibler, 1951)
<i>Tetylenchus</i> sp.	(Taylor and Schleder, 1959)
<i>Trichodorus</i> spp.	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Trophurus minnesotensis</i>	(Caveness, 1958)
<i>Trophurus</i> sp.	(Taylor and Schleder, 1959)
<i>Tylenchorhynchus acutus</i>	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Tylenchorhynchus brevidens</i>	(Taylor et al., 1958)
<i>Tylenchorhynchus clarus</i>	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Tylenchorhynchus claytoni</i>	(Anonymous, 1984)
<i>Tylenchorhynchus cylindricus</i>	(Taylor and Schleder, 1959)
<i>Tylenchorhynchus dubius</i>	(Taylor et al., 1958)
<i>Tylenchorhynchus fischeri</i>	(Taylor and Schleder, 1959)
<i>Tylenchorhynchus latus</i>	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Tylenchorhynchus leptus</i>	(Taylor et al., 1958)
<i>Tylenchorhynchus martini</i>	(Anonymous, 1984)
<i>Tylenchorhynchus maxinus</i>	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Tylenchorhynchus nudus</i>	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Tylenchorhynchus striatus</i>	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Tylenchus</i> spp.	(Taylor et al., 1958; Taylor and Schleder, 1959)
<i>Xiphinema americana</i>	(Crow and MacDonald, 1978; MacDonald, 1979; Taylor et al., 1958; Taylor and Schleder, 1959; Wallace et al., 1993)
<i>Xiphinema chambersi</i>	(Taylor and Schleder, 1959)

3.2 Plant-parasitic nematodes of corn and soybean in the Midwest

In Minnesota, the nematodes thought to be most important on corn (in approximate order of importance) are *Pratylenchus*, *Xiphinema*, *Longidorus*, *Helicotylenchus*, *Hoplolaimus*, *Trichodorus/Paratrichodorus*, *Tylenchorrhynchus*, *Mesocriconema*, and *Pratylenchus* (Chen et al., 2010; Tylka, 2007; Tylka, 2011) (Tables 1.2 & 1.3). Other nematodes that are important on corn but are not found in Minnesota include *Belonolaimus*, *Meloidogyne*, and *Heterodera oryzae* (Norton, 1984; Windham, 1998). In the Midwest, the nematodes thought to be most important on soybean (in approximate order of importance) are SCN, *Pratylenchus*, *Xiphinema*, *Helicotylenchus*, and *Hoplolaimus*. Other nematodes, such as *Belonolaimus*, *Meloidogyne*, *Hoplolaimus columbus*, and *Rotylenchulus* are important on soybean in the southern United States, but are not present in the Midwest (Kinloch, 1998; Schmitt and Noel, 1984).

3.2.1 *Pratylenchus* (lesion nematode)

There are many species of *Pratylenchus*, most of which have wide host ranges including both corn and soybean. In the Midwest, *P. hexincisus*, *P. scribemi*, and *P. penetrans* are thought to be the most common *Pratylenchus* species in corn fields, but species composition varies from field to field with multiple species generally present in a single field (Windham, 1998). All three species are also common in soybean fields (Schmitt and Noel, 1984), although soybean is reported to be a poor host for *P. hexincisus* (MacDonald, 2010). *Pratylenchus* are migratory endoparasites that enter the root near the root cap and cause physical damage to cells as they penetrate the roots and induce changes in plant physiology by feeding on plant cells. *Pratylenchus* have life cycles as short as 3 to 4 weeks on corn (Windham, 1998) so they can build up to high densities in the soil or in plant roots (Todd and Oakley, 1996). Because *Pratylenchus* eggs may be laid inside roots and all life stages feed on or in roots, *Pratylenchus* may have high density in corn or soybean roots while soil density remains low (Schmitt and Noel, 1984; Windham, 1998). Thus, especially in corn, damage by *Pratylenchus* may be high even when soil populations are low (Norton, 1984; Tylka, 2011; Windham, 1998).

Table 1.2. Frequency and damage potential of plant-parasitic nematodes on corn in the Midwest†					
Scientific name	Common name	Feeding type	Estimated Frequency	Damage potential	Damage threshold
<i>Pratylenchus</i>	lesion	migratory endoparasite	nearly all fields	moderate-high	1,000/g root
<i>Xiphinema</i>	dagger	sedentary ectoparasite	common	moderate	30-40
<i>Longidorus breviannulatus</i>	needle	sedentary ectoparasite	rare	very high	1
<i>Trichodorus</i>	stubby root	migratory ectoparasite	low	high	unknown
<i>Hoplolaimus</i>	lance	migratory endoparasite	low	moderate-high	300-400
<i>Helicotylenchus</i>	spiral	migratory ectoparasite	nearly all fields	low	500-1000+
<i>Paratylenchus</i>	pin	ectoparasite		low	unknown
<i>Tylenchorhynchus</i>	stunt	migratory ectoparasite	common	low	100
<i>Mesocriconema</i>	ring	ectoparasite	low	low-moderate	100
<i>Belonolaimus</i>	sting	ectoparasite	‡absent or rare	high	1
<i>Heterodera zea</i>	cyst	sedentary endoparasite	absent	high	
<i>Meloidogyne</i>	Root-knot	sedentary endoparasite	absent	moderate to high	

† Table is adapted from: (Chen et al., 2010; Tylka, 2011) and information is drawn from references in accompanying review section
‡ *Belonolaimus* is not reported in Minnesota

Table 1.3. Summary of soil samples (75 samples) submitted for analysis of corn nematodes from Minnesota corn fields during 2009-2012. †

Nematode	Number of samples infested ‡	Nematodes/100 cm ³ soil		
		Mean	Maximum	Standard deviation
<i>Pratylenchus</i> (Lesion nematode)	71	333	2680	489
<i>Hoplolaimus</i> (Lance nematode)	18	13	100	16
<i>Helicotylenchus</i> (<i>Spiral nematode</i>)	67	299	2070	422
<i>Tylenchorhynchus</i> (Stunt nematode)	5		20	
<i>Paratylenchus</i> (Pin nematode)	9	9	55	13
<i>Mesocriconema</i> (Ring nematode)	14	11	400	53
<i>Trichodorus</i> (Stubby nematode)	4		10	
<i>Paratrichodorus</i> (Stubby nematode)	1		5	
<i>Longidorus</i> (Needle nematode)	1			
<i>Paralongidorus</i> (Needle nematode)	0			
<i>Xiphinema</i> (Dagger nematode)	20	12	110	19

† Table is adapted from an unpublished table by Chen, S.Y. The samples were collected from fields with suspected corn nematode damage and do not represent average population densities or frequencies in Minnesota.

‡ Samples were submitted either to University of Minnesota Southern Research and Outreach Center (67 samples) or UM Plant Disease Clinic (8 samples)

Groups of *Pratylenchus* nematodes may feed in the same area of corn or soybean roots forming lesions which facilitate secondary infection by fungal and bacterial pathogens (Schmitt and Noel, 1984; Windham, 1998). Extent of damage caused by *Pratylenchus* varies by species for both soybean (Schmitt and Noel, 1984) and corn (Norton, 1984; Windham, 1998) although the nematode is more consistently damaging on corn than soybean (Kinloch, 1998; Norton, 1984; Windham, 1998). In an Iowa corn field only infested with *P. hexincisus*, 26% yield reduction occurred (Norton and Hinz, 1976). A Kansas study in irrigated corn suggested corn yield was reduced by 1% for each 1 000 *Pratylenchus*/g root (Todd and Oakley, 1996). Although other

nematodes cause more acute damage, *Pratylenchus* is present in most Midwest corn and soybean fields and causes moderate damage (Kinloch, 1998; Windham, 1998), so it is probably the most important nematode on corn and second most important nematode on soybean, behind SCN, in the Midwest.

3.2.2 *Xiphinema* (dagger nematode)

Xiphinema are large (up to 2 mm) ectoparasitic nematodes with long stylets. *Xiphinema* may feed on single cells for days at a time, so they are considered sedentary ectoparasites. Although *Xiphinema* is relatively common in soybean and corn fields in the Midwest, the information on its relationship with these crops is limited (Chen et al., 2010; Norton, 1984; Schmitt and Noel, 1984; Windham, 1998). Some studies have suggested that *Xiphinema americanum* reproduction is lower on corn than soybean in the field with populations reduced when corn is continuously cropped (Evans et al., 2007; Ferris and Bernard, 1971) although one study in southern Minnesota found that *X. americanum* populations reached very high levels when corn was planted for ten years (MacDonald, 1979). Additionally, its density has been negatively correlated with corn yields (Norton et al., 1978) and caused significant yield loss in a sandy North Carolina soybean field (Schmitt and Noel, 1984). *Xiphinema* is thought to be moderately damaging on corn and is relatively common in Minnesota corn field samples submitted by farmers (Table 1.3) suggesting it is one of the more important nematodes on corn (Chen et al., 2010; Koening et al., 1999; Tylka, 2011). As a member of the *Longidoridae* family, *Xiphinema* can transmit viruses causing damage in some crops including *Soybean Severe Stunt Virus* which is found in soybean crops in the eastern United States (Evans et al., 2007).

3.2.3 *Longidorus* (needle nematode)

One *Longidorus* species, *Longidorus breviannulatus*, is associated with corn in the Midwest. *Longidorus* is the largest (up to 5 mm long) plant-parasitic nematode and feeds on plant roots ectoparasitically using its long stylet. In response to infection, corn roots may form galls at the tips, be discolored or stunted (Windham, 1998).

L. breviannulatus is only present in soils with sand content over 50% and only reproduces well at 90% sand or higher, so it is not common in the Midwest (Malek et al., 1980). However, in locations where it does occur, *L. breviannulatus* is highly damaging on corn even with low populations (Malek et al., 1980; Norton, 1984; Tylka, 2007). Populations are highest early in the season with most damage occurring as early as two

weeks after corn is planted (Norton, 1984; Windham, 1998). In Minnesota corn field samples, *L. breviannulatus* is rare (Table 1.3), but it is one of the more important nematodes on corn because of its high damage potential with up to 62% yield loss observed in some Midwest fields (Malek et al., 1980). *L. breviannulatus* is thought to infect only Gramineae species (Windham, 1998) and has not been reported on soybean (Malek et al., 1980).

3.2.4 *Hoplolaimus* (lance Nematode)

Hoplolaimus are medium-sized (around 1 mm) nematodes that are migratory endoparasites or semiendoparasites on corn and soybean (Kinloch, 1998; Norton, 1984; Schmitt and Noel, 1984; Windham, 1998). Common symptoms of *Hoplolaimus* infection in corn and soybean include stunted, spindly plants with discolored, tunneled root system devoid of secondary roots (Kinloch, 1998; Norton, 1984; Schmitt and Noel, 1984; Windham, 1998). *H. galeatus* has a wide host range with high reproduction on both soybean and corn (Ahmad and Chen, 1980; Norton, 1984). *H. galeatus* is highly damaging on corn with yields losses of 26% exclusively from this species observed in nematicide trials in Iowa (Norton and Hinz, 1976; Norton, 1984). However, *H. galeatus* is not considered damaging on soybean (Schmitt and Noel, 1984). While *H. columbus* (Columbia lance nematode) can cause significant yield loss on soybean, it is only moderately damaging on corn and currently restricted to the southern United States (Kinloch, 1998; Norton, 1984; Schmitt and Noel, 1984; Windham, 1998). Additionally, *Hoplolaimus* tends to be a problem mainly in sandy soils (Ahmad and Chen, 1980) and is not very common in Minnesota (Chen et al., 2010). However, it is a concern for corn production because of the high potential for yield loss when it does occur. In contrast, the *Hoplolaimus* species present in Minnesota are of little concern for soybean production since they cause little damage on soybean (Schmitt and Noel, 1984) and do not seem to be widely distributed (Table 1.3).

3.2.5 *Helicotylenchus* (spiral nematode)

Helicotylenchus are medium-sized nematodes that have very wide host ranges and are probably present in every soybean and corn field (Kinloch, 1998; Norton, 1984; Schmitt and Noel, 1984; Windham, 1998). *H. dihystra*, *H. digonicus*, and *H. pseudorobustus* are among the most common species in corn (Norton, 1984; Windham, 1998) with *H. dihystra* and *H. pseudorobustus* especially prolific in soybean (Kinloch, 1998). *Helicotylenchus* are migratory ectoparasites or semi-endoparasites that do not

penetrate or only partially penetrate the root although it has been found inside corn roots (Norton, 1984; Windham, 1998). Symptoms of infection include small brownish root lesions (Norton, 1984). They are not considered very pathogenic to corn or soybean, with very high numbers necessary to cause yield loss (Kinloch, 1998; Windham, 1998) although yield increases of 15 to 20% have been reported in nematicide-treated, *Helicotylenchus*-infested corn fields (Norton et al., 1978). Despite their low damage potential (Niblack, 1992; Tylka, 2011), spiral nematodes are important in Minnesota because they are present in nearly every sampled corn field, often in high numbers (Table 1.3).

3.2.6 *Trichodorus* and *Paratrichodorus* (stubby root nematode)

Paratrichodorus minor is the main Trichodorid reported to cause problems on corn. Trichodorids are migratory ectoparasites that feed exclusively at corn root tips which may terminate root growth leading to the short, coarse, “stubby” roots (Norton, 1984; Windham, 1998). Stubby root nematodes are problematic in sandy soils and the southeastern United States, but are not common in Minnesota corn fields samples (Table 1.3). However, they are of some concern because they are highly damaging on corn even with low populations (Norton, 1984; Windham, 1998). *P. minor* has been reported on soybean, but did not seem to cause yield loss (Schmitt and Noel, 1984).

3.2.7 *Tylenchorhynchus* (stunt nematode)

Tylenchorhynchus are medium-sized, ectoparasitic nematodes. In the Midwest, the most common species is thought to be *T. maximus* with *T. claytoni* more common in the Southeast. Both *Tylenchorhynchus* species have been shown to damage corn in greenhouse trials with damage occurring with high populations (Norton, 1984; Windham, 1998). In one trial, *T. claytoni* suppressed soybean yields 21% (Ross et al., 1967), but did not suppress yield in another trial (Schmitt and Noel, 1984). In Midwest corn fields, *Tylenchorhynchus* are relatively common, but of low concern because of their low damage potential (Tylka, 2011). *Tylenchorhynchus* has been observed in soybean fields, but had minimal population increase in a southern Illinois soybean field suggesting soybean may be a poor host (Lawn and Noel, 1986).

3.2.8 *Mesocriconema* (ring nematodes)

Ring nematodes belong to the genera *Mesocriconema* and *Criconemella*. They are medium sized (0.2 to 1mm) nematodes with distinct “ring” annulations on their cuticle. Ring nematodes are common in the Southeast, but their pathogenicity on corn

and impact on corn yield is not well established (Norton, 1984; Windham, 1998). Similarly, ring nematodes are found in soybean fields and have reproduced well on soybean in the greenhouse (McGawley and Chapman, 1983), but have not been associated with yield loss (Schmitt and Noel, 1984). In the Midwest, ring nematodes are not common and of low concern in both corn and soybean.

3.2.9 *Paratylenchus* (pin nematodes)

Paratylenchus are tiny (< 0.3 mm), ectoparasitic nematodes. Little is known about them although they are known to feed on corn (Norton, 1984) and thought to cause damage only at high populations (MacDonald, 2010). *Paratylenchus* has been observed in soybean fields including at high numbers in a ten year monoculture in southern Minnesota (MacDonald, 1979) and has reproduced well on soybean in the greenhouse (McGawley and Chapman, 1983). However, in contrast, there was minimal *Paratylenchus* population increase in a southern Illinois soybean field (Lawn and Noel, 1986).

3.3 Management of plant-parasitic nematodes of corn and soybean

Except for SCN, there are few economically viable options for nematode management in corn and soybean. Possible options for control include use of resistant cultivars, crop rotation, nematicide application, and biocontrol.

Generally, use of resistant cultivars is the most effective and flexible option for nematode management (Kinloch, 1998; Windham, 1998). However, commercial cultivars of corn or soybean with resistance to the plant-parasitic nematodes (other than SCN) in the Midwest are not available. There is germplasm for lesion nematode resistance in corn, but it is not incorporated into any commercial cultivar (Wicks et al., 1990a; Wicks et al., 1990b). Similarly, perennial teosinte (*Zea diploperennis*), a close relative of corn, is a good source of resistance for *Helicotylenchus pseudorobustus* (Norton et al., 1985), but has not been incorporated into commercial cultivars. Resistance to purely ectoparasitic nematodes has not been developed in corn (Windham, 1998). Since these nematodes have not been proven to cause widespread yield loss, there is little economic incentive for seed companies to develop nematode-resistant cultivars (Windham, 1998). Selection of cultivars tolerant to nematodes is an option, although little information is available about tolerance of modern cultivars (MacDonald, 2010).

Crop rotation may be an option depending on the nematode that is causing the problem. For nematodes with restricted host range, such as *Longidorus*, crop rotation may help reduce nematode population densities (Windham, 1998). However, for nematodes with wide host range, such as *Pratylenchus*, crop rotation is unlikely to be effective. Additionally, especially in fields with multiple problem nematodes, it may be difficult to find an economically viable crop that will reduce nematode population densities. However, one study in Minnesota did show that avoiding monoculture helps reduce plant-parasitic nematode population densities in corn (MacDonald, 1979).

There is evidence that nematicides can help reduce nematode populations and alleviate yield loss (Malek et al., 1980; Norton and Hinz, 1976; Norton et al., 1978). However, positive results are not consistent and nematicide application is generally not cost effective (Niblack, 1992). There are also few available nematicides for corn or soybean and they carry environmental and human health concerns (Chen, 2011; Tylka, 2007). But for fields with highly virulent nematodes, such as *Longidorus* or high plant-parasitic nematode populations, nematicide application may be viable (Malek et al., 1980). Various bionematicides, green manures, and biocontrol organisms applied as soil amendments, cover crops, or spores of organisms are effective for population reduction of various nematodes in some crops (Matthiessen and Kirkegaard, 2006; Oka, 2010). However, there is limited research on biocontrol of most corn and soybean nematodes. Additionally, for some soil amendments and cover crops—particularly in northern climates-- there are agronomic and economic limitations.

There is evidence that tillage intensity affects plant-parasitic nematode population density, although there is not a consistent trend across studies. In some cases, minimizing tillage increased populations of some plant-parasitic nematode (Govaerts et al., 2007; Thompson, 1992; Tylka, 2007). In other cases, however, minimizing tillage reduced populations of certain plant-parasitic nematodes (Rahman et al., 2007) or had no consistent effect (McSorley and Gallaher, 1994; Okada and Harada, 2007). This suggests that tillage may have different impacts on different plant-parasitic nematodes or have varying impact depending on cropping system, soil type, or other factors. More research is needed in Midwestern fields to determine how tillage affects specific plant-parasitic nematodes.

Nematodes affect plant roots and thus the plant's ability to uptake water and nutrients. Thus, yield loss due to nematodes is often higher when moisture is limited. In

some cases, alleviating water stress with irrigation or other practices may reduce yield loss, although it will not reduce nematode populations (Windham, 1998).

Overall, there is need to assess plant-parasitic nematode populations and characteristics in corn and soybean in the Midwest including Minnesota. One priority should be an unbiased, random survey of plant-parasitic nematodes in Minnesota corn fields including population levels to determine more confidently which nematodes are most prevalent. Of similar importance are nematicide trials or similar studies to determine the extent of yield damage that various nematode genera cause to modern cultivars at various population levels. Knowing at what population levels crop loss occurs and to what extent would help determine future course of action for researchers, agronomists, and farmers. Finally, the effect of management practices, particularly tillage and crop rotation, as well as soil factors on nematode populations needs to be better evaluated.

4. Nematode community

4.1 Nematode community as bioindicators

In recent years, there has been increased interest in soil health, particularly as it relates to sustainable agriculture. While chemical measures are one way of measuring soil health, biological components can also be used. One such biological measure that is sensitive to many environmental factors is nematode community analysis. Compared to other microbes, nematodes are relatively large and morphologically distinct, making them relatively easy to identify (Bongers 1990). Additionally, nematodes span a wide range of trophic groups including herbivores/plant parasites, fungivores (feed on fungi), bacterivores (feed on bacteria), predators (of other invertebrates), and omnivores (combination of food sources), allowing them to be used as indicators of various processes in the soil (Yeates et al., 1993). Due to their range of life habits and ability to be counted relatively easily, they can be used to analyze trophic structure and other measures of soil health (Bongers and Bongers, 1998).

General ecological indices including Shannon-Weaver diversity, evenness, and Simpson's dominance indices provide basic information about the richness, abundance, and diversity of the nematode community. However, they do not differentiate among nematode life strategies (Neher and Darby, 2009).

More information about soil health can be derived from the abundance or relative abundance (percent of total nematode population) of individual trophic groups based on

their roles in the soil environment and ecosystem. Bacterivores feed on bacteria and may be indicative of an increase in nutrients/bacteria or environmental stress depending on species (Ferris et al., 2001). Additionally, bacterivores can increase nutrient cycling in soil by mineralizing nutrients immobilized in bacteria providing more nutrients for plants and other organisms. Similarly, fungivores participate in nutrient cycling, although they are associated with a more stable, developed environment as fungi are more advanced decomposers (Ferris et al., 2001). Omnivores and predators often indicate a more healthy soil with more biota as they feed on lower trophic groups therefore relying on high populations of organisms at lower trophic levels to maintain their population (Ferris et al., 2001). A high abundance of beneficial (non-plant-parasitic) nematodes is associated with higher biological activity and resources in the soil.

Since plants are their food source, a high abundance of herbivores may indicate a diverse or productive plant community which is generally desired in an ecological setting (Bongers, 1990). In an agricultural setting, a soil with high abundance of major plant-parasitic nematodes is considered unhealthy because plant growth will be reduced. However, other plant parasites (such as root-hair or algal feeders) (Yeates et al., 1993) have negligible effect on plant growth and indicate better soil health in an agricultural setting as well.

Despite this, even within trophic groups different nematode taxa have very different life cycles and sensitivity to environmental stress, so it is difficult to make inferences about soil health based solely on trophic group abundances. To more accurately and sensitively measure various aspects of soil health, a number of nematode community indices have been developed. These indices are calculated based on both trophic group and life history strategy as measured by the colonizer-persister (c-p) value (1 to 5 scale) of each nematode in the community (Bongers, 1990). Nematodes with low c-p values (colonizers) are similar to ecological “r-strategists” and have short life cycles, high reproductive rate, small size, and high tolerance to environmental stress. In contrast, nematodes with high c-p values (persisters) are similar to “k-strategists” and have long life cycles, low reproductive rates, large size, and low tolerance to environmental stress.

The Maturity Index (MI) measures disturbance (any disruption of the ecosystem) of the soil system based on the average c-p value in the community, with higher values indicating less disturbance or later stages of succession (Bongers, 1990). Thus, a higher

MI is generally associated with more healthy soil. Bongers (1990) did not include plant-parasitic nematodes in the original MI because they are reflective of plant abundance and development, not solely soil condition. Instead, a Plant-Parasitic Index (PPI) including only herbivores was created which may have a positive (Bongers et al., 1997; Bongers and Ferris, 1999) or negative (Neher and Campbell, 1996) relationship with disturbance. In contrast, Yeates (1994) proposed that plant community composition and production is part of soil community succession and health. Therefore, Yeates (1994) included all nematodes in an additional modified maturity index (Σ MI). Additional indices (Σ MI25, MI25) exclude opportunistic nematodes because these nematodes generally indicate recent enrichment. These indices examine soil disturbance ignoring recent enrichment effects (Bongers and Korthals, 1993; Yeates, 1994).

Maturity indices, as weighted averages, show if there are more persisters or colonizers, but do not inform about the absolute abundance of either group. So, two soils could have the same MI, but one soil could be much more biologically active with ten-fold more nematodes. However, to more sensitively detect soil processes and conditions, Ferris (2001) developed the following food web indices. The first three indices sensitively detect three common food web conditions: structured, enriched, and basal using weighted, modified relative abundances (percent of total nematode abundance) of nematode guilds (based on trophic group and c-p value) indicative of each condition. The structure index (SI) is a measure of the structure or number of links in the nematode trophic system with higher values indicating more structure. Nematodes at higher trophic levels (omnivores and predators) and following persister life strategies are more common in structured systems. The enrichment index (EI) measures soil enrichment, defined as disturbance that causes an influx of nutrients or substrates including influxes due to organism death. Higher EI values indicate more enrichment with the characteristic group being very low c-p value (1 or 2) fungivores and bacterivores. The basal index (BI) indicates stressed, low resource conditions with higher BI indicating more basal conditions. Stress-tolerant fungivores and bacterivores (cp value 2) that are present in almost any soil are the characteristic basal group (Ferris et al., 2001).

In addition to the three indices corresponding to food web conditions, Ferris (2001) developed the Channel Index (CI) to determine if decomposition pathways are primarily fungal or bacterial. CI is a modified ratio of the weighted abundance (more

weight for higher c-p values) of fungivores to bacterivores. Higher CI values indicate decomposition by fungi while lower values indicate decomposition by bacteria (Ferris et al., 2001). A similar index takes the abundance of fungivores and divides it by fungivore plus bacterivore abundance (Neher and Campbell, 1996). This index, referred to as FFB, is more statistically robust than the fungivore to bacterivore ratio (FB) (Neher et al., 1995), but less refined than CI (Ferris et al., 2001). Whichever index is used, decomposition through fungal pathways is equated with a more advanced or healthy soil system (Ferris et al., 2001; Neher et al., 1995; Neher and Campbell, 1996).

A final index takes the abundance of fungivores and bacterivores and divides it by plant-parasitic nematodes. Higher values of this index (FBPP) indicate a more healthy soil with values greater than one suggesting the benefits outweigh the drawbacks of the nematode community on plants (Wasilewska, 1989).

4.2 Effects of crop management on the nematode community

Agronomic settings are a primary area of interest for nematode community analysis. Interest in sustainable agronomic practices has increased in recent years (Holland, 2004; Snapp et al., 2005). Additionally, the biology and fertility of soil, as indicated by the nematode community, has direct impact on crop production. The three management practices relevant to the thesis research are reviewed here: tillage, fertilizer, and pesticide application. The reviews of tillage and fertilizer are restricted to studies of row crops in temperate climates except when the crop rotation or fertilizer treatment is exceptionally similar to the research presented in this thesis. For pesticide application, the scope of research reviewed is broader since there are fewer studies in this area and pesticides tend to affect nematodes directly and are less dependent on the environment.

4.2.1 Effects of tillage on the nematode community

Conservation tillage is commonly implemented as a strategy to conserve soil and minimize production costs (Holland, 2004), so its impact on soil characteristics particularly the nematode community is of great interest. Among studies on the nematode community, only one found no effects of tillage. In the study, Bulluck et al. 2002 compared a low-till (tilled once) and high-till (tilled four times) in a North Carolina tomato monoculture with tillage regimes implement for only two years. Tillage alone had no effects on nematode abundance or nematode community indices possibly because the tillage intensity was too similar between treatments.

Two studies found that tillage affects only plant-parasitic nematodes. In one such study, Govaerts et al. (2007) compared no till to conventional tillage with treatments applied in various corn and wheat rotations over a twelve year period in Mexico. Nematodes were only divided into free-living, *Pratylenchus thornei* (a major plant parasite), and “other” plant parasites with no community indices calculated. At the end of the twelve year treatment period, tillage did not affect free-living nematodes, but increased *Pratylenchus thornei* and other plant parasitic nematodes in corn plots under no-till (NT) compared with conventional till (CT) plots (Govaerts et al., 2007). There was some evidence that plant-parasitic nematode populations were related to plant growth, although their populations were not significantly correlated with crop yield. The lack of tillage effects on free-living nematodes may be partly due to the wide depth range sampled (0-20 cm) since other studies found that tillage effects vary over this range (Sanchez-Moreno et al., 2006; Treonis et al., 2010).

