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# Soil food web structure and function in annual row-crop systems: How can nematode communities infer soil health?



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#### ABSTRACT

Increased agricultural management intensity is known to degrade soil food web structure and function, however, the impact of 50+ years of contrasting agricultural management practices on soil food webs is largely unknown. The relationship between soil food web structure and sensitive soil health indicators has seldom been quantified in row crop agricultural systems in the Midwest, USA. This study aims to 1) quantify soil food web structure and function in long-term systems that range in tillage intensity and perenniality over the course of a single growing season; and 2) identify the relationship between soil health indicators and soil food web structure. Nematode feeding groups and nematode indices were used to indicate soil food web structure and soil food web function, respectively. Additionally, a suite of soil health indicators including permanganate oxidizable carbon (POXC), β-glucosidase (GLU), and N-Acetyl-β-glucosaminidase (NAG) were measured in two identical long-term experimental trials with contrasting soil types (clay versus silt loam) under two tillage intensities (chisel till (CT) vs. notill (NT)) combined with two crop rotations (corn-soy (CS) vs. corn-forage-forage (CFF)). All samples were collected in the corn phase of the rotation. Non-metric multidimensional scaling of nematode community assemblage and vector analysis demonstrated that soil food web structure was significantly related to POXC, GLU, and NAG within CFF systems. Nematode families Thornenematidae and Rhabditidae served as indicators of improved and degraded soil food web function, respectively. Our findings indicate that including perennials within a crop rotation fosters a soil food web structure that can enhance carbon and nitrogen cycling in large scale row-crop agriculture. Moreover, this study emphasizes the potential for specific nematode families to serve as indicators of soil food web function, demonstrating that nematodes should be integrated into the soil health framework

# 1. Introduction

Soil food webs maintain ecosystem functions that are essential for enhanced soil health within agricultural systems (Breure, 2004; Ferris, 2010; Schmidt et al., 2020; van den Hoogen et al., 2019; Verhoef and Brussaard, 1990). Nematodes are soil microfauna that serve as effective representatives of the soil food web as they can reflect both soil food web structure and function (Bongers, 1990). Nematodes can serve as indicators of soil food web structure as their population abundances fluctuate based on predator-prey relationships with bacteria, fungi, nematodes, and plants (Ferris et al., 1996; Sánchez-Moreno et al., 2006, 2008). Additionally, nematodes can reflect soil food web function through nematode indices, which are calculated from the classification of nematodes into functional guilds that represent colonizers and persisters within the soil food web (Ferris et al., 2001; Neher, 2001; Ritz and Trudgill, 1999; Sánchez-Moreno et al., 2011; Yeates, 2003; Yeates and Bongers, 1999). Nematode indices can infer a vast array of ecosystem functions including, nutrient enrichment (Enrichment Index), disturbance (Basal Index), bacterial or fungal dominated decomposition pathways (Channel Index), soil food web succession and complexity (Structure and Maturity Index), and plant parasitic infestation (Plant Parasitic Index) (Bongers, 1990; Ferris et al., 2001; Ferris and Bongers, 2009).

Soil food web structure and function have been extensively quantified within agricultural systems under varying tillage intensity and crop rotations worldwide (DuPont et al., 2009; Ferris and Matute, 2003; Leslie et al., 2017; Mikkelsen and Coyne, 2015; Moore, 1994; Sánchez-Moreno et al., 2008; van den Hoogen et al., 2019; Wardle et al., 1995). However, the quantification of beneficial nematode communities is understudied in row-crop agriculture in the Midwest, USA (van den

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F-statistics of the effect of System, Time, and the interaction between System and Time on the percent of Bacterivore, Fungivore, Predator-Omnivore, and Plant Parasitic in Corn-Soy Chisel, Corn-Soy No-till, Corn-Forage-Forage Chisel, and Corn Forage-Forage No-till systems from a repeated measures ANOVA at Hoytville. n = 3.\*\*\*

Source	Bacterivore	Fungivore	Predator-Omnivore	Plant parasite
System (S)	10.41**	11.75*	7.08*	2.77
Time (Ti)	1.38	4.55	39.07**	0.60
S × Ti	4.16	6.57*	7.08*	0.85

<sup>\*</sup> Significance level: p < 0.05.

\*\* Significance level: p < 0.01.

<sup>\*\*\*</sup> Significance level: p < 0.001

Hoogen et al., 2019). Moreover, the long-term effect of varying management intensities on soil food web structure and function have yet to be investigated in row-crop agriculture. As a result, there is a deep lack of understanding surrounding soil food web structure and function in corn and soybean systems under long-term management across the upper Midwest (Melakeberhan et al., 2021). Past findings from other regions demonstrate that increased tillage intensity and annual monoculture systems are known to degrade soil food web function (de Vries et al., 2013; Ferris et al., 2001; Postma-Blaauw et al., 2010; Tsiafouli et al., 2015). For example, Wardle et al. (1995) reported that intensive tillage stimulates bacterivore abundances and inhibits predatoromnivore nematode abundances. In contrast, no-till systems enhance the formation of nematode habitat within macro-aggregates and increases the interactions between nematodes in varying trophic guilds (Hassink et al., 1993; Zhang et al., 2021; Zhong et al., 2017). In addition, a reduction of diverse root inputs from annual monoculture systems can reduce nematode abundance and diversity (Kostenko et al., 2015). The incorporation of perennial legumes into annual systems may pose as a solution, given that perennials are known to enhance soil food web structure and function (Culman et al., 2010; Sprunger et al., 2019, 2020). However, it is unclear if perennial legumes rotated with annual row-crops have the same effect.

