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Crop rotation and tillage affect nematode communities more than biocides in monoculture soybean

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ABSTRACT

Long-term monoculture of susceptible soybeans naturally develops suppressiveness to soybean cyst nematode (SCN) *Heterodera glycines* if fields are not tilled or biocides applied. Nematode community indices, that integrate the responses of different taxa and trophic groups to perturbation, provide a tool to monitor the ecological status of soil communities. We tested the hypothesis that soil suppressiveness to *Heterodera glycines* is correlated positively to management practices that favor both greater trophic diversity (food web complexity) and a later stage ecological succession (less disturbance) within free-living nematode communities. A factorial combination of cultivation, crop rotation, and biocide application treatments were monitored for four years in a field with a history of no-till and monoculture of susceptible soybean for 15 years. Crop rotation had the greatest impact on nematode community index values followed by descending order of cultivation and biocides. Suppressive soils did have greater food web complexity, but not necessarily ecological succession. Nematode community composition was influenced by covariables nitrogen and organic matter content (mean 6.6%), but not pH or salinity. The study is novel by using a food web approach that includes multiple trophic levels rather than simply population ecology.

1. Introduction

Soybean cyst nematode (SCN) *Heterodera glycines* Ichinohe has become a major pest problem in the soybean (*Glycine max*) producing regions in the world (Riggs, 2004). It causes an estimated annual crop loss that ranges from \$500 million to \$1.5 billion in the USA (Koenning and Wrather, 2010). A rotation of corn (*Zea mays*) as a nonhost in years alternating with susceptible soybean is practiced in the North Central region as a management strategy for SCN, but pathogen populations may continue to increase. Resistant cultivars of soybean generally increase yields in SCN-infested fields, but not if SCN population densities exceed 5000 eggs/100 cm^3 soil (Warnke et al., 2008).

Continuous cultivation of susceptible soybean exceeding five years may exhibit a natural suppression for SCN, as demonstrated in the southern USA (Hartwig, 1981) and several locations in China (Sun and Liu, 2000). Populations of SCN increased in the first few years and, thereafter, declined to a level that resulted in no economic damage to soybean. So far, this phenomenon has been found for at least six diseases caused by cyst nematode species including *Heterodera avenae* in cereals, *H. glycines* in soybeans, *H. schachtii* in sugar beets, *H. cruciferae* in cabbage, *Globodera pallida* and *G. rostochiensis* in potato (Kerry, 1988). Hyperparasites are believed responsible for the decline in nematode populations, but types or species of hyperparasites involved have not been determined (Kerry, 1988). Alternative mechanisms behind the natural suppressive soils are also poorly documented.

A field under monoculture of soybean with no-tillage system for > 15 years, found to be naturally suppressive to SCN, is the platform for this study. Short-term, greenhouse experiments suggest that biological factors contributed to nematode suppression. *Hirsutella rhossiliensis* was observed in the soils and parasitized a large percentage of SCN second-stage juveniles (J2) (Chen, 2007). Both biocide treatments and mixing soil (to mimic cultivation) increased SCN egg population density and reduced the proportion of J2 parasitized by fungi (Bao et al., 2011). In addition, values of nematode community diversity index decreased and values of trophic group dominance and maturity indices increased with mixing of soil (Bao et al., 2011). These indices require a minimum of trophic or family level identification (Cheng et al., 2018; Grabau and Chen, 2016b). Genus level investigation is more powerful and meaningful than trophic group, because genera within a trophic group or family can respond differently to the same disturbance (Fiscus and

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Neher, 2002).

Most of the nematode-suppressive soils research has been conducted in the greenhouse and not in the field (Chen, 2007). Herein, we determine whether observations in the greenhouse can be repeated in a field environment. Furthermore, most nematology research has focused on nematodes as pathogens or parasites rather than the contribution of free-living nematodes and their role in nutrient cycling, decomposition and other beneficial ecological processes (Neher, 2010). To our knowledge, this study represents the first to determine whether communities of free-living nematodes in soils naturally suppressive to a major plant-parasitic nematode differ from soils that are conducive to disease.

The major objective of this study was to compare composition of free-living nematode communities associated with suppression of *Heterodera glycines*. The hypothesis tested was that free-living nematode communities change when natural suppression is disrupted. A companion study provided evidence that the non-treated (no-till, soybean monoculture) control was suppressive to SCN (Kidane et al., 2012a,b). Treatments in this study were chosen as management practices expected to disrupt suppression, as a means to deduce other mechanisms.

2. Material and methods

2.1. Site description

The research was conducted in a field exhibiting natural suppression to SCN at the Southern Research and Outreach Center in Waseca, Minnesota, USA. The field site has been managed as no-till and planted to susceptible soybean monoculture for > 15 years. The soil was a Nicollet clay loam (fine loamy, mixed, mesic Aquic Hapludoll). The soil pH was 6.5 ± 0.43 (here and further SD is reported), total nitrogen content was 20.7 ± 10.16 mg/kg of soil, and organic matter content was 6.64 ± 0.97%. The mean SCN egg population density at planting in 2009 was 4326 eggs/100 cm^3 soil. The soil was demonstrated (by a greenhouse bioassay) to be suppressive to SCN (Bao et al., 2011; Kidane et al., 2012a,b). Autoclaving or formalin application removed suppressiveness suggesting it was microbial in nature, and suppression could be restored by adding 10% untreated field soil (Chen, 2007). Yield did not differ significantly across treatments (Kidane et al., 2012a,b).

2.2. Experimental design

The experiment was designed as a full factorial split-plot with no-till and conventional tillage as main plots, and five crop sequence-biocide treatments as subplots. Each experimental unit was 7.6 m long and 4.57 m wide, each containing six rows of crops. Each treatment combination was replicated four times per year. Experimental plots were sampled at three times during the cropping season (planting, midseason and harvesting) for duration of four years (2009, 2010, 2011, and 2012). In total, there were 480 samples (10 treatment combinations × 4 replications × 3 seasons × 4 years). Main plots were either conventional tillage (CT) or remained as no-till (NT). All the agronomical practices were the same in CT and NT except plowing. The conventional tillage treatment was fall chisel plowing after harvesting soybean (2008 and 2010), moldboard plowing (including both corn and soybean plots in 2009 and 2011) after harvesting corn and soybean, and field cultivation followed by a finishing implement prior to planting.

Subplots were five-fold, including one crop rotation, three biocide and one control treatments. Corn (cultivar KD 4661) was planted in rotation with susceptible soybean (cultivar Pioneer brand 92B13). Three different biocide treatments, bactericide (streptomycin), fungicide (captan) and broad spectrum biocide (formalin) were applied to quantify the effect of bacteria and/or fungi on the suppression of SCN. Captan (*N*-trichloromethylthio-4-cyclohexene-1, 2-dicarboximide) as was applied at 27 g per 200 L that delivered 11.6 g active ingredient of 80% wettable powder (Ingham and Coleman, 1984; Ingham et al., 1991). Streptomycin (streptomycin sulfate Sigma S 5601) at 18 g per 200 L of water to give 7.75 kg active ingredient/ha (Ingham and Coleman, 1984; Ingham et al., 1991), and formalin (38% formaldehyde) at 6.8 L per 220 L water (Williams, 1969).

Captan and streptomycin were applied manually in the four central rows two weeks before planting and every two weeks after planting for two months (five times per year). Formalin was applied by irrigation in the four central rows (3 m wide) three weeks before planting. The irrigation system was set up before applying biocides. In each plot, two 180 L tanks were used and three irrigation pipes emerged from each tank and were positioned on the ground. Tanks were placed at 1.2 m height so that there was sufficient pressure for water to go through the pipe and distribute the solution evenly in the plot (Kidane et al., 2012a,b). Formalin irrigation was applied to bare ground without any plastic sealing. Soil samples were taken in a systematic pattern across the two central rows in each subplot in each season. Crop residues were removed from the surface before sampling and a soil sample consisting of 25 to 30 soil cores (2 cm diameter, 20 cm deep) from each plot. The number of soil cores depended on season and tillage. Soil samples were mixed thoroughly and subsamples of 300 cm^3 soil/plot were sent to the University of Vermont by 2-day express delivery to avoid the temperatures fluctuation during transit. Soil samples were stored at 15 °C to maintain consistent nematode community composition (Barker et al., 1969) until extraction of fauna was completed.

