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Effects of Plasticulture and Conservation Tillage on Nematode Assemblage and Their Relationships with Nitrous Oxide Emission following a Winter Cover Cropping and Vegetable Production System

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Abstract: Agriculture production emits significant amounts of nitrous oxide (N_2O), a greenhouse gas with high global warming potential. The objectives of this study were to examine whether different husbandry practices (tillage and plasticulture) following winter cover cropping would influence soil food web structure and whether a change in the soil community could help mitigate N_2O emission in vegetable plantings. Three consecutive field trials were conducted. A winter cover crop mix of forage radish (*Raphanus sativus*), crimson clover (*Trifolium incarnatum*) and cereal rye (*Secale cereale*) were planted in all plots. Winter cover crop was terminated by flail mowing followed by (1) conventional till without surface residues [Bare Ground (BG)], (2) conventional till with black plastic mulch (BP) without surface residues, (3) strip-till (ST) with partial surface residues, or (4) no-till (NT) with surface residues. The cash crop planted subsequently were eggplant (*Solanum melongena*) in 2012 and 2014 and sweet corn (*Zea mays*) in 2013. The soil food web structure was consistently disturbed in the BP compared to other treatments as indicated by a reduction in the abundance of predatory nematodes in 2012 and 2014, and nematode maturity index in 2013 in BP. Changes in soil food web structure in the conservation tillage (NT or ST) treatments based on the weight abundance of nematode community analysis were not consistent and did not improve over the 3-year study; but were consistently improved based on functional metabolic footprint calculation at termination of cover crops of 2013 and 2014. None-the-less, the N_2O emissions increased as the abundance of fungivorous nematodes increased during all three trials. It was also found that improved soil food web structure [higher abundance of omnivorous in 2012 or predatory nematodes in 2013 and 2014, and structure index (SI) in all