The concept of soil health has gained momentum because of its holistic approach to integrating chemical, biological, and physical properties of soil to sustain plant productivity, plant and animal health, and regulate air and water quality (Doran and Zeiss, 2000). Soil health indicators that are promoted by the National Resource Conservation Service (NRCS) can indicate differences in ecosystem services between agricultural systems that vary in management intensity, however little is known on how these indicators relate to soil food web structure (Nunes et al., 2020; Williams et al., 2020). Key soil health indicators including permanganate oxidizable carbon (POXC), soil autoclaved-citrate extractable (ACE) soil protein, mineralizable carbon (MinC), and enzyme activities are known to be enhanced in agricultural systems with reduced management intensity (Culman et al., 2013; Franzluebbers et al., 2000; Franzluebbers and Stuedemann, 2008; Haney et al., 2018; Schomberg et al., 2009; Vahdat et al., 2010; García-Ruiz et al., 2009; Tabatabai, 1994). The integration of the biological, chemical, and physical properties of the soil can allow for a more comprehensive assessment of soil health (Wade et al., 2020). For example, the assimilation of sensitive soil health indicators with soil biological indicators, such as nematode communities, can serve as an indicator of the

#### Table 2

Nematode family and cp class found in Corn-Soy Chisel Till (CS CT), Corn-Soy No-till (CS-NT), Corn-Forage-Forage Chisel till (CFF CT), and Corn-Forage-Forage No-till (CFF NT) systems during Hoytville Planting and Hoytville Harvest. "X" denotes that the family was found in the system.

Family		Hoytville Planting				Hoytville Harvest			
	cp-class	CS CT	CS NT	CFF CT	CFF NT	CS CT	CS NT	CFF CT	CFF NT
Herbivores									
Hoplolaimidae	3					Х	Х	Х	Х
Tylenchidae	2	Х	Х	Х	Х	Х	Х	Х	Х
Longidoridae	5	Х	Х	Х	Х				
Anguinidae	2	Х		Х	Х	Х	Х	Х	Х
Dolichodoridae	3	Х	Х	Х	Х				
Belondiridae	5		Х			Х		Х	
Paratylenchidae	3	Х	Х			Х	Х	Х	Х
Trichodoridae	4								х
Osstellidae	1						Х	Х	х
Ecphyadophoridae	2	Х			Х		Х	Х	
Neotylenchidae	2					х	Х	Х	Х
Fungivores									
Leptonchidae	4						х	Х	
Iotonchiidae	2					х	х	Х	х
Aphelenchoididae	2	Х	Х	Х	х	х	х	Х	х
Aphelenchidae	2	х		Х	х				
*									
Bacterivores									
Rhabditidae	1	Х	Х	Х		х			
Panagrolaimidae	1	Х	Х	Х	х	х	х	Х	х
Cephalobidae	2	Х	Х	х	Х	х	х	Х	х
Prismatolaimidae	3	Х					х		х
Diploscapteridae	1							Х	
Rhabdolaimidae	3								х
Leptolaimidae	3					х			
Plectidae	2					Х		Х	Х
Predators and omnivores									
Qudsianematidae	4	Х		Х					
Mononchidae	4	х	Х	Х	Х				
Thornenematidae	5		Х		Х				
Nordiidae	4	х	Х		Х				
Cyatholaimidae	3	Х							



**Fig. 1.** A) Hoytville and B) Wooster percent of Plant Parasitic, Predator-Omnivore, Fungivore, and Bacterivore nematodes in Corn-Soy Chisel, Corn-Soy No-till, Corn-Forage-Forage Chisel, and Corn Forage-Forage No-till systems over the course of two sampling periods planting, and harvest. Error bars represent one standard error from the mean for each feeding group. n = 3.

F-statistics of the effect of System, Time, and the interaction between System and Time on the percent of Bacterivore, Fungivore, Predator-Omnivore, and Plant Parasitic nematodes in Corn-Soy Chisel, Corn-Soy No-till, Corn-Forage-Forage Chisel, and Corn Forage-Forage No-till systems from a repeated measures ANOVA at Wooster. n = 3.\*\*

Source	Bacterivore	Fungivore	Predator-Omnivore	Plant Parasitic
System (S)	1.47	0.36	2.15	2.79
Time (Ti)	10.69*	0.50	35.76***	1.12
$S \times \text{Ti}$	0.64	2.53	1.76	0.27

<sup>\*</sup> Significance level: p < 0.05.

\*\* Significance level: p < 0.01.

\*\*\*\* Significance level p < 0.001

functioning of essential ecosystem services (Culman et al., 2010; Martin and Sprunger, 2021a; Okada and Ferris, 2001; Ripoche et al., 2021; Sprunger et al., 2019; Zhang et al., 2019).

This study assessed the relationship between soil food web structure and function and sensitive soil health indicators on two 50-year-old trials that varied in perennial diversity and tillage intensity. The objectives were to 1) quantify soil food web structure and function in longterm systems that range in tillage intensity and perenniality over the course of the growing season; and 2) identify the relationship between soil health indicators and soil food web structure in systems that vary in management intensity. We hypothesized that; 1) systems of reduced tillage intensity and increased perenniality would display enhanced soil food web structure and function over the course of the growing season; and 2) soil food web structure would relate better with soil health indicators in reduced tillage/increased perenniality systems compared to intensive tillage/annual crop rotation systems.