2.3. Data collection

2.3.1. Nematode extraction and community structure

Nematodes were extracted from 200 ± 3.1 g of fresh soil from each experimental unit using a modified Cobb's decanting and sieving method. A water slurry of nematodes was passed three times through each of six different USA standard testing sieves (A.S.T.M. E-11 specifications): No. 20-mesh sieve (840 µm), No. 60-mesh sieve (250 µm), No. 100-mesh sieve (140 µm), No. 200-mesh sieve (73 µm), and No. 325-mesh sieve (43 µm) and final pass was through a No. 400-mesh sieve (38 µm). This was followed by placing the nematode solution on a double cotton-wool filter extraction tray for 48 h (s'Jacob and van Bezooijen, 1984). This method requires that nematodes actively swim through the fine spaces in the filter into the water below.

Collected samples were allowed to settle by gravity for 24 h at 15 °C and the volume adjusted to 100 ml in Nalgene bottles prior to nematode enumeration. Ten ml of subsample was taken from each 100 ml sample (10%) to estimate total abundance per sample using an Olympus CX41 light compound microscope with Hoffman modulation with 100 to 200× magnification. A minimum of 150 random individuals per sample were identified using the keys of Andrássy (1983), Bongers (1988), Jairajpuri and Ahmad (1992), Maggenti et al. (1987), Siddiqi (2000), and Thorne (1974). If fewer than 150 nematodes were harvested in a sample, all recoverable nematodes were identified. Identifications were performed using an upright Olympus (Model B5ITF) compound microscope with differential interference contrast (DIC) and observed at 100 to 400× magnification.

Taxonomic families were assigned to trophic groups (Yeates et al., 1993). Families of nematodes were assigned CP values, reflecting life history characteristics associated with stages ecological succession (Bongers, 1990; Bongers et al., 1991, 1995; Table 1). Additional samples taken from the same plot were dried at 60 °C to provide the dry weight to determine gravimetric moisture. Abundance of nematodes was expressed as number per gram of dry soil.

Indices to estimate tropic diversity, generic diversity, and successional maturity indices of nematode communities (plant-parasitic and/or free-living) were calculated. As a measure of food web complexity, trophic diversity Hills N1 index was computed as exp - $\Sigma[Pi(\ln Pi)]$ where Pi is the proportion of trophic group i in the total nematode community (Neher and Darby, 2006). Genus diversity (*N1* genus) was

Table 1
Nematode genera assigned to family and colonizer-persister value. All genera were included in the analysis of covariance, but only those genera found in at least 5% of total samples ($n = 440$) were included in the principal response curves (PRC) analysis. The right-most column is abundances of genera in the naturally suppressive soil (no-till, soybean monoculture, not treated with biocides) at midseason.

Genus	Family	*c-p* value[a]	*Incidence* (% of samples) $n = 440$	*PRC*	*Suppression control* mean ± 1 SE (#/100 g) $n = 16$
Bacterivores					**632.0 ± 129.89**
Acrobeles	Cephalobidae	2	3.4		0 ± 0
Acrobeloides	Cephalobidae	2	99.1	√	355.8 ± 78.20
Acrolobus	Cephalobidae	2	3.2		0 ± 0
Alaimus	Alaimidae	4	2.7		0 ± 0
Cephalobus	Cephalobidae	2	35.9	√	9.5 ± 5.25
Cervidellus	Cephalobidae	2	72.0	√	41.2 ± 20.40
Chiloplacus	Cephalobidae	2	29.1	√	3.3 ± 3.28
Chronogaster	Leptolaimidae	2	27.3	√	12.7 ± 4.12
Eucephalobus	Cephalobidae	2	93.0	√	101.0 ± 18.8
Eumonohystera	Monhysteridae	2	24.3	√	1.8 ± 1.21
Mesorhabditis	Rhabditidae	1	44.8	√	0 ± 0
Panagrolaimus	Panagrolaimidae	1	62.0	√	7.0 ± 5.85
Plectus	Plectidae	2	80.9	√	42.4 ± 8.2
Prismatolaimus	Prismatolaimidae	2	45.9	√	8.3 ± 3.02
Pristionchus	Neodiplogasteridae	1	49.1	√	11.6 ± 4.81
Rhabditis	Rhabditidae	1	36.1	√	37.1 ± 20.2
Wilsonema	Plectidae	2	8.0	√	0.47 ± 0.32
Fungivores					**433.2 ± 92.19**
Aphelenchoides	Aphelenchoidae	2	99.5	√	260.3 ± 64.10
Aphelenchus	Aplelenchidae	2	96.8	√	143.4 ± 28.09
Boleodorus	Tylenchidae	2	5.5	√	0 ± 0
Diphtherophora	Diphtherophoridae	3	3.2		0 ± 0
Ditylenchus	Anguinidae	2	90.5	√	26.7 ± 8.02
Filenchus	Tylenchidae	2	45.0	√	1.8 ± 0.90
Pseudaphalenchus	Anguinidae	2	14.8	√	0 ± 0
Tylenchus	Tylenchidae	2	13.2	√	1.0 ± 0.86
Plant-parasites					**239.2 ± 56.11**
Anguina	Anguinidae	2	3.9		0 ± 0
Basiria	Tylenchidae	2	2.5		0.58 ± 0.39
Cephalenchus	Tylodoridae	2	2.5		0 ± 0
Helicotylenchus	Hoplolaimidae	3	90.5	√	115.0 ± 21.61
Heterodera	Heteroderidae	3	91.8	√	118.6 ± 39.37
Longidorus	Longidoridae	5	3.4		1.0 ± 0.86
Pratylenchus	Pratylenchidae	3	5.2	√	0 ± 0
Psilenchus	Psilenchidae	2	32.5	√	4.1 ± 2.07
Rotylenchus	Hoplolaimidae	3	3.6		0 ± 0
Trichodorus	Trichodoridae	4	2.0		0 ± 0
Omnivores-predators					**41.2 ± 11.23**
Aporcelaimium	Aporcelaimidae	5	25.2	√	2.8 ± 1.53
Aporcelaimus	Aporcelaimidae	5	9.3	√	0.46 ± 0.32
Axonichium	Belondiridae	5	37.7	√	3.6 ± 1.52
Clarkus	Mononchidae	4	10.5	√	0 ± 0
Diplogaster	Diplogasteridae	1	25.0	√	0.46 ± 0.32
Discolaimus	Discolaimidae	4	17.5	√	6.1 ± 4.27
Dorylaimoides	Leptonchidae	4	15.2	√	0.38 ± 0.38
Epidorylaimus	Qudsianematidae	4	12.3	√	5.7 ± 3.88
Eudorylaimus	Qudsianematidae	4	47.3	√	3.4 ± 1.59
Mesodorylaimus	Thornenematidae	4	11.8	√	0 ± 0
Paraxonichium	Aporcelaimidae	5	9.5	√	0.97 ± 0.53
Seinura	Aphelenchoidae	2	23.2	√	0.47 ± 0.32
Thonus	Qudsianematidae	4	32.3	√	6.7 ± 3.05
Tripyla	Tripylidae	3	46.6	√	10.2 ± 2.76
Total nematodes					**1345.7 ± 244.71**

[a] Colonizer-persister (*c-p*) values of 1–5 were assigned according to Bongers (1990) with Monhysteridae re-assigned as *c-p* group 2 (Bongers and Bongers, 1998).

computed similarly but *Pi* represented proportion of genus *i* in the total nematode community. Hills indices are simpler to interpret ecologically than commonly used Shannon forms. N1 values represent the number of abundant *i* groups. Successional maturity indices were computed three ways, i.e., fungivores/{fungivores + bacterivores} (F:B), free-living nematodes with CP2 through CP5 (MI25), plant-parasitic nematodes (PPI), and the combination of free-living and plant-parasitic nematodes (ΣMI25). These are standard names of the mentioned indices (Neher and Darby, 2006). Maturity indices are weighted means computed as Σ[CP-value (*i*) * f(*i*)]/[total numbers of nematodes] where (*i*) is the individual taxon and f(*i*) is the frequency of the taxa in a sample (Bongers, 1990). Two extensions of the maturity index were also computed, i.e., channel index (CI) and enrichment index (EI) (Ferris et al., 2001). Given the dearth of CP = 1 and omnivores, we chose not to calculate the structural index (Ferris et al., 2001).