Similarly, a study in Wagga Wagga, Australia (Rahman et al., 2007) tillage only affected plant parasites. Conventional tillage (3 pre-plant cultivations) and no-till regimes were implemented for 24 years in a long-term study that also included crop rotation (wheat monoculture vs. wheat-lupin) with samples taken at 0 to 10 cm. Only bacterivores, omnivores, and plant parasites were consistently observed in the study. Plant-parasites, primarily *Pratylenchus* and *Paratylenchus*, were the only group with significant differences between tillage treatments with abundance greater under CT than NT (Rahman et al., 2007).

Of the remaining studies, one (Overstreet et al., 2010) did not measure nematode community indices, but found effects on nematode trophic group abundance. Overstreet et al. (2010) compared conventional tillage (moldboard) and a type of minimum tillage (strip till) in either continuous tomato or a rotation of various vegetables. Treatments were applied for ten years before nematode abundances in the soil were analyzed. Bacterivores, plant parasites, and predators were more abundant under minimum than conventional tillage at various times while there were no significant differences in fungivore or omnivore abundance. Similarly, the ratio of fungivores to bacterivores was not affected suggesting tillage did not affect microbial decomposition pathway with pathways primarily bacterial in this agricultural setting.

The remaining studies found various effects on both nematode trophic group abundance and community indices. Villenave et al. (2009) studied effects of

conventional vs. no-till and various mulch treatments under a corn-soybean rotation in Madagascar at 0-5 cm soil depth with treatments applied for 25 years. Total free-living nematode abundance was lower under no-till than conventional tillage although tillage did not significantly affect abundance of any individual trophic group. However, maturity index (MI) and structure index (SI) were significantly higher under no-till than conventional tillage indicating a less disturbed, more structured community without tillage. The enrichment index (EI) was not affected by tillage. Based on decreased free-living nematode abundance, but increased community structure, the authors suggest that in this system, there were more trophic levels but fewer nematodes within trophic levels in NT compared to CT (Villenave et al., 2009). This could be a result of the tropical climate, although another study in a tropical location and a relatively similar cropping system (corn and wheat) found no effect of tillage on free-living (Govaerts et al., 2007).

Okada and Harada (2007) also compared conventional tillage (plowing before planting and after harvest) to no-tillage, except in a soybean monoculture in Fukushima, Japan. Treatments were applied for nine years with the nematode community analyzed in the final two years. Overall, tillage had minimal effect on nematode abundance, but many effects on nematode community indices. Although there was variation among seasons, the abundance of omnivores, *Meloidogyne* (root-knot nematodes) and the combined facultative plant parasite-fungivore group was generally greater under no-tillage compared to conventional tillage. Additionally, there were no differences in total plant-parasitic nematodes, *Pratylenchus* or soybean yield among tillage treatments. *Pratylenchus* population abundance was very high regardless of tillage likely because of the soybean monoculture although the authors also suggest *Pratylenchus* were protected from tillage by residing in plant roots (Okada and Harada, 2007). Among nematode community indices MI, CI, SI, and diversity indices were higher while EI was lower under NT compared to CT (Okada and Harada, 2007). Overall, this suggests that no-till communities were more mature and structured, but less enriched with a tendency toward fungal decomposition.

A study conducted in Davis, California by Sanchez-Moreno et al. (2006) compared the nematode community under conventional tillage (residues disked and incorporated to 20 cm between crops) and no tillage in either continuous cropping (tomato-sorghum-cover crop-garbanzo) or intermittent fallow (tomato-fallow-fallow-garbanzo) systems following one year of treatments. Sanchez-Moreno et al. (2006)

used Wardle's (1995) methodology and terminology for inferring resilience of soil organisms to tillage with positive V index indicating population increase and negative numbers indicate population decrease with tillage (Wardle, 1995). According to this terminology, bacterivores and fungivores were *mildly stimulated by tillage* (V index= 0.29 and .03) while plant parasites and predator-omnivores were *mildly inhibited by tillage* (V=-0.06 and -.004). However, based on nematode abundance data, tillage effects were not significant for predator-omnivores (*Dorylaimidae*), major plant parasites (*Pratylenchus* or *Tylenchorrynchus*), or most bacterivores (*Panagrolaimus*, *Acrobeloides*, and *Plectus*).

Additionally, SI, Basal Index (BI), and EI were not affected by tillage. One major bacterivore (Rhabditidae) and fungivore (*Aphelenchus*) group was significantly more abundant under conventional than no-tillage. However, Tylenchidae (including both fungivores and weak plant parasites) were more abundant under no-till than conventional tillage. Similarly, channel index (CI) was significantly higher under no-till than conventional tillage suggesting a shift toward fungal decomposition pathways in no-till compared with conventional tillage. Based on increased fungivore and bacterivore populations, the initial nutrient flush under conventional tillage may have stimulated fungal and bacterial growth although there was no indication of enrichment of soil resources based on EI (Sanchez-Moreno et al., 2006).

Treonis et al. (2010) also compared the nematode community after relatively short periods (three years) of conventional tillage (pre-plant and post-harvest) and no-tillage. In this Maryland tomato-soybean-corn rotation system, nematodes were sampled at the 0-5 and 5-25 cm depths with significant differences between the two depths. At the 0-5 cm soil depths, fungivores and plant parasites were more abundant under no-till compared to conventional tillage while bacterivores were more abundant under conventional tillage than no-till. At 5-25 cm depth, omnivore-predators were more abundant under no-till than conventional tillage. At the 0-5 cm range, the basal and channel indices were increased while the enrichment index was decreased under no-till compared to conventional tillage indicating a more basal, less enriched community with more fungal decomposition pathways under no-till.

Although results varied by study, there were some consistent effects of tillage on the nematode community, particularly nematode community indices. The channel, structure, and maturity indices were increased under reduced- or no-till compared to conventional tillage in about half of the relevant studies (Okada and Harada, 2007;

Sanchez-Moreno et al., 2006; Treonis et al., 2010). This suggests reduced tillage favors fungal decomposition pathways while fostering a more stable, less disturbed nematode community with higher trophic levels. Generally, this is considered a more balanced, healthy soil. Additionally, the enrichment index was generally greater under conventional tillage (Okada and Harada, 2007; Treonis et al., 2010) suggesting the flush of nutrients from tillage leads to an increase in enrichment opportunist nematodes although EI was unaffected by tillage in some studies (Sanchez-Moreno et al., 2006; Villenave et al., 2009).

In contrast, tillage effects on trophic group abundances were not very consistent. Omnivores were increased under no-tillage in two studies (Okada and Harada, 2007; Treonis et al., 2010), but unaffected by tillage in five studies (Bulluck et al., 2002; Govaerts et al., 2007; Overstreet et al., 2010; Rahman et al., 2007; Sanchez-Moreno et al., 2006; Villenave et al., 2009) with similar lack of effects on predators (Bulluck et al., 2002; Govaerts et al., 2007; Rahman et al., 2007; Sanchez-Moreno et al., 2006; Villenave et al., 2009). While omnivores and predators are more sensitive to disturbance as a group (Ferris et al., 2001), Sanchez-Moreno et al. (2006) suggest that taxa of omnivores and predators present in agricultural systems are generally tolerant of disturbance resulting in low populations and explaining why additional disturbance (tillage) does not affect them. Similarly, fungivore abundance was increased with reduced tillage in two studies (Okada and Harada, 2007; Treonis et al., 2010), unaffected in four studies (Bulluck et al., 2002; Overstreet et al., 2010; Rahman et al., 2007; Villenave et al., 2009) and affected differently for different genera in another study (Sanchez-Moreno et al., 2006). Bacterivores were also unaffected overall by tillage (Bulluck et al., 2002; Okada and Harada, 2007; Overstreet et al., 2010; Rahman et al., 2007; Sanchez-Moreno et al., 2006; Villenave et al., 2009). Plant-parasitic nematode abundance was increased under minimum tillage (Govaerts et al., 2007; Overstreet et al., 2010; Treonis et al., 2010) as often as it was unaffected (Okada and Harada, 2007; Sanchez-Moreno et al., 2006; Villenave et al., 2009), although it was also increased with minimum tillage in one case (Rahman et al., 2007). Additionally, there was evidence that tillage effects varied by taxa of plant-parasitic nematode (Okada and Harada, 2007; Sanchez-Moreno et al., 2006).

4.2.2 Effect of fertilizers on the nematode community

Fertilization is one of the common agronomic practices and is essential for good crop production. In addition to rate, producers must choose the type of inorganic or organic fertilizer. Organic fertilizers have gained attention as part of sustainable management practices. This review focuses primarily on organic and inorganic fertilizer application in temperate row crops.

In addition to the tillage effects described previously, the study by Okada and Harada (2007) compared an organic fertilizer (rice-straw compost with inorganic N supplement), inorganic fertilizer (NPK at 158-158-158 kg/ha), and no fertilizer control in a long-term study. In this study, fertilizer strongly affected nematode trophic group abundance but not nematode community indices which is the opposite of its results for tillage. Bacterivores, facultative herbivores/fungivores, plant-parasites, *Pratylenchus* and predators were more abundant under both inorganic and organic fertilizers than control but similarly abundant under inorganic compared to organic fertilizer. However, fertilizer did not significantly affect omnivore or *Meloidogyne* populations. Part of the increase in plant parasitic nematodes under fertilizer treatment can be attributed to a significant increase in soybean yield with fertilizers (Okada and Harada, 2007). One modified maturity index (ΣMI_{25}) of several tested was decreased under inorganic fertilizer than other treatments suggesting a more disturbed community with inorganic fertilizer application. However, other maturity indices (MI, MI₂₅, ΣMI) as well as EI and CI were not affected by fertilizers. Overall, these results suggest the effects of fertilizer on community structure were limited and that inorganic and organic fertilizers had similar effects on trophic groups possibly due to excess composting of the organic fertilizer (Okada and Harada, 2007)(Okada and Harada, 2007)(Okada and Harada, 2007).

In addition to and in combination with the tillage treatments previously described, the short-term Treonis et al. (2010) study compared an organic amendment (various low C:N amendments in spring, high C:N straw amendment in fall) to a non-amended control. There were few effects of fertilizer at either depth measured (0 to 5 and 5 to 25 cm) on nematode abundance and none on nematode community indices. At 0 to 5 cm, bacterivores were less abundant and fungivores were more abundant under organic fertilizer treatments than control. At 5 to 25 cm, bacterivore abundance was lower and total nematode abundance was higher in organic fertilizer treatments. There were no significant effects of fertilizer on plant-parasite or omnivore-predator abundance.

Rahman et al. (2007) also compared inorganic fertilizer (urea at 100 kg/Ha) to no fertilizer control in a wheat monoculture under conventional tillage with crop residue burnt as part of the long-term study described previously. Under inorganic fertilizer, plant-parasitic nematode (*Pratylenchus* and *Paratylenchus*) abundance decreased while free-living nematode (bacterivores and omnivores) abundance increased. Rahman et al. (2007) suggest that compounds in or derived from the urea may have been toxic to plant parasites, while the flush of nutrients increased food supply for free-living nematodes, especially bacterivores.

Hu and Cao (2008) compared the effects of a 60% wheat and 30% livestock manure compost, an inorganic N-P (298-101-0 kg/ha) fertilizer and a non-amended control applied for nine years in a north China winter wheat-summer corn system. In general, nematode abundance was greater under organic fertilizer than inorganic fertilizer or control treatments especially at 0 to 10 cm soil depths. At the 0 to 10 cm soil depth, nematode abundance was significantly greater in organic fertilizer than inorganic fertilizer or control plots for bacterivores, fungivores, plant parasites, and omnivores/predators. At 10 to 20 cm soil depth, organic fertilizer increased omnivore-predator abundance compared to inorganic fertilizer or control. At the same depth, organic fertilizer decreased bacterivore abundance compared to inorganic fertilizer, but not control. Inorganic fertilizer decreased plant-parasitic nematode abundance compared to control. At the 10 to 20 cm depth, fungivores were not affected by fertilizer. While no indices were calculated, this study showed that the effects of organic fertilizer may vary by soil depth with greater effects in shallow soil, and that organic fertilizer can stimulate nematode populations in ways that inorganic fertilizers do not (Hu and Cao, 2008).

Leroy et al. (2009) studied the effects of various organic amendments, chemical fertilizer, and two no fertilizer controls (fallow and with crop) in a winter wheat-*Phaecelia* cover crop-red cabbage rotation in Belgium. Organic amendments included farmyard manure, cattle slurry supplemented with separate crop residue, vegetable compost, and two types of farm compost all of which were applied over three years. In general, there were few effects of fertilizer on nematode trophic group abundance, but more on nematode community indices. Bacterivore abundance tended to be higher with cattle slurry and farmyard manure than all other treatments. Plant-parasitic nematode abundance was lower for cattle slurry than the control with crops present at some times.

In the third year of application, the Channel Index was greater for farmyard manure, farm compost, and cattle slurry than the control with crop. In the final year of application, the Channel and Maturity indices were lower for farmyard manure and cattle slurry than chemical fertilizer suggesting more bacterial decomposition and disturbance in the manure treatments. The enrichment index was higher in the farmyard manure and cattle slurry than vegetable or farm compost indicating more enriched food web in the manure treatments. Overall, these results suggest that manure has a more pronounced effect on the soil food web in the short-term than compost or synthetic fertilizers do. Also, since there were no differences in SI, fertilizer treatment did not seem to affect food web structure in the short term (Leroy et al., 2009).

In contrast, Liang et al. (2009) studied the long-term effects of organic (pig compost), inorganic (urea), and 50% organic-50% inorganic (organic+inorganic) fertilizers in a conventional tillage, corn monoculture system in Northeast China in a twenty year long-term study before analyzing the nematode community at four times at the end of the study. Generally, organic or organic +inorganic treatments resulted in greater nematode abundance than inorganic fertilizer or control treatments across trophic groups although there was variation among sampling dates and groups. In particular, bacterivore abundance was greater under organic than non-organic treatments only early in the season due to a rapid increase in bacterivore abundance in organic plots after fertilization and a gradual increase in chemical plots throughout the season (Liang et al., 2009). Similarly, early in the season, the enrichment index was higher and the channel index was lower in both organic treatments compared to non-organic treatments indicating enrichment of the food web and bacterial decomposition with organic treatments.

In addition to tillage, the Bulluck et al. (2002) two-year study compared various organic and inorganic fertilizer treatments in a North Carolina tomato crop system. Fertilizer treatment only affected abundance of bacterivores, fungivores, and certain species of plant parasites. Fungivores were consistently more abundant under organic compared to inorganic fertilizer treatment. Bacterivores were more abundant in swine manure or cotton gin compost than inorganic fertilizer at various seasons. This suggests organic fertilizers stimulated the microbial community. *Pratylenchus* populations were higher with vetch cover crop and manure treatments while *Helicotylenchus* populations were higher with cotton gin compost treatments and *Meloidogyne* populations were

unaffected by fertilizers. Swine manure and cotton gin compost had significantly higher EI and, early in the season, lower Σ MI than other fertilizers indicating an enriched, disturbed community. Early in the season, CI was greater under inorganic fertilizer and rye cover crop than other treatments indicating fungal decomposition pathways.

In another study, Villenave et al. (2010) compared various organic and inorganic fertilizers in a Sorghum monoculture in a long-term experiment (26 years of treatment) in Burkina Faso with the nematode community analyzed in the final year of the study. Overall, there were few significant effects of inorganic fertilizer. Total nematode and plant-parasitic nematode abundance was higher in plots with inorganic fertilizer (urea, straw+urea, manure+urea) than plots without inorganic fertilizer (control, straw, manure), but there was no significant effect of inorganic fertilizer on free-living nematode (non-plant-parasitic), bacterivore, fungivore, or omnivore-predator abundance.

In contrast, there were many effects of organic fertilizer. Both bacterivore and fungivore abundance was greatest in straw treatments (straw alone or with urea), intermediate in manure treatments (manure alone or with urea), and lowest in non-organic treatments (control or urea) (Villenave et al., 2010). Plant-parasitic nematode abundance was higher in manure treatments than any others while omnivore-predator abundance was not affected by organic fertilizer. The effects on plant-parasitic nematodes may have been related to plant growth as yield was highest under manure. Among nematode community indices EI, SI, and CI were measured with CI unaffected by fertilizers. EI was only affected early in the season with EI higher under straw treatments than any other treatment. SI was generally highest with inorganic fertilizer, intermediate in manure, and lowest in straw treatments. Decreased structure under organic fertilizer may have been due to disturbance through enrichment of lower trophic groups (Villenave et al., 2010).

There were some strong, consistent trends for fertilizer treatments across studies. Organic fertilization consistently increased bacterivore abundance compared to control (Hu and Cao, 2008; Leroy et al., 2009; Okada and Harada, 2007; Villenave et al., 2010) and inorganic fertilization (Bulluck et al., 2002; Hu and Cao, 2008; Leroy et al., 2009; Liang et al., 2009; Okada and Harada, 2007; Villenave et al., 2010). Additionally, fungivore abundance was generally greater (Hu and Cao, 2008; Liang et al., 2009; Okada and Harada, 2007; Treonis et al., 2010; Villenave et al., 2010) under organic fertilizer than control treatments. Both the enrichment and the channel indices were

increased with organic fertilization in about half of studies where EI and CI were measured (Bulluck et al., 2002; Leroy et al., 2009; Liang et al., 2009; Villenave et al., 2010).

However, the maturity index was generally decreased under organic compared with inorganic fertilizers (Bulluck et al., 2002; Leroy et al., 2009). This suggests organic fertilizers are a rich source of nutrients for microbes and can shift decomposition pathways toward fungi. This influx of nutrients can act as a form of disturbance that benefits opportunistic, colonizer-type nematodes and microbes at the expense of persister-type nematodes. Organic fertilizer also tended to increase plant-parasitic nematode populations (Hu and Cao, 2008; Liang et al., 2009; Okada and Harada, 2007; Villenave et al., 2010), likely by increasing plant growth leading to more food resources for these nematodes.

4.2.3 Effect of pesticides on nematode community

As with nematode community studies on tillage and fertilizer, the results of studies of pesticide effects on the nematode community vary. This can be partly attributed to the variety of locations, crops, environmental conditions, pesticide types, and pesticide application rates/methods used in these studies. Additionally, nematode populations are spatially heterogeneous and affected by many edaphic factors, so results tend to be variable. Since different pesticides affect different types of organisms, this section is split by pesticide type including granular nematicides, and soil fumigants or biocides.

4.2.3.1 Granular nematicides

Granular nematicides are commonly used in agriculture to control pests as they affect nematodes and, to varying degrees depending on the agent, insects. Therefore, granular nematicides generally affect the nematode community directly, and its effects are generally not mediated by changes in populations of other microbes. Here, four studies that tested a total of six granular nematicides, mostly in field studies, are reviewed. Only two of these studies measured any nematode community indices. One of them, a field study in the Negev desert tested fenamiphos nematicide (400 ppm) applied with water in comparison to non-treated, water only, and biocide treatments over one year. Fenamiphos decreased MI, genus diversity, trophic diversity, and species richness but did not affect \sum MI or FBPP compared to water control. In the study, fenamiphos also decreased total nematode, fungivore, and bacterivore abundance but

did not affect herbivore or omnivore/predator abundance (Pen-Mouratov and Steinberger, 2005).

Wada (2011) also measured nematode community indices in a Japanese radish field. It tested the effects of the granular nematicide imicyafos (3 kg a.i./ha) on the nematode community and *P. penetrans* populations. It measured community structure and diversity using PCR-DGGE with different taxa creating different bands on the DGGE. Imicyafos nematicide decreased the number of PCR-DGGE bands suggesting fewer taxa and less community structure, but did not affect community diversity. While trophic group abundances were not measured, total nematode abundance was not affected by nematicide (Wada et al., 2011).

Among studies that only examined trophic group and total nematode abundance, carbofuran, a nematicide/insecticide was used in two studies (Chelinho et al., 2011; Smolik, 1983). Chelinho (2011) tested the effects of various rates of carbofuran in agricultural soil that was defaunated, then treated, re-inoculated with nematodes, and incubated in laboratory conditions. Carbofuran decreased total nematode, herbivore, fungivore, and bacterivore abundance but not omnivore/predator abundance. Enrichment opportunistic nematodes from the families Rhabditidae and Panagrolaimidae were increased by carbofuran application probably due to increased organic matter in the form of decaying organisms killed by the nematicide application (Chelinho et al., 2011).

Smolik (1983) examined the effects of three granular nematicides on free-living Dorylaimida (an order of mainly omnivores) and microbivores (combined fungivores and bacterivores) in a number of South Dakota corn fields. Carbofuran (1.12 and 2.24 kg a.i./ha) decreased Dorylaimida and microbivore densities in 25 to 46% and 13 to 31% of fields respectively depending on season and rate. In the same study, terbufos (1.12, 1.68, and 2.24 kg a.i./ha) decreased Dorylaimida and microbivore densities in 40 to 60% and 20 to 50% of fields respectively depending on season and rate. Of the three nematicides tested, aldicarb (1.12 and 2.24 kg a.i./ha) had the most effect, reducing Dorylaimida densities in 50 and 100% of fields at midseason and harvest respectively while reducing microbivore densities in 25 to 50% of fields (Smolik, 1983).

Since so few studies on granular nematicides examined nematode community indices or the same trophic groups, it is difficult to make generalizations about their effects. However, granular nematicide consistently decreased bacterivore and fungivore

population densities (Chelinho et al., 2011; Pen-Mouratov and Steinberger, 2005; Smolik, 1983; Wada et al., 2011). Although omnivores and predators are very sensitive to disturbance (Bongers, 1990; Yeates et al., 1993), nematicide did not affect omnivore-predator populations in most instances (Chelinho et al., 2011; Pen-Mouratov and Steinberger, 2005; Smolik, 1983) although both terbufos and aldicarb nematicides affected *Dorylaimida* in one study (Smolik, 1983). It may be difficult to detect changes in omnivore-predator abundance due to low populations in agricultural settings (Chelinho et al., 2011; Pen-Mouratov and Steinberger, 2005). Additionally, nematicide application generally decreased total nematode abundance (Chelinho et al., 2011; Pen-Mouratov and Steinberger, 2005) although not in all cases where it was measured (Wada et al., 2011). Similarly, nematicides generally decreased total herbivore (Chelinho et al., 2011) or target plant-parasitic nematode species (Sato et al., 2009) although not in all instances (Pen-Mouratov and Steinberger, 2005).

4.2.3.2 Soil fumigants and biocides

Soil fumigants are also used in agriculture to control soil pests although they have broader range of activity than granular nematicides. Fumigants affect nematodes, fungi, weeds, and, to some extent, bacteria (Harris, 1991; Sanchez-Moreno et al., 2010; Thomas, 1996). Therefore, soil fumigants may affect the nematode community directly as their chemical compounds kill nematodes and indirectly because these compounds may also kill nematode food sources (fungi and bacteria). Three studies on soil fumigants measured nematode community indices. A Florida study examined the effects of methyl bromide and chloropicrin (both fumigants) applied three months before establishing a pepper crop (Wang et al., 2006). FFB, SI, and taxa richness were all decreased by soil fumigant application suggesting bacterial decomposition pathways, less community structure, and fewer taxa. Bacterivore, herbivore, omnivore, and fungivore densities were all decreased with soil fumigation. Soil fumigations affected the nematode community for at least 3 and in some instances 7 months after fumigation. Generally, fumigation affected herbivores most strongly with nearly no herbivores present 7 months after fumigation. Similarly, fungivores were decreased through 7 months after fumigation while decreases in bacterivore population were not as dramatic or persistent.

Sanchez-Moreno (2010) studied the effects of the fumigants 1,3-dichloropropene (1,3-D) and chloropicrin in various combinations and rates in strawberry fields at two sites

in southern Spain over 35 weeks. Generally, EI and SI were decreased while BI was increased in fumigated compared with non-fumigated plots with differences detected up to 35 weeks after fumigation. At one site, FFB and diversity were decreased by fumigation suggesting bacterial decomposition pathways, but only at one month after fumigation. At the other site, CI was higher in fumigated plots at 35 weeks after fumigation only, suggesting fungal decomposition pathways. Fumigation decreased fungivore, herbivore, omnivore, predator and total nematode density at various times. Fungivores were most consistently and omnivores were least consistently affected by fumigation. This may be due to low omnivore populations and the fact that fumigants kill fungivores directly and generally also kill their food source, fungi (Sanchez-Moreno et al., 2010). However, while 1,3-D is thought to have minimal effects on fungi (Noling and Becker, 1994; Timper et al., 2012), 1,3-D only was also consistently effective against fungivores.

Similar to the previous study, a study by Timper (2012) tested the fumigant 1,3-dichloropropene (66.2 kg a.i./ha) except in combination with the granular nematicide aldicarb (6.7 kg a.i./ha) in a Georgia cotton field over three years. Generally, SI and MI were lower with fumigant/nematicide suggesting less structured, more disturbed soil community. CI was decreased with fumigant/nematicide application suggesting bacterial decomposition pathways, but only at 1 month after application with effects dissipating by 3 months after application. EI was not affected by the pesticides. Pesticide application reduced populations of all trophic groups (bacterivores, fungivores, herbivores, omnivores, and predators) up to 3 months after application with bacterivore and herbivore populations reduced up to a year after pesticide application. Compared to other trophic groups, overall omnivore and predator density was affected for a shorter time despite relatively high populations. This suggests some of the genera present were adapted to agricultural disturbance (Fiscus and Neher, 2002; Sanchez-Moreno et al., 2010; Timper et al., 2012).

Among studies that did not measure nematode community indices, Harris (1991) tested three fumigants (chloropicrin, dazomet, and methyl bromide) in a strawberry field. Chloropicrin (150 L/ha), methyl bromide (450 kg/ha), and dazomet (570 kg/ha) effectively reduced nematode density when applied to soil after strawberry beds were constructed with chloropicrin reducing densities below detectable levels (Harris, 1991). Dazomet (380 kg a.i./ha) and methyl bromide (750 kg a.i./ha) also reduced nematode

densities when beds were plowed after soil fumigant although to lesser extent than when applied after strawberry bed construction.

Finally, Ettema and Bongers (1993) applied the fumigant metam sodium (sodium methyl-dithiocarbamate at 2550 kg product/ha) alone and in combination with cow manure to fallow soil in Wageningen, Netherlands. Treatments were not compared to control since nematode populations varied between sites before treatment. Plots were monitored for 60 weeks with samples taken weekly until 13 weeks after treatment and at decreasing intervals thereafter. In plots that were only fumigated, nematodes were not detected until 3 weeks after treatment and were extremely low until 33 weeks after treatment at which time it reached 1500 nematodes/100 g soil. A limited number of taxa became established in the fumigated soil consisting primarily of cp1 and 2 bacterivores with one omnivore (*Microdorylaimus*) and one fungivore (*Aphelenchoides*) also present. At first, Rhabditina was the most dominant group, but by 33 weeks after treatment *Acrobelloides* was the most dominant with *Aphelenchoides* and *Plectus* also common. In fumigated and manure-treated plots, nematodes were not detected until 3 weeks after treatment, but increased rapidly after that until peaking at 8500 nematodes/100 g soil at 8 weeks after treatment. By 10 weeks after treatment, nematode population had decreased and stabilized at 2000 nematodes/100 g soil. Even fewer taxa were established in the fumigated-manure than the fumigated only plots with Rhabditina and *Acrobelloides* in high numbers and *Aphelenchoides* and *Panagrolaimus* in low numbers through most of the experiment. Trends in which species were dominant were similar for fumigated and fumigated-manure plots. In both fumigation treatments, MI and diversity steadily increased throughout the experiment (Ettema and Bongers, 1993).