# 2. Methods

# 2.1. Site description

This study was conducted at two sites that are a part of The Ohio State University Triplett Van Doren Long-Term Research Trial. The Northwest Crop Rotation Trial was established in 1963 and will be referred to as Hoytville. The Hoytville site is in Wood County, Ohio at 41°13′ N, 83°45′W. The Hoytville soil series is a Hoytville Clay Loam, and the taxonomic class is a fine, illitic, mesic Mollic Epiaqualf (Soil Survey Staff, NRCS, USDA). This soil is a deep poorly drained clay loam with a slope range of 0-1 % with high shrink swell potential. The second site is the Wooster Crop Rotation Trial established in 1962. The Wooster Crop Rotation Trial, will be referred to as Wooster, and is located in Wayne County, Ohio at 40°45′N, 81° 54′ W. The soil is a Wooster Silt Loam, and the taxonomic class is a fine-loamy, mixed active, mesic Oxyaquic Fragiudalf (Soil Survey Staff, NRCS, USDA). The Wooster site is well drained and has a slope range of 2-6 % with low to no shrink-swell potential. The 30-year monthly average precipitation from April-October was 81 mm and 83 mm at the Hoytville and Wooster Site, respectively (Ohio Agriculture Research and Development Center) (Supplementary Fig. S1).

# 2.2. Experimental design

The Hoytville and Wooster sites are full factorial randomized

Nematode family and cp class found in Corn-Soy Chisel Till (CS CT), Corn-Soy No-till (CS-NT), Corn-Forage-Forage Chisel till (CFF CT), and Corn-Forage-Forage No-till (CFF NT) systems during Wooster Planting, and Wooster Harvest. "X" denotes that the family was found in the system.

Family		Wooster Planting				Wooster Ha			
	cp-class	CS CT	CS NT	CFF CT	CFF NT	CS CT	CS NT	CFF CT	CFF NT
Herbivores									
Hoplolaimidae	3					Х	Х		
Tylenchidae	2	Х	Х	Х	Х	Х	Х	Х	Х
Longidoridae	5		Х		Х		Х		Х
Anguinidae	2	Х	Х		Х	Х	Х	Х	
Dolichodoridae	3	Х	Х	Х	Х				
Neotylenchidae	2				Х				
Fungivores									
Leptonchidae	4					х		х	х
Iotonchiidae	2			х					
Aphelenchoididae	2	Х	Х	Х	Х				
Aphelenchidae	2			Х					
Diphtherophoridae	3					Х	Х	Х	
<b>D</b> ( )									
Bacterivores	1	v	V	V	V	Y	V	V	v
Rhabditidae	1	А	X	X	X	X	Х	А	Х
Panagrolaimidae	1	v	X	X	X	X	V	V	v
Cephalobidae	2	X	А	А	А	А	Х	А	Х
Neodipiogasteridae	1	X	v	V					
Brevibuccidae	1	А	А	А		V	V	V	v
Plectidae	2					Х	А	X	Х
Predators and omnivores									
Qudsianematidae	4		Х	Х	Х	Х			Х
Mononchidae	4	Х		Х	Х	Х			Х
Aporcelaimidae	5						Х		
Thornenematidae	5		Х	Х	Х				
Chrysonematidae	5	Х							
Dorylaimidae	4	Х							
Nordiidae	4			Х					

#### Table 5

F-statistics of the effect of System, Time, and the interaction between System and Time on the Maturity Index (MI), Structure Index (SI), Enrichment Index (EI), Basal Index (BI), Channel Index (CI), and Plant Parasitic Index (PPI) from repeated measures ANOVA at Hoytville.  $n = 3.^{***}$ 

Source	MI	SI	EI	BI	CI	PPI
System (S)	1.40	1.40	1.92	0.61	6.46*	2.01
Time (Ti)	13.43**	13.43**	9.91*	12.35**	23.98**	1.65
S × Ti	1.72	1.72	2.64*	1.33	3.68	0.61

<sup>\*</sup> Significance level: p < 0.05.

\*\* Significance level: p < 0.01.

\*\*\* Significance level p < 0.0001.

complete block design experiments. Hoytville plot sizes are 30.5 m  $\times$ 6.4 m and Wooster plot sizes are 22.3 m  $\times$  4.5 m. Each trial has four systems that consist of: corn (Zea Mays)-forage-forage (CFF) no-till (NT), CFF chisel till (CT), corn soybean (Glycine max) (CS) NT, and CS CT. Each system has three replicate plots, making twelve plots total at each site. The NT treatment is zero tillage, and the residue of the previous year is left on the field. Additionally, the CT treatment is performed in the spring with 30 % of the residue from the previous crop left on the field. The forage crops in the CFF rotation consisted of an oats (Avena sativa) and red clover (Trifolium pretense) mix in Hoytville, and alfalfa (Medicago sativa) in Wooster. During Spring 2020 of the pre-planting corn phase of the rotation, starter fertilizer was applied (34-45 kg  $ha^{-1}$ ) at both sites. Additionally, urea fertilizer was added during the V5 corn stage (202 kg  $ha^{-1}$ ). Phosphorus (P) and potassium were also applied as necessary depending on soil health test results. The corn seeding rate was approximately 84,014 seeds ha<sup>-1</sup> at both sites.