2.3.2. Soil chemistry

Soil chemical properties were measured as co-variables. Soil pH was determined on 1:5 soil/water extract with a pH meter, and electrical conductivity (EC) was determined by 1:5 water using an EC meter (Smith et al., 1996). Soil organic matter content (OM) was determined by loss-on-ignition in a GS Blue metric furnace at 360 °C (Konen et al., 2002). Available nitrogen was extracted with 1 M KCl and filtered through Ahlstrom 642 paper. Ammonium-N (NH_4-N) was quantified by salicylate method (QuikChem Method 10-107-06-2-O), and nitrate-N (NO_3-N) was quantified by first reducing nitrate to nitrite and diazotizing with sulfanilamide (QuikChem Method 10-107-04-1-B). NH_4-N and NO_3-N were read at 660 and 520 nm, respectively, on a Latchat analyzer (Hach, Colorado, USA).

2.4. Statistical analysis

A full model, repeated measures split-plot analysis of covariance (ANCOVA) was performed on mid-season samples with year as the repeated variable using the MIXED procedure. Mid-season was chosen because this is the only sampling time each year in which the soil properties were also measured on each sample. A split-plot model was used treating main and subplots as fixed variables, and block and the 2-way interaction of block and tillage as random variables. Soil chemical properties (pH, EC, NH_4-N, NO_3-N and OM) were included as co-variables. NH_4-N, NO_3-N and OM were transformed as ln (x + 0.01). Convergence was met without any autoregressive adjustments (using default).

Proportions of nematodes by trophic groups, MI25, ΣMI25, PPI, F:B, N1 genus, N1 trophic, CI and EI values were included as dependent variables. Orthogonal contrasts were performed, regardless of ANOVA results, to test effects of subplot management treatments in comparison with no biocide as a control: 1) effect of crop rotation, 2) application of formalin, 3) application of streptomycin, and 4) application of captan. Data were analyzed for normality using the UNIVARIATE procedure prior to ANOVA using SAS software version 9.4. Proportions were transformed as the arcsine of the square root to meet assumptions of a Gaussian distribution. Given their scarcity, it was necessary to combine omnivores and predators before the transformation to meet assumptions of parametric statistics.

Principal response curve (PRC) analysis was used as multivariate repeated measures analysis, to quantify and represent the impact of tillage, rotation and biocide on nematode genus as a function of three seasons per year of the experiment (van den Brink et al., 2003). PRC is based on redundancy analysis (RDA), and adjusted for overall changes in community response through time, defining the naturally suppressive soil as the x-axis (van den Brink et al., 2003). The treatments and seasons were treated as nominal (0, 1) environmental and co-variables, respectively to allow the significance of the treatment regime to be tested per season. This can be achieved by modeling the abundance of each particular nematode variable as a sum of three terms, namely its mean abundance in the control, a time-specific treatment effect, and an error (van den Brink et al., 2003). For simplicity, biplots were restricted to illustrate only the 20 genera that explained the most variation. PRC was performed using 'CANOCO' software, version 5.0 (Šmilauer and Lepš, 2014). Statistical significance was computed by Monte Carlo permutation of both first ordination axis and all axes together.

3. Results

3.1. Nematode community composition

Of the 49 genera detected at the site, 39 were present in at least 5% of the samples (Table 1). Overall 17, 8, 10, and 14 genera of bacterivores, fungivores, plant-parasites, and omnivores-predators were enumerated, respectively. Of the trophic groups, bacterivores were most abundant, followed progressively by fungivores and plant-parasites (Table 1). As a main effect, tillage increased relative abundance of bacterivores and decreased plant-parasites (Table 2). Effect of tillage on relative abundance of fungivores and omnivore-predators depended on crop-biocide treatment. Relative abundance of fungivores decreased with tillage without biocide and application of captan or formalin, but increased with rotation to corn or application of streptomycin (Table 2). Tillage without biocide increased their abundance, but tillage with streptomycin decreased their abundance (Table 2).

Heterodera and *Helicotylenchus* dominated the plant-parasitic nematodes (Table 1), and responded inversely to treatment combinations (Fig. 1). Free-living nematodes that characterized the natural suppressive soil across years and seasons contained a common core of bacterivores (*Wilsonema*) and omnivore-predators (*Aporcelaimus* or *Aporcelaimium*, *Clarkus*, *Dorylamoides*, *Eudorylaimus*, and *Paraxonchium*) (Figs. 2–4). Genera of fungivores were inconsistent in the suppressive control.

As covariables, OM and the form of nitrogen affected nematode community composition, but not pH or salinity (Table 2). Both MI25 and ΣMI25 values were associated positively with NH_4-N. Genus N1 was associated positively with NH_4-N, negatively with NO_3-N, and negatively with OM. CI was associated negatively NH_4-N and positively with NO_3-N (Table 2).

3.2. Crop rotation

Crop rotation had the greatest impact on nematode community index values followed by descending order of cultivation and biocides (Table 2). Rotation to corn decreased food web complexity (trophic N1), genus N1, and PPI values (Table 2). Relative abundance of fungivores increased and bacterivores decreased with rotation to corn, compared to the suppressive control (Table 2). These shifts are reflected as increased values of F:B, CI and EI when rotation was applied, compared to the suppressive control (Table 2). Compared to monoculture soybean, rotation to corn reduced *Acrobeloides* and *Heterodera*, and increased *Aphelenchoides*, *Aphelenchus*, and *Ditylenchus* (Fig. 2). These changes increased when corn was planted in 2009 and 2011, and drifted back toward the monoculture in the years that soybean was planted, but never quite reached the soybean monoculture baseline.

3.3. Tillage

Over the four years, tillage consistently affected the relative abundance of trophic groups, plant-parasites (decreased) and bacterivores (increased) but did not affect community indices of nematodes (Table 2). Compared to no-till, conventional tillage increased abundances of *Aphelenchoides*, *Aphelenchus*, *Acrobeloides*, and *Ditylenchus*, especially at harvest in the first three years of the experiment (Fig. 3). Abundance of both *Helicotylenchus* and *Heterodera* increased temporarily at planting in tilled treatments (Fig. 3).

3.4. Biocides

Application of streptomycin decreased values of ΣMI25, and captan increased genus N1. All three of the biocides increased EI (Table 2). Biocides had no effects on trophic N1, MI25, F:B, PPI, or CI. Biocide application generated seasonal fluctuations within nematode communities (Fig. 4). Temporal patterns of captan and formalin appeared relatively synchronous (with peaks at harvest) and counter to streptomycin (peak at planting).

3.5. Interaction between tillage and biocides

A two-way interaction of tillage and crop-biocide affected abundance of omnivore-predators and EI values (Table 2). Relative abundance of omnivore-predators increased with tillage in the no-biocide suppressive soil and decreased with tillage when streptomycin was applied. EI values decreased with tillage in plots without biocide or formalin application, but increased with tillage when streptomycin or captan was applied. Genus N1 values decreased when no-biocide suppressive soils were tilled, but increased with tillage when captan was applied.