The limited number of studies on soil fumigants once again makes it difficult to draw definite conclusions about their effects on the nematode community. All studies that measured community indices, however, found that soil fumigation decreases SI indicating a decrease in nematode community structure (Sanchez-Moreno et al., 2010; Timper et al., 2012; Wang et al., 2006). Additionally, decomposition pathways tend to shift toward bacterial with soil fumigation (Timper et al., 2012; Wang et al., 2006) and nematode diversity tends to decrease (Ettema and Bongers, 1993; Sanchez-Moreno et al., 2010; Wang et al., 2006). EI was increased in one (Sanchez-Moreno et al., 2010) of two studies that measured it (Timper et al., 2012). This suggests soil fumigation may create nutrient resources, potentially in the form of decaying organisms (Bongers and

Korthals, 1993; Yeates, 1994) which is also demonstrated by the dominance of enrichment opportunists in fumigated fallow soil (Ettema and Bongers, 1993).

Soil fumigation had strong effects on nematode populations as total abundance was decreased in all six instances (Harris, 1991; Sanchez-Moreno et al., 2010; Timper et al., 2012; Wang et al., 2006). Similarly, fungivore, herbivore, and omnivore abundances were decreased in all three instances they were measured (Sanchez-Moreno et al., 2010; Timper et al., 2012; Wang et al., 2006) with bacterivores decreased in two of three instances (Sanchez-Moreno et al., 2010; Wang et al., 2006). Overall bacterivore density may be less affected by soil fumigation because decreases in sensitive bacterivores are offset by increases in opportunistic bacterivores (Timper et al., 2012), although bacterivores can still be strongly affected in some cases (Sanchez-Moreno et al., 2010). Overall, soil fumigation tended to affect herbivores most intensely and for the longest periods (Timper et al., 2012; Wang et al., 2006).

In limited studies, the most apparent difference between fumigants and granular nematicides was the more consistent effects of fumigants on omnivores and predators. This may reflect stronger effects of fumigants on non-target nematodes and the soil community in general or it may be a byproduct of higher populations omnivore-predator populations in some soil fumigant studies (Sanchez-Moreno et al., 2010; Timper et al., 2012) allowing for better detection of changes. Both soil fumigants and granular nematicides consistently decreased fungivore, bacterivore, and herbivore populations.

Chapter 2: Efficacy of Organic Soil Amendments for Control of Soybean Cyst Nematode in Greenhouse Experiments

1. Introduction

Soybean Cyst Nematode (SCN), *Heterodera glycines*, is the major yield-limiting pathogen in United States soybean production causing an estimated 3 billion kilograms of soybean yield loss in 2009, accounting for one quarter of total soybean yield loss from disease (Koenning and Wrather, 2010). Current management strategies rely on SCN-resistant cultivars and crop rotation. While resistant soybean cultivars are effective (Chen, 2007b; Chen et al., 2001b), there are few available resistance sources (Kim et al., 2011b), and the SCN population is adapting and diversifying to overcome these sources of resistance (Niblack et al., 2008; Zheng et al., 2006). Annual rotation of SCN-susceptible soybean with corn, the primary crop in the Midwest, does not adequately manage SCN (Chen et al., 2001a; Porter et al., 2001). Additionally, while some chemical fumigants and non-fumigants can effectively reduce nematode populations, most of them are not cost effective for soybean production systems in the Midwest. So, there is an increasing interest in reasonable-cost SCN management strategies with low environmental pollution to alleviate reliance on current management strategies (Matthiessen and Kirkegaard, 2006; Oka, 2010; Rich et al., 2004).

One alternative strategy is applying organic soil amendments. Effective soil amendments reduce nematode populations by killing or paralyzing nematodes directly (bionematicide) or altering the microbial community to suppress pathogen growth (green manure) (Matthiessen and Kirkegaard, 2006; Oka, 2010). Amendments could serve as alternatives to synthetic fumigant biocides, pesticides and fertilizers especially in organic production systems (Moore, 2011; Oka, 2010). Use of soil amendments for nematode control would also increase their value and marketability, benefiting producers of these products. In this study, various organic soil amendments were screened for SCN population control in a greenhouse setting (Table 2.1). These amendments were selected because they or similar amendments show potential as a fertilizer/biofertilizer, biofumigant, or rotation crop. Amendments were chosen from three general categories: byproducts of ethanol or electricity production, fresh plant material of alternative crops, or processed plant products.

Soil amendment	Application rates (w:w)	Further description	Source
Non-amended control	0		
Condensed distiller's solubles (CDS)	1%, 4.30%	Liquid/paste byproduct of ethanol production	Corn Plus ethanol plant, Winnebago, MN
Ash of CDS	0.20%, 1.00%	Raw combusted CDS ash	Corn Plus ethanol plant, Winnebago, MN
Turkey manure ash (TMA)	0.20%, 1.00%	Raw combusted turkey manure ash	Fibrominn power plant, Benson, MN
Marigold powder	0.20%, 1.00%	<i>Calendula officinalis</i> dry plant matter ground in electric blender	Marigold plant residue from Russ Gesch, ARS, Morris, MN
Canola meal	0.20%, 1.00%	byproduct of Canola oil extraction	CHS, Inver Grove Heights, MN
Field pennycress seed powder	0.10%, 0.50%	<i>Thlaspi arvense</i> seeds ground in coffee grinder	Seeds from Don Wyse, UMN, St.Paul, MN
Field pennycress plant	1.07%	Fresh <i>T. arvense</i> plant cut in 2 cm sections	Seeds from Don Wyse, UMN, St.Paul, MN
<i>Camelina</i> plant	1.07%	Fresh <i>Camelina sativa</i> cut in 2 cm sections	Seeds from Russ Gesch, ARS, Morris
Marigold plant	1.07%, 2.86%	Fresh marigold plant (<i>Calendula officinalis</i> var Carolla); cut in 2 cm sections	Seeds from Russ Gesch, ARS, Morris, MN
<i>Cuphea</i> plant	2.86%, 0.64%	Fresh <i>Cuphea</i> plant (inter-species hybrid 'MNPSR23'); cut in 2 cm sections	Seeds from Russ Gesch, ARS, Morris, MN

The first category of soil amendments was chosen partially because the recent surge in Midwest ethanol production has increased availability of ethanol byproducts. According to a US Energy Information Administration report (2012), 52.6 billion liters of ethanol were produced in the US up from 7.9 billion liters in 2002. Additionally, corn grain is used for nearly all US ethanol production with 40% of the 315 billion kilogram 2010 corn crop being used for ethanol production (US Energy Information Administration, 2012). Since so much land, labor, and industrial resources are devoted to ethanol production it is important-- especially for farmers and ethanol producers-- to maximize production value by finding meaningful uses for byproducts. The two main byproducts of the most common ethanol production method, dry grind, are dried distiller's grain (DDG) and condensed distiller's solubles (CDS). In some dry grind

processes these byproducts are combined as a product called dry distiller's grain with solubles (DDGS) (Bhadra et al., 2010).

Ethanol byproducts have high value as foodstuffs for cattle (Bhadra et al., 2010; Erickson et al., 2006; Kononoff and Janicek, 2006), swine (Hanson et al., 2012; McDonnell et al., 2011; Miller et al., 2006a) and poultry (Cozannet et al., 2010; Oryschak et al., 2010; Scheideler, 2006) due to their high fat, protein, vitamin, and phosphorus content (Bhadra et al., 2010; Cozannet et al., 2010; Erickson et al., 2006; Nelson et al., 2009; Spiels et al., 2002). However, when byproducts are available in excess of animal feed demand, they are applied to agricultural fields as fertilizer (Maslakow, 2011), a circumstance more common with increased ethanol production. Byproducts are valuable land fertilizers due to their high N,P, and K contents with reported values of 38 to 48, 3 to 8.9, and 4 to 11.5 g/kg respectively (Moore et al., 2010; Nelson et al., 2009; Spiels et al., 2002). Ethanol byproducts are also one of the few approved nitrogen fertilizers for organic production (Moore, 2011). Additionally, use of ethanol byproducts for land fertilization rather than animal feed may be preferred for corn with high aflatoxin levels, which is concentrated in DDG and toxic to animals (Nelson et al., 2009). Despite these benefits, few studies have examined ethanol byproduct use as fertilizer with a lone study on DDG showing increased corn yield (Nelson et al., 2009) and no results of CDS use as a fertilization reported.

However, CDS has been tested as a soil amendment with reported reductions of fungal pathogen populations and corresponding root diseases in potato, radish, and cucumber (Abbasi et al., 2007; Abbasi et al., 2009). In one study, pathogen control seemed to be a result of increased pathogen-antagonistic soil microbes (Abbasi et al., 2007), although various organic acids in CDS were shown to be toxic to fungal pathogens in another investigation (Abbasi et al., 2007; Abbasi et al., 2009). Additionally, DDG can be used as a weed herbicide at very high application rates (Boydston et al., 2008). Similarly, corn gluten meal, a byproduct of wet mill ethanol production, and hydrolyzed corn gluten meal extracts inhibit plant growth and may be used as alternative herbicides (Christians, 1993; Liu and Christians, 1994; Liu et al., 1994). Although ethanol byproducts have not been tested against nematodes, it is possible that the same compounds that inhibit plant and fungal growth also affect nematodes.

Some ethanol plants burn CDS in a fluidized bed reactor to generate heat for operating the ethanol plants. An ash is left over after burning the CDS. Vetsch (2009) reported that this ash of CDS has an NPKS content of 0–17.6–16.2–5.7. Despite having somewhat less P available in the first year, ash of CDS had similar value to triple super phosphate as a corn fertilizer (Vetsch, 2010). Similar to ash of CDS, turkey manure ash (TMA) is the product of combusting organic material. It is a byproduct of “co-fire” process used at Fibrominn power plant in Benson, MN to burn turkey manure and other biomass to generate electricity. Turkey manure ash was also analyzed by Vetsch (2009) and had an NPKS content of 0-13.5-6.6-1.6. It was an agronomically valuable corn fertilizer at high application rates (Vetsch, 2010).

Four alternative Midwest oilseed crops were tested in this study: *Cuphea* (interspecific hybrid MNPSR23), pot marigold (*Calendula officinalis*), spring camelina (*Camelina sativa*), and field pennycress (*Thlaspi arvense*). *Cuphea* seed oil is a replacement for palm oil and other tropical oils (Graham, 1989) with the advantage that *Cuphea* yields well in temperate regions of the US and is a viable rotation crop for soybean, corn and wheat (Gesch et al., 2010; Kim et al., 2011a) that may have benefits for western corn rootworm (Coleoptera: Chrysomelidae) management (Behle and Isbell, 2005). The impact of *Cuphea* on SCN and other nematodes is not reported.

Pot marigold seed oil can be used as a US-produced substitute for harmful volatile organic compounds (VOC) in paints, adhesives, and similar products (Biermann et al., 2010). While pot marigold is still being established as an agricultural crop, its value for nematode management is well known, particularly for *Tagetes*, a marigold genus closely related to *Calendula*. Various *Tagetes* species and cultivars have been shown to reduce nematode populations including *Meloidogyne* (root-knot nematodes) when rotated with tomato (Ploeg, 1999; Ploeg, 2002), *Xiphinema index* (dagger nematode) as a grape vineyard cover crop (Villate et al., 2012), *Pratylenchus penetrans* (lesion nematode) when rotated with tobacco (Reynolds et al., 2000), *P. penetrans* when rotated with potato (LaMondia, 2006), and *P. penetrans* when rotated with *Narcissus tazetta* in the field (Slootweg, 1956). In an *in vitro* study seed exudates of two *Tagetes* varieties were nematicidal to *Meloidogyne hapla*, *P. penetrans*, and *Heterodera schachtii* (sugar beet cyst nematode) (Riga et al., 2005).

However, even within the *Tagetes* genus, effectiveness varies by marigold variety and target nematode (Chitwood, 2002; Douda et al., 2010; Insunza et al., 2001;

McSorley et al., 2009; Ploeg, 1999). Some studies have investigated the effects of *Calendula* specifically on nematodes. Flower and leaf extracts of *C. officinalis* were an effective nematostatic agent *in vitro* on *Xiphinema americana sensu lato* (Insunza et al., 2001) and flowers from *C. officinalis* and two other species effectively reduced *Meloidogyne artiellia* populations when applied as a soil amendment at 1% weight/volume (w/v) (Perez et al., 2003). *In vitro* studies using *C. officinalis* extracts, specifically oleanolic acid and its glucuronide derivatives, reduced survival of *Heligmosomoides* species, an animal parasitic nematode (Doligalska et al., 2011; Szakiel et al., 2008). This suggests *Calendula* may contain a different nematotoxic compound than the polythienyls in *Tagetes* (Chitwood, 2002; Douda et al., 2010) suggesting their effectiveness as nematode-management amendments may be different from one another. Additionally, the powderized *C. officinalis* plant residue tested in this study has not been previously tested on nematode populations.

Spring camelina, field pennycress, and canola (*Brassica napus*), from which canola meal is derived, are members of the *Brassicaceae* family. *Brassicaceae* members contain glucosinolates that are converted to isothiocyanates in the soil (Morra and Kirkegaard, 2002) which can be toxic to plants, microbes, and nematodes depending on the dose (Balesh et al., 2005; Bending and Lincoln, 1999; Gimsing and Kirkegaard, 2009; Hu et al., 2011; Matthiessen and Kirkegaard, 2006; Morra and Kirkegaard, 2002; Oka, 2010; Vaughn et al., 2006a). Other sulfur containing compounds may also contribute to biofumigation by *Brassica* plants (Bending and Lincoln, 1999). Therefore, *Brassicaceae* plants and their products have been commonly applied to agricultural land as biofumigants (Gimsing and Kirkegaard, 2009; Matthiessen and Kirkegaard, 2006; Morra and Kirkegaard, 2002; Walker, 1997; Walker and Morey, 1999) and less commonly as fertilizer (Balesh et al., 2005; Moore, 2011).

Spring camelina is a low input crop that performs well in the Midwest and arid north regions of the US (Gesch and Cermak, 2011; Lenssen et al., 2012). Camelina has been tested for use primarily as a biodiesel source (Ciubota-Rosie et al., 2013; Lebedevas et al., 2012; Soriano and Narani, 2012), but also as a biokerosene source (Llamas et al., 2012), animal food (Cappelozza et al., 2012; Kakani et al., 2012), and biodegradable paper (Kim and Netravali, 2012).

Three specific types of glucosinolates have been identified in camelina (Berhow et al., 2013), suggesting it should have biofumigant properties typical of *Brassica* plants,

although few studies have investigated camelina specifically. In field trials, camelina seed meal (byproduct of seed oil extraction) reduced growth of *Phymatotrichopsis omnivora* (causal agent of cotton root rot) hyphae and sclerotia growth or germination (Hu et al., 2011). Camelina has been tested as a cover crop for *X. index* and SCN control in greenhouse studies, but did not significantly reduce populations of either nematode (Villate et al., 2012; Warnke et al., 2006).

Field pennycress is an agricultural weed (Warwick et al., 2002) that has recently been considered as a low-input, high yield alternative crop used for biodiesel (Moser et al., 2009), and industrial lubricants or oils (Cermak et al., 2013; Evangelista et al., 2012). Thiocyanates in pennycress seed meal exhibit biofumigant activity *in vitro* against plant seedlings (Vaughn et al., 2005; Vaughn et al., 2006a) although neither pennycress plant material nor seed meal has been tested for nematode management.

Canola meal is the dry cake leftover from extracting oil from canola seeds. Canola meal has been used as a cattle, hog, and poultry feed due to its high nutritional value (Huhtanen et al., 2011; Seneviratne et al., 2011; Snyder et al., 2010; Thacker and Widyaratne, 2012) although its high glucosinolate and erucic acid content are harmful to some animals (Moore, 2011; Sharma et al., 2009). Since canola meal is also an animal feed, it is relatively expensive for fertilizer use, although its negative side effects on animals and some key benefits as a fertilizer may make it economically viable as a fertilizer in some cases. Canola meal is valuable as an organic fertilizer because it has relatively high N (51 to 63 g/kg), P (6 to 14 g/kg), and K (7.75-15 g/kg) content as well as a C:N ratio (around 8), that is uncommonly low for organic fertilizers (Banuelos and Hanson, 2010; Gale et al., 2006; Moore, 2011; Snyder et al., 2010). Depending on application rate, it can also be an effective biofumigant of weeds (Banuelos and Hanson, 2010; Vaughn et al., 2006b; Walker, 1996), insects (Borek et al., 1997; Elberson et al., 1996) and microbial pathogens (Chung et al., 2002; Mazzola et al., 2012).

Additionally, canola plants have been effective as a biofumigant against *Tylenchulus semipenetrans* (citrus nematode) in the greenhouse when used as compost (Walker, 1996), *Pratylenchus thornei* (lesion) when rotated with wheat (Owen et al., 2010), and *Rotylenchulus reniformis* (reniform nematode) when applied as a compost to a cowpea field (Wang et al., 2001). Canola was also tested as a rotation crop for control of SCN in greenhouse and *in vitro* assays by Warnke et al. (2006, 2008). One set of greenhouse assays simulating a crop season followed by fallow for potential rotation

crops showed reduction of SCN populations in canola-incorporated soil compared with Illinois bundleflower, ryegrass, fallow, or corn-incorporated soil (Warnke et al., 2008). However a similar set of greenhouse assays showed no difference in SCN population density between canola-incorporated soil and fallow (Warnke et al., 2006). Laboratory assays showed that neither fresh nor residual canola plant extracts stimulate SCN hatch making it a poor trap crop. While fresh canola extract had no effect on SCN infective second stage juvenile (J2), canola residue extract showed nematotoxic effects on J2 *in vitro* (Warnke et al., 2008). Canola meal itself has been effective against *Meloidogyne arenaria* (RKN) as an *in vitro* nematicide, *M. arenaria* as a soil amendment in a tomato greenhouse trial (Walker, 1996), *P. penetrans* as a soil amendment in an apple rootstock greenhouse assay (Mazzola et al., 2009), and *P. penetrans* and *Meloidogyne incognita* (RKN) in soil laboratory tests (Zasada et al., 2009).

2. Methods

Ten organic soil amendments at various concentrations for a total of nineteen treatments (Table 2.1) were screened for efficacy in controlling SCN under greenhouse conditions. For fresh plant amendments, plants were grown in UMN greenhouses in St. Paul, MN. Plants were cut on the same day as they were incorporated into soil. Plants were mature when harvested and all aboveground plant parts except seeds were incorporated into soil after cutting plant material into 2 cm sections by hand.

For each treatment except CDS, the soil amendment was added to 1.4 kg of a 3:1 (soil:sand weight) mixture of untreated SCN-free field soil and sterile sand. The mixture was inoculated with SCN eggs at a rate of 2,000 eggs/100 cm³ soil, and mixed thoroughly in two-gallon plastic bags. This mixture was placed in 16-cm-diameter pots and planted with 6 seeds of SCN-susceptible soybean cultivar (Sturdy) which were thinned to 3 plants per pot after germination (seven days). As a semi-liquid paste, CDS could not be mixed with soil in bags, so CDS treatments were added to the top of the soil after planting. Pots were arranged on greenhouse benches in a randomized complete block design with four replicates.

At 40 days after planting (DAP), corresponding to about one SCN reproductive cycle, plant heights were recorded, and seven 1-cm soil cores per pot were collected. Cysts were extracted from the entire soil sample by hand decanting and then sucrose centrifugation, crushed using a mechanical crusher (Faghihi and Ferris, 2000), and SCN population density (eggs/100 cm³ soil) was determined. At 70 DAP, corresponding to

about two SCN reproductive cycles, plant heights were recorded, and soybeans were cut at the soil line. Aboveground soybean plant dry mass was recorded after drying for 48 hours at 90 °C. At 70 DAP, soil was removed from pots and mixed thoroughly by hand. Soybean roots were rubbed gently by hand to dislodge cysts into the soil mixture. Cysts were extracted and crushed from a 100 cm³ sample of the soil mixture to determine SCN egg population density. The experiment was conducted twice, with Experiment 1 planted April 22, 2011 and Experiment 2 planted January 11, 2012, both in St. Paul, Minnesota. During Experiment 1, soil temperature was measured from May 13 to the end of the experiment. During this time, mean soil temperature was 27.7 °C with standard deviation 3.34 °C and range 22.3 to 43.7 °C. Soil temperature during Experiment 2 averaged 25.9 °C with standard deviation 2.27 °C and range 19.5 to 32 °C.

3. Statistical analysis

At 40 DAP, there were no significant interactions between experiment and treatment effects for SCN population ($P=0.1388$) or mean plant height ($P= 0.5015$), therefore results from the two experiments were combined. At 70 DAP, there was significant interaction between experiment and treatment effect for SCN population ($p<0.01$), so results were analyzed separately by experiment.

Each response variable (SCN egg population density at 40 and 70 DAP; mean plant height at 40 and 70 DAP; and soybean shoot mass) was analyzed using two-way ANOVA . ANOVA models were checked for homogeneity of variance using Levene's test and normality of residuals graphically. When necessary, response variables were transformed to meet these assumptions. SCN egg population at 40 DAP for combined experiments was transformed to the one third power. Mean plant height at 40 DAP for combined experiments was natural log transformed. For Experiment 1 at 70 DAP, no variables were transformed. For Experiment 2 at 70 DAP, plant height was squared.

Due to significant treatment effects for plant height and shoot dry mass, further analysis was done on plant growth factors in relation to SCN egg population. For 40 DAP data, an additional ANOVA was conducted with SCN egg population as response variable and mean plant height included as a covariate before treatment. For 70 DAP experiment 1 data, ANOVA with SCN egg population as response and mean plant height as covariate was conducted. Additionally for 70 DAP Experiment 1 data, ANOVA was performed with SCN egg population as response and plant dry mass as covariate. Transformation of variables was same as for standard ANOVA.

At 40 DAP, linear regression of SCN egg population on experiment and plant height (disregarding treatments and blocking) was conducted for the two experiments combined. The same analysis was conducted on 70 DAP experiment 2 data. Also for 70 DAP experiment 2 data, linear regression of SCN egg population density on plant mass (disregarding treatments and blocking) was conducted. Linear regression models were checked for homogeneity of variance (using Levene's test) and normality of residuals (graphically) and transformed when necessary. SCN egg population density at 40 DAP of the two experiments combined was transformed to the 1/3 power, while SCN egg population density at 70 DAP for Experiment 1 was square-root transformed for linear regression. Data was analyzed using R version 2.15.

4. Results

4.1 Experiments 1&2 combined 40 DAP

For the two experiments combined, soil amendment treatment significantly affected SCN population density at 40 DAP ($P < 0.001$). Marigold plant, pennycress seed powder, canola meal, and CDS (all at high rate) were the most effective with SCN population density reductions of 46.6%, 46.7%, 73.2%, and 73.3%, respectively, from the control (Fig. 2.1). Soil amendment treatment also significantly affected plant height at 40 DAP ($P < 0.001$). Addition of soil amendments resulted in similar or reduced plant height compared with control. Cuphea plant, CDS, Pennycress seed powder, and marigold plants all at their higher rate resulted in the lowest plant height with reductions of 22%, 24%, 29%, and 39% from control (Fig. 2.2).

Due to significant effects of treatment on plant height, the effect of plant height on SCN population was also examined at 40 DAP. A linear model of SCN egg population based on mean plant height (both at 40 DAP) and Experiment (a factor with value of 0 for Experiment 1 and value of 1 for Experiment 2) was fit resulting in a linear model with separate intercepts for the two experiments. The linear model was significant ($P < 0.001$) with an equation of

$$(\text{SCN eggs})^{(1/3)} = 5.15 + (11.67 * \text{Experiment}) + (0.2725 * \text{plant height})$$

Which, for Experiment 1 is equivalent to an equation of

$$(\text{SCN eggs})^{(1/3)} = 5.15 + (0.2725 * \text{plant height})$$

Or for Experiment 2, an equation of

$$(\text{SCN eggs})^{(1/3)} = 11.67 + (0.2725 * \text{plant height})$$

The coefficients for Experiment and plant height were also significant ($P < 0.001$). The positive coefficient for Experiment reflects the higher SCN population in Experiment 2 (17,923 eggs/ 100 cm³ soil) than Experiment 1 (1,842 eggs/100 cm³ soil) (Fig. 2.3). Additionally, the positive coefficient for plant height suggests a positive relationship between SCN population and mean plant height at 40 DAP. Adjusted R-squared was 0.72 suggesting Experiment and mean plant height accounted for 71.7% of variation in SCN egg population density. When mean plant height was included as a covariate preceding soil amendment in ANOVA, soil amendment treatment ($P < 0.001$) still significantly affected SCN population (Table 2.2).

4.2 Experiment 1: 70 DAP & Experiment 2: 70 DAP

In Experiment 2, there were no significant differences in SCN egg population at 70 DAP ($P=0.5029$). The average egg population density was 163000 eggs/100 cm³ soil. Similarly, treatment did not affect plant mass ($P=0.4420$) or plant height ($P=0.0676$).

In Experiment 1, soil amendment treatment significantly affected SCN egg population density at 70 DAP ($P < 0.01$). Canola meal at 1% rate significantly decreased SCN population (70%) compared with control (Fig. 2.4). In contrast, CDS at the lower rate of 1% increased egg population 61% compared with control.

Plant height at 70 DAP was also significantly affected by soil amendment treatment ($P < 0.05$). Only 1% pennycress seed powder and 4.3% CDS resulted in significant differences from control with 20% and 22% reductions, respectively (Fig. 2.5). In addition, soybean aboveground dry mass at 70 DAP was significantly affected by soil amendment treatment ($P < 0.001$). CDS at 4.3%, ash of ethanol at 0.2%, and ash of turkey manure at 1% rates resulted in 42%, 34% and 28% increases in plant mass compared with control (Fig. 2.6).

Due to significant effects of treatment on plant height, plant mass, and SCN population for experiment 1 at 70 DAP, the relationships between plant height and SCN population as well as plant mass and SCN population were examined. The linear regression of SCN population on mean plant height produced a significant model ($P < 0.001$) with an equation of:

$$(\text{SCN eggs})^{(1/2)} = 87.62 + (10.80 * \text{Plant Height})$$

The slope coefficient for plant height was also significant ($P < 0.001$) suggesting a positive relationship between plant height and SCN population (Fig. 2.7). However, adjusted R squared was 0.175 suggesting the model accounted for only 17.5% of

variation in SCN egg population. When mean plant height was included as a covariate preceding soil amendment treatment in ANOVA, treatments still significantly ($P < 0.001$) affected SCN population (Table 2.3).

The regression of SCN population on plant mass also produced a significant ($P < 0.001$) model :

$$(\text{SCN eggs})^{(1/2)} = 184.6 + (39.54 * \text{plant mass})$$

The slope coefficient for plant mass was also significant ($P < 0.001$) suggesting a positive relationship between plant mass and SCN egg population density (Fig. 2.8). However, adjusted R-squared was only 0.198 meaning the model accounted for only 19.8% of variation in SCN population density. When mean plant height was included as a covariate preceding soil amendment treatment in ANOVA, treatments still significantly ($P < 0.001$) affected SCN population density (Table 2.4).

Figure 2.1. SCN egg population density at 40 DAP for Experiments 1 and 2 combined. * indicates treatment is significantly different from control (LSD, $\alpha = 0.05$).