# 2.3. Soil sampling and processing

Soil sampling for this study occurred in the corn phase of the rotation. Two weeks post corn emergence (May 2020) and two weeks prior to corn harvest (October 2020) fifteen soil cores were taken at the 10 cm depth in each plot using stratified random sampling to make one composite sample. Soil samples were then subsampled for elemental and nutrient analysis (5 g), soil moisture (SM) (45 g), soil texture (50 g), POXC, ACE soil protein (protein), MinC (40 g), enzyme activity (10 g), and nematode identification (100 g). In addition, soils for enzyme analysis were sieved to 4 mm and stored at 4 °C. Soils for nematode identification were sieved to 2.54 cm to filter out large rocks and roots. After, soil samples were stored at 4 °C until further processing.

# 2.4. Soil nutrient analysis

A portion of the soil sample was sent to Spectrum Analytics (Washington Court, Ohio, USA) for elemental and nutrient analyses (Supplementary Table S1). Soil moisture was assessed through weighing out 45 g of fresh soil, drying the soil at 105 °C for 24 h, and weighing the soil after drying (Supplementary Table S2). Soil texture was assessed using the protocol adapted from Gee and Bauder (1986) (Supplementary Table S1). In summary, the soil was air dried for 24 h, sieved (< 2 mm), pretreated with sodium hexametaphosphate, and a hydrometer was used to measure the density of soil particles in suspension at specific periods of time.

# 2.5. Nematode extraction and identification

Free-living nematodes were extracted using the elutriation method (Oostenbrink, 1960). In summary, soil (100 g) was soaked in 450 mL of



**Fig. 2.** Nematode indices of the A) Maturity B) Structure C) Enrichment D) Basal E) Channel F) Plant Parasitic Index at the Hoytville Site during planting and harvest. Color represents systems of Corn-Soy Chisel, Corn-Soy No-till, Corn-Forage-Forage Chisel, and Corn Forage-Forage No-Till. Error bars represent one standard error from the mean. Different letters represent significant differences between main effects of time or system. n = 3.

water for 24 h. Next, samples were passed through the elutriator, where nematodes in soil solution were collected on 150  $\mu$ m and 38  $\mu$ m sieves. The soil and water solution was collected in 50 mL centrifuge tubes. The solution was centrifuged at 2800 rpm for 5 mins to collect the nematodes in the soil, and the supernatant was discarded. The samples were processed using the centrifugal sugar flotation method to bring the nematodes into solution (Hooper et al., 2005). However, the use of centrifugal sugar flotation may have biased nematode community assemblage against larger bodied predators and omnivores. This process was conducted by adding 30 mL of 45 % sucrose solution to the soil sample containing the nematodes. This solution was centrifuged at 1500 rpm for 2 min, which allowed nematodes to be collected in the supernatant. Nematodes were collected on a 25  $\mu$ m sieve and washed into a 50 mL tube for live identification. Nematodes were identified live to allow for clearer visualization of certain nematode attributes (Southey, 1985).

The total number of nematodes were counted in each sample under a microscope at  $50 \times$  magnification and at least 100 individuals were identified to family at  $100-400 \times$  (Bongers, 1988). Nematode counts were expressed as the number of nematodes in 100 g of dry soil. Each nematode was classified as an adult or dauer larvae to allow the determination of the population stage. Nematode taxa were assigned to trophic groups (Yeates et al., 1993) and colonizer-persister groups based on Bongers and Bongers (1998). Nematode indices were calculated using Nematode Indicator Joint Analysis (NINJA), a software program that utilizes identified nematode family abundances to calculate nematode indices based on colonizer-persister values (Sieriebriennikov et al., 2014).

## 2.6. Soil health indicators

Enzyme activity is a recommended NRCS soil biological indicator

and measures the potential of microbes within the soil to breakdown particular substrates within the soil. Enzyme activity was determined colometrically using the protocol adapted from Deng and Popova (2011), and Tabatabai (1994). This study measured enzyme activities of β-glucosidase (GLU), acid phosphatase (AP), N-Acetyl-β-glucosaminidase (NAG), and arylsulfatase (AS), which assesses carbon (C), P, N, and sulfur(S) cycling, respectively. These assays measured the amount of pnitrophenol released when the soil is incubated with the appropriate buffer and substrate (Tabatabai, 1994). In short, a specific buffer that corresponded to the enzyme measured was mixed with soil (1 g). Buffers that were used for GLU, NAG, AS, and AP were modified universal buffer (pH 6.0), acetate buffer (0.1 M, pH 5.5), acetate buffer (pH 5.8), and modified universal buffer (pH 6.5), respectively. Enzyme substrate for the specific enzyme measured was added to the soil-buffer mixture. Substrates that were used for GLU, NAG, AS, and AP activity were pnitrophenyl-β-D-glucoside (0.05 M), p-nitrophenyl-N-Acetyl-β-D glucopyanoside (0.01 M), p-nitrophenyl sulfate (0.05 M), and p-nitrophenyl phosphate, respectively. Controls for each sample were prepared without the addition of the substrate. All samples were incubated at 37 °C for 1 h. The reaction was then stopped by adding 0.5 M CaCl<sub>2</sub> and 4 mL of Tris (hydroxymethyl) aminomethane (THAM) (0.1 M, pH 12). All control samples received the addition of the appropriate substrate after incubation. All soil suspensions were filtered through Whatman #2 filter paper and absorbance was measured at 415 nm. Enzyme activity was calculated using a calibration curve of standards containing 0, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nmol of *p*-nitrophenol.