Table 2

Repeated measures two-way analysis of covariance of soil nematode trophic group (relative abundance) and community indices (means ± 1 SE). Only mid-season was analyzed, 4 years combined (n = 160). Planting and harvest data are available in Table S1. pH and electrical conductivity were additional covariables but not significant so not illustrated. Values in the treatment columns (rotation and monoculture soybean) are untransformed means (n = 16). *Italics* represent contrasts that differ significantly from the naturally suppressive soil (bold).

Response[d]	Expt. design[a]			Orthogonal contrasts[b]				Rotation		Monoculture soybean
								No biocide		No biocide
	T[a]	CB	T*CB	R	S	C	F	NT	CT	NT
% PlPar	*							14.5 ± 2.2	7.7 ± 1.2	**17.6 ± 2.0**
% Bact	*	***		***				*23.5 ± 4.1*	*26.9 ± 3.1*	**44.4 ± 2.4**
% Fung		***	*	***				*58.4 ± 4.3*	*61.0 ± 4.1*	**34.1 ± 3.2**
% Omni-Pred			*		*			3.6 ± 0.4	4.4 ± 0.7	**3.9 ± 1.1**
F:B		***		***				*0.71 ± 0.05*	*0.69 ± 0.04*	**0.43 ± 0.03**
PPI		***	***	**				*2.98 ± 0.01*	*2.76 ± 0.06*	**2.99 ± 0.01**
MI25								2.07 ± 0.01	2.11 ± 0.02	**2.09 ± 0.03**
ΣMI25					*			2.20 ± 0.03	2.16 ± 0.02	**2.25 ± 0.03**
% CI		***	*	***				*86.9 ± 4.12*	*85.4 ± 5.17*	**72.8 ± 5.88**
% EI		***	*	***	*	*	*	*76.5 ± 4.25*	*75.1 ± 2.59*	**53.3 ± 2.45**
Trophic N1		***		**				*2.6 ± 0.11*	*2.6 ± 0.13*	**3.1 ± 0.09**
Genus N1		***		***		*		*6.3 ± 0.50*	*7.3 ± 0.63*	**8.6 ± 0.49**

Response[d]	Monoculture soybean							Covariables[c]		
	No biocide	Streptomycin		Captan		Formalin				
	CT	NT	CT	NT	CT	NT	CT	OM	NH_4	NO_3
% PlPar	11.3 ± 2.8	13.8 ± 1.6	8.6 ± 1.6	14.6 ± 1.7	11.6 ± 1.4	12.1 ± 0.01	10.4 ± 1.6			
% Bact	51.7 ± 2.8	40.7 ± 3.5	47.6 ± 3.0	37.5 ± 3.8	48.3 ± 2.0	38.6 ± 2.3	49.4 ± 3.1			
% Fung	32.5 ± 2.3	34.3 ± 4.0	38.8 ± 3.9	43.1 ± 3.4	35.4 ± 2.5	45.5 ± 2.2	32.8 ± 3.4			
% Omni-Pred	4.6 ± 0.8	*11.2 ± 4.0*	*5.0 ± 1.6*	4.7 ± 0.7	4.7 ± 0.7	3.8 ± 0.5	7.3 ± 1.2			
F:B	0.38 ± 0.02	0.44 ± 0.045	0.44 ± 0.04	0.54 ± 0.04	0.42 ± 0.03	0.5 ± 0.03	0.4 ± 0.04			
PPI	2.91 ± 0.03	3.0 ± 0.01	2.9 ± 0.03	3.0 ± 0.01	3.0 ± 0.04	3.0 ± 0.02	3.0 ± 0.01			
MI25	2.10 ± 0.02	2.1 ± 0.02	2.1 ± 0.01	2.1 ± 0.01	2.1 ± 0.01	2.1 ± 0.01	2.1 ± 0.02		* pos	
ΣMI25	2.19 ± 0.04	*2.2 ± 0.02*	*2.1 ± 0.02*	2.2 ± 0.02	2.2 ± 0.02	2.2 ± 0.02	2.2 ± 0.02		* pos	
% CI	75.8 ± 5.73	75.5 ± 6.85	66.9 ± 4.96	86.1 ± 4.22	64.3 ± 5.96	74.1 ± 4.04	71.5 ± 5.09		* neg	*** pos
% EI	48.7 ± 4.03	*53.7 ± 3.52*	*61.6 ± 2.81*	*58.9 ± 3.61*	*57.4 ± 2.18*	*64.7 ± 2.30*	*50.3 ± 4.01*			
Trophic N1	2.8 ± 0.13	3.2 ± 0.11	2.8 ± 0.12	3.0 ± 0.12	3.0 ± 0.06	3.0 ± 0.08	3.0 ± 0.10			
Genus N1	7.8 ± 0.75	8.2 ± 0.47	8.7 ± 0.59	*8.3 ± 0.47*	*9.1 ± 0.52*	7.1 ± 0.31	7.5 ± 0.46	* neg	** pos	** neg

[a] Split plot model with tillage (NT: no-till, CT: conventional till) and main effect and crop-biocide (CB) as subplot, and the two-way interaction (T*CB) with *: $p \le .05$, **: $p \le .01$, and ***: $p \le .001$.
[b] Single degree of freedom contrasts between each subplot treatment and the untreated control (no-till monoculture without biocide).
[c] Direction (neg: negative correlation with response variable, pos: positive correlation with response) of covariables with *: $p \le .05$, **: $p \le .01$, and ***: $p \le .001$. Empty cells represent $p > .05$.
[d] Trophic groups were arcsine ($\sqrt{x} + 0.01$) and soil properties as ln (x + 0.01) transformed before analysis: plant-parasites (% PlPar), bacterivores (% Bact), fungivores (% Fung), and omnivore-predators (% Omni-Pred). Soil properties are abbreviated as nitrate (NO_3), and ammonium (NH_4), and percent organic matter (OM). Community indices are abbreviated as fungivores / (fungivores + bacterivores) ratio (F:B), maturity index of plant-parasitic nematodes (PPI), maturity index of free-living nematodes (MI25), maturity index of free-living and plant-parasitic nematodes (ΣMI25), channel index (CI), enrichment index (EI), Hills 1 diversity of trophic groups (trophic N1) and Hills 1 diversity of genera (genus N1).

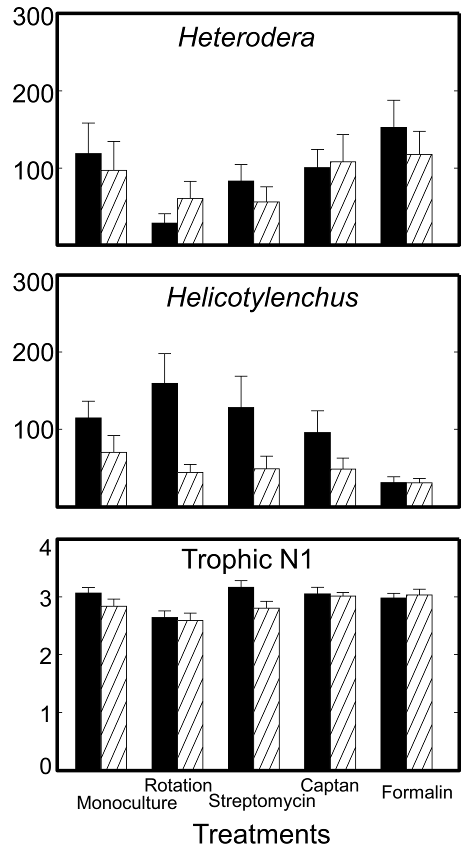

Fig. 1. Mean (± SE) abundance (individuals 100 g soil^{-1}) of vermiform *Heterodera*, *Helicotylenchus*, and trophic diversity (Trophic N1) from soil extractions in factorial combinations of tillage (solid: no-till, hatched: conventional till) and crop rotation-biocide treatments (n = 16). Abundance was measured mid-season across four years. The leftmost bar in each panel is the naturally suppressive soil.