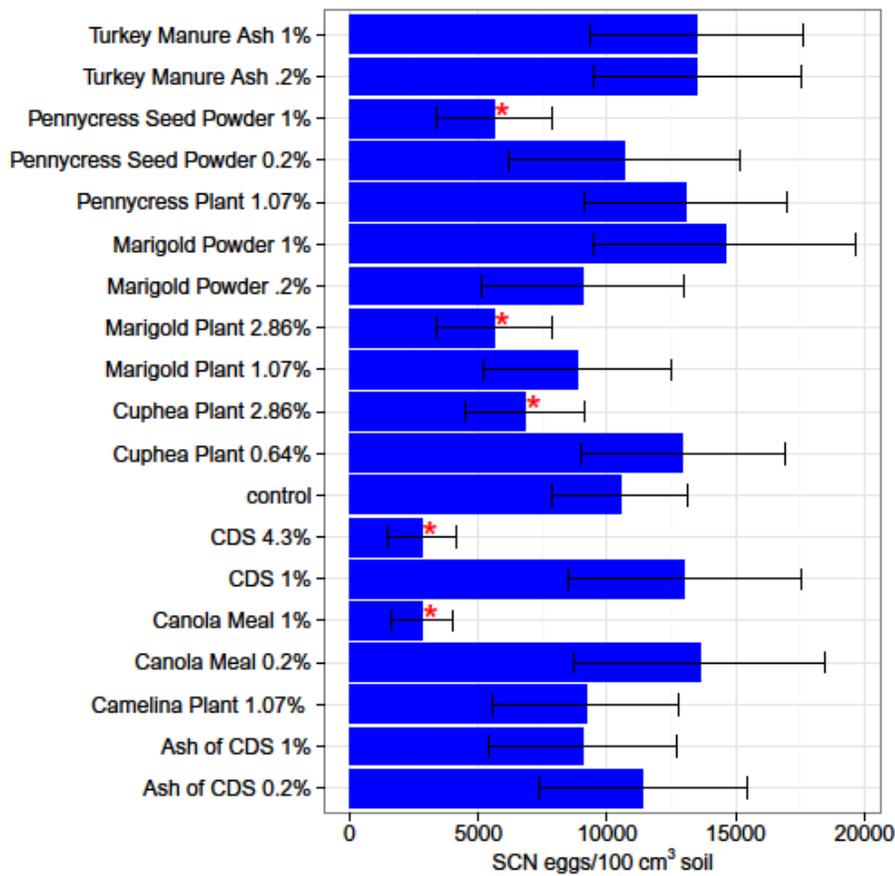


Table 2.2. 40 DAP Experiments 1 & 2 combined: Analysis of Covariance (ANCOVA) for SCN egg population density with mean plant height as covariate.

Source of Variation	Df	Mean Square	F value	
Trial	1	8110.8	694.1	***
Block/Trial	6	75.2	6.4	***
Mean Plant Height	1	224.7	19.2	***
Treatment	18	74.4	6.4	***
Trial x Treatment	18	17.2	1.5	
Residuals	102	11.7		

*** indicates values are significant at $P < 0.001$

Figure 2.2. Mean plant height at 40 DAP for Experiments 1 and 2 combined.

“*” indicates treatment is significantly different from control (LSD, $\alpha=0.05$).

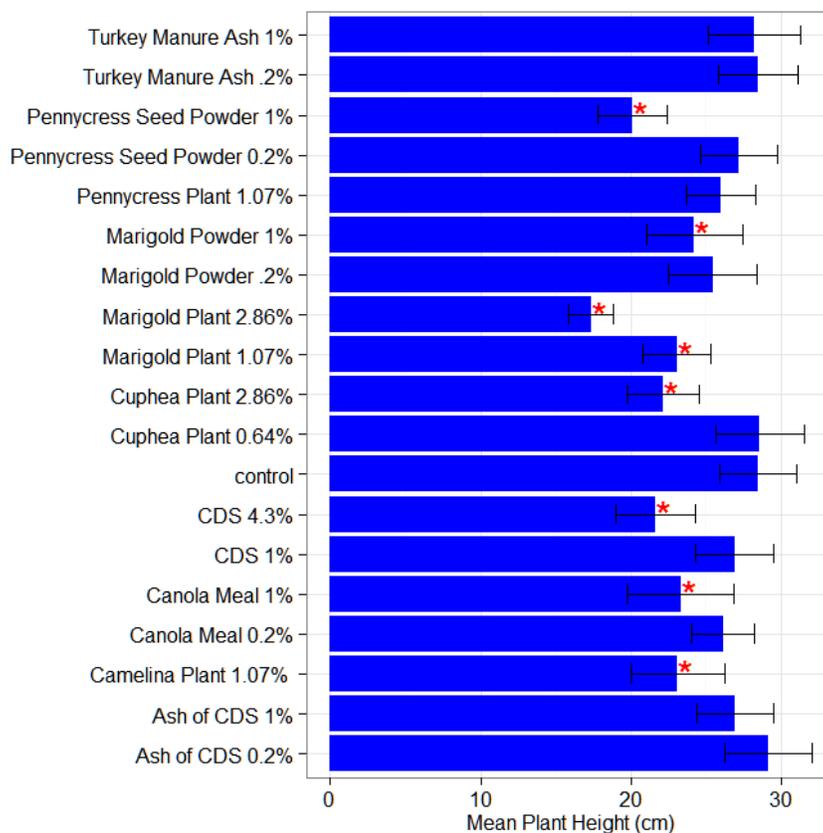


Figure 2.3. Linear regression of SCN egg population density ((eggs/100 cm³ soil)^{1/3}) on mean plant height (both at 40 DAP) with separate intercepts for each Experiment. Solid and dashed lines are equations for Experiment 1 and 2 respectively.

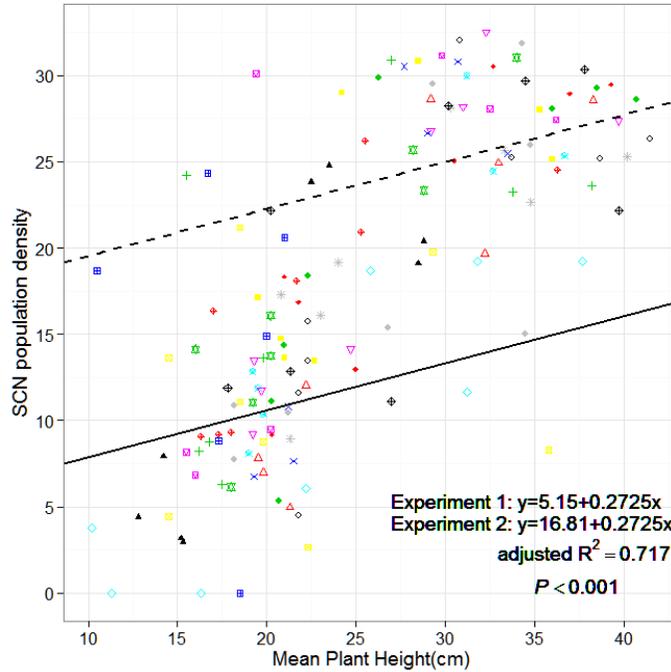


Figure 2.4. SCN egg population density at 70 DAP for Experiment 1.

* indicates treatment is significantly different from control (LSD, $\alpha = 0.05$).

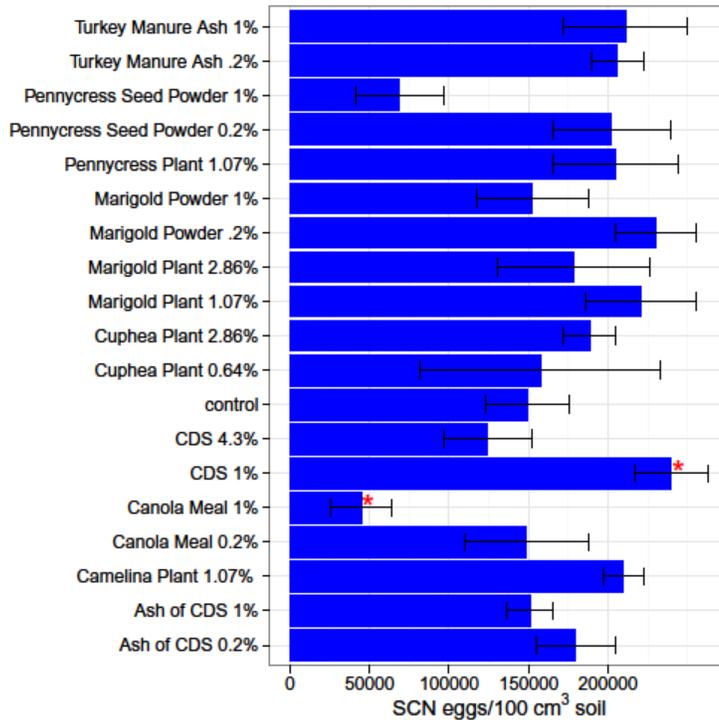


Figure 2.5. Soybean plant height at 70 DAP for Experiment 1

* indicates treatment is significantly different from control (LSD, $\alpha = 0.05$)

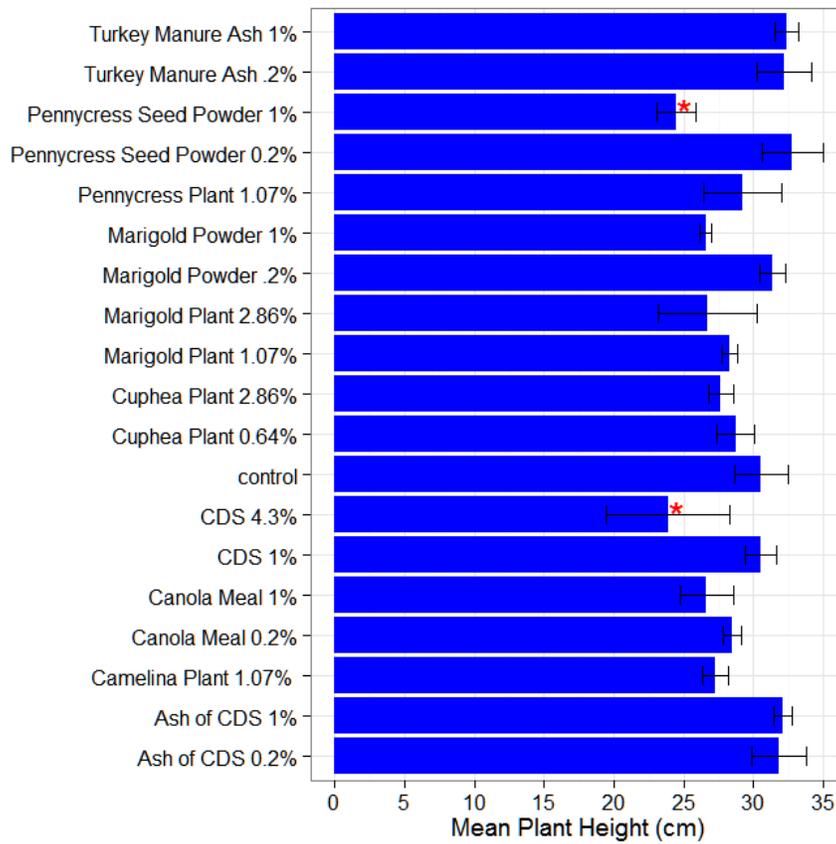


Figure 2.6. Soybean shoot dry mass at 70 DAP for Experiment 1

* indicates treatment is significantly different from control (LSD, $\alpha = 0.05$).

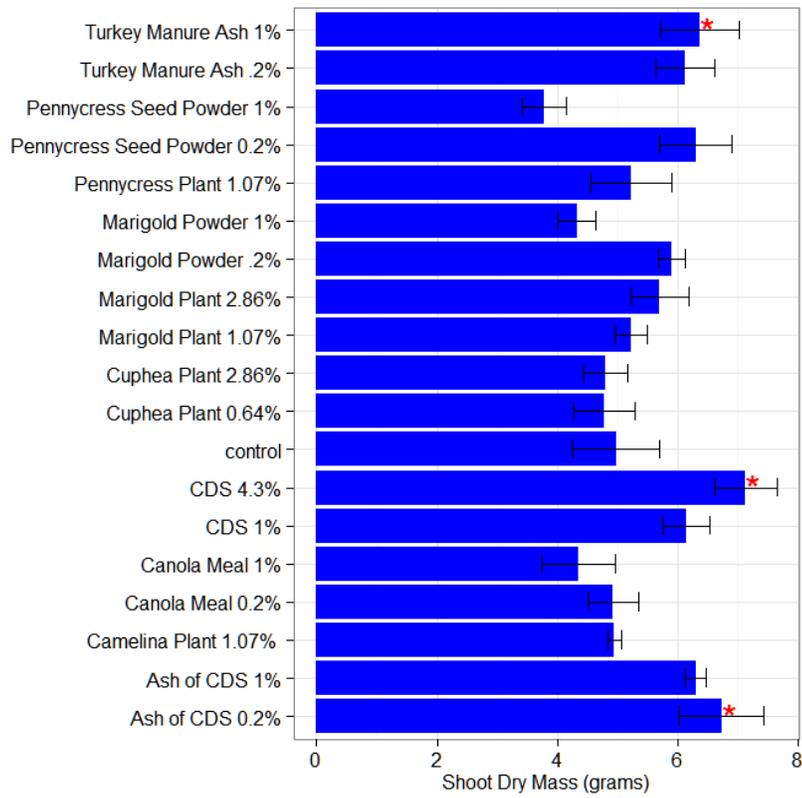


Figure 2.7. Linear regression of SCN egg population density on mean plant height for Experiment 1 at 70 DAP. Points of the same shape and color represent pots with the same treatment.

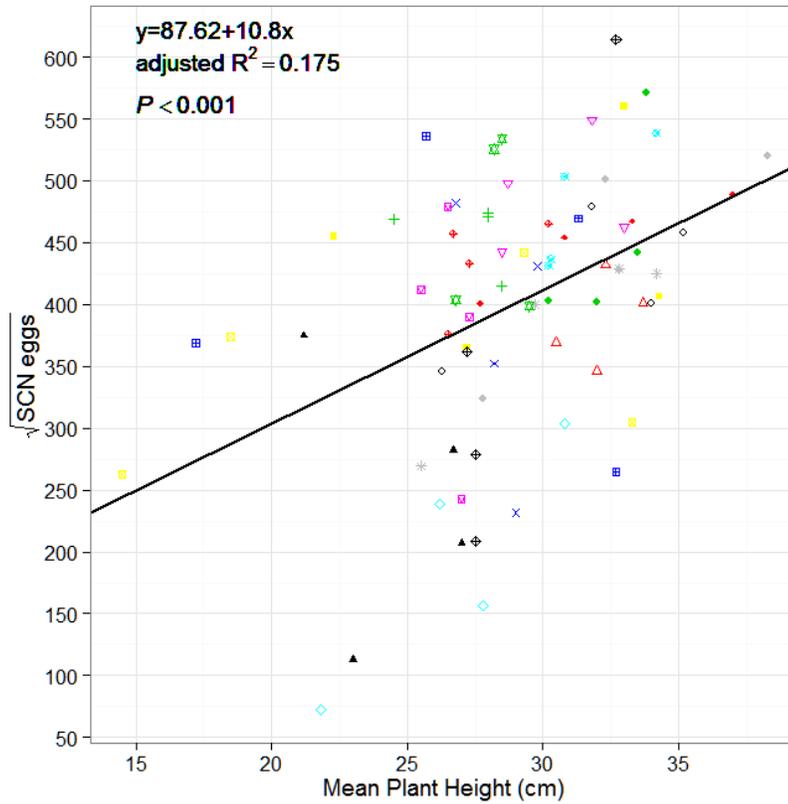


Table 2.3. 70 DAP Experiment 1: Analysis of Covariance (ANCOVA) for SCN egg population density with plant height as covariate.

Source of Variation	DF	Mean square	F value	
Block	3	5.3×10^{10}	5.3	**
Mean Plant Height	1	8.4×10^{10}	25.0	***
Treatment	18	1.41×10^{-11}	2.3	*
Residuals	53	1.8×10^{-11}		

*, **, and *** indicate values are significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively

Figure 2.8. Linear regression of SCN egg population density ((eggs/100 cm soil)^{1/2}) on plant mass for Experiment 1 at 70 DAP. Points of the same shape and color represent pots with the same treatment.

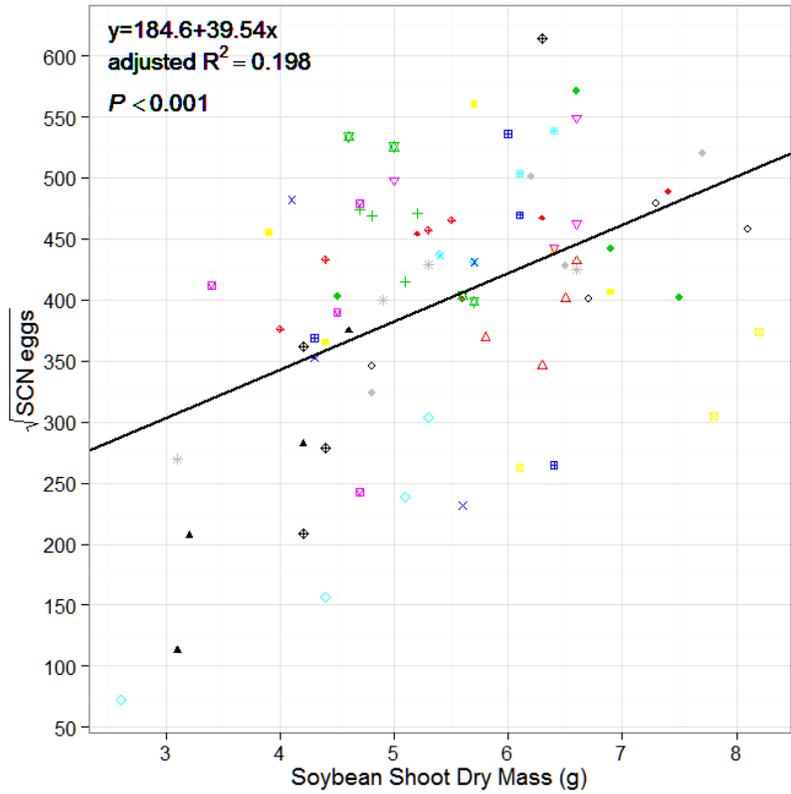


Table 2.4. 70 DAP Experiment 1: Analysis of Covariance (ANCOVA) for SCN egg population density with soybean shoot mass as covariate.

Source of Variation	DF	Mean Square	F value	
Block	3	1.77×10^{10}	5.5	**
Plant mass	1	7.91×10^{10}	24.8	***
Treatment	18	8.41×10^9	2.6	**
Residuals	53	3.19×10^9		

** , and *** indicate values are significant at $P < 0.01$, and $P < 0.001$, respectively.

5. Discussion

Results suggest some organic soil amendments can effectively reduce SCN population density within one generation cycle of application. Pennycress seed powder, canola meal, and CDS were the most effective. However, for most amendments, efficacy dissipated after two generations especially in Experiment 2. This suggests amendments cause an acute SCN population density reduction event, but the SCN population rebounds after two generations. In the second experiment, the SCN egg population density was higher overall likely allowing it to recover more quickly leading to the absence of significant treatment effects on the population density of the second generation. Based on these results, SCN management by soil amendments may require multiple applications or have limited duration. Nematode control by soil amendments may be better with relatively lower SCN population densities.

The observed overall SCN egg population density trend is consistent with a model of introduction of nematotoxic compounds through soil amendments which kill a portion of nematodes or paralyze them temporarily, but after which remaining or recovered nematodes continue to reproduce and the nematode population rebounds (Oka, 2010). Degradation or removal (leaching) of nematotoxic compounds over time would also fit this model. However, mechanisms of action were not examined in this study and other explanations are possible. Mechanisms of action may be different for different amendments and there may be multiple contributing mechanisms for a single amendment.

In particular, some SCN population reduction may be caused by the observed reduction in plant growth under some treatments as this would decrease food resources for SCN. A possible explanation for reduced plant growth is the presence of phytotoxic compounds in the soil amendments. While no obvious signs of phytotoxic effects such as scorching or stunting were observed in this study, other studies have proven or strongly suggested some of the tested amendments have phytotoxic compounds. Pennycress seed meal has been shown to be phytotoxic to plant seeds at rates as low as 0.1% (w/w) (Vaughn et al., 2005; Vaughn et al., 2006a) due to isothiocyanates (Vaughn et al., 2005). Phytotoxic dipeptides in corn gluten meal, a similar product to CDS, inhibited perennial ryegrass root growth *in vitro* (Liu and Christians, 1994). Canola meal at 3% (w/w) caused stunting, scorching, and death of tomato plants (Walker, 1996). DDG at 5% (w/w) caused phytotoxic symptoms in a bioherbicide trial on weeds (Boydston et al.,

2008). Given that the compounds in the products above are both phytotoxic and nematotoxic, and compounds in marigold and *Brassica* plants have proven toxic to various microbes (Bending and Lincoln, 1999; Doligalska et al., 2011; Hu et al., 2011; Morra and Kirkegaard, 2002; Szakiel et al., 2008), it is possible that marigold and *Brassica* amendments in this study were also phytotoxic. However, these soil amendments could have reduced plant growth by affecting soil physical, chemical, or biological properties.

Whatever the cause of plant growth reduction, the positive relationship between SCN population and plant growth (Figs. 2.6, 2.7 & 2.8) suggests that decreased plant growth contributed to SCN control by amendments to some degree. The dissipation of treatment effects on SCN population at 70 DAP could therefore be partially related to more uniform plant growth at that time. However, this could also be a mostly spurious correlation with treatments affecting both plant and nematode growth, but plant growth having only minor effects on nematode populations in this case. This assertion is supported by the disproportionate decrease in nematode population compared with plant growth reduction. For example, while canola meal and CDS (high rates) reduced plant height 18% and 24%, they caused concurrent SCN population reductions of 73% which are unlikely to be caused solely by the observed plant growth reduction. This is validated for the study at large in that treatments significantly affected SCN population even after accounting for plant growth at both 40 DAP for combined data (plant height, Table 2.2) and 70 DAP for Experiment 1 (plant height and plant mass, Tables 2.3 and 2.4). Additionally, the fact that plant height and mass only accounted for less than 20% of variation in egg populations in linear models suggests that plant growth only accounts for part of the effects of soil amendments on egg populations.

These pieces of evidence suggests that soil amendments reduced SCN populations both directly using nematotoxic compounds and indirectly by decreasing soybean growth possibly due to phytotoxic compounds although the contribution of each is impossible to determine precisely from this study. Other recognized mechanisms of nematode population reduction are also possible, such as increased microbial antagonists to SCN or induced plant defenses (Oka, 2010).

Although most amendments reduced plant growth, some amendments showed promise as soybean fertilizers. In particular, turkey manure ash and CDS ash showed similar or greater growth compared with control throughout the experiment. Despite

decreasing plant height, CDS at 4.3% also exhibited value as a fertilizer since it increased shoot mass at harvest while also decreasing SCN population at 40 DAP. Since many treatments had similar plant height and shoot mass at 70 DAP after reducing plant height compared with control at 40 DAP (Figs. 2.2 & 2.5) and have previously exhibited value as fertilizer, they may be useful fertilizers when strategies to mitigate phytotoxic effects are employed. One successful example of this is mustard meal (*Brassica carinata*), similar to canola meal, which was applied 20 days before planting to allow phytotoxic compounds to degrade and successfully increased tef (*Eragrostis tef*) yields up to 116% over control in one study (Balesh et al., 2005).

In conclusion, some of the soil amendments screened—particularly pennycress seed powder, canola meal, and CDS—showed potential as nematode management agents although phytotoxicity is a concern. Additionally, some amendments show potential as soybean fertilizers—particularly CDS, TMA, and ash of CDS. Further research is needed to determine mechanisms of SCN population reduction by specific amendments with particular emphasis on showing nematotoxicity or phytotoxicity directly and identifying causal compounds. Research should also focus on application timing and rate to maximize nematode population reduction while minimizing phytotoxic effects.

Chapter 3

Impacts of Fertilizer, Nematicide and Tillage on Soil Ecology and Agronomy in Corn and Soybean Field Experiments

1. Introduction

Plant-parasitic nematodes significantly suppress yield in many crops growing throughout the world (Koenning et al., 1999). However, symptoms of nematode infestation (including yield suppression, chlorosis, and stunted roots) are not easily seen and can go undetected or be attributed to other diseases or nutrient deficiencies (Dickerson et al., 2000; Jackson, 2006; Koenning et al., 1999; Niblack, 1992; Tylka, 2007; Tylka, 2011). Therefore, while some diseases caused by nematodes are well known, others may go undetected and thus unmanaged (Koenning et al., 1999). In the Midwest, soybean cyst nematode (SCN), *Heterodera glycines*, is well-known to producers and agronomists because it is the major yield-limiting pathogen in soybean (Koenning and Wrather, 2010). Much effort is made to manage SCN, mainly through crop rotation (Chen et al., 2001a; Porter et al., 2001) and use of resistant cultivars (Chen, 2007b; Chen et al., 2001b). However, these strategies have biological and agronomic limitations (Chen et al., 2001a; Niblack et al., 2008; Porter et al., 2001; Zheng et al., 2006)(Chen et al., 2001a; Niblack et al., 2008; Porter et al., 2001; Zheng et al., 2006)(Chen et al., 2001a; Niblack et al., 2008; Porter et al., 2001; Zheng et al., 2006), so it is important to identify supplemental management strategies for SCN.

One area of interest for SCN management is biocontrol. Fungal antagonists of SCN, including egg-parasitic fungi (Chen and Chen, 2002), nematode trapping fungi (Liu and Chen, 2000), and endoparasitic fungi on juveniles (Chen and Liu, 2007; Chen and Reese, 1999; Chen et al., 2000a; Liu and Chen, 2000), are present in Minnesota soils. Some of these organisms, particularly endoparasitic fungi, contribute to biological suppression of SCN populations in Minnesota soils (Bao et al., 2011; Chen and Liu, 2005; Chen, 2007a), and there is a great deal of interest in determining how management strategies impact populations of these fungi.

In contrast to the well-studied SCN, there are questions and concerns among producers and agronomists about corn yield loss caused by plant-parasitic nematodes, but relatively little current information is available. Carbamate and organophosphate insecticide application has declined since the introduction of Bt corn and reduced tillage

has widespread implementation. There is concern that these changes in management practices may allow plant-parasitic nematode populations to increase resulting in greater crop damage in more locations (Jackson, 2006; Tylka, 2007). However, it is difficult to determine yield loss from nematodes in a production setting thus there is little information on nematode population thresholds for crop damage and information that is available for the Midwest is not based on current agronomic practices (Tylka, 2011). There is pressing need to assess yield loss from corn nematodes and determine how management practices such as fertilization, tillage, and nematicide application affect them as well as SCN.

However, particularly in recent years, there is concern about environmental impacts of nematicide (Matthiessen and Kirkegaard, 2006; Oka, 2010; Rich et al., 2004; Thomas, 1996). Additionally, good soil health is always desired in order to have high yields, sustain production over years, and maintain a strong ecosystem. While chemical measures are one way of assessing soil health, biological components can also be used. One such biological measure that is sensitive to many environmental factors is nematode community analysis. Compared with other microbes, nematodes are relatively large and morphologically distinct, making them relatively easy to identify (Bongers 1990). Additionally, nematodes span a wide range of trophic groups including herbivores/plant parasites, fungivores (feed on fungi), bacterivores (feed on bacteria), predators (of other invertebrates), and omnivores (combination of food sources), allowing them to be used as indicators of various processes in the soil (Yeates et al., 1993). Nematode life strategy and sensitivity to disturbance varies, so nematodes can be conveniently classified on a colonizer-persister scale with colonizers having high reproduction rates, shorter life spans, and low sensitivity to chemical or physical disturbance while persisters have low reproductive rate, long life span, and high sensitivity to disturbance (Bongers, 1990).

Based on these properties, various indices and measures have been developed to assess activity in, processes of, and conditions of the soil based on the nematode community (Table 3.1). These include indices that measure diversity such as Shannon-Weaver diversity, evenness, and Simpson's dominance indices. They also include abundance and relative abundance (proportion of total nematode abundance) of major trophic groups including bacterivores, fungivores, herbivores, omnivores, and predators which can reflect populations or quantity of their food source (Ferris et al., 2001; Yeates

et al., 1993). Maturity indices measure the amount of disturbance caused to the nematode community and include the maturity index (MI), Σ MI, MI25, Σ MI25, and PPI with some indices including only free-living nematodes (MI and MI25) or nematodes with c-p values 2 to 5 (Bongers, 1990; Bongers and Korthals, 1993; Bongers et al., 1997; Yeates, 1994). Other indices, including the enrichment index (EI), basal index (BI), and structure index (SI), inform about soil food web condition (Ferris et al., 2001). Finally, a few indices provide information on decomposition pathways (FFB and channel index) (Ferris et al., 2001; Neher et al., 1995) or general soil health (FBPP) (Wasilewska, 1989).