Soil samples for POXC, protein, and MinC quantification were air dried for two days and on the third day dried in a desiccator at 40  $^{\circ}$ C for 24 h. Post air drying, the soil samples were ground and sieved to 2 mm. Mineralizable C is a recommended NRCS soil biological health indicator (USDA-NRCS, 2015) and reflects the pool of C that is most available to



**Fig. 3.** Faunal profile representing system N enrichment and soil food web maturity under A) planting and B) harvest at Hoytville. Color represents the observations for each of Corn-Soy Chisel, Corn-Soy No-till, Corn-Forage-Forage Chisel, and Corn Forage-Forage No-Till. Error bars represent one standard error from the mean. n = 3.

F-statistics and significance of the effect of System, Time, and the interaction between System and Time on the Maturity Index (MI), Structure Index (SI), Enrichment Index (EI), Basal Index (BI), Channel Index (CI), and Plant Parasitic Index (PPI) from repeated measures ANOVA at Wooster.  $n = 3.^{***}$ 

Source	MI	SI	EI	BI	CI	PPI
System (S)	0.13	1.35	0.56	0.31	1.43	3.53
Time (Ti)	14.48*	43.69**	3.83	2.80	0.34	0.48
S × Ti	0.14	4.87*	1.41	5.33*	1.33	4.89*

<sup>\*</sup> Significance level: p < 0.05.

\*\* Significance level: p < 0.01.

<sup>\*\*\*\*</sup> Significance level p < 0.0001.

microbial communities. The MinC procedure used was from Franzluebbers et al. (2000) and Hurisso et al. (2018). Specifically, soils were rewetted to 50 % water-holding capacity with deionized water, incubated at 25 °C for 24 h, and the carbon dioxide (CO<sub>2</sub>) concentration was determined through injecting 1 mL of headspace air into a LI-820 infrared gas analyzer (LI-COR, Biosciences, Lincoln, Nebraska, USA). The POXC analysis was adapted from Culman et al. (2012) and reflects a more processed pool of C. Permanganate oxidizable carbon is a recommended NRCS soil biochemical health indicator and represents the pool of C oxidized by potassium permanganate solution, which is C bound to soil minerals (Lucas and Weil, 2012). In summary, 0.02 M potassium permanganate (KMnO4) solution (20 mL) was added to the soil (2.5 g), shaken, and then allowed to settle. Next, the supernatant was mixed with deionized water and read on a 96 well plate reader spectrophotometer at 550 nm. Soil protein is a NRCS recommended soil biological health indicator and reflects the organic pool of N. Soil protein represents the largest pool of organic nitrogen (N) which is available for microbial mineralization and plant uptake (Kleber et al., 2007). Soil protein was measured through methods adapted from Hurisso et al. (2018). Briefly, sodium citrate solution (24 mL) was added to the soil (3 g), autoclaved at 121 °C for 40 mins, and measured at 562 nm in a 96well spectrophotometric plate reader using the colorimetric bicinchoninic-acid (BCA) assay (Thermo Scientific, Pierce, Rockford, Illinois, USA).

#### 2.6.1. Statistical analyses

Normality of data was assessed through the use of studentized residuals plots with *MASS* in R 4.1.0 (R Core Team, 2021; Venables and Ripley, 2002). Homogeneity of data was assessed with Bartlett's test in R (Arsham and Lovric, 2011). A randomized complete block design with repeated measures ANOVA was conducted using the PROC MIXED procedure in SAS v.9.0, with time treated as a repeated measure to account for sampling variances during each time period. Treatments of tillage and crop rotational diversity were combined into one system e.g. (CS CT) in the model to understand the effect of management intensity rather than the singular effect of crop rotation or tillage intensity. Thus, for both sites, system, timepoint, and the interaction between system and year were treated as fixed factors and block was treated as a random



Fig. 4. Nematode indices of the A) Maturity B) Structure C) Enrichment D) Basal E) Channel F) Plant Parasitic Index at the Wooster Site during planting and harvest. Color represents system of Corn-Soy Chisel, Corn-Soy No-till, Corn-Forage-Forage Chisel, and Corn Forage-Forage No-Till. Error bars represent one standard error from the mean. Different letters represent significant differences between main effects of time or system.

factor. Means separation was conducted using lsmeans and significant differences were determined at p = 0.05. Graphing was performed with *ggplot2* in R (Wickham, 2016).

#### 2.6.2. Permutational analysis of variance

Permutational analysis of variance (perMANOVA) with parameters for Bray-Curtis distance and 100 permutations was conducted on nematode communities using the adonis function from the *vegan* package in R to gauge the effect of system on nematode community structure (Oksanen et al., 2013).

# 2.6.3. Nematode faunal profiles

Faunal profiles were created to illustrate the enrichment and maturity of the soil food web (Ferris, 2010). For each system the enrichment index (EI) was graphed on the y-axis and the structure index (SI) on the x-axis, where each graph is divided into four quadrats that represent level of N enrichment and system disturbance (Ferris et al., 2001). Quadrants can represent soil food webs that are: disturbed and Nenriched, maturing and N-enriched, degraded and N-depleted, or mature and fertile.