4. Discussion

The study is novel by using a food web approach that includes multiple trophic levels rather than simply population ecology. Results support the hypothesis that free-living nematode communities change when natural suppression is disrupted. These disruptions are common management practices to reduce disease and, thus, increase yield for soybean. Tillage, crop rotation, and general biocides may reduce SCN populations and increase soybean yield but they alter free-living nematode communities in soil uniquely. Therefore, the differences among nematode communities in unamended and treated soils in this study represent a true test of differences among suppressive and conducive soil. Sensitivity of free-living nematode communities to various types of disruption factors (Fiscus and Neher, 2002; Zhao and Neher, 2013) reflect the relative importance of food web complexity and natural suppressiveness in monoculture soybean. Management practices that favor later ecological succession and greater trophic diversity of nematode communities in soils without tillage, absence of pesticides that target microbes, and avoidance of excess fertility (Neher, 2010).

Nematode community indices that integrate the responses of different taxa and trophic groups to perturbation provides a powerful basis for analysis of fauna assemblages in soil as in situ environmental assessment systems (Bongers, 1990; Bongers and Ferris, 1999; Ferris et al., 2001; Neher, 2001). However, the relationship between nematode community attributes and antagonists of plant-feeding nematodes is poorly documented (Neher, 2010), and we are unaware of any publication quantifying relationships between the nematode community indices and soil suppressiveness to plant-parasitic SCN nematodes. This study helps to reduce this gap in knowledge by testing the ability of indices of nematode community composition and structure to predict disease suppression.

Traditionally, monocultures of a susceptible host are a recipe for escalating disease. In response, rotation to a non-host is recommended. Indeed, crop rotation reduced populations of vermiform *Heterodera glyines* but it also reduced food web complexity (trophic N1) of the nematode community compared to the naturally suppressive soil. However, indices of food web complexity contradicted that of

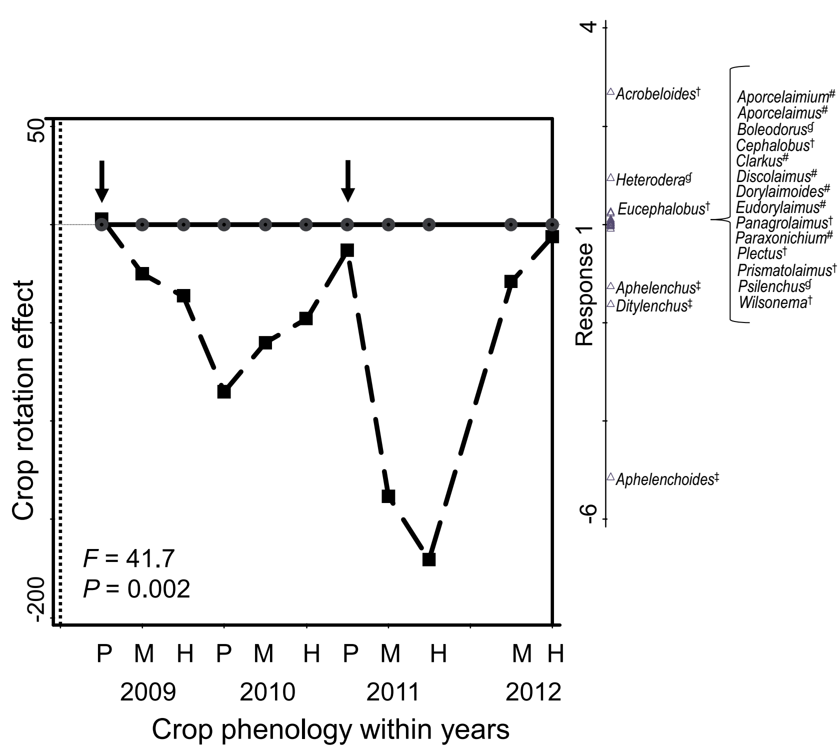

Fig. 2. Effect of crop rotation on nematode genus distribution compared to monoculture soybean. Neither crop has a biocide application. Principal response curve bi-plot of PRC (y-axis, crop rotation effect) and crop phenology (P: planting, M: midseason, H: harvest in 2009 to 2011, and M and H in 2012) are shown. Dashed line represents crop rotation and solid line for soybean monoculture as reference line. Years of corn planted are marked with arrows. Genus names are coded by trophic group (†: bacterivores, ‡: fungivores, ǥ: plant-parasites, #: omnivores-predators).

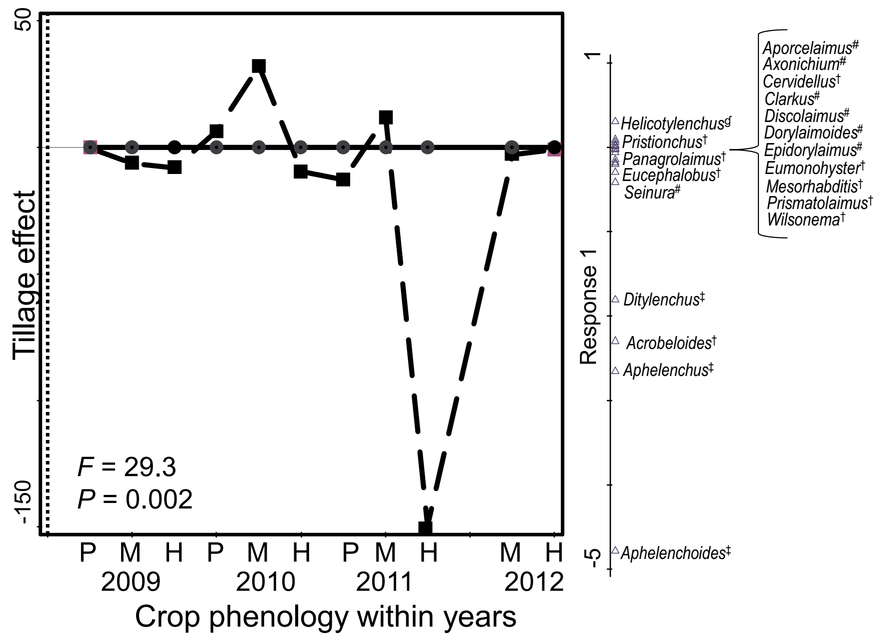

Fig. 3. Effect of tillage on nematode genus distribution. Principal response curve bi-plot of PRC (y-axis, till) and crop phenology (P: planting, M: midseason, H: harvest in 2009 to 2011, and M and H in 2012) are shown. Dashed line represents conventional tillage and solid line for no-tillage as reference line. Genus names are coded by trophic group (†: bacterivores, ‡: fungivores, ƍ: plant-parasites, #: omnivores-predators).