Using these tools, this study integrates assessment of agronomic and ecological impact of management practices in a corn-soybean system. In particular, the objectives of this study were to: (i) Determine effect of fertilizer/nematicide and tillage practices on SCN, other plant-parasitic nematodes, and fungal parasites of SCN. (ii) Analyze effects of fertilizer/nematicide, tillage, and plant-parasitic nematodes on corn and soybean yield. (iii) Investigate effects of fertilizer/nematicide and tillage on soil health through soil chemical/physical measures and nematode community analysis.

2. Materials and Methods

2.1 Field Site

An experiment was established at two adjacent sites in the same field in Waseca, MN in 2011. Based on data taken in 1999, the soil at these sites is Webster clay loam (fine-loamy, mixed, superactive, mesic Typic Endoaquolls) with an average of 38.5% sand, 31.0 % silt, and 30.5% clay (Chen, 2007b).

The history of these sites prior to this experiment is well-known. The sites have been in corn and soybean in various sequences since 1997. From 1997 to 2003, annual soybean and corn rotation was maintained at both sites with soybean and corn the initial crops at the north and south sites respectively such that different crops were planted at two sites each year. The sites were also divided into plots with various plots getting SCN susceptible or resistant soybean cultivars in appropriate years. In 2004, both sites were planted to soybean with the north planted only with SCN-susceptible and the south planted to SCN susceptible or resistant depending on plot. In 2005, the whole site was planted to corn. From 2006 to 2008, the whole site was planted to susceptible soybean except in 2006 when select plots were planted with SCN-resistant soybean. In 2009, the

north half of the field was planted to susceptible soybean and the south half to corn with corn and susceptible soybean rotated annually since then.

From 1997 to 2005, corn plots received uniform N fertilization (152 to 168 kg/hectare (ha)) while soybean plots were not fertilized. In 2006, select plots received N and K fertilization. In 2007, select plots received nitrogen treatment as part of a previous study. In the three following years, each half was fertilized uniformly with no fertilizer applied in 2008 and N applied to corn in 2009 (180 kg N/ha) and 2010 (191 kg N/ha). SCN was present at the field site with initial population density around 3000 eggs/100 cm³ soil.

The site has been separated into blocks of conventional and minimum tillage continuously since 1997. Conventional tillage blocks were moldboard plowed in fall and field cultivated in spring from 1997 to 2005. From 2006 on, it was chisel plowed in fall and field cultivated in spring. Minimum till blocks were not tilled or cultivated from 1997 until 2010, but were strip tilled in spring from 2011 onward.

2.2 Experimental Design

The experiment was a randomized complete block design with split-plot arrangement and 3 replicates. Separate experiments in soybean to corn (soybean-corn, north site) and corn to soybean (corn-soybean, south site) annual rotation were conducted from 2011 to 2012. Main plot treatments were two tillage regimes while subplots had six fertilizer/nematicide treatments. There were 36 plots in each experiment for a total of 72 plots. The two tillage regimes were conventional tillage (CT) and minimum tillage (MT). Conventional tillage plots were chisel plowed in the fall (2010 & 2011), cultivated in the spring before fertilizer application (2012 only), strip tilled and field cultivated between fertilization and planting (2011 & 2012). Minimum tillage plots were strip tilled after fertilization but before planting in the spring of each year.

At the soybean-corn site, the six fertilizer-nematicide treatments were: 1) no amendments (control); 2) Nitrogen-Phosphorous-Potassium (NPK); 3) N-P-K-Sulfur (NPKS); 4) anaerobically digested swine manure with triple super phosphate supplement (manure); 5) nematicide only; and 6) NPKS and nematicide (NPKS+nematicide). NPK, NPKS, and NPKS+nematicide treatments received 157 kg N/ha, 112 kg P₂O₅/ha, and 124 kg K/ha in the form of ammonium nitrate (34-0-0), triple super phosphate (TSP) (0-46-0), and potash (0-0-60) respectively. NPKS and NPKS+nematicide treatments also received 18 kg S/ha in the form of gypsum (CaSO₄). Swine manure was applied at a rate

of 28062 L/ha and supplemented with 56 kg/ha P_2O_5 from triple super phosphate to make first year available nutrients roughly equivalent to synthetic NPKS rates. At this application rate, total nutrients (NPKS) in swine manure were 225-62-124-18 kg/ha (UW Soil & Forage Analysis Lab, Marshfield, WI) based on sample of the field-applied manure. Using University of Minnesota guidelines (Hernandez and Schmitt, 2012), first year available nutrients in swine manure under CT treatment (surface broadcast, incorporated within 4 days) were 124-49-111-18 for a total of 124-106-111-18 kg/ha including TSP supplement. First-year available nutrients for swine manure under MT (considered no incorporation) were 79-49-111-18 for a total of 79-106-111-18 kg/ha including TSP supplement (Hernandez and Schmitt, 2012).

Plots received described fertilizer treatments in 2011 only. In 2012, a uniform urea application (112 kg/ha) was applied to the soybean-corn site to provide minimum nutrient requirements for corn, and the residual effects of 2011 fertilizer application were monitored for both sites. Based on UM guidelines and 2011 manure analysis, 34 kg/ha of residual N was available in 2012 for manure treatment plots under both CT and MT (Hernandez and Schmitt, 2012). Nematicide was applied to appropriate plots (treatments 5 & 6) in both 2011 and 2012. In 2011, Counter (AMVAC Chemical Corporation, Newport Beach, CA), active ingredient terbufos, at 2.44 kg active ingredient (a.i.)/ha was applied in furrow at plant using a pesticide applicator. In 2012, Bolster-Temik (Bayer Crop Sciences, Pittsburgh, PA), active ingredient aldicarb, was applied in furrow at plant using Smartbox pesticide applicator at 2.94 kg a.i./ha.

At corn-soybean site the six fertilizer-nematicide treatments were: 7. low nitrogen (control); 8. Nitrogen-Phosphorous-Potassium (NPK); 9. N-P-K-Sulfur (NPKS); 10. anaerobically digested swine manure and synthetic phosphorus (manure); 11. nematicide with low nitrogen supplement (nematicide only); and 12. NPKS and nematicide (NPKS+nematicide). In 2011; NPK, NPKS, manure, and NPKS+nematicide treatments at the corn-soybean site were fertilized identically to corresponding treatments at the soybean-corn site. At the corn-soybean site, control and nematicide treatments received 56 kg N/ha from ammonium sulfate in 2011 to meet minimal nutrient requirements for corn. In 2012, no treatments at the corn-soybean site received any fertilizer in order to observe residual fertilizer impacts. Nematicide treatments at the corn-soybean site received Counter and Bolster-Temik in 2011 and 2012 in the same manner as nematicide treatments at the soybean-corn site.

2.3 Site management

Plots were 9.1 meters long by 4.6 meters wide with six plant rows (76 cm row spacing) in each plot. Fertilizer was applied to appropriate plots on May 17 and incorporated with strip tillage only (MT plots) or strip tillage and cultivation (CT plots) on May 18. Corn and soybean were planted, with concurrent nematicide application to appropriate plots, on May 19. Corn had *Bt* insect- and glyphosate-resistance (DeKalb 46-61) while soybean was SCN-susceptible and glyphosate-resistant (Pioneer 91Y90). Weeds were managed using glyphosate herbicide application (corn: June 9 and July 1; soybean: June 9 and July 6). Conventional tillage plots were chisel plowed in fall 2011 after harvest.

In 2012, conventional tillage plots were field cultivated on April 26, and plots were strip tilled (MT) or strip tilled and field cultivated again (CT) on May 18. Corn (DeKalb 46-61) and soybean (Pioneer 92Y12, SCN-susceptible & glyphosate-resistant), were planted, with concurrent nematicide application to appropriate plots, on May 17 and 18 respectively. Urea (with agrotain [Koch Agronomic Services LLC] nitrogen stabilizer) was broadcast applied to corn plots without incorporation on June 6. Weeds were managed with applications of glyphosate herbicide on June 4 and July 2.

2.4 Data Collection

2.4.1 Plant stand and crop yield

In 2011, soybean and corn were harvested October 3 and 13, respectively. In 2012, soybeans and corn were harvested on September 28 and October 12 respectively. Soybean yield was measured at 13% seed moisture and corn yield at 15% seed moisture. In 2012, stand counts were taken from 5 feet in each of the two central soybean rows and 10 feet from each of the two central corn rows on June 22 (34 DAP).

2.4.2 Soil and plant sampling

In 2011, soil samples were taken from all plots on May 4 before fertilization (Spring 2011), July 5 (47 days after planting (DAP)), July 31 (Midseason 2011, 73 DAP), and October 28/November 1 (Fall 2011). In 2012, soil samples were taken from all plots May 14 (Spring 2012), July 30 (Midseason 2012), and September 17-18 (Fall 2012). All of these soil samples were processed for both nematode community analysis and SCN egg population density. In 2011, soil samples were also taken from control and manure plots at the soybean-corn site on July 28 (68 DAP) for determination of fungal parasites of SCN. At each soil sampling, 20 soil cores at a depth of 15 cm were taken from each

plot using a 2.5-cm-diameter soil probe and bulked in plastic bags. At spring and fall, soil cores were taken in central two plant rows and in bare soil between central four plant rows. At midseason, all soil cores were taken from in central two plant rows. Soil samples were stored at 4 to 10 °C and processed within 2 days. Each soil sample was hand screened through a metal screen (6 mm square aperture) and mixed thoroughly. Subsamples were used to determine nematode soil population and fungal parasite densities. On June 9 (21 DAP) in 2011, plant samples were collected from manure and control plots to determine nematode density in plant roots. Eighteen corn or 12 soybean plants with intact root system were dug from six locations in appropriate plots.

2.4.3 Soil Processing for Nematode Community

From all soil samples collected at spring, midseason, and fall 2011 and 2012, separate subsamples were processed for population densities of vermiform nematodes and SCN eggs. Soil samples were processed for vermiform nematodes using sucrose floatation and centrifugation (Jenkins, 1964) within a week of collection from the field. A 100 cm³ (185g) subsample of homogenized moist soil was soaked in water for at least 15 minutes then gently stirred for 3-5 minutes using a drill press stirrer at low speed. The suspension was decanted through a food strainer (approximately 800-µm aperture) into a metal pitcher. After settling for one minute, the suspension was decanted through a 40-µm-aperture sieve. Sieve contents were collected in water and centrifuged for 4 minutes at 1100 g. After discarding supernatant, a 38% (w/v) sucrose solution was added and the suspension was centrifuged again for 4 minutes at 1100 g. The supernatant, containing vermiform nematodes, was collected in water (Jenkins, 1964).

The nematode community was analyzed using this sample. For each plot, a subsample of at least 100 nematodes was identified to genus level and counted. For initial (Spring 2011) identification, temporary glass slides of a known volume of nematode suspension were made and viewed using a compound microscope. For subsequent seasons, subsamples of nematode suspension were measured into lined tissue culture wells, allowed to settle and counted using an inverted compound microscope. Nematodes were identified to genus level based on Bongers's classification scheme, except for family Rhabditidae which was identified to family level (Bongers, 1994). Based on this data, abundance per 100 cm³ soil for each observed genus was calculated for each plot. Subsequently, trophic group abundance, trophic group relative

abundance (abundance relative to total nematode abundance) and nematode community indices were calculated.

For SCN egg extraction, a 100 cm³ soil subsample was taken from each plot soil sample after weeks to months of storage at 4 to 10 °C. Soil was soaked in 1.76% dishwasher detergent solution for at least 15 minutes, then cysts (females) were extracted from the soil using a semiautomatic elutriator (Byrd et al., 1976), collected on nested 250-µm-aperture and 850-µm-aperture sieves, and centrifuged in 63% sucrose solution for 5 min at 1100 g. Cysts were emaciated with a mechanical crusher to release eggs (Faghihi and Ferris, 2000), which were collected in water and stored at 4 °C before being counted and summarized as number of SCN eggs/100 cm³ soil.

For this study, nematode variables that were statistically analyzed included: (i) nematode community indices: Shannon-Weaver diversity index, evenness index, Simpson's dominance index, MI, MI25, Σ MI, Σ MI25, PPI, EI, BI, SI, and CI (summarized in Table 3.1); (ii) trophic group variables: abundance (nematodes /100 cm³ soil) of each trophic group (herbivores, bacterivores, fungivores, and omnivores), FFB, FBPP, total nematode abundance, and relative abundance (percent of total nematodes) of each trophic group (herbivores, bacterivores, fungivores, and omnivores); and (iii) plant-parasitic nematode variables (per 100 cm³ soil): vermiform SCN (2nd-stage juveniles (J2) and males from soil), SCN eggs, *Helicotylenchus*, *Pratylenchus*, and *Xiphinema*.

2.4.4 Endoparasitic nematodes in plant roots

Endoparasitic nematodes were extracted from roots of the corn and soybean plants collected at 21 DAP in 2011. After collecting, whole plants were soaked in water then roots were rinsed with water to remove soil, shoots were removed, roots were bulked by plot, excess water on roots was drained, and the roots were weighed by plot. Roots were softened by 3 cycles of freezing at -20 °C for 24 hours followed by thawing (Ruan et al., 2012). Roots were macerated in water with an electric food blender for 30 seconds and decanted through nested 850- and 25-µm-aperture sieves. After rinsing both sieves, materials in the 25-µm-aperture sieve were collected in 38% sucrose solution and centrifuged for 5 minutes at 1100 g. The supernatant, containing any nematodes present in the roots, was collected in water. For each plot, *Pratylenchus*, SCN J2 and SCN J3 or J4 nematodes in a subsample was identified and counted. From this, the density of each group per gram of root was calculated.

Table 3.1. Summary of nematode community indices.			
Variable	Symbol	Calculation	Greater value Indicates
Shannon-Weaver Diversity Index		(genera relative abundance * ln(relative abundance)), summed for all genera	more diverse nematode community (more genera with more similar abundance)
Evenness		diversity divided by ln(# genera)	similar abundance among genera
Simpson's Dominance Index		relative abundance ² summed for all genera	less diverse nematode community
Maturity Index	MI	average nematode c-p value excluding herbivores	less disturbed soil community
MI25	MI25	same as MI, but nemas with c-p of 1 excluded	less disturbance excluding enrichment
Σ MI	Σ MI	same as MI, but also includes herbivores	less disturbance, more established plant community
Σ MI25	Σ MI25	same as Σ MI, but nemas with c-p 1 excluded	less disturbance excluding enrichment; more established plant community
Plant Parasitic Index	PPI	average herbivore c-p value	more mature herbivore community; more plant production/diversity; less/more disturbed soil
Enrichment Index	EI	weighted‡, modified relative abundance of opportunistic nematodes	soil has more food and nutrient resources (enriched condition)
Basal Index	BI	weighted, modified relative abundance of stress-tolerant nematodes	more environmental stress, fewer resources (basal condition)
Structure Index	SI	weighted, modified relative abundance of high c-p nematodes	more trophic links (structured condition); later succession
Channel Index	CI	weighted ratio of fungivores to bacterivores	decomposition mediated by fungi more than bacteria (more advanced condition)
F/(F+B)	FFB	# fungivores/(# fungivores +bacterivores)	similar to CI
(F+B)/PP	FBPP	(# fungivores +bacterivores)/# herbivores	more favorable plant growth conditions
‡weights give more value to nematodes that are larger (consume more resources) or more strongly representative of the index (ex: more extreme enrichment opportunists have a larger weight in EI)			

2.4.5 Soil processing for fungal parasites of SCN

In 2011, soil samples collected at 68 DAP from each control and manure plot at soybean-corn site were homogenized and subsampled to analyze fungal parasites of SCN. In 2012, subsamples from each control and manure plot at both sites were taken

from soil sampled at 73 DAP for analysis of fungal parasites of SCN following homogenization. Soil samples were processed for fungal parasites of nematodes including trapping fungi, fungal endoparasites of J2, and fungal parasites of eggs.

2.4.5.1 Nematode-trapping fungi

Nematode-trapping fungi density in soil was determined using dilution plating and most probable number procedures similar to that used in previous studies (Blodgett, 2010; Eren and Pramer, 1965; Jaffee et al., 1996; Jaffee et al., 1998; Timm et al., 2001). A subsample of 50 g of moist soil from each manure and control plot was weighed into sterilized 50-mL flasks the same day as soil was sampled. 50 mL of sterilized, deionized water and a sterilized magnetic stir bar were added to flask and stirred for 5 minutes on a magnetic stir plate. This soil suspension of 1 g soil/mL water was immediately poured into a sterile 50-mL centrifuge tube. A 10-fold dilution was made by adding 3 mL of original soil suspension to 27 mL sterile, deionized water in a centrifuge tube yielding a 0.1 g/mL suspension. A subsequent 10-fold dilution was performed to yield a 0.01 g/mL suspension. The same day, suspensions were plated on ¼ strength corn meal agar (CMA) amended with 100 ppm streptomycin and 50 ppm chlortetracycline to limit bacterial growth. For each dilution (1, 0.1, and 0.01 g soil/mL for each plot), 0.2 mL suspension was pipetted onto each plate with five plates per dilution.

One day after plating suspensions, vermiform nematodes were added to plates to stimulate nematode-trapping fungal growth. In 2011, freshly hatched SCN J2 in sterile water were plated at a rate of 205 J2 in 0.22 mL suspension per plate. In 2012, vermiform *Caenorhabditis elegans* (acquired from *Caenorhabditis* Genetics Center, Minneapolis, MN) grown on pure culture of *Escherichia coli* strain OP50 (*Caenorhabditis* Genetics Center) on Nematode Growth Medium (NGM) were used due to greater motility compared with SCN. *C. elegans* were added at a rate of 121 nematodes in 0.20 mL suspension per plate. Plates were stored in sterile containers at 25 °C until assessment (positive or negative) of trapping fungi growth by visualization of fungal traps, trapping fungi conidiophores, or trapping nematodes using a dissecting microscope (Jaffee and Muldoon, 1995; Jaffee et al., 1998; Timm et al., 2001). In 2011, plates were assessed twice from 21-26 days after plating soil. In 2012, plates were assessed once from 32-38 days after plating soil. For each plot, trapping fungi density (colony forming units (cfu)/g soil) was determined using most probable number technique based on trapping fungi growth at each dilution (Blodgett, 2010).

2.4.5.2 Fungal parasites of SCN eggs

At both 68 DAP 2011 and 73 DAP 2012, cysts were extracted from a unique 100 cm³ soil subsample of each plot by collecting cysts in water after first centrifugation in the described egg extraction. Cysts were extracted 4 days after soil sampling in both 2011 and 2012. Cysts were stored at 4 °C until egg parasitism was assessed 3 and 5 days after extraction in 2011 and 2012 respectively. From each plot, individual cysts were picked using forceps, placed on a water drop on a glass microscope slide (up to a dozen cysts/slide), and gently crushed by pressing a cover glass onto the slide (Chen and Chen, 2002; Chen et al., 1996; Chen and Chen, 2003). For each cyst, egg-parasitic index (EPI) was recorded on a 0 to 10 scale (0=no eggs colonized, 10= 91-100% eggs colonized) (Chen and Chen, 2002; Chen et al., 1996; Chen and Chen, 2003). At least 20 cysts or all extracted cysts were assessed for each plot. In addition to mean EPI, the percent of cysts colonized by egg parasitic fungi was calculated for each plot (Chen and Chen, 2002; Chen and Chen, 2003).

2.4.5.3 Fungal endoparasites of SCN J2

At 68 DAP 2011, vermiform nematodes were extracted from a unique 100 cm³ soil subsample from each sampled plot (manure and control at soybean-corn site) to assess fungal endoparasites of SCN J2. At midseason 2012, manure and control plots were assessed for endoparasites using the sample extracted for nematode community assessment. For soybean plots, 100 SCN J2 were assessed, but for corn plots all J2 in the sample were assessed due to lower SCN population. Signs of infection included mycelium growth in or protruding from body and fungal spores attached to body (Chen et al., 1996; Chen et al., 2000a; Liu and Chen, 2000). Infected J2 also tended to be shrunken and disfigured. For each plot, percent of J2 infected by fungal endoparasites was calculated (Liu and Chen, 2000).

2.4.6 Soil processing for soil properties

In spring 2011 (only soybean-corn site sampled), midseason 2011, and fall 2011; 100 g subsamples of soil from each plot were air-dried and assessed for soil properties (University of Minnesota Research Analytical Laboratory, St. Paul, MN). In spring 2011 and fall 2011; organic matter (OM), pH, P, K, Zn, Cu, Mn, and Fe were determined. At the corn-soybean site, Olsen-P extract test was used to determine soil P because pH was above 7.4. At the soybean-corn site, soil P was determined using Bray P-1 for 2 blocks, but using Olsen-P extract for the third block based on soil pH. Because two

separate tests were used, soil P for the soybean-corn site was not analyzed statistically. At midseason 2011, nitrate-nitrogen was determined.

3. Statistical Analysis

Data were analyzed separately for each experiment and at each season. For each experiment-season, two-way (split plot) ANOVA was conducted for each response variable that was measured across all factors. Data on variables (nematode-parasitic fungi, endoparasitic nematodes in roots) measured only for certain fertilizer treatments (manure and control) were analyzed using two-way ANOVA under appropriate treatments. ANOVA models were checked for homogeneity of variance (using Levene's test) and normality of residuals (graphically). When necessary, response variables were transformed to meet these assumptions (Table 3.2). For variables with significant fertilizer-nematicide effects ($P \leq 0.05$), fertilizer-nematicide treatment means were separated using Fischer's protected LSD. All analysis was performed using R (version 2.15).

4. Results

4.1 Soil Properties

There were no significant tillage effects at either site or fertilizer-nematicide effects on soil properties at the soybean-corn site ($P > 0.05$). There were significant fertilizer-nematicide treatment effects on soil P levels at the corn-soybean site in fall 2011 ($P < 0.001$). Soil P levels were significantly greater under manure than all other treatments. Soil P levels were also greater under NPKS or NPKS with nematicide than control, NPK, or nematicide-only treatments. At the corn-soybean site in fall 2011, there were significant fertilizer-nematicide treatment effects for Zn ($P \leq 0.05$), but also significant fertilizer-nematicide by tillage interaction ($P \leq 0.05$). There were significant fertilizer-nematicide effects on Zn under CT ($P \leq 0.05$), but not MT ($P > 0.1$). Under conventional tillage, Zn soil levels were greater with manure treatment than all other treatments except NPKS with nematicide. Under CT, Zn soil levels were also greater under NPKS with nematicide than NPK or nematicide only treatments. Soil nutrients and physical properties for both sites are summarized in Table 3.3.

Table 3.2. Summary of response variable transformations by site and season.

Variable	Soybean-corn site						Corn-soybean site							
	2011			2012			2011		2012					
	Pi	Pm	Pf	Pi	Pm	Pf	Pm	Pf	Pi	Pm	Pf			
Diversity														
Evenness					x^2									x^2
Dominance						$\ln(x)$				$\ln(x)$				$\ln(x)$
MI														$\ln(x)$
MI25														$\ln(x)$
Σ MI			x^4				x^2	$1/x$					$1/x$	
Σ MI25														
PPI					$1/x$	$1/x$								
EI	x^3	x^2												
BI	$\ln(x)$	$\ln(x)$												
SI	$\ln(x+1^{-f})$		$\ln(x+1^{-f})$											$\ln(x+1^{-f})$
CI		$\ln(x)$	$\ln(x)$						$\ln(x)$	$\ln(x)$				
FFB					$1/x$									
FBPP		$1/x$	x^3						$1/x$					
# Herbivores				$x^{(3/2)}$						$\ln(x)$				
# Fungivore			x^2							$\ln(x)$				
# Bacterivores		$\ln(x)$		$\ln(x)$					$1/x$	$1/x$				
# Omnivores	$\ln(x+1^{-f})$	$\ln(x+1^{-f})$		\sqrt{x}										
# Nematodes		$\ln(x)$				$\ln(x)$			$1/x$	$1/x$				
% Herbivores				$1/x$	$1/x$				x^2					
% Fungivores		$\ln(x)$			$1/x$									
%Bacterivores			$\ln(x)$	$1/x$					$\ln(x)$					
% Omnivores	$\ln(x+1^{-f})$	$\ln(x)$								$\ln(x+1^{-f})$				
<i>Pratylenchus</i>	$\ln(x+1^{-f})$		$\ln(x+1^{-f})$	$\ln(x+1^{-f})$	$\ln(x+1^{-f})$	$\ln(x+1^{-f})$								
<i>Helicotylenchus</i>	$\ln(x+1^{-f})$		x^3	$\ln(x+1^{-f})$	$\ln(x+1^{-f})$	$\ln(x+1^{-f})$				$\ln(x+1^{-f})$				
<i>Xiphinema</i>		$\ln(x+1^{-f})$	\sqrt{x}	$\ln(x+1^{-f})$	$\ln(x+1^{-f})$	$\ln(x+1^{-f})$			x^2	$\ln(x+1^{-f})$				
SCN eggs		\sqrt{x}	$x^{(2/3)}$	$\ln(x)$		\sqrt{x}			$\ln(x)$					$\ln(x)$
SCN J2		\sqrt{x}		x^2		$\ln(x+1^{-f})$			$\ln(x)$	$\ln(x)$				
Yield														$\ln(x)$
P (Olsen-P)										x^2				
pH			$\ln(x)$											
K			x^2											
cysts colonized		$1/x$												
trapping fungi		$\ln(x)$												

Table 3.3. Soil properties by site and season in 2011.†

Property	Soybean-corn site			Corn-soybean site	
	Vi	Vm	Vf	Vm	Vf
pH	7.13 ± 0.09		7.07 ± 0.11		7.89 ± 0.03
OM	6.83 ± 0.13		6.77 ± 0.13		7.88 ± 0.10
NO ₃		54.2 ± 1.62		58.9 ± 1.50	
Cu	1.00 ± 0.02		1.03 ± 0.02		0.83 ± 0.02
Zn	3.19 ± 0.17		1.93 ± 0.10		1.23 ± 0.05
Mn	19.0 ± 0.97		19.3 ± 0.96		12.2 ± 0.56
Fe	38.4 ± 4.77		48.7 ± 6.32		9.7 ± 0.45
K	94.8 ± 2.3		113 ± 2.4		113 ± 3.4
P (Bray P-1) ‡	9.08 ± 0.30		10.57 ± 0.76		
P (Olsen-P) ‡	4.5 ± 0.17		5.08 ± 0.37		3.88 ± 0.16

† Vi, Vm, and Vf are mean values (± standard error) prior to applying fertilizer and nematicide, 47 days after planting, and at harvest in 2011, respectively.

‡ At the soybean-corn site, soil P was from Bray P-1 extract for 2 blocks, but Olsen-P extract for 1 block based on soil pH.

4.2 Plant growth and crop yield

In 2011, soybean yield was significantly affected by both tillage and fertilizer-nematicide ($P \leq 0.05$). Soybean yield was greater under minimum (3 386 kg/ha) than conventional tillage (2 902 kg/ha). Among fertilizer-nematicide treatments, soybean yield was highest under manure, although only statistically different from NPK and NPKS treatments which had lowest yields. While terbufos nematicide increased yield 21% in combination with NPKS, it decreased yield 5.3% without fertilizer compared with equivalent treatments without nematicide (Fig. 3.1).