# 2.6.4. Non-metric multidimensional scaling

Non-metric multidimensional scaling (NMDS) with Bray-Curtis distance measures was used to visualize the similarities of soil food web structure within system of varying management intensity using the metaMDS function in R with the *vegan* package (Fiscus and Neher, 2002; Ito et al., 2015; Zhong et al., 2017). As nematode taxa are representative of soil food web structure NMDS was performed on 18, 25, 21, and 18 nematode taxa for Hoytville planting, Hoytville harvest, Wooster planting, and Wooster Harvest, respectively. Default parameters were used except that the data was not auto transformed. The final stress values were 0.12, 0.15,0.12, 0.15 for Hoytville planting, Hoytville harvest, Wooster planting, and Wooster Harvest, respectively. Additionally, correlations between the nematode communities and soil health indicators were analyzed through a vector analysis with 1000 permutations using the scores function in the *vegan* package in R. The length and angle of the vectors represent the strength and direction of the relationship between soil health indicators and nematode community structure.

# 3. Results

# 3.1. Soil food web structure

System type had a significant main effect on bacterivore, fungivore, and predator-omnivore feeders at the Hoytville site (p < 0.05; Table 1). Additionally, a total of 28 nematode families were identified across all systems at the Hoytville site (Table 2). Bacterivore populations in the CS CT system were greater than the rest of the systems at harvest (p < 0.05; Fig. 1). Bacterivore nematodes of the family Rhabditidae were only found in CT or CS systems (Table 2). Fungivore populations were considerably lower within the CS NT systems compared to all other systems at planting (p < 0.05; Fig. 1). By harvest, fungivore populations were on average 79 % greater in CFF NT systems compared to all other systems (p < 0.05; Fig. 1). Fungivore nematodes of the family *Leptonchidae* were only found in systems of reduced disturbance (Table 2). Predatoromnivore feeding group abundance in CFF NT and CS NT systems was double that of CFF CT and CS CT systems at planting (p < 0.05). Predator-omnivore nematodes of the family Thornenematidae were only found in NT systems (Table 2). Time was found to have a significant main effect on predator-omnivore feeding groups at the Hoytville site. Predator-omnivore nematodes were present in all systems at planting,



Fig. 5. Faunal profile representing system N enrichment and soil food web maturity under A) planting and B) harvest at Wooster. Color represents the observations for each of Corn-Soy Chisel, Corn-Soy No-till, Corn-Forage-Forage Chisel, and Corn Forage-Forage No-Till. Error bars represent one standard error from the mean.

however, predator-omnivore nematodes were not found at harvest in any of the systems (p < 0.05; Table 1; Fig. 1). Predator-omnivore nematode families of *Qudsianematidae*, *Mononchidae*, *Thorenematidae*, *Nordiidae*, and *Cythaloaimidae* were present only at planting (Table 2).

Bacterivore and predator-omnivore nematode feeding groups were significantly affected by time at Wooster (p < 0.05; Table 3). Bacterivore abundances increased on average by three-fold between planting and harvest across all systems (p < 0.05; Fig. 1). Predator-omnivore populations decreased by 94 % between planting and harvest, when averaged across system (p < 0.05; Fig. 1). Corn-forage-forage CT and CS NT systems had no identified predator-omnivore feeding groups at harvest. In CFF NT and CS CT systems predator-omnivore abundance decreased three-fold between planting and harvest.

A total of 24 nematode families were observed across all systems at Wooster (Table 4). Predator-omnivore families of *Nordiidae and Dorylaimidae* were only present at planting (Table 4). Herbivores of the family *Hopololaimidae* and *Longidoridae* were only found in CS and NT systems, respectively (Table 4). Additionally, predator-omnivore nematodes of the family *Thornenematidae* were present in all systems except CS CT systems (Table 4).

# 3.2. Soil food web function

Generally, system had little effect on nematode indices at Hoytville. The Channel Index (CI) was the only nematode index significantly affected by system (p < 0.05; Table 5). The CI was 74 % greater in CFF

NT systems at harvest than compared to all other treatments and time points (Fig. 2E). The maturity index (MI), SI, CI, basal index (BI), and EI were affected by time at Hoytville (p < 0.05; Table 5). When averaged across system the MI and SI were relatively greater at planting than compared to harvest (Fig. 2A; Fig. 2B). Similarly, the EI was 26 % greater in systems at planting relative to harvest (Fig. 2C). Additionally, time had a significant effect on the BI, where the BI increased three-fold between planting and harvest when averaged across system (Fig. 2D). Faunal profiles at Hoytville indicated temporal trends, where all systems during planting were shown to be mature, N-enriched, bacterial dominated, and low in C:N (Fig. 3A). At harvest CS NT systems shifted towards a more mature state (Fig. 3B).

System did not have a main effect on nematode indices at Wooster (Table 6). However, time had a significant main effect on the MI and SI at Wooster (p < 0.05; Table 6). The MI was 28 % greater at planting than compared to harvest systems (p < 0.05; Fig. 4A). The SI decreased two-fold between planting and harvest (Fig. 4B). Faunal profiles for Wooster displayed temporal trends (Fig. 5). Specifically, at planting all systems were found to be in a more mature state, however, at harvest CFF systems demonstrated a shift towards a disturbed environment (Fig. 5A; Fig. 5B).