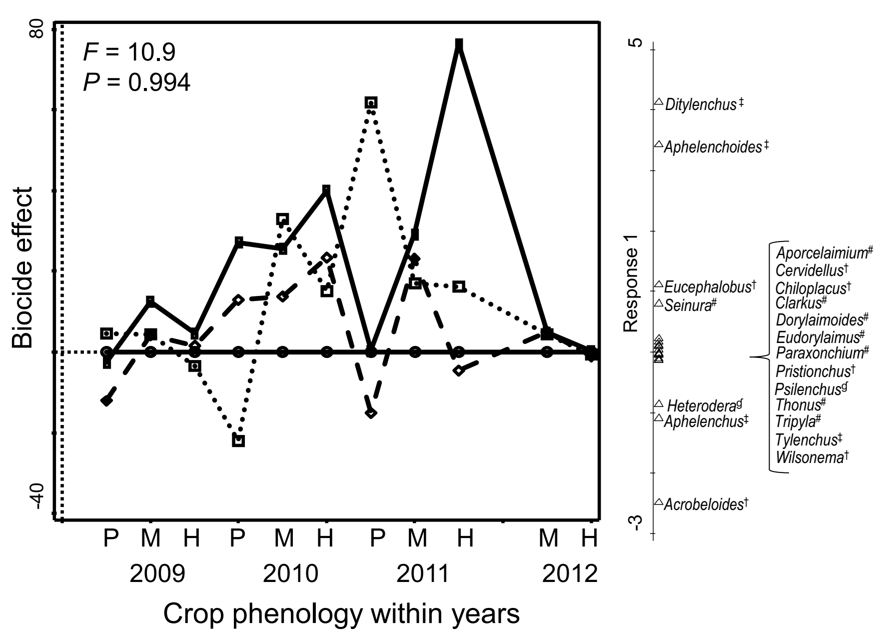

Fig. 4. Effect of biocide on nematode genus distribution. Principal response curve of PRC (y-axis, treatments) and crop phenology (P: planting, M: midseason, H: harvest in 2009 to 2012) are shown. Symbol represent biocide treatments (dotted: streptomycin, solid: captan, dashed: formalin) with no-biocide as horizontal reference line. Genus names are coded by trophic group (†: bacterivores, ‡: fungivores, ƍ: plant-parasites, #: omnivores-predators).

ecological succession in their prediction of disease suppressiveness to *Heterodera glycines*. Food webs dominated by bacterivores or relatively small values of F:B and CI, and large values of EI are considered early successional (Ferris et al., 2001; Neher and Campbell, 1994). Based on F:B and CI, food webs were less complex but successionally more mature in corn-soybean rotation than naturally suppressive soils. This finding supports a related study that also demonstrated relatively abundant fungivores in corn and more abundant bacterivores in soybean (Grabau and Chen, 2016b). Inconsistent with this statement are relatively high values of EI with corn rotation in this study. These values suggest that rotation to corn generated an enrichment effect comparable to fertility amendments.

These values suggest that rotation to corn generated an enrichment effect comparable to fertility amendments. Fertility-based enrichment depends on the chemical formulation, with inverse impacts for NH_4 and NO_3.

Relatively later stages of succession in the nematode community is congruent with hypotheses about soils with high OM (6.64 ± 0.97%) supporting high densities or diversities of soil microbes (Ghorbani et al., 2008; Grabau et al., 2018; Messiha et al., 2007; Weller et al., 2002), and, most notably, high densities, frequencies, or diversities of antagonistic populations (Adesina et al., 2007; Bonanomi et al., 2010; Renčo, 2013; Weller et al., 2002). Abundance of plant-parasitic nematodes is correlated negatively with OM (Norton et al., 1971). Organic amendments, including swine manure, add not only nutrients but microbes that matriculate through the food chain to support increased abundance of bacterivorous nematodes (Grabau et al., 2018). Amounts of organic matter affect predators of SCN which supports large amounts of saprophytic fungi (Ginitis et al., 1983). Antagonists with saprophytic abilities can be prey for microbial-feeding nematodes (Linford et al., 1938; Oka, 2010; McSorley, 2011). Many studies report additions of organic matter increase antagonists of nematodes, but few show that these organisms are responsible for suppression of plant-parasitic nematodes (Oka, 2010; McSorley, 2011). Others have proposed predacious nematodes would feed on plant-parasitic nematodes (Sánchez-Moreno and Ferris, 2007; Steel and Ferris, 2016; Tyler et al., 1987), but abundance of predaceous nematodes was neither associated with tillage treatment nor associated with suppressiveness of *H. glycines* (Kidane et al., 2012a,b).

Cultivation is destructive to soil foodwebs by disrupting not only the physical structure of soil but shifting the community to an earlier stage of ecological succession with greater dominance of the bacterial than fungal pathway (Cheng et al., 2018; Grabau et al., 2018; Neher and Campbell, 1994; Treonis et al., 2010). Tillage is confounded by its effects on abiotic and biotic properties of soil. Generally, tillage reduces soil moisture and increases temperature and penetration but decreases organic matter in the surface 20 cm of soil (Bernard et al., 1996; Doran, 1980; Griffith et al., 1975). As poikilotherms, higher temperatures translate into faster development and shorter generation times resulting in population growth for nematodes. No-till favors increase in facultative anaerobes (Doran, 1980). Anaerobic bacteria may also affect nematode survival. Certain anaerobes produce toxic substances that kill nematodes (Hollis and Johnston, 1957; Johnston, 1957). *Bacillus* spp. are facultative anaerobes and been shown to antagonize plant-parasitic nematodes, forming the basis of commercial biopesticides registered for nematode control (Xiang et al., 2018).

This study is one of the first to identify and report genera of free-living nematodes correlated with microbial suppressiveness of the *H. glycines*. Taxa that increased when suppression was broken were those already known to be tolerant to disturbance. Our results support a meta-analysis suggesting *Ditylenchus* increasing with cultivation (Zhao and Neher, 2013). *Aphelenchoides* has been reported as a common genus in corn fields, as well as *Acrobeloides* and *Aphelenchus* (Čerevková et al., 2018). Other investigations infer that microbe-feeding fauna are involved in or correlated to SCN-suppression (Kidane et al., 2012a,b). To our knowledge, there are no prior reports of bacterivorous or omnivorous nematode genera associated with suppressive soils.

The more targeted biocide treatment response suggests that fungal antagonists play a more important role in SCN suppression than bacteria. This was validated by an increased number of SCN eggs in response to captan compared to streptomycin (Kidane et al., 2012a,b). A follow-up study of the microbiome in the SCN cysts from this site indicated that both bacteria and fungi play important roles in the soil suppression (Hu et al., 2017). There was some inconsistency of biocide treatments from year to year in the experiment. This can at least partly be explained by the application procedure. It was necessary to supply sufficient water through irrigation to insure the chemicals would penetrate into the root zone.

The single year rotation to corn appears to reduce the antagonists that coevolved with the soybean monoculture. This type of coevolution in the rhizosphere has been reported as natural suppression of other soilborne pathogens. Take-all decline is a well-characterized example of induced-specific suppression, occurs on average 4–6 years continuous monoculture or wheat or barley (Kwak and Weller, 2013). Bare patch of wheat (*Rhizoctonia solani* AG-8, syn. *Thanatephorus cucumeris*) decreased after five years of continuous no-till wheat in Australia (Schlatter et al., 2017). Decline of bare patch is associated with long-term inputs of carbon as organic matter, analogous to the organic matter content of the soils naturally suppressive to SCN in this study.

Soybean is host to both *Heterodera glycines* and *Helicotylenchus* (Grabau and Chen, 2016a; Niblack, 1992; Yan et al., 2017). However, the inverse relationship of these two genera held across all treatments, suggesting it was more than simply a host response. Inverse relationships between two genera of plant-parasitic nematodes have been reported elsewhere. For example, a similar observation was observed for potato cyst nematode (*Globodera rostochiensis*) and *Helicotylenchus* (Kerry et al., 2009). This type of relationship is called niche differentiation or niche exclusion due to coevolutionary displacement (Kinkel et al., 2011). It results in the elimination of one species from habitat(s) where another species or set of species is present, specifically in cases where one population may lack the capacity to respond to a novel antagonistic phenotype in another.

5. Conclusion

Relatively complex food webs, containing fungi and fungivorous nematodes, correspond with natural suppression in this field with no-till monoculture soybean. It appears that fungi are important antagonists, but not hyperparasites of *H. glycines*. The next step is to investigate which saprophytic fungi are involved and their functional mechanism in these soils. This can lead to identification of management regimes that foster the presence and function of fungal communities that antagonize SCN.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2019.03.016.