In 2012, there were significant tillage effects ($P \leq 0.05$) on soybean yield with 23.7% greater yield under CT (1369) than MT (1106 kg/ha). There were also significant fertilizer effects ($P \leq 0.05$) on 2012 soybean yield. 2012 soybean yields were increased with manure, NPKS and NPKS+nematicide compared with control. Aldicarb nematicide application did not change yields compared with corresponding fertilizer treatments (Fig. 3.2). In 2012 soybean, there were no significant fertilizer-nematicide or tillage ($P > 0.1$) on stand counts (371 600 plants/ha).

In 2011, corn yield was significantly ($P \leq 0.05$) greater under conventional

(10176 kg/ha) than minimum tillage (9548 kg/ha). There were significant fertilizer-nematicide treatment effects on 2011 corn yield ($P < 0.001$). Manure, NPKS alone, and NPK alone all increased yield compared with control (Fig. 3.3). Yield was also greater under manure than NPK treatment. Terbufos nematicide did not affect corn yield (Fig. 3.3).

In 2012, tillage effects on corn yield were not significant ($P > 0.1$). There were significant fertilizer-nematicide effects on corn yield ($P \leq 0.05$). Fertilizer alone treatments (manure, NPK, and NPKS) increased yield compared with control (Fig. 3.4). Yield was also greater under manure than NPKS treatment. Aldicarb nematicide alone increased yield 16% compared with control, but did not significantly increase yield in combination with NPKS. There were no significant tillage or fertilizer-nematicide effects on stand count in 2012 corn. Average corn stand count was 84860 plants/ha.

4.3 Plant-parasitic nematodes

The four major plant-parasitic nematodes consistently present at the site were: *Heterodera glycines*, *Helicotylenchus* (spiral nematode), *Pratylenchus* (lesion nematode), and *Xiphinema* (dagger nematode). While SCN only damages soybean, the other genera can suppress yield on either corn or soybean (Koenning et al., 1999; Niblack, 1992; Warnke et al., 2006).

Average SCN egg population at both sites (Fig. 3.5) exceeded the nematode management advisory damage thresholds of 200 eggs/100 cm³ soil (Chen, 2011). While *Pratylenchus* damage thresholds for corn are generally based on nematodes in root, average soil population (Fig. 3.6) was below estimated damage thresholds (300 nematodes/100 cm³ soil) (Tylka, 2011; Tylka, 2011). Similarly, *Helicotylenchus* and *Xiphinema* average site population densities (Figs. 3.7 & 3.8) were below estimated corn damage thresholds (500 *Helicotylenchus*/100 cm³ soil; 30 *Xiphinema*/100 cm³ soil) (Tylka, 2011).

Figure 3.1. Effect of fertilizer and nematicide on 2011 soybean yield. Treatments with different letters indicate significantly different mean yields using LSD at $P \leq 0.05$.

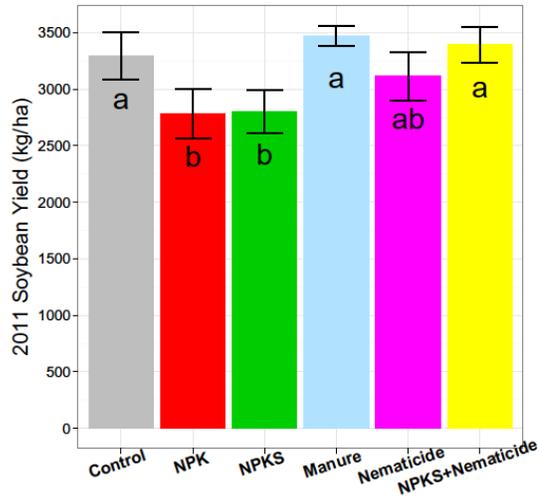


Figure 3.3. Effect of fertilizer and nematicide on 2011 corn yield. Treatments with different letters indicate significantly different mean yields using LSD at $P \leq 0.05$.

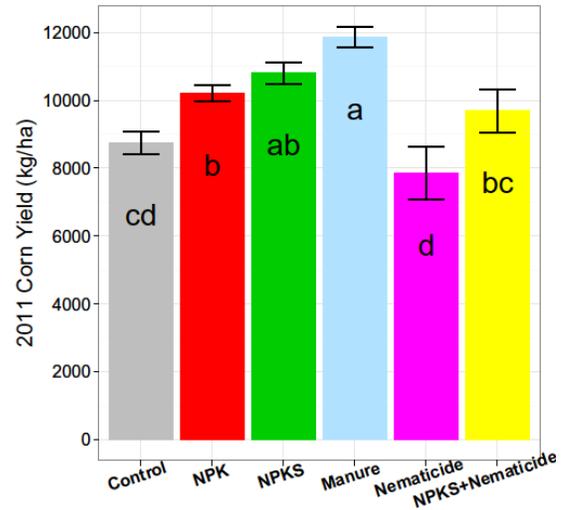


Figure 3.2. Effect of fertilizer and nematicide on 2012 soybean yield. Treatments with different letters indicate significantly different mean yields using LSD at $P \leq 0.05$.

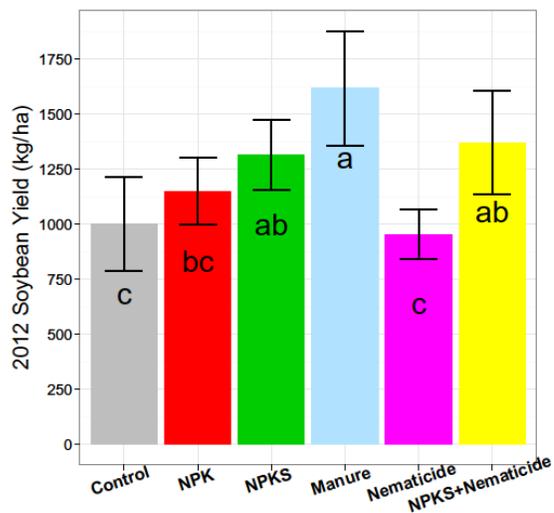


Figure 3.4. Effect of fertilizer and nematicide on 2012 corn yield. Treatments with different letters indicate significantly different mean yields using LSD at $P \leq 0.05$.

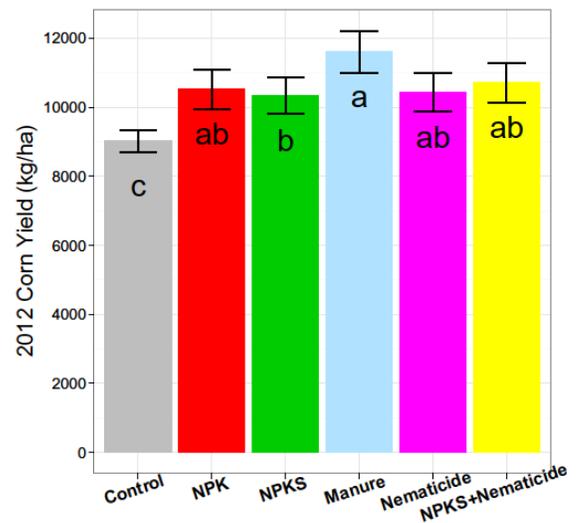


Figure 3.5. Soybean cyst nematode egg population density in the soil by rotation/site and season.

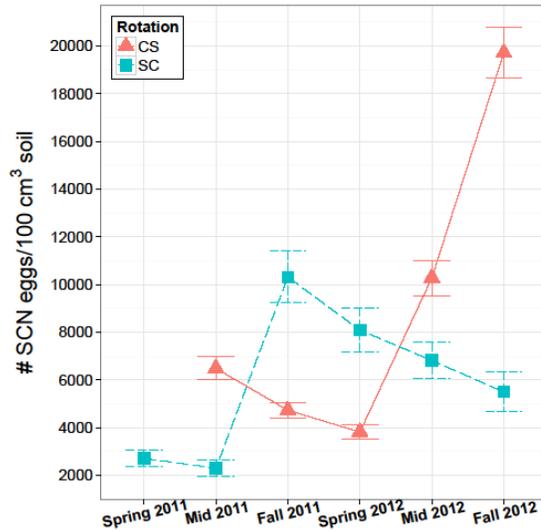


Figure 3.7. *Helicotylenchus* population density in the soil by rotation/site and season.

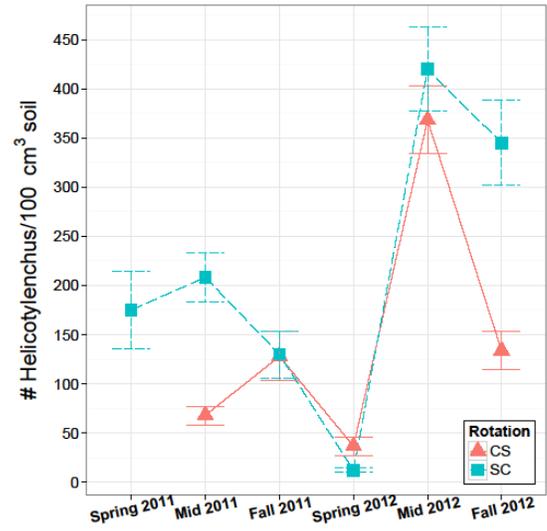


Figure 3.6. *Pratylenchus* population density in the soil by rotation/site and season.

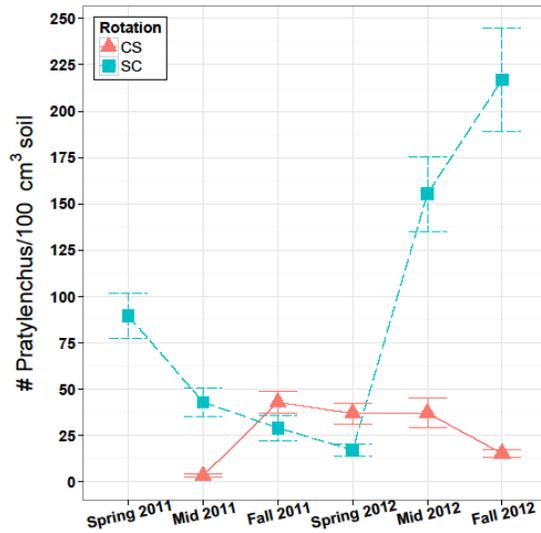
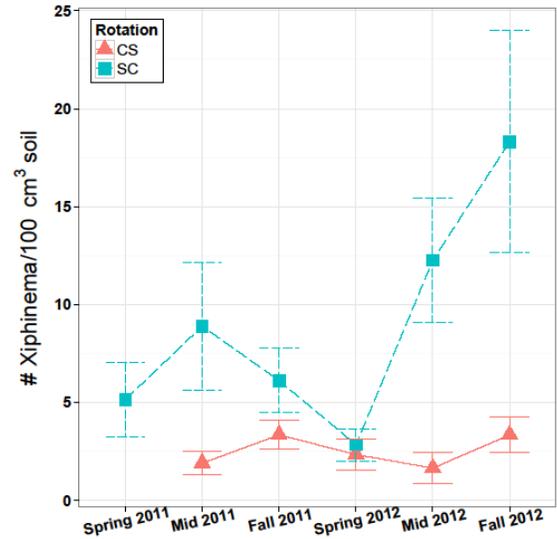


Figure 3.8. *Xiphinema* population density in the soil by rotation/site and season.



4.3.1 *Pratylenchus*, *Helicotylenchus*, and *Xiphinema*

There were no significant fertilizer-nematicide or tillage effects on *Pratylenchus* in corn or soybean roots ($P > 0.1$). *Pratylenchus* density was low in both corn (3 ± 1 nematodes/g root) and soybean roots (12 ± 6 nematodes/g root). Neither *Xiphinema* nor *Pratylenchus* soil populations were affected by tillage or fertilizer-nematicide at any season or site ($P > 0.05$).

Helicotylenchus soil population was affected by tillage in fall 2011 with greater population under minimum than conventional tillage (Table 3.4), although this was only marginally significant ($P = 0.065$). *Helicotylenchus* soil population was significantly affected by fertilizer-nematicide treatment ($P \leq 0.05$) in fall 2011 corn and midseason 2012 soybean. In fall 2011, *Helicotylenchus* population densities were greater with NPKS than any other treatment. In midseason 2012, *Helicotylenchus* population densities were greater with fertilizers alone than NPKS+nematicide (Table 3.4).

4.3.2 Soybean cyst nematode

There was not significant fertilizer, nematicide, or tillage treatment effects on SCN J2, combined SCN 3rd-stage (J3) and 4th-stage (J4) juvenile, or combined SCN juvenile (2nd, 3rd, and 4th stage) densities in soybean roots ($P > 0.1$). SCN egg population density in the soil was not significantly affected by tillage (Table 3.5). However, vermiform SCN (J2 + male) soil population densities were significantly affected by tillage in fall 2012 soybean ($P \leq 0.01$) with greater population density under minimum (610) than conventional tillage (381 nematodes/100 cm³ soil).

Vermiform SCN soil populations were not affected by fertilizer-nematicide treatments ($P > 0.05$). At the soybean-corn site, SCN egg populations were significantly affected by fertilizer-nematicide treatments in spring, midseason, and fall 2012 ($P \leq 0.05$) with populations significantly decreased under control and nematicide alone compared with fertilizer treatments (Table 3.5). In midseason 2012 soybean, SCN egg population was significantly affected by fertilizer-nematicide treatment ($P \leq 0.05$) with aldicarb nematicide decreasing SCN egg population density compared to corresponding fertilizer treatments.

Table 3.4. Effects of fertilizer, tillage, and nematicide on *Helicotylenchus* population density in the soil. †

Treatment	2011			2012		
	Pi	Pm	Pf	Pi	Pm	Pf
Soybean – corn site						
Tillage:						
Conventional tillage	109	183	77	11	438	320
Minimum tillage	242	233	183	13	402	370
ANOVA (<i>F</i> value)						
Tillage (T)	0.28	0.87	13.84 •	0.53	0.21	0.75
Fertilizer-nematicide (Fn)	1.06	1.29	1.26	1.67	0.94	1.11
T × Fn	0.80	0.83	1.70	1.19	0.41	0.93
Corn-soybean site						
Tillage:						
Conventional tillage		62	175	38	371	157
Minimum tillage		74	81	35	366	110
Fertilizer-nematicide‡:						
Control		74	129 b	21	336 ab	162
NPK		42	86 b	28	494 a	144
NPKS		58	224 a	20	442 a	183
Manure		110	164 b	52	484 a	141
Nematicide		78	69 b	58	284 ab	75
NPKS+Nematicide		46	97 b	41	170 b	97
ANOVA (<i>F</i> value)						
Tillage (T)		0.16	2.89	1.11	0.02	0.66
Fertilizer-nematicide (Fn)		1.52	2.68 *	0.87	3.16 *	1.15
T × Fn		0.83	2.86 *	0.28	1.47	1.88

† Pi, Pm, Pf are population densities (nematodes per 100 cm³ soil) prior to applying fertilizer and nematicide, 47 days after planting, and at harvest in 2011, and prior to applying nematicide, 68 days after planting, and harvest in 2012, respectively.

‡ Values followed by different letters in the same column are significantly different according to LSD test at $P < 0.05$.

* indicates $P \leq 0.05$, and • indicates $P = 0.065$ for Pf 2011 tillage at soybean-corn site.

Table 3.5. Effects of fertilizer, tillage, and nematicide on the soybean cyst nematode egg population density.†

Treatment	2011			2012		
	Pi	Pm	Pf	Pi	Pm	Pf
Soybean – corn site						
Tillage:						
Conventional tillage	2881	2515	9181	8750	6900	6151
Minimum tillage	2531	2069	11438	7417	6714	4833
Fertilizer-nematicide‡:						
Control	2092	1252	8817	6442	4092	3017
NPK	2225	2365	11033	9808	7250	5463
NPKS	2534	2956	10333	8550	7958	7129
Manure	2740	2058	13363	8792	8625	6742
Nematicide	2805	2182	7417	5925	5200	3779
NPKS+Nematicide	3840	2938	10892	8983	7717	6825
ANOVA (<i>F</i> value)						
Tillage (T)	1.07	1.00	1.07	0.00	0.02	0.04
Fertilizer-nematicide (Fn)	1.81	2.31	1.88	3.28 *	2.64 *	4.39 **
T × Fn	0.78	0.73	1.65	0.71	0.24	1.19
Corn-soybean site						
Tillage:						
Conventional tillage		6050	4914	3831	9903	17197
Minimum tillage		6906	4531	3792	10636	22217
Fertilizer-nematicide‡:						
Control		6275	4650	4025	12017	19433
NPK		7283	4500	3558	14025	21542
NPKS		6417	5042	3408	10867	21108
Manure		6942	5342	4850	10108	20367
Nematicide		5558	4217	3400	8708	15300
NPKS+Nematicide		6392	4583	3625	5892	20492
ANOVA (<i>F</i> value)						
Tillage (T)		2.03	0.17	0.00	0.15	3.68
Fertilizer-nematicide (Fn)		0.24	0.24	0.55	4.18 **	1.95
T × Fn		0.42	0.76	0.45	1.78	0.82

Pi, Pm, and Pf are population densities before planting, at midseason, and at harvest respectively * indicates $P \leq 0.05$, ** indicates $P \leq 0.01$

‡Values followed by different letters in the same column are significantly different according to LSD test at $P < 0.05$.

4.4 Fungal parasites of soybean cyst nematode

At the soybean-corn site, there were no tillage or manure effects ($P > 0.1$) on fungal endoparasites of SCN juveniles in either 2011 or 2012. In 2011, 7% ($\pm 2\%$) of J2 were parasitized while in 2012, 22.6% ($\pm 4.2\%$) of J2 were parasitized. Similarly, at the corn-soybean site in 2012, there were no significant manure or tillage effects with 36.5% ($\pm 3.1\%$) of J2 parasitized.

At the soybean-corn site, there were no tillage or manure effects on egg-parasitic index (EPI) in 2011 or 2012 ($P > 0.1$). EPI was very low in both 2011 (1.22 ± 0.22) and 2012 (0.65 ± 0.14) at the soybean-corn site. At the corn-soybean site in 2012 there was significant tillage by manure interaction ($P \leq 0.05$) for EPI. However, when ANOVA was performed for individual factors, there were no significant manure effects under MT or CT, or overall ($P > 0.1$). There were no significant effects of tillage ($P > 0.1$) on EPI with mean EPI of $0.60 (\pm 0.17)$ in 2012 corn.

In 2011, at the soybean-corn site, there were no manure or tillage effects ($P > 0.1$) on mean percentage of cysts colonized. Overall, 23% ($\pm 2.1\%$) of cysts were colonized. In 2012 corn, there were no significant tillage ($P > 0.1$) or manure effects ($P > 0.05$) on percent cysts colonized with 18.3% ($\pm 2.8\%$) cysts colonized. In 2012 soybean 2012, there was not significant treatment or tillage effects ($P > 0.1$) on % cysts colonized with 16.6% ($\pm 4.0\%$) cysts colonized overall. There were no significant tillage or manure effects on nematode-trapping fungi in 2011 soybean (0.648 ± 0.32 CFU/g), 2012 soybean plots (3.39 ± 0.96 CFU/g), or 2012 corn (3.24 ± 0.55 CFU/g).

4.5 Nematode community

4.5.1 Taxonomy and summary statistics

Over six seasons in 72 plots, a total of 88,724 nematodes spanning 72 genera and 36 families were identified and counted. The nematode genera observed at the field sites are summarized in Table 3.6. Across all seasons, plots, and sites; trophic group composition was: 48.7% herbivores, 31.2% bacterivores, 18.2% fungivores, 1.7% omnivores, and 0.2% predators. Among herbivores, *Heterodera* was the most common at 20.8% of all nematodes counted (relative abundance). Members of the family Rhabditidae were the most common bacteria-feeders at 19.49% relative abundance. *Aphelenchus* was the most common fungivore at 7.7% relative abundance. Among omnivores, *Eudorylaimus* was most common at 0.47% relative abundance while *Discolaimus* was the most common predator at 0.18% relative abundance.

Table 3.6. Average relative abundance over all samples and descriptions for nematode genera observed at field sites.

Genus	Family	c-p value	Trophic group	Relative abundance
<i>Acrobeles</i>	Cephalobidae	2	Bacterivore	1.11%
<i>Acrobeloides</i>	Cephalobidae	2	Bacterivore	0.01%
<i>Acrolobus</i>	Cephalobidae	2	Bacterivore	0.01%
<i>Alaimus</i>	Alaimidae	4	Bacterivore	0.26%
<i>Allodorylaimus</i>	Qudsianematidae	4	Omnivore	0.01%
<i>Anaplectus</i>	Plectidae	2	Bacterivore	0.13%
<i>Anatonchus</i>	Anatonchidae	4	Predator	0.01%
<i>Aphelenchoides</i>	Aphelenchoididae	2	Fungivore	7.48%
<i>Aphelenchus</i>	Aphelenchidae	2	Fungivore	7.70%
<i>Aporcelaimellus</i>	Aporcelaimidae	5	Omnivore	0.35%
<i>Aporcelaimium</i>	Aporcelaimidae	5	Omnivore	0.01%
<i>Aporcelaimus</i>	Aporcelaimidae	5	Omnivore	0.05%
<i>Aprutides</i>	Aphelenchoididae	2	Fungivore	<0.01%
<i>Aulolaimus</i>	Aulolaimidae	3	Bacterivore	0.04%
<i>Axonchium</i>	Belondiridae	5	Herbivore	0.25%
<i>Basiria</i>	Tylenchidae	2	Herbivore	0.04%
<i>Boleodorus</i>	Tylenchidae	2	Herbivore	0.19%
<i>Bunonema</i>	Bunonematidae	1	Bacterivore	0.01%
<i>Cephalenchus</i>	Tylenchidae	2	Herbivore	0.01%
<i>Cephalobus</i>	Cephalobidae	2	Bacterivore	4.20%
<i>Chiloplacus</i>	Cephalobidae	2	Bacterivore	0.31%
<i>Chronogaster</i>	Leptolaimidae	2	Bacterivore	0.25%
<i>Cobbonchus</i>	Monochidae	4	Predator	0.02%
<i>Criconemoides</i>	Criconematidae	3	Herbivore	<0.01%
<i>Diphtherophora</i>	Campydoridae	4	Fungivore	0.35%
<i>Diplogaster</i>	Diplogasteridae	1	Bacterivore	4.19%
<i>Discolaimium</i>	Actinolaimidae	5	Predator	0.01%
<i>Discolaimus</i>	Actinolaimidae	5	Predator	0.18%
<i>Ditylenchus</i>	Anguinidae	2	Herbivore	1.41%
<i>Dorydorella</i>	Qudsianematidae	4	Omnivore	0.43%
<i>Dorylaimellus</i>	Belondiridae	5	Herbivore	<0.01%
<i>Dorylaimoides</i>	Discolaimidae	5	Omnivore	0.01%
<i>Enchodelus</i>	Nordiidae	4	Omnivore	<0.01%
<i>Epidorylaimus</i>	Qudsianematidae	4	Omnivore	<0.01%
<i>Eucephalobus</i>	Cephalobidae	2	Bacterivore	2.07%
<i>Eudorylaimus</i>	Qudsianematidae	4	Omnivore	0.47%
<i>Filenchus</i>	Tylenchidae	2	Herbivore	6.33%
<i>Granonchulus</i>	Monochidae	4	Predator	<0.01%

Table 3.6 continued. Average relative abundance over all samples and descriptions for nematode genera observed at field sites.

<i>Genus</i>	Family	c-p value	Trophic group	Relative abundance
<i>Helicotylenchus</i>	Hoplolaimidae	3	Herbivore	13.22%
<i>Heterocephalobus</i>	Cephalobidae	2	Bacterivore	0.03%
<i>Heterodera</i>	Heteroderidae	3	Herbivore	20.76%
<i>Hoplolaimus</i>	Hoplolaimidae	3	Herbivore	0.01%
<i>Leptolaimus</i>	Leptolaimidae	2	Bacterivore	0.05%
<i>Leptonchus</i>	Leptonchidae	4	Fungivore	0.01%
<i>Longidorella</i>	Nordiidae	4	Omnivore	<0.01%
<i>Mesodorylaimus</i>	Thornenematidae	5	Omnivore	0.02%
<i>Michonchus</i>	Monochidae	4	Predator	<0.01%
<i>Microdorylaimus</i>	Qudsianematidae	4	Omnivore	0.05%
<i>Miculenchus</i>	Tylenchidae	2	Herbivore	0.04%
<i>Monochus</i>	Monochidae	4	Predator	0.01%
<i>Nygolaimus</i>	Nygolaimidae	5	Predator	<0.01%
<i>Panagrobelus</i>	Panagrolaimidae	1	Bacterivore	0.10%
<i>Panagrolaimus</i>	Panagrolaimidae	1	Bacterivore	0.04%
<i>Paramphidelus</i>	Alaimidae	4	Bacterivore	0.03%
<i>Paratrichodorus</i>	Trichodoridae	4	Herbivore	<0.01%
<i>Plectus</i>	Plectidae	2	Bacterivore	1.10%
<i>Pratylenchus</i>	Pratylenchidae	3	Herbivore	5.15%
<i>Prismatolaimus</i>	Prismatolaimidae	3	Bacterivore	0.10%
<i>Psilenchus</i>	Psilenchidae	2	Herbivore	0.46%
<i>Pungentus</i>	Nordiidae	4	Herbivore	0.03%
<i>Rhabditis</i>	Rhabditidae	1	Bacterivore	19.49%
<i>Rhabditophanes</i>	Alloionematidae	1	Bacterivore	0.04%
<i>Tobrilus</i>	Tobrilidae	3	Bacterivore	<0.01%
<i>Thonus</i>	Qudsianematidae	4	Omnivore	0.44%
<i>Trichodorus</i>	Trichodoridae	4	Herbivore	<0.01%
<i>Trophurus</i>	Dolichodoridae	3	Herbivore	0.03%
<i>Tylencholaimellus</i>	Leptonchidae	4	Fungivore	<0.01%
<i>Tylencholaimus</i>	Leptonchidae	4	Fungivore	0.05%
<i>Tylenchorhynchus</i>	Dolichodoridae	3	Herbivore	0.03%
<i>Tylenchus</i>	Tylenchidae	2	Herbivore	0.12%
<i>Wilsonema</i>	Plectidae	2	Bacterivore	0.10%
<i>Xiphinema</i>	Longidoridae	5	Herbivore	0.51%

4.5.2 Trophic groups and total nematode populations

4.5.2.1 Total nematode population

Nematode abundance in the soil was significantly greater under conventional than minimum tillage ($P \leq 0.05$) at the corn-soybean site in spring 2012 (Table 3.7). Tillage never significantly affected nematode abundance at the soybean-corn site. In 2011 soybean at midseason, there were significant fertilizer-nematicide effects on nematode abundance ($P \leq 0.01$) (Table 3.7), but also significant fertilizer-nematicide by tillage interaction with significant fertilizer-nematicide effects under both MT and CT ($P \leq 0.01$). Under minimum tillage, nematode abundance was greater with manure application compared to any other treatment. Under conventional tillage, nematode abundance under manure was similar to control, but greater than any other treatment although the magnitude of this difference was smaller than under MT (data not shown).

4.5.2.2 Fungivore population

Fungivore abundance was significantly ($P \leq 0.05$) greater under CT than MT at the corn-soybean site in midseason 2011 and spring 2012 (Table 3.8). At the soybean-corn site, fungivore abundance was not significantly affected by tillage ($P > 0.1$). In 2011 soybean at midseason, there was significant fertilizer-nematicide by tillage interaction ($P \leq 0.05$), but there were no fertilizer-nematicide effects under MT or CT ($P > 0.05$). In fall 2011 corn, there was significant tillage by fertilizer-nematicide interaction ($P \leq 0.05$), but no significant fertilizer-nematicide effects under MT ($P > 0.05$), or CT ($P > 0.1$). In 2012, fungivore abundance was significantly affected by fertilizer-nematicide at the corn-soybean site at midseason and fall. In midseason, fungivore abundance was generally decreased with nematicide application. In fall, there were fewer fungivores under nematicide alone than NPKS or manure treatment (Table 3.8).