# 3.3. Soil food web structure and soil health indicators

At Hoytville, perMANOVA  $R^2$  were 0.27 and 0.23 in planting and harvest, respectively. Thus, system accounted for 23 % and 27 % of



**Fig. 6.** Non-metric Dimensional Scaling and a vector analysis of nematode communities in relationship to soil health indicators of:  $\beta$ -Glucosidase (GLU), N-Acetyl- $\beta$ -Glucosaminidase (NAG), Aryl Sulfatase (AS), Acid Phosphatase (AP), protein, Permanganate Oxidizable Carbon (POXC), Mineralizable Carbon (MinC), and Soil Moisture (SM) at A) Hoytville planting B) Hoytville harvest C) Wooster planting D) Wooster Harvest. Red vectors indicate significance at p < 0.05.

nematode community variation during planting and harvest, respectively (Fig. 6A; Fig. 6B). At planting all systems were clustered together indicating no difference in nematode community assemblage (Fig. 6A). At harvest, nematode community taxa were unique within each system, as there was little overlap between system points (Fig. 6B). At Wooster, perMANOVA R<sup>2</sup> were 0.31 and 0.43 in planting and harvest, respectively. Therefore, system accounted for 31 % and 43 % of the variation between nematode communities during planting and harvest, respectively (Fig. 6C; Fig. 6D). Overlap between standard error bars of system points that represent CFF NT and CS CT systems at planting indicated similar nematode taxa within these systems (Fig. 6C). At harvest, nematode community taxa within all systems appeared to be distinctly unique (Fig. 6D).

Soil health indicators were correlated with nematode community taxa in systems with greater perenniality at Hoytville and Wooster. Vectors that represented GLU and NAG indicated a strong correlation with the nematode community taxa of the CFF systems at Hoytville during planting (p < 0.05; Fig. 6A). At harvest, soil moisture was correlated with the nematode community taxa in CFF systems at the Hoytville site (p < 0.05; Fig. 6B). At Wooster, POXC was correlated with the nematode community taxa in the CFF CT system at planting (p < 0.05; Fig. 6C). At harvest, GLU and soil moisture were correlated with nematode community taxa within CFF systems at the Wooster site (p < 0.05; Fig. 6D).

# 4. Discussion

# 4.1. Soil food web structure is sensitive to management intensity and temporal dynamics

We hypothesized that systems of reduced tillage intensity and increased perenniality would have enhanced soil food web structure and

function over the course of the growing season. Results partially corroborated our first hypothesis as soil food web structure was enhanced in systems of greater perenniality and reduced tillage intensity. Bacterivore abundances were greatest in the CS CT system during harvest at Hoytville. This finding is not surprising, given that intensive tillage has been found to increase r-strategist nematode populations and decrease K-strategist nematode populations, which are larger in size and thus more susceptible to disturbance (Fiscus and Neher, 2002; Ito et al., 2015; Zhong et al., 2017). Similarly, Ferris et al. (2004) found that bacterivores often had greater abundances in systems with mechanical disturbance and less crop diversity, as bacterivore nematode feeding groups are resilient to environmental stressors. Fungivore populations demonstrated opposite trends, with greater abundance found in the CFF NT systems. Greater fungivore abundance implicates the prevalence of a fungal dominated microbial community and thus, a slower decomposition pathway (Rahman et al., 2007). Predator-omnivore populations were greater in all NT systems regardless of crop rotation during planting at Hoytville. Likewise, sensitive predator-omnivore nematode families such as the Thornematidae and Qudsianematidae family were found only in systems of reduced disturbance in Hoytville and Wooster. The greater predator-omnivore abundance in systems of less disturbance signifies enhanced soil food web structure, which may provide greater benefits to sustained ecosystem function (Conti et al., 2020; Ferris et al., 2004; Ferris, 2010; Wardle et al., 1995; Zhong et al., 2017).

Results showed that the soil food web became more disturbed over the course of the growing season, which partially disproved our first hypothesis. For instance, we found that predator-omnivore abundances were significantly greater at planting compared to harvest at Hoytville and Wooster. These results indicate that the structure of the soil food web may be more disturbed at harvest in all systems. Furthermore, the disturbance to the soil food web at harvest may be caused by fluctuations of nutrient input over the course of the growing season (Griffiths, 1994). Specifically, the abundances of sensitive nematodes may decline as resource availability becomes greater at the end of the growing season, which stimulates an increased bottom-up effect and results in greater *r*-strategist abundances (Baird and Bernard, 1984; DuPont et al., 2009; Ferris, 2010; Ugarte et al., 2013; Zhang et al., 2019). Faunal profiles also supported this theory, particularly at the Wooster site where systems shifted to be more N-enriched and disturbed over the course of the growing season. Likewise, du Preez et al. (2018) also found similar results where *K*-strategists decreased and *r*-strategists increased over the course of two sampling time points.