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References

Adesina, M.F., Lembke, A., Costa, R., Speksnijder, A., Smalla, K., 2007. Screening of bacterial isolates from various European soils for in vitro antagonistic activity towards *Rhizoctonia solani* and *Fusarium oxysporum*: site-dependent composition and diversity revealed. Soil Biol. Biochem. 39, 2818–2828. https://doi.org/10.1016/j.soilbio.2007.06.004.

Andrássy, I., 1983. A Taxonomic Review of the Suborder Rhabditina (Nematode: Secernentia). ORSTROM, Paris.

Bao, Y., Neher, D.A., Chen, S.Y., 2011. Effect of soil disturbance and biocides on nematode communities and extracellular enzyme activity in soybean cyst nematode suppressive soil. Nematology 13, 687–699. https://doi.org/10.1163/138855410X541230.

Barker, K.R., Nusbaum, C.J., Nelson, L.A., 1969. Effects of storage temperature and extraction procedure on recovery of plant parasitic nematodes from field soils. J. Nematol. 1, 240–247.

Bernard, E.C., Self, L.H., Tyler, D.D., 1996. Fungal parasitism of soybean cyst nematode, *Heterodera glycines* (Nemata: Heteroderidae), in differing cropping-tillage regimes. Appl. Soil Ecol. 5, 57–70. https://doi.org/10.1016/S0929-1393(96)00125-4.

Bonanomi, G., Antignani, V., Capodilupo, M., Scala, F., 2010. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. Soil Biol. Biochem. 42, 136–144. https://doi.org/10.1016/j.soilbio.2009.10.012.

Bongers, T., 1988. De Nematoden van Nederland. KNNV-Bibliotheekuitgave, Pirola, Schoorl, Netherlands.

Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83, 14–19. https://doi.org/10.1007/BF00324627.

Bongers, T., Bongers, M., 1998. Functional diversity of nematodes. Appl. Soil Ecol. 10, 239–251. https://doi.org/10.1016/S0929-1393(98)00123-1.

Bongers, T., Ferris, H., 1999. Nematode community structure as a bio-indicator in environmental monitoring. TREE 14, 224–228. https://doi.org/10.1016/S0169-5347(98)01583-3.

Bongers, T., Alkemade, R., Yeates, G.W., 1991. Interpretation of disturbance-induced maturity decrease in marine nematode assemblages by means of the maturity index. Mar. Ecol. Prog. Ser. 76, 135–142. https://www.jstor.org/stable/24825556.

Bongers, T., de Goede, R.G.M., Korthals, G.W., Yeates, G.W., 1995. Proposed changes of cp classification for nematodes. Russ. J. Nematol. 3, 61–62.

Čerevková, A., Miklisová, D., Szoboszlay, M., Tebbe, C.C., Cagáň, L., 2018. The responses of soil nematode communities to Bt maize cultivation at four field sites across Europe. Soil Biol. Biochem. 119, 194–202. https://doi.org/10.1016/j.soilbio.2018.01.023.

Chen, S., 2007. Suppression of *Heterodera glycines* in soils from fields with long-term soybean monoculture. Biocontrol Sci. Tech. 17, 125–134. https://doi.org/10.1080/09583150600937121.

Cheng, Z., Melakeberhan, H., Mennan, S., Grewal, P.S., 2018. Relationship between soybean cyst nematode, *Heterodera glycines*, and soil nematode communities under long-term tillage and crop rotation systems. Nematropica 48, 101–115.

van den Brink, P.J., van den Brink, N.W., ter Braak, C.J.F., 2003. Multivariate analysis of

ecotoxicological data using ordination: demonstrations of utility on the basis of various examples. Aust. J. Ecotoxicol. 9, 141–156.
Doran, J.W., 1980. Soil microbial and biochemical changes associated with reduced tillage. Soil Sci. Soc. Am. J. 44, 765–771. https://doi.org/10.2136/sssaj1980.03615995004400040022x.
Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Appl. Soil Ecol. 18, 13–29. https://doi.org/10.1016/S0929-1393(01)00152-4.
Fiscus, D.A., Neher, D.A., 2002. Distinguishing nematode genera based on relative sensitivity to physical and chemical disturbances. Ecol. Appl. 12, 565–575. https://doi.org/10.1890/1051-0761(2002)012[0565:DSOFLS]2.0.CO;2.
Ghorbani, R., Wilcockson, S., Koocheki, A., Leifert, C., 2008. Soil management for sustainable crop disease control: a review. Environ. Chem. Lett. 6, 149–162. https://doi.org/10.1007/s10311-008-0147-0.
Ginitis, B., Morgan-Jones, G., Rodriguez-Kabana, R., 1983. Fungi associated with several developmental stages of *Heterodera glycines* from an Alabama soybean field soil. Nematropica 13, 181–200.
Grabau, Z.J., Chen, S., 2016a. Determining the role of plant-parasitic nematodes in the corn-soybean crop rotation yield effect using nematicide application: I. corn. Agron. J. 108, 782–793. https://doi.org/10.2134/agronj2015.0431.
Grabau, Z.J., Chen, S., 2016b. Influence of long-term corn-soybean crop sequences on soil ecology as indicated by the nematode community. Appl. Soil Ecol. 100, 172–185. https://doi.org/10.1016/j.apsoil.2015.12.016.
Grabau, Z.J., Vetsch, J.A., Chen, S., 2018. Swine manure, nematicides, and long-term tillage change soil ecology in corn and soybean production. Agron. J. 110, 1–14. https://doi.org/10.2134/agronj2018.04.0252.
Griffith, D.R., Mannering, J.V., Galloway, H.M., Parsons, S.D., Richey, C.B., 1975. Effect of eight tillage-planting systems on soil temperature, percent stand, plant growth, and yield of corn on five Indiana soils. Agron. J. 65, 321–326. https://doi.org/10.2134/agronj1973.00021962006500020040x.
Hartwig, E.E., 1981. Breeding productive soybean cultivars resistant to the soybean cyst nematode for the southern United States. Plant Dis. 65, 303–307.
Hollis, J.P., Johnston, T., 1957. Microbiological reduction of nematode populations in water-saturated soils. Phytopathology 47, 16.
Hu, W., Samac, D.A., Liu, X., Chen, S., 2017. Microbial communities in the cysts of soybean cyst nematode affected by tillage and biocide in a suppressive soil. Appl. Soil Ecol. 119, 396–406. https://doi.org/10.1016/j.apsoil.2017.07.018.
Ingham, R.E., Coleman, D.C., 1984. Effects of streptomycin, cycloheximide, fungizone, captan, carbofuran, cygon, and PCNB on soil microorganisms. Microb. Ecol. 10, 345–358. https://doi.org/10.1007/BF02015559.
Ingham, R.E., Parmelee, R.W., Coleman, D.C., Crossley, D.A., 1991. Reduction of microbial and faunal groups following application of streptomycin and captan in Georgia no-tillage agro ecosystems. Pedobiologia 35, 297–304.
Jairajpuri, M.S., Ahmad, W., 1992. Dorylaimidae: Free-living, Predacious and Plant-parasitic Nematodes. Oxford and IBH, New Delhi.
Johnston, T., 1957. Further studies on microbiological reduction of nematode population in water-saturated soils. Phytopathology 47, 525–526.
Kerry, B.R., 1988. Fungal parasites of cyst nematodes. Agric. Ecosyst. Environ. 24, 293–295. https://doi.org/10.1016/0167-8809(88)90073-4.
Kerry, B., Davies, K., Esteves, I., 2009. Managing potato cyst nematode through maximizing natural decline and population suppression. In: PCN Final Project Report (Rothamsted Research). United Kingdom, Agriculture and Horticulture Development Board (AHDB).
Kidane, E., Hu, W., Chen, S., Neher, D.A., 2012a. Ecology of soils suppressive to the soybean cyst nematode: I. Effect of tillage and crop-biocide treatments on soil suppressiveness to nematode and soybean yield. J. Nematol. 44, 471.
Kidane, E., Hu, W., Chen, S., Liu, X., Neher, D.A, 2012b. Ecology of soils suppressive to the soybean cyst nematode: II. Effect of tillage and crop-biocide treatments on nematophagous fungi. J. Nematol. 44, 472.
Kinkel, L.L., Bakker, M.G., Schlatter, D.C., 2011. A coevolutionary framework for managing disease-suppressive soils. Annu. Rev. Phytopathol. 49, 47–67. https://doi.org/10.1146/annurev-phyto-072910-095232.
Koenning, S.R., Wrather, J.A., 2010. Suppression of soybean yield potential in the continental United States by plant diseases from 2006-2009. Online. Plant Health Progress. https://doi.org/10.1094/PHP-2010-1122-01-RS.
Konen, M.E., Jacobs, P.M., Burras, C.L., Talaga, B.J., Mason, J.A., 2002. Equations for predicting soil organic carbon using loss-on-ignition for north central US soils. Soil Sci. Soc. Am. J. 66, 1878–1881. https://doi.org/10.2136/sssaj2002.1878.
Kwak, Y.-S., Weller, D.M., 2013. Take-all of wheat and natural disease suppression: a review. Plant Pathol. J. 29, 125–135. https://doi.org/10.5423/PPJ.SI.07.2012.0112.
Linford, M.B., Yap, F., Oliveira, J.M., 1938. Reduction of soil populations of the root-knot nematode during decomposition of organic matter. Soil Sci. 45, 127–142.
Maggenti, A., Luc, M., Raski, D., Fortuner, R., Geraert, E., 1987. A reappraisal of Tylenchina (Nemata). 2. Classification of the suborder Tylenchina (Nemata: Diplogasteria). Revue Nématol. 10, 135–142.
McSorley, R., 2011. Overview of organic amendments of management of plant-parasitic nematodes, with case studies from Florida. J. Nematol. 43, 69–81.
Messiha, N.A.S., van Bruggen, A.H.C., Diepeningen, A.D., Vos, O.J., Termorshuizen, A.J., 2007. Potato brown rot incidence and severity under different management and amendment regimes in different soil types. Eur. J. Plant Pathol. 11, 367–381. https://doi.org/10.1007/s10658-007-9167-z.
Neher, D.A., 2001. Role of nematodes in soil health and their use as indicators. J. Nematol. 33, 161–168.
Neher, D.A., 2010. Ecology of plant and free-living nematodes in natural and agricultural soil. Annu. Rev. Phytopathol. 48, 371–394. https://doi.org/10.1146/annurev-phyto-073009-114439.
Neher, D.A., Campbell, C.L., 1994. Nematode communities and microbial biomass in soils with annual and perennial crops. Appl. Soil Ecol. 1, 17–28.
Neher, D.A., Darby, B.J., 2006. Computation and application of nematode community indices: general guidelines. In: Abebe, E., Andrássy, I., Traunspurger, W. (Eds.), Freshwater Nematodes: Ecology and Taxonomy. CABI, Wallingford, pp. 211–222.
Niblack, T.L., 1992. Pratylenchus, Paratylenchus, Helicotylenchus, and other nematodes on soybean in Missouri. J. Nematol. 24, 738–744.
Norton, D.C., Frederick, L.R., Ponchillia, P.E., Nyhan, J.W., 1971. Correlations of nematodes and soil properties in soybean fields. J. Nematol. 3, 154–163.
Oka, Y., 2010. Mechanisms of nematode suppression by organic soil amendments – a review. Appl. Soil Ecol. 44, 101–115.
Renčo, M., 2013. Organic amendments of soil as useful tools of plant parasitic nematodes control. Helminthologica 50, 3–14. https://doi.org/10.2478/s11687-013-0101-y.
Riggs, R.D., 2004. History and distribution. In: Schmitt, D.P., Wrather, J.A., Riggs, R.D. (Eds.), Biology and Management of the Soybean Cyst Nematode. Schmitt and Associates, Marceline, MO, pp. 9–40.
Sánchez-Moreno, S., Ferris, H., 2007. Suppressive service of the soil food web: effects of environmental management. Agric. Ecosyst. Environ. 119, 75–87. https://doi.org/10.1016/j.agee.2006.06.012.
Schlatter, D., Kinkel, L.L., Thomashow, L.S., Weller, D.M., Paulitz, T.C., 2017. Disease suppressive soils: new insights from the soil microbiome. Phytopathology 107, 1284–1297. https://doi.org/10.1094/PHYTO-03-17-0111-RVW.
Siddiqi, M.R., 2000. Tylenchida: Parasites of Plants and Insects, second ed. CABI, Wallingford.
s'Jacob, J.J., van Bezooijen, J., 1984. Manual for Practical Work in Nematology, revised edition. Department of Nematology, Agricultural University, Wageningen, Netherlands.
Šmilauer, P., Lepš, J., 2014. Multivariate Analysis of Ecological Data Using Canoco 5, second edition. Cambridge University.
Smith, J.L., Doran, J.W., Jones, A.J., 1996. Measurement and use of pH and electrical conductivity for soil quality analysis. In: Doran, J.W. (Ed.), Methods for Assessing Soil Quality. Soil Science Society of America, Madison, WI, pp. 169–185.
Steel, H., Ferris, H., 2016. Soil nematode assemblages indicate the potential for biological regulation of pest species. Acta Oecol. 73, 87–96. https://doi.org/10.1016/j.actao.2016.03.004.
Sun, M.H., Liu, X.Z., 2000. Suppressive soils of soybean cyst nematode in China. Acta Phytopathol. Sin. 30, 353–356.
Thorne, G., 1974. Nematodes of the northern Great Plains. Part II. Dorylaimoidea in part (Nemata: Adenophorea), Techical Bulletin 41.
Treonis, A.M., Austin, E.E., Buyer, J.S., Maul, J.E., Spicer, L., Zasada, I.A., 2010. Effects of organic amendment and tillage on soil microorganisms and microfauna. Appl. Soil Ecol. 46, 103–110.
Tyler, D.D., Chambers, A.Y., Young, L.D., 1987. No-tillage effects on population dynamics of soybean cyst nematode. Agron. J. 79, 799–802. https://doi.org/10.2134/agronj1987.00021962007900050008x.
Warnke, S.A., Chen, S.Y., Wyse, D.L., Johnson, G.A., Porter, P.M., 2008. Effect of rotation crops on hatch, viability, and development of *Heterodera glycines*. Nematology 10, 869–882. https://doi.org/10.1163/156854108786161391.
Weller, D.M., Raaijmakers, J.M., Gardener, B.B.M., Thomashow, L.S., 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu. Rev. Phytopathol. 40, 309–348. https://doi.org/10.1146/annurev.phyto.40.030402.110010.
Williams, T.D., 1969. The effects of formalin, nabam, irrigation and nitrogen on *Heterodera avenae* and *Ophiobolus graminis* and the growth of spring wheat. Ann. Appl. Biol. 64, 325–334. https://doi.org/10.1111/j.1744-7348.1969.tb02882.x.
Xiang, N., Lawrence, K.S., Donald, P.A., 2018. Biological control potential of plant growth-promoting rhizobacteria suppression of *Meloidogyne incognita* on cotton and *Heterodera glycines* on soybean: a review. J. Phytopathol. 166, 449–458. https://doi.org/10.1111/jph.12712.
Yan, G., Plaisance, A., Huang, D., Handoo, Z.A., 2017. First report of the spiral nematode *Helicotylenchus microlobus* infecting soybean in North Dakota. J. Nematol. 49, 1.
Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding habits in soil nematodes families and genera - an outline for soil ecologists. J. Nematol. 25, 315–331.
Zhao, J., Neher, D.A., 2013. Soil nematode genera that predict specific types of disturbance. Appl. Soil Ecol. 64, 135–141. https://doi.org/10.1016/j.apsoil.2012.11.008.