Fungivore relative abundance was significantly ($P \leq 0.05$) greater under CT than MT in spring and fall 2012 at the corn-soybean site (Table 3.9). At the soybean-corn site, there were significant ($P \leq 0.05$) fertilizer-nematicide treatment effects in midseason 2011, fall 2011, and midseason 2012, but no tillage effects. In midseason 2011, fungivore relative abundance was decreased under manure compared with all other treatments. In fall 2011, fungivore relative abundance was increased under NPKS+nematicide and control compared to NPKS and manure. In midseason 2012, fungivore relative abundance was decreased with nematicide treatment and increased with manure application (Table 3.9).

Table 3.7. Effects of fertilizer, tillage, and nematicide on total nematode population density in the soil.†

Treatment	2011			2012				
	Pi	Pm	Pf	Pi	Pm	Pf		
Soybean – corn site								
Fertilizer-nematicide‡:								
Control	990	1255	1379	b	847	1892	ab	1826
NPK	879	1163	2014	ab	759	1528	b	1705
NPKS	846	1093	1778	ab	695	1433	b	1851
Manure	885	2483	2472	a	781	2485	a	1554
Nematicide	822	1243	1349	b	724	1741	b	1548
NPKS+Nematicide	799	1157	1347	b	685	1490	b	1351
ANOVA (<i>F</i> value)								
Tillage (T)	3.64	2.11	0.00		0.14	0.53		0.24
Fertilizer-nematicide (Fn)	0.35	18.62	**	3.63	*	0.69	3.11	*
T × Fn	1.08	8.42	**	0.55		1.10	0.65	1.57
Corn-soybean site								
Tillage:								
Conventional tillage		827	1062		667	a	2866	1238
Minimum tillage		561	499		390	b	2564	1104
ANOVA (<i>F</i> value)								
Tillage (T)		10.39	9.86		42.92	*	0.47	1.97
Fertilizer-nematicide (Fn)		0.80	1.94		1.98		1.87	2.24
T × Fn		0.47	1.89		1.12		2.19	2.59

† Pi, Pm, Pf are population densities (nematodes/100 cm³ soil) prior to applying fertilizer and nematicide, 47 days after planting, and at harvest in 2011, and prior to applying nematicide, 68 days after planting, and harvest in 2012, respectively.

‡ Values followed by different letters in the same column are significantly different according to Fischer's LSD at $P < 0.05$.

* indicates $P \leq 0.05$, and ** indicates $P \leq 0.01$ for adjoining *F* value.

Table 3.8. Effects of fertilizer, tillage, and nematicide on fungivore density in the soil.†

	2011			2012		
	Pm	Pf	Pi	Pm	Pf	
Corn-soybean site						
Tillage:						
Conventional tillage	165 a	264	153 a	392	314	
Minimum tillage	87 b	115	66 b	349	207	
Fertilizer-nematicide‡:						
Control	151	182	113	422 ab	246 abc	
NPK	108	189	106	419 ab	216 bc	
NPKS	144	205	119	397 ab	321 ab	
Manure	180	167	154	531 a	395 a	
Nematicide	83	178	88	212 c	173 c	
NPKS+Nematicide	90	217	77	244 bc	212 bc	
ANOVA (<i>F</i> value)						
Tillage (T)	74.90 *	15.76 •	43.48 *	0.47	3.29	
Fertilizer-nematicide (Fn)	1.08	1.13	0.53	3.75 *	3.12 *	
T × Fn	0.75	2.74 *	1.71	0.67	1.83	

† Pi, Pm, Pf are population densities (nematodes/100 cm³ soil) prior to applying fertilizer and nematicide, 47 days after planting, and at harvest in 2011, and prior to applying nematicide, 68 days after planting, and harvest in 2012, respectively.

‡ Values followed by different letters in the same column are significantly different according to Fischer's LSD at $P < 0.05$.

* indicates $P \leq 0.05$, and • indicates $P = 0.058$ for Pf 2011.

Table 3.9. Effects of fertilizer, tillage, and nematicide on fungivore relative abundance in the soil. †

Treatment	2011			2012					
	Pi	Pm	Pf	Pi	Pm	Pf			
	Soybean-corn site			Corn- soybean site					
Tillage:									
Conventional tillage	0.219	0.210	0.142	0.220	a	0.133	0.128	a	
Minimum tillage	0.186	0.123	0.160	0.151	b	0.137	0.062	b	
Fertilizer-nematicide‡:									
Control	0.217	0.172	a	0.200	a	0.204	0.153	ab	0.113
NPK	0.174	0.197	a	0.141	ab	0.190	0.131	abc	0.110
NPKS	0.229	0.180	a	0.108	b	0.196	0.151	ab	0.098
Manure	0.165	0.088	b	0.117	b	0.195	0.167	a	0.086
Nematicide	0.218	0.193	a	0.146	ab	0.160	0.094	c	0.084
NPKS+Nematicide	0.211	0.168	a	0.194	a	0.168	0.144	bc	0.079
ANOVA (<i>F</i> value)									
Tillage (T)	2.09	1.94	0.07	21.30	*	0.36	25.37	*	
Fertilizer-nematicide (Fn)	0.95	2.70	*	2.66	*	0.57	4.04	*	0.61
T × Fn	0.42	0.12	0.41	1.56		1.36	0.67		

† Pi, Pm, Pf are population relative abundances prior to applying fertilizer and nematicide, 47 days after planting, and at harvest in 2011, and prior to applying nematicide, 68 days after planting, and harvest in 2012, respectively.

‡ Values followed by different letters in the same column are significantly different according to Fischer's LSD at $P < 0.05$.

* indicates $P \leq 0.05$ for adjoining *F* value.

Table 3.10. Effects of fertilizer, tillage, and nematicide on bacterivore population density in the soil.†

Treatment	2011			2012		
	Pi	Pm	Pf	Pi	Pm	Pf
Soybean-corn site						
Tillage:						
Conventional tillage	338	442	834	439	770	259
Minimum tillage	309	688	694	417	556	280
Fertilizer-nematicide‡:						
Control	272	332	576 bc	496	668 b	247
NPK	357	375	839 b	437	532 bc	294
NPKS	278	298	840 b	364	381 c	259
Manure	361	1703	1395 a	491	1169 a	280
Nematicide	320	372	546 bc	417	635 bc	344
NPKS+Nematicide	353	310	388 c	363	592 bc	194
ANOVA (<i>F</i> value)						
Tillage (T)	0.16	0.21	0.32	0.01	11.30	0.10
Fertilizer-nematicide (Fn)	0.46	15.34 **	5.96 **	0.98	8.13 **	0.86
T × Fn	0.58	4.50 **	0.94	0.85	0.36	0.74
Corn-soybean site						
Tillage:						
Conventional tillage		360	226	258 a	673	161 a
Minimum tillage		275	75	140 b	479	72 b
Fertilizer-nematicide‡:						
Control		211	118	187	686	149
NPK		208	97	205	569	126
NPKS		256	140	190	618	147
Manure		863	257	247	790	127
Nematicide		189	118	196	415	67
NPKS+Nematicide		180	172	171	379	81
ANOVA (<i>F</i> value)						
Tillage (T)		1.00	9.14	220.93 **	1.88	315.69 **
Fertilizer-nematicide (Fn)		0.74	1.13	0.67	2.61	1.63
T × Fn		0.85	1.17	0.43	1.29	1.95

† Pi, Pm, Pf are population densities in spring, midseason, and at harvest respectively. ** indicates $P \leq 0.01$ for adjoining *F* value.

4.5.2.3 Bacterivore population

In spring and fall 2012 soybean, bacterivore abundance was significantly ($P \leq 0.05$) greater under CT than MT (Table 3.10). There were significant fertilizer-nematicide effects on bacterivore abundance only at the soybean-corn site. In midseason 2011 at the soybean-corn site, there were significant fertilizer-nematicide effects ($P \leq 0.01$), but also significant fertilizer-nematicide by tillage interaction ($P \leq 0.01$). Bacterivore abundance was greater under manure treatment compared with any other treatment with the differences more pronounced in minimum than conventional tillage (Table 3.11). In fall 2011 at the soybean-corn site, there were significant fertilizer-nematicide effects ($P \leq 0.05$) with bacterivore abundance increased with manure treatment compared with all other treatments. NPKS+nematicide decreased bacterivore abundance compared to NPKS. At the soybean-corn site in midseason 2012, there were fertilizer effects ($P \leq 0.05$) with bacterivore abundance increased under manure compared with all other treatments and decreased under NPKS compared to control (Table 3.10).

Tillage significantly ($P \leq 0.05$) affected bacterivore relative abundance in fall 2012 corn with bacterivore relative abundance greater under conventional than minimum tillage (Table 3.12). There were significant fertilizer-nematicide effects on bacterivore relative abundance only at the soybean-corn site (midseason and fall 2011; midseason 2012). In all three seasons, bacterivore relative abundance was increased under manure treatment (Table 3.12).

Table 3.11. Interactive effects of fertilizer, nematicide and tillage on bacterivore population density in the soil.†

Treatment ‡	Soybean-corn 2011	
	Pm under MT §	Pm under CT §
	Mean	Mean
Control	210 b	453 b
NPK	507 b	243 c
NPKS	257 b	340 bc
Manure	2517 a	890 a
Nematicide	357 b	387 bc
NPKS+ Nematicide	283 b	337 bc

† Pm is population density (nematodes/100 cm³ soil), 47 days after planting in 2011.

§ “under MT” and “under CT” indicate means under minimum and conventional tillage, respectively.

‡ Values followed by different letters in the same column are significantly different according to Fischer’s LSD at $P < 0.05$.

Table 3.12. Effects of fertilizer, tillage, and nematicide on bacterivore relative abundance in the soil.†

Treatment	2011			2012		
	Pi	Pm	Pf	Pi	Pm	Pf
Soybean – corn site						
Tillage:						
Conventional tillage	0.380	0.347	0.449 a	0.563	0.405	0.315
Minimum tillage	0.347	0.344	0.387 b	0.575	0.339	0.301
Fertilizer-nematicide‡:						
Control	0.285	0.262 b	0.402 b	0.603	0.354 bc	0.314
NPK	0.394	0.310 b	0.413 b	0.568	0.359 bc	0.248
NPKS	0.316	0.268 b	0.471 ab	0.517	0.279 c	0.268
Manure	0.414	0.655 a	0.556 a	0.609	0.487 a	0.339
Nematicide	0.356	0.313 b	0.380 bc	0.589	0.369 bc	0.339
NPKS+Nematicide	0.415	0.265 b	0.284 c	0.528	0.385 b	0.338
ANOVA (<i>F</i> value)						
Tillage (T)	0.11	0.02	20.88 *	1.44	2.97	0.08
Fertilizer-nematicide (Fn)	2.58	15.72 **	5.97 **	0.86	4.05 *	1.82
T × Fn	1.13	2.54	1.37	0.83	0.44	1.62

† Pi, Pm, and Pf are population relative abundances prior to applying fertilizer and nematicide, 47 days after planting, and at harvest in 2011, and prior to applying nematicide, 68 days after planting, and harvest in 2012, respectively.

‡ Values followed by different letters in the same column are significantly different according to Fischer's LSD at $P < 0.05$.

* indicates $P \leq 0.05$ and ** indicates $P \leq 0.01$ for adjoining *F* value.

4.5.2.4 Herbivore population

At the soybean-corn site, herbivore abundance was significantly ($P \leq 0.01$) greater under MT (703 herbivores/100 cm³ soil) than CT (532) in midseason 2011. There were no significant tillage effects on herbivore abundance at the corn-soybean site or fertilizer-nematicide effects at either site.

There were no significant tillage effects on herbivore relative abundance at either site ($P > 0.05$). Fertilizer-nematicide application affected herbivore relative abundance in year of fertilizer application (2011) at the corn-soybean site only. In midseason 2011, there were significant fertilizer-nematicide effects ($P \leq 0.01$) with herbivore relative abundance decreased under manure application compared with all other treatments (Table 3.13). In fall 2011, there was significant fertilizer-nematicide by tillage interaction ($P \leq 0.05$) with significant fertilizer-nematicide effects under CT ($P \leq 0.05$) but not MT ($P > 0.05$). Under CT, herbivore relative abundance was greater in NPKS+nematicide than other fertilizer treatments.

Table 3.13. Interactive effects of fertilizer, nematicide and tillage on herbivore relative abundance in the soil.†

Treatment ‡	Soybean-corn 2011	
	Pm	Pf under CT
Control	55.8% a	42.2% ab
NPK	49.3% a	36.4% b
NPKS	53.7% a	28.5% b
Manure	25.2% b	32.0% b
Nematicide	48.5% a	40.9% ab
NPKS+ Nematicide	56.0% a	54.0% a

† Pm and Pf under CT are population relative abundances 47 days after planting (both tillage types) and at harvest (conventional tillage only), respectively, in 2011.

‡ Values followed by different letters in the same column are significantly different according to Fischer's LSD at $P < 0.05$.

4.5.2.5 Omnivore and predator population

There were no tillage effects ($P > 0.05$) on omnivore abundance or relative abundance. Nematicide only treatment significantly decreased omnivore abundance ($P \leq 0.05$) and relative abundance ($P \leq 0.05$) compared with control or NPKS treatments in midseason 2011 soybean plots, although populations were quite low at under 15 nematodes/100 cm³ soil (data not shown). Similarly, predator populations were generally

around 5 nematodes/100 cm³ soil and were not statistically analyzed due to their uneven distribution throughout the sites.

4.5.3 Diversity indices

At the corn-soybean site, there were significant tillage effects ($P < 0.05$) on Shannon-Weaver diversity, evenness, and Simpson's dominance in fall 2012. Conventional tillage decreased dominance (Table 3.14), but increased diversity and evenness (data not shown).

There were few fertilizer-nematicide effects on diversity indices. In 2011 soybean at midseason there were significant fertilizer effects on diversity indices ($P \leq 0.05$), with Simpson's dominance increased while Shannon-Weaver diversity and evenness were decreased under manure compared with other treatments (data not shown). In soybean plots in the residual year, Simpson's dominance under aldicarb nematicide was similar to NPK, but greater than any other treatment only at midseason (Table 3.14).

Table 3.14. Effects of fertilizer, tillage, and nematicide on Simpson's Dominance Index.†

Treatment	2012			
	Vi	Vm	Vf	
corn-soybean site				
Tillage:				
Conventional tillage	0.184	0.198	0.184	b
Minimum tillage	0.163	0.259	0.359	a
Fertilizer-nematicide‡:				
Control	0.147	0.205	b	0.243
NPK	0.163	0.223	ab	0.269
NPKS	0.171	0.198	b	0.248
Manure	0.191	0.196	b	0.263
Nematicide	0.178	0.273	a	0.286
NPKS+Nematicide	0.189	0.275	a	0.320
ANOVA (<i>F</i> value)				
Tillage (T)	1.05	13.95	•	48.69 *
Fertilizer-nematicide (Fn)	0.85	3.00	*	1.22
T × Fn	0.17	1.40		1.48

† Vi, Vm, and Vf are values prior to applying nematicide, 68 days after planting, and harvest in 2012, respectively.

‡ Values followed by different letters in the same column are significantly different according to Fischer's LSD at $P < 0.05$.

* indicates $P \leq 0.05$ for adjoining *F* value. • indicates $P = 0.065$ for Vm tillage.

4.5.4 Maturity indices

4.5.4.1 MI

There were no significant tillage effects ($P > 0.1$) on the maturity index at any time. At both sites, there were significant ($P \leq 0.05$) fertilizer-nematicide effects in both the year of fertilizer application (midseason and fall 2011) and the year following application (midseason 2012). For both sites in these seasons, MI was decreased under manure treatment as compared to most treatments with differences more pronounced earlier in the study and at the soybean-corn site (Table 3.15). In fall 2011 at both sites, MI was generally increased under nematicide treatments compared to corresponding fertilizer treatments not treated with nematicide.

4.5.4.2 MI25

There were no significant tillage effects on MI25 at either site ($P > 0.1$). In fall 2011 corn, there were significant fertilizer-nematicide effects ($P \leq 0.05$), with MI25 significantly increased under nematicide only treatment compared with control, NPK, NPKS, and manure. In spring 2011 corn, there were significant fertilizer-nematicide effects ($P \leq 0.05$) with MI25 under nematicide similar to NPKS+nematicide, but significantly greater than any other treatment. In spring 2012 corn, there were significant ($P \leq 0.05$) fertilizer-nematicide effects with MI25 significantly increased under manure compared with control and NPKS while also significantly decreased under control compared with all treatments except NPKS. In fall 2012 corn, there was significant fertilizer-nematicide by tillage interaction ($P \leq 0.05$) with significant fertilizer-nematicide effects under MT ($P \leq 0.05$), but not CT ($P > 0.1$). Under MT, MI25 was significantly increased with manure compared to any other treatment.

4.5.4.3 Σ MI

At the corn-soybean site, Σ MI was significantly ($P \leq 0.05$) greater under minimum than conventional tillage in spring and fall 2012 (Table 3.16). There were no significant tillage effects at the soybean-corn site. There were no fertilizer-nematicide effects at the corn-soybean site, but at the soybean-corn site, there were fertilizer-nematicide effects in every season except spring 2011 and 2012. Throughout these seasons, Σ MI was generally decreased with manure application (Table 3.16).

Table 3.15. Effects of fertilizer, tillage, and nematicide on Maturity Index.†

Treatment	2011			2012					
	Vi	Vm	Vf	Vi	Vm	Vf			
Soybean-corn site									
Fertilizer-nematicide‡:									
Control	1.77	2.00	a	1.64	bc	1.35	1.65	ab	1.76
NPK	1.61	1.92	a	1.64	bc	1.38	1.72	a	1.70
NPKS	1.80	1.96	a	1.53	cd	1.36	1.75	a	1.83
Manure	1.63	1.52	b	1.40	d	1.35	1.51	b	1.57
Nematicide	1.73	1.92	a	1.82	ab	1.38	1.66	ab	1.71
NPKS+Nematicide	1.70	1.86	a	1.85	a	1.38	1.80	a	1.66
ANOVA (<i>F</i> value)									
Tillage (T)	0.19	0.07		0.50		0.14	0.83		0.75
Fertilizer-nematicide (Fn)	1.97	8.21	**	6.84	**	0.05	2.88	*	2.27
T × Fn	2.43	1.64		1.08		1.05	0.71		0.31
Corn-soybean site									
Fertilizer-nematicide‡:									
Control		2.11	ab	1.96	bc	1.70	1.98	abc	1.93
NPK		2.04	b	1.97	bc	1.53	2.12	ab	1.91
NPKS		1.95	b	1.84	bc	1.64	1.90	bc	1.72
Manure		1.67	c	1.72	c	1.51	1.78	c	1.76
Nematicide		2.27	a	2.26	a	1.64	1.95	abc	1.80
NPKS+Nematicide		1.97	b	2.01	ab	1.58	2.15	a	1.89
ANOVA (<i>F</i> value)									
Tillage (T)		1.11		0.54		1.77	5.31		0.06
Fertilizer-nematicide (Fn)		8.54	**	3.41	*	1.12	3.25	*	0.30
T × Fn		0.52		0.78		0.43	0.43		0.63

† Vi, Vm, and Vf are values prior to applying fertilizer and nematicide, 47 days after planting, and at harvest in 2011, and prior to applying nematicide, 68 days after planting, and harvest in 2012, respectively.

‡ Values followed by different letters in the same column are significantly different according to Fischer's LSD at $P < 0.05$

* indicates $P \leq 0.05$ and ** indicates $P \leq 0.01$ for adjoining *F* value.

Table 3.16. Effects of fertilizer, tillage, and nematicide on ΣMI .†

Treatment	2011			2012		
	Vi	Vm	Vf	Vi	Vm	Vf
Soybean-corn site						
Fertilizer-nematicide‡:						
Control	2.29	2.38 a	2.10 bc	1.71	2.26 a	2.34 ab
NPK	2.13	2.29 a	2.15 ab	1.82	2.28 a	2.38 a
NPKS	2.25	2.32 a	2.02 bc	1.86	2.30 a	2.43 a
Manure	2.15	1.63 b	1.85 c	1.79	2.01 b	2.19 b
Nematicide	2.15	2.24 a	2.26 a	1.76	2.21 a	2.16 b
NPKS+Nematicide	2.07	2.39 a	2.37 a	1.93	2.28 a	2.28 ab
ANOVA (<i>F</i> value)						
Tillage (T)	0.28	0.63	0.19	0.02	1.33	0.24
Fertilizer-nematicide (Fn)	1.17	10.54 **	5.98 **	1.13	2.84 *	2.82 *
T × Fn	0.83	1.87	1.66	0.67	0.96	1.81
Corn-soybean site						
Tillage:						
Conventional tillage		1.96	2.29	1.96 b	2.49	2.37 b
Minimum tillage		2.13	2.56	2.19 a	2.61	2.65 a
ANOVA (<i>F</i> value)						
Tillage (T)		1.02	4.19	30.52 *	6.62	18.27 *
Fertilizer-nematicide (Fn)		1.09	1.08	0.50	2.50	1.01
T × Fn		0.97	0.78	0.40	0.64	0.87

† Vi, Vm, and Vf are values prior to applying fertilizer and nematicide, 47 days after planting, and at harvest in 2011, and prior to applying nematicide, 68 days after planting, and harvest in 2012, respectively.

‡ Values followed by different letters in the same column are significantly different according to Fischer's LSD at $P < 0.05$.

* indicates $P \leq 0.05$ and ** indicates $P \leq 0.01$ for adjoining *F* value.

4.5.4.4 Σ MI25

At the corn-soybean site, Σ MI25 was significantly ($P \leq 0.05$) greater under MT than CT for all seasons except midseason 2012 (Fig. 3.9). At the soybean-corn site, there were no significant tillage effects on Σ MI25. The only significant fertilizer-nematicide treatment effect was in spring 2012 corn ($P \leq 0.05$) when Σ MI25 was increased by fertilizer and nematicide treatments (data not shown).

4.5.4.5 PPI

At the corn-soybean site, PPI was significantly greater under MT than CT during most seasons (Fig. 3.10). There were no significant tillage effects at the soybean-corn site. There was significant tillage-fertilizer-nematicide interaction ($P \leq 0.05$) in fall 2012 corn with significant fertilizer-nematicide effects under CT ($P \leq 0.05$), but not MT ($P > 0.1$). Under CT, PPI was increased under NPKS+nematicide compared with control, NPK, and nematicide only, but decreased under nematicide alone compared with NPKS+nematicide and NPKS (data not shown).

4.5.5 Food web indices

4.5.5.1 EI

There were no significant tillage at either site or fertilizer-nematicide treatment effects at the corn-soybean site ($P > 0.05$). At the soybean-corn site, there were significant fertilizer-nematicide treatment effects or significant tillage by fertilizer-nematicide interactions in all seasons after treatment application except for spring and midseason 2012. In midseason 2011 soybean, EI was significantly increased under manure compared with all other treatments (Table 3.17). In fall 2011 soybean, there were significant fertilizer-nematicide effects on EI ($P \leq 0.01$), but also significant tillage by fertilizer-nematicide interaction ($P \leq 0.01$). Under MT, EI was decreased under nematicide treatments compared with NPK, NPKS and manure while increased under manure compared with control or nematicide treatments (data not shown). Under CT, EI was decreased under NPKS+nematicide compared with any other treatment (data not shown). In fall 2012 corn, EI was greater under manure than control, NPK, NPKS, or nematicide only (Table 3.17).

Figure 3.9. Σ MI25 at the corn-soybean site over time.

* indicates significant tillage effects at $P \leq 0.05$

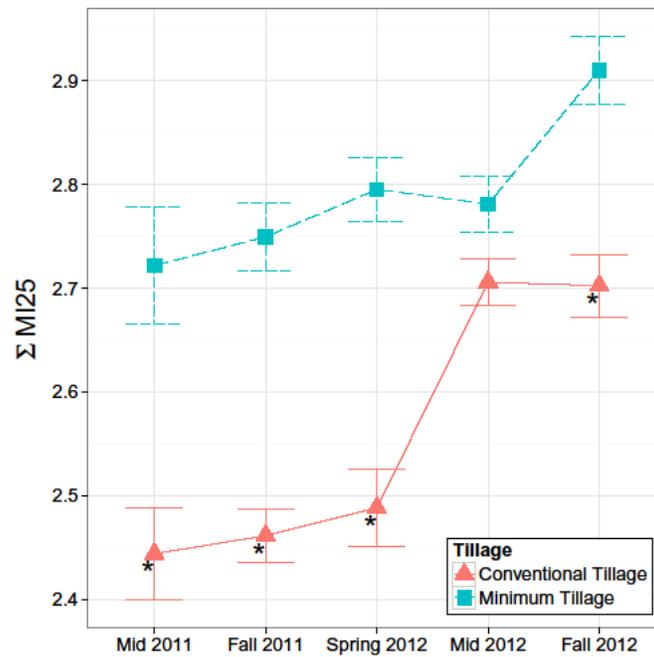


Figure 3.10. Plant-parasitic index at the corn-soybean site over time.

* indicates significant tillage effects at $P \leq 0.05$, • $P = 0.056$ Fall 2011

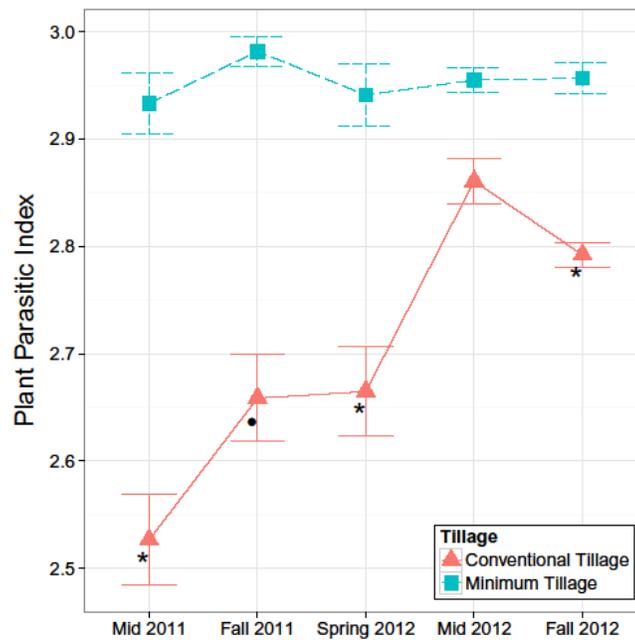


Table 3.17. Effects of fertilizer, tillage, and nematicide on Enrichment Index.†

Treatment	2011			2012		
	Vi	Vm	Vf	Vi	Vm	Vf
Soybean – corn site						
Tillage:						
Conventional tillage	79.3	63.3	88.6	93.6	76.1	77.8
Minimum tillage	72.7	59.1	83.3	92.3	70.4	73.4
Fertilizer-nematicide‡:						
Control	70.1	50.4 b	85.0	91.1	74.9	74.1 bc
NPK	79.5	58.4 b	88.4	93.9	70.6	74.4 bc
NPKS	73.9	58.9 b	90.5	92.0	69.8	71.0 c
Manure	80.3	82.3 a	93.3	95.5	83.2	82.3 a
Nematicide	75.5	54.9 b	81.6	92.1	74.4	73.1 bc
NPKS+Nematicide	76.8	62.3 b	77.0	93.1	66.4	78.5 ab
ANOVA (<i>F</i> value)						
Tillage (T)	0.95	0.15	1.27	0.43	1.05	0.69
Fertilizer-nematicide (Fn)	1.47	10.13 **	9.38 **	1.23	2.53	2.72 *
T × Fn	0.58	2.09	1.40 **	1.05	0.39	1.28

† Vi, Vm, and Vf are values prior to applying fertilizer and nematicide, 47 days after planting, and at harvest in 2011, and prior to applying nematicide, 68 days after planting, and harvest in 2012, respectively.