# 4.2. Variability of nematode indices in annual cropping systems ranging in management intensity

Results partially disproved our first hypothesis as nematode indices were highly variable and were unable to detect differences in soil food web function between systems that have been managed in substantially different ways for over 50 years. Our results support those of Wall et al. (2002) which report inconsistent results from the use of univariate indices when comparing between sandy beach and dune systems. Additionally, given that all systems were sampled during the corn phase of the rotation, the presence of the same crop type may have diluted the differences that management practice had on soil food web function (Olusanya et al., 2019; Zhang et al., 2012). Low number of replicates within the experimental design of this study could have also led to increased variability of the nematode indices. Nematode indices shifted over the growing season and were potentially influenced by seasonal nutrient dynamics rather than management practice. Specifically, the establishment of plant roots between planting and harvest has been shown to mask the effect of management practices on nematode communities (Degrune et al., 2017; Pausch et al., 2017; Spedding et al., 2004).

#### 4.3. Soil food web structure is related to soil health indicators

We hypothesized that soil food web structure would be closely related to soil health indicators in systems of reduced tillage and increased perenniality. Results showed that NRCS soil health indicators were correlated to nematode community taxa within systems of greater perenniality, which supports our second hypothesis. Through the visualization of nematode community taxa using NMDS we found that enzyme activities of GLU and NAG had significant relationships with soil food web structure in the CFF systems during planting at Hoytville. β-glucosidase was also correlated to soil food web structure within CFF NT systems at harvest in Wooster. These results indicate that specific enzymes that represent C and N mineralization are closely related to soil food web structure in systems of greater perenniality. García-Ruiz et al. (2009) reported similar results with the geometric mean of enzyme activities positively correlated to nematode richness and diversity. Our results showed that soil health indicators that measure the stable C pool (POXC) and organic N pool (protein) are significantly correlated to soil food web structure in systems with greater perenniality. Culman et al. (2010) also reported a significant relationship between nematode communities and readily oxidizable C in perennial systems. The strong relationship between nematode community composition and protein in CFF systems is corroborated by Martin and Sprunger (2021b) who report increased nematode enrichment opportunist abundance and protein in perennial crop rotations. The relationship between nematode community structure and NRCS soil health indicators has potential to inform the role of soil food web structure in augmenting ecosystem services. However, the relationship between NRCS recommended soil health indicators and nematode community taxa was inconsistent between timepoint and site. The variability of results as well as the breadth left for interpretation severely limits the potential for soil food web structure derived from NMDS to be used as a soil health indicator.

Given the limitations of NMDS, specific nematode families rather than nematode community assemblage may serve as a better soil health indicator of soil food web function. Specifically, our study showed that the predator-omnivore, Thornenematidae, family has the potential to serve as an indicator of reduced disturbance and increased perenniality across a suite of landscapes, as this trend was apparent at both Hoytville and Wooster. Moreover, the Thornenematidae family may indicate improved soil food web function as this nematode family is found in undisturbed conditions with narrow ecological amplitude (Ferris and Bongers, 2006). Furthermore, the Thornenematidae family has previously been reported to represent increased levels of soil food web structure and trophic level interactions (Ferris et al., 2001; Reichel et al., 2017). The presence of the Rhabditidae family in systems with reduced perennial diversity or increased disturbance suggests that this nematode family may indicate degraded soil food web function within cropping systems (Sprunger et al., 2019). This finding is corroborated by Karpinska et al. (2021) who found increased Rhabditidae abundances in systems with degraded soil food web structure and greater rates of N mineralization. We recommend that future research should work to incorporate specific nematode families into the soil health framework, given that these families have the ability to indicate soil biological health and soil food web function.

# 4.4. Long-term management practices foster enhanced soil health and mature soil food webs

Through the analysis of nematode faunal footprints this study found that long-term tillage and crop rotation management has the potential to create optimal soil health conditions. Nematode faunal profiles offer valuable insight regarding the impact of nematode communities on soil health (Ferris, 2010). In our study, most systems were consistently Nenriched, demonstrating that consistent management over a 50-year period likely leads to greater nutrient enrichment. Furthermore, Nenrichment indicates a greater abundance of enrichment opportunist nematodes, which is caused by increased labile organic matter inputs (Ferris et al., 2001; Ferris and Tuomisto, 2015). Our results may also indicate that long-term management has the potential to build labile organic matter pools for microbial decomposition and plant nutrient uptake. Greater organic N-enrichment has important implications for improved soil health as increased organic N pools can reduce inorganic N inputs (Blesh and Drinkwater, 2013). We also found that long-term management facilitated a mature soil food web in most systems. Maturing soil food webs represent a greater abundance of nematodes higher up in the trophic food web (Ferris, 2010). The presence of sensitive nematodes in the higher levels of the soil food web can enhance soil health through greater C sequestration (Ingham et al., 1985; Martin and Sprunger, 2021a) and improved ecosystem resilience (Yeates, 2003). Nematode faunal footprints and NMDS point to the potential of long-term management practices under reduced tillage intensity and increased perenniality to improve soil food web structure and functioning for enhanced soil health.

### 5. Conclusion

This study integrated measures of nematode community composition with soil health indicators to understand how soil food web structure and function shift over the growing season. First, our results indicated that systems with increased perenniality, reduced tillage intensity, and long-term management can foster a soil food web that enhances C and N cycling. Second, our results showed that nematode feeding groups and families, rather than nematode indices, should be used as indicators of soil food web function within row crop systems over the course of a single growing season. Moreover, *Thornenematidae* and *Rhabditidae* nematode families proved to be indicators of structured and degraded soil food web systems, respectively. Our study demonstrates that nematode families should be further developed as indicators of soil health within row crop agriculture.

# Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

# Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2022.104553.

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