‡ Values followed by different letters in the same column are significantly different according to Fischer's LSD at $P < 0.05$.

* indicates $P \leq 0.05$ and ** indicates $P \leq 0.01$ for adjoining *F* value.

4.5.5.2 BI

There were no significant tillage effects on BI at either site. In midseason and fall 2011 at the soybean-corn site, there were significant fertilizer-nematicide effects ($P < 0.001$), but also fertilizer-nematicide by tillage interaction ($P \leq 0.05$). In midseason 2011 soybean, under minimum tillage, BI was significantly ($P \leq 0.05$) decreased under manure compared with any other treatment (data not shown), but not affected under conventional tillage ($P > 0.1$). In fall 2011 soybean, there were significant fertilizer-nematicide effects under MT ($P \leq 0.05$) but not CT ($P > 0.05$). Under MT, BI was significantly decreased under manure compared with control and nematicide treatments, and increased under nematicide treatments compared with equivalent non-nematicide treatments. In fall 2012 corn, there were significant fertilizer-nematicide effects ($P < 0.05$) with BI decreased under manure compared with all treatments except NPKS+nematicide (Table 3.18).

In corn plots at fall 2011, there was significant tillage by fertilizer-nematicide interaction ($P \leq 0.05$) with significant fertilizer-nematicide effects on BI under both MT and CT ($P \leq 0.05$). Under MT, BI was decreased by nematicide treatments compared with control, NPK, or NPKS. Under CT, BI was greater under nematicide, NPKS+nematicide and NPK than manure (data not shown).

4.5.5.3 SI

There were no significant tillage effects on SI at either site ($P > 0.05$). For SI, fertilizer-nematicide treatment effects or interactions were present only at the soybean-corn site and only in the residual year (spring and fall 2012). In spring 2012 corn, SI was greater in all fertilizer and nematicide treatments except NPKS than control, and in manure than NPKS (data not shown). In fall 2012 corn, there were significant fertilizer-nematicide by tillage interactions for SI ($P \leq 0.05$) with fertilizer-nematicide effects under MT ($P \leq 0.05$), but not CT ($P > 0.1$). Under minimum tillage, SI was greater under NPKS+nematicide than NPK or nematicide treatment (data not shown).

4.5.5.4 CI

There were no significant tillage effects at either site ($P > 0.05$). There were only significant fertilizer-nematicide treatment effects ($P \leq 0.05$) in soybean plots and only in 2011 (all seasons) (Table 3.19). In midseason 2011 soybean, there was significant fertilizer-nematicide effects, but also fertilizer-nematicide by tillage interaction ($P \leq 0.01$).

Table 3.18. Effects of fertilizer, tillage, and nematicide on Basal Index.†

Treatment	2011			2012		
	Vi	Vm	Vf	Vi	Vm	Vf
Soybean – corn site						
Tillage:						
Conventional tillage	19.1	34.1	17.8	6.0	22.6	21.2
Minimum tillage	25.1	37.8	26.2	7.3	28.0	25.4
Fertilizer-nematicide‡:						
Control	27.2	43.7	21.9	8.6	24.3	24.3 a
NPK	19.4	38.4	17.7	5.6	27.5	24.6 a
NPKS	23.5	37.3	15.5	7.7	28.7	26.7 a
Manure	18.2	17.2	10.7	4.2	16.0	17.0 b
Nematicide	22.7	43.3	33.4	7.4	24.9	26.5 a
NPKS+Nematicide	21.7	35.8	32.7	6.5	30.3	20.5 ab
ANOVA (<i>F</i> value)						
Tillage (T)	1.24	0.01	2.07	0.39	1.44	0.79
Fertilizer-nematicide (Fn)	1.20	15.73 **	8.34 **	1.42	2.34	2.73 *
T × Fn	0.44	2.81 *	2.67 *	0.95	0.33	1.70

† Vi, Vm, and Vf are values prior to applying fertilizer and nematicide, 47 days after planting, and at harvest in 2011, and prior to applying nematicide, 68 days after planting, and harvest in 2012, respectively.

‡ Values followed by different letters in the same column are significantly different according to Fischer's LSD at $P < 0.05$.

* indicates $P \leq 0.05$ and ** indicates $P \leq 0.01$ for adjoining *F* value.

• indicates $P = 0.059$ for Vf 2011 tillage.

There were significant fertilizer-nematicide effects under MT ($P \leq 0.05$), with CI decreased under manure compared with any other treatment, but no effects under CT ($P > 0.1$). In fall 2011 soybean, there were significant fertilizer-nematicide effects ($P < 0.01$) with CI increased under NPKS+nematicide compared with NPK, NPKS, or manure treatments.

Table 3.19. Effects of fertilizer, tillage, and nematicide on Channel Index.†

Treatment	2011					
	Vi		Vm		Vf	
	Soybean-corn site					
Fertilizer-nematicide‡:						
Control	30.9	a	57.3	a	18.8	ab
NPK	17.0	b	44.7	a	13.7	bc
NPKS	23.8	a	42.3	a	11.3	c
Manure	13.9	b	15.6	b	7.3	c
Nematicide	21.9	ab	45.4	a	23.7	ab
NPKS+Nematicide	17.8	ab	37.6	a	30.9	a
ANOVA (<i>F</i> value)						
Tillage (T)	0.31		0.37		0.28	
Fertilizer-nematicide (Fn)	3.16	*	12.21	**	5.21	**
T × Fn	1.22		4.23	**	0.56	

† Vi, Vm, and Vf are values prior to applying fertilizer and nematicide, 47 days after planting, and at harvest in 2011

‡ Values followed by different letters in the same column are significantly different according to Fischer's LSD at $P < 0.05$.

* indicates $P \leq 0.05$ and ** indicates $P \leq 0.01$ for adjoining *F* value.

4.5.5.5 FFB & FBPP

There were no significant tillage effects on FFB at either site ($P > 0.05$). Fertilizer-nematicide effects were only present in soybean plots and only in 2011 (all seasons). In midseason 2011 soybean, FFB was significantly affected by fertilizer-nematicide ($P \leq 0.01$) with FFB lower under manure than other treatments (Table 3.20). In fall, FFB was lower under manure and NPKS alone than NPKS with nematicide or control. FBPP was not significantly affected by tillage or fertilizer-nematicide ($P > 0.05$).

5. Discussion

5.1 Plant growth and yield

Based on contradicting 2011 and 2012 results, the effects of tillage on soybean yield are inconsistent. This discrepancy may be due to site differences, interaction with year's environment (drier in 2012), or interaction with fertilizer-nematicide application.

Most literature suggests no effect on yield (Chen et al., 2001a; Conley et al., 2011; Donald et al., 2009; Hershman and Bachi, 1995) or yield reduction (Chen, 2007b; Koenning et al., 1995; Noel and Wax, 2003; Westphal et al., 2009) for soybean under minimum tillage in the Midwest.

Application of NPK and NPKS suppressed soybean growth in the year it was applied, possibly due to excess soil nitrogen inhibiting nodule formation and nitrogen fixation (Hungria et al., 2005; Salvagiotti et al., 2008). However, similar studies on pre-plant nitrogen application to soybean did not find decreased soybean yield with nitrogen application at similar rates (Sorensen and Penas, 1978), although other studies suggest that impacts depend on initial soil nitrogen levels (Alithawi et al., 1980; Stone et al., 1985). While swine manure also contained N it did not suppress yield in this study, possibly due to the slightly lower rate and delayed release compared with the inorganic fertilizers (Salvagiotti et al., 2008).

Table 3.20: Effects of fertilizer, tillage, and nematicide on F/(F+B) ratio.†

Treatment	2011					
	Vi		Vm		Vf	
	Soybean-corn site					
Fertilizer-nematicide‡:						
Control	0.447	a	0.385	a	0.342	ab
NPK	0.315	cd	0.396	a	0.265	bc
NPKS	0.427	ab	0.389	a	0.200	c
Manure	0.283	d	0.132	b	0.192	c
Nematicide	0.391	abc	0.380	a	0.280	bc
NPKS+Nematicide	0.337	bcd	0.366	a	0.413	a
ANOVA (<i>F</i> value)						
Tillage (T)	0.09		0.79		0.41	
Fertilizer-nematicide (Fn)	3.25	*	5.79	**	5.11	**
T × Fn	1.22		0.19		0.23	

† Vi, Vm, and Vf are values prior to applying fertilizer and nematicide, 47 days after planting, and at harvest in 2011, and prior to applying nematicide, 68 days after planting, and harvest in 2012, respectively.

‡ Values followed by different letters in the same column are significantly different according to Fischer's LSD at $P < 0.05$.

* indicates $P \leq 0.05$ and ** indicates $P \leq 0.01$ for adjoining *F* value.

Terbufos nematicide only had positive impact on plant growth in combination with NPKS. Other studies have suggested nematicides are more beneficial in combination with fertilizer because fertilizers stimulate plant growth while nematicides simultaneously

decrease plant-parasitic nematode populations allowing plants to avoid early-season damage from nematodes (Burris et al., 2010; Thompson et al., 2012). However, in this study NPK and NPKS suppressed plant yield and SCN populations were not significantly affected by terbufos in soybean, so this explanation is unlikely. It is possible that decreased yield due to NPKS was anomalous, and there was no benefit of nematicide application, particularly since there was no nematicide yield benefit without fertilizer. Based on these inconsistencies, more research is needed to make definite conclusions about the impacts of terbufos nematicide.

Manure application had strong residual positive effects on soybean yield. NPKS, but not NPK had residual benefits on yield suggesting residual benefit of sulfur for soybean yield although this may be due to increased corn biomass the previous year. Aldicarb nematicide did not improve soybean yields despite decreasing SCN soil population density, which causes sizeable yield loss (Chen, 2011; Koening and Wrather, 2010; Koening et al., 1999). Generally, aldicarb has successfully reduced SCN population densities and increased soybean yields or growth in Midwest studies although varying degrees of inconsistency from year to year and field to field were also observed (Niblack et al., 1992; Noel, 1987; Rotundo et al., 2010; Smith et al., 1991). Additionally, there is some evidence that aldicarb stimulates plant growth under some conditions and application rates even when nematodes are absent, so phytotoxicity is not a concern at the rates applied in this study (Barker et al., 1988).

Overall, conventional tillage increased corn yield compared to minimum tillage. This is consistent with similar studies in the Midwest as most found corn yield increases with increased tillage (Iragavarapu and Randall, 1995; Kumar et al., 2012; Lal and Ahmadi, 2000; West et al., 1996; Wilhelm and Wortmann, 2004) although some showed no differences (Kwaw-Mensah and Al-Kaisi, 2006; Linden et al., 2000) or yield decrease (Hussain and Olson, 2012).

Manure was the most effective fertilizer for corn yield in the first year of fertilizer application although NPK and NPKS alone were also effective. Sulfur application was beneficial based on results. Terbufos nematicide was detrimental to corn yield, but based on the lack of effects on the soil nematode community, the reason for this is speculative. This suggests terbufos nematicide is not a good option for corn nematode management, but more studies are needed to make a conclusion.

Manure has the most residual benefit for corn yield while all fertilizers had some residual benefits compared with control. Aldicarb nematicide benefited corn yield to some degree regardless of fertilization scheme. This suggests corn nematodes may have affected yield and aldicarb can have value as a nematicide on corn. However, corn nematode populations were below damage thresholds (Tylka, 2007) and aldicarb did not significantly reduce populations at midseason or fall. Nematicide could have decreased plant-parasitic nematode populations early in the season before midseason sampling resulting in increased yield. However, corn yield benefits with aldicarb application could also be due to effects on insect parasites of corn (Barker et al., 1988).

5.2 Plant-parasitic nematodes

5.2.1 *Helicotylenchus*, *Pratylenchus*, and *Xiphinema*

Overall, tillage did not strongly impact populations of plant-parasitic nematodes and populations did not build up to damaging levels despite long-term regimes. This suggests tillage did not strongly impact *Helicotylenchus*, *Pratylenchus*, or *Xiphinema*. While few tillage studies have included *Xiphinema* or *Helicotylenchus*, there is more evidence of *Pratylenchus* decrease under conventional tillage (Govaerts et al., 2007; McKeown et al., 1998; Rahman et al., 2007) than increase (Thompson, 1992) or no effects (Okada and Harada, 2007; Sanchez-Moreno et al., 2006).

While *Xiphinema* and *Pratylenchus* were not affected by fertilizers or nematicide, *Helicotylenchus* was, possibly because it had the largest population. Aldicarb nematicide decreased *Helicotylenchus* population densities especially in combination with NPKS with corn yield also increased by this treatment. While this suggests *Helicotylenchus* had negative impact on corn yield, other work suggests *Helicotylenchus* is weakly virulent on corn (Norton et al., 1978). In contrast, fertilizer-nematicide effects on *Helicotylenchus* population in 2012 soybeans generally mirrored yield suggesting a correlation with plant growth.

5.2.2 Soybean cyst nematode

Overall, tillage had little impact on SCN populations under this study's conditions although there was limited evidence that conventional tillage restricted vermiform SCN population. Other studies on SCN have generally found no effects of tillage (Chen et al., 2001a; Chen, 2007b; Conley et al., 2011; Hershman and Bachi, 1995), or population decrease (Donald et al., 2009; Koenning et al., 1995; Westphal et al., 2009) although one study corroborated SCN increase under minimum tillage (Noel and Wax, 2003).

Fertilizer application increased SCN egg population density at the soybean-corn site, possibly due to increased plant biomass. This suggests fertilizers had limited value for managing the SCN population although manure has been effective in minimizing soybean yield loss to SCN damage in another study (Bao et al., 2013).

While terbufos nematicide did not affect SCN soil populations, aldicarb effectively reduced SCN egg population density in 2012 soybean. However, this SCN reduction did not correspond to increased soybean yields despite occurring in early season when SCN has highest impact on yield (Chen, 2011). SCN population densities were relatively high at midseason (> 5500 eggs/100 cm³ soil) with and without nematicide treatment, so they may have caused a similar amount of damage. Additionally, in this study, aldicarb was not effective for long-term SCN management, as SCN egg populations rebounded to similarly high levels (>15000 eggs/100 cm³ soil) under all treatments by fall. Generally, aldicarb has successfully reduced SCN populations in studies in the Midwest (Niblack et al., 1992; Noel, 1987; Smith et al., 1991) although it was ineffective in one study (Rotundo et al., 2010) and inconsistent in all studies. Aldicarb has also been effective against SCN in the Southeastern United States (Koenning et al., 1998; Schmitt et al., 1983; Schmitt et al., 1987) although not under all conditions (Koenning et al., 1998; Young, 1998).

5.3 Fungal parasites of SCN

Overall, both sites had low incidence of nematode-trapping fungi and SCN-egg parasitic fungi while long-term tillage had little impact on them. In 2011, parasitism of SCN juveniles in this study was low compared with other Minnesota studies (Chen and Liu, 2007), but in 2012 parasitism was greater in this study compared with other studies. The reasons for this year to year difference are unclear. Additionally, in 2012, parasitism of juveniles was greater in soybean than corn which is consistent with other studies (Chen and Liu, 2007) and the observation that fungal parasitism is density-dependent with respect to nematode population (Chen and Liu, 2007; Jaffee et al., 1989). Overall, results of this study suggest fungal antagonism of SCN was low with manure and tillage having minimal short- and long-term impacts respectively on fungal antagonists at this site. However, one study suggested that physical disturbance reduces fungal parasitism of SCN juveniles in suppressive soil in a greenhouse study (Bao et al., 2011) although another study suggested any reduction of fungal parasitism is site-specific and inconsistent under field conditions (Chen and Liu, 2007).

5.4 Nematode community

Both fertilizer/nematicide and tillage effects were site-specific as tillage effects were more predominant at the corn-soybean site while fertilizer-nematicide effects were more common at the soybean-corn site. Since fertilizer-nematicide application was short-term and applied only at the start of this study, differences in fertilizer-nematicide effects between sites may be due to cropping differences. For example, nutrient uptake and utilization differs between corn and soybean so there will be some differences in nutrients available for the soil microbial community between the two crops. However, differing fertilizer-nematicide effects may have been due to differences in soil properties between the two sites since they cannot be completely distinguished from cropping effects. Tillage regimes were conducted for a long period, so it is unlikely that cropping differences within this study would impact tillage effects, especially to the extent observed.

5.4.1 Trophic group and total nematode abundance

Tillage effects on nematode and trophic groups were site-specific with most effects at the corn-soybean site. Based on nematode populations, conventional tillage increased fungal, bacterial, and overall microbial activity in various seasons which is consistent with some studies (Okada and Harada, 2007; Treonis et al., 2010). Increased microbial and beneficial nematode growth may be due to increased resources created when conventional tillage oxygenates and breaks up soil releasing nutrients resulting in lower organic matter in the long run (Holland, 2004; Hussain and Olson, 2012; Kumar et al., 2012). In contrast, conventional tillage impeded herbivore population growth at the soybean-corn site, although this was inconsistent. Many other studies have also shown increases in herbivore populations (Govaerts et al., 2007; Overstreet et al., 2010; Sanchez-Moreno et al., 2006; Treonis et al., 2010). Tillage effects on herbivore populations were probably due to changes in soil properties since herbivore populations did not seem to be related to plant growth in this case.

Fertilizer-nematicide effects on trophic group populations were much more pronounced at the soybean-corn site than the corn-soybean site. At the corn-soybean site aldicarb nematicide had negative impact on soil health as it reduced total nematode, and fungivore populations suggesting fungal and overall biological activity was also decreased which is consistent with other studies (Sanchez-Moreno et al., 2010; Timper et al., 2012; Wang et al., 2006). However, nematicide impacts were inconsistent as

aldicarb did not affect the nematode community at the soybean-corn site, and terbufos did not clearly affect the nematode community at either site.

At the soybean-corn site in the year fertilizers were applied, manure clearly affected the nematode community much differently than any other treatment with very strong effects at midseason that were still detectable by fall. At both midseason and fall, there was greater total nematode and bacterivore populations under manure than other treatments suggesting higher biological activity due to the influx of diverse compounds and nutrients available for microbial growth (Hernandez et al., 2007). Particularly, as an organic material, manure contains organic carbon sources which fuel microbial metabolism and thus population growth (Wolf and Wagner, 2005) while NPK and NPKS contained no carbon sources. This suggests manure application generates a nutrient flush and subsequent microbial population increase leading to the observed bacterivore population increase, specifically the enrichment opportunists Rhabditidae, through fall. Other studies on swine manure found similarly increased bacterivore abundance compared with inorganic fertilizers (Bulluck et al., 2002; Hu and Cao, 2008; Leroy et al., 2009; Liang et al., 2009; Villenave et al., 2010). These results suggest organic fertilizers provide resources to the soil community that inorganic fertilizers do not. Additionally, bacterivore population remained increased under manure treatment in midseason 2012 suggesting manure had residual benefits for the nematode community.

The effects of manure on the soil community at the soybean-corn site in initial year of application were enhanced by minimizing tillage as manifested by stronger differences in bacterial activity and biological activity at midseason under minimum compared with conventional tillage. Contrary to the results of this study, incorporation of manure into the soil with tillage maximizes retention of nutrients in the soil (Hernandez and Schmitt, 2012) which should enhance the effects of manure on the nematode community. In this study, nutrients may have been more concentrated in the upper soil, where sampling occurred, under minimum tillage because manure was not incorporated into lower soil layers as done with conventional tillage. This tillage-manure interaction also dissipated through the season as it was less apparent at fall.

5.4.2 Diversity Indices

In 2011 soybean, manure application resulted in a community with less diversity than any other treatment due to its dramatic enhancement of enrichment opportunistic bacterivores. By fall, manure effects had dissipated such that while bacterivores were

generally more prevalent than other trophic groups under manure compared with other treatments, there was not significant change in overall community diversity. Aldicarb nematicide application increased dominance of nematodes in soybean plots at the 2012 midseason, suggesting that the nematicide had uneven effects, reducing abundance of some genera while having minimal effect on others which is similar to the effects of other nematicides (Pen-Mouratov and Steinberger, 2005; Sanchez-Moreno et al., 2010; Wang et al., 2006).

5.4.3 Maturity Indices

At the corn-soybean site, conventional tillage consistently decreased overall and herbivore nematode community stability (shift toward colonizers) and succession as shown by PPI and Σ MI, but did not affect stability of the free-living (non-herbivores) nematode community as shown by MI. This suggests tillage is more disruptive to herbivores than other nematodes in contrast to other studies, which suggest tillage disrupts the entire community (Okada and Harada, 2007; Villenave et al., 2009). Even among nematodes classified as persisters, sensitivity to specific types of disturbance varies (Fiscus and Neher, 2002), so it is possible tillage selected for free-living nematodes throughout the colonizer-persister scale that are adapted to soil physical disturbance. Since herbivores are dependent on host plants, they may be strongly adapted to infect their host rather than to survive physical disturbance. Tillage effects on maturity indices did not appear to be reflective of plant growth. The absence of tillage effects at the soybean-corn site again suggests tillage effects are site-specific and may depend on soil characteristics.

As with other measures, fertilizer effects on maturity indices were predominantly observed at the soybean-corn site. In the initial year of application in the soybean plots, manure disturbed soil mainly by enrichment as disturbance was not detected when enrichment was ignored. This suggests the number of enrichment opportunists increased, but the rest of the community remained stable implying bottom-up rather than top-down impacts on nematode community structure (Bongers and Korthals, 1993; Treonis et al., 2010; Yeates, 1994). The soil community was also disturbed by manure at the corn-soybean site although not as strongly as at the soybean-corn site. Similar studies found little effect of manure on nematode community structure (Bulluck et al., 2002; Leroy et al., 2009; Liang et al., 2005; Villenave et al., 2010). Through fall of the residual year at the soybean-corn site only, residual enrichment resulted in more

disturbed soil communities under manure than synthetic fertilizer at both midseason and fall. As organic matter in manure is decomposed over time, nutrients are released into inorganic, plant-available forms (Diaz et al., 2012; Hernandez and Schmitt, 2012; Myrold, 2005; Wolf and Wagner, 2005) and complex carbon structures such as lignin and cellulose are decomposed into simpler carbohydrates available to a wider range of microbes (Hernandez et al., 2007; Wolf and Wagner, 2005) resulting in residual benefits for plants and microbes.

Nematicides generally have negative impacts on soil health by disturbing nematode community structure as they kill nematodes (Bongers, 1990; Ettema and Bongers, 1993; Sanchez-Moreno et al., 2010; Timper et al., 2012; Wang et al., 2006). Terbufos nematicide had different effects at the two sites resulting in a more disturbed soil nematode community in soybean, but less disturbed soil nematode community in corn. Aldicarb did not impact soil maturity in 2012 corn, but had positive impacts on nematode community maturity in 2012 soybean. It is possible that nematicide was more effective, in the short term, against colonizer than persister-type herbivores, possibly because nematicide was more acutely toxic to the active colonizers than the more sedentary persisters, resulting in more highly structured herbivore community. By definition persisters should be more susceptible to disturbance including nematicide, although this may reflect the long period required for persisters to recover from nematicide rather than greater acute susceptibility to nematicide (Bongers, 1990; Bongers et al., 1997). Another explanation is that increased herbivore community structure reflects increased plant production in this case.

5.4.4 Food web indices

Tillage had minimal impact on soil web condition based on nematode community indices despite strong impacts on nematode populations. Tillage breaks up and releases nutrients from organic matter and oxygenates the soil which can cause a short-term nutrient flush, but long-term depletion of organic matter (Holland, 2004) which should impact food resources for nematodes and soil web condition. However, other studies did not detect tillage effects on soil enrichment (Bulluck et al., 2002; Sanchez-Moreno et al., 2006; Villenave et al., 2009) although some suggested conventional tillage enriches the nematode community (Okada and Harada, 2007; Treonis et al., 2010).

Nematicides had various effects on the food web at different sites and crops. At the corn-soybean site in fall 2011, terbufos nematicide produced a less stressed

environment (basal) compared with other treatments under CT, but a less basal community than others under MT, suggesting that tillage may change the soil properties in ways that make nematicide more impactful. At the soybean-corn site in fall under minimum tillage, terbufos had a negative impact on soil health creating a more basal soil nematode community with fewer resources and more environmental stress which is similar to other studies (Ettema and Bongers, 1993; Sanchez-Moreno et al., 2010; Timper et al., 2012; Wang et al., 2006). However, nematicide with NPKS had seemingly positive impact as it promoted fungal pathways which can indicate a more mature soil (Ferris et al., 2001; Neher et al., 1995; Neher and Campbell, 1996) although in this case it may reflect lack of resources decreasing bacterial population.

Fertilizer application had greater impact on food web indices at the soybean-corn site than the corn-soybean site. At the soybean-corn site in year of application, the soil food web was more enriched under manure than other treatments due to the previously described influx of resources for microbial growth. As would be expected with the implied increase in bacterial population, decomposition pathways were much more strongly bacterial as opposed to fungal under manure at midseason. Similar studies also suggested swine manure enriches the nematode community (Bulluck et al., 2002; Liang et al., 2009; Villenave et al., 2010), but suggested decomposition pathways were shifted toward fungi not bacteria (Leroy et al., 2009; Liang et al., 2009; Liang et al., 2005). As with trophic group abundance, the effects of manure on food web condition were enhanced by minimum compared with conventional tillage at midseason in the initial year.

Results also suggest there are some residual effects of previous year fertilizer on the soil food web, especially at the soybean-corn site, although diminished from first-year effects. At fall 2012, nematode communities at the soybean-corn site remained more enriched under manure than synthetic fertilizer treatments suggesting lingering enrichment of soil nutrient resources. As organic matter in manure is decomposed over time, nutrients are released, resulting in residual benefits for plants and microbes. Additionally, the number of trophic links/stage of succession was later under manure than some treatments at spring. A possible explanation is manure induced a bottom-up effect on the nematode community with the populations of lower trophic groups and colonizers increasing rapidly after initial influx of resources in 2011, while higher trophic groups or persister-type nematode populations grew in 2012 as resources gradually

moved up the food chain leading to a more highly structured community (Treonis et al., 2010).

6. Conclusions

Objective 1: Determine effect of fertilizer/nematicide and tillage practices on SCN, fungal parasites of SCN and other plant-parasitic nematodes. Conventional tillage had minimal benefits for managing plant-parasitic nematode populations. SCN and *Helicotylenchus* populations were increased by fertilization, probably due to increased plant growth. Aldicarb application effectively reduced both SCN and *Helicotylenchus* population densities in soybean while there was no evidence that terbufos reduced plant-parasitic nematode population densities. Management practices did not affect fungal parasites of SCN at this site.

Objective 2: Analyze effects of fertilizer/nematicide, tillage, and plant-parasitic nematodes on corn and soybean yield. Conventional tillage increased corn yields, but had inconsistent impact on soybean yield. Manure fertilization was beneficial to corn and soybean yield in both initial and residual years of application. NPK fertilization was beneficial for corn and soybean yield, except as a starter fertilizer in soybean. Sulfur fertilization had some benefits for corn and soybean yield. Terbufos nematicide was not beneficial for corn yield and inconsistent for soybean yield. While aldicarb was not beneficial for soybean yield, it increased corn yield under some conditions. Additionally, aldicarb nematicide did not affect plant-parasitic nematode soil populations in corn plots, so the impact of plant-parasitic nematodes on corn yield was unclear from this study.

Objective 3: Investigate effects of fertilizer/nematicide and tillage on soil health using soil chemical/physical measures and the nematode community. In this study, tillage and fertilizer treatments had minimal impact on soil chemical/physical properties. Manure application had the strongest impact on the nematode community with strong enrichment and overall positive impact on soil health despite some disruption of the nematode community. In contrast, synthetic fertilizer had little impact on the soil nematode community. Nematicide had inconsistent impacts on the nematode community with seemingly positive and negative impacts on soil health with effects potentially mediated by plant growth. Tillage also had mixed effects on the nematode community including increased beneficial nematode population densities but decreased community structure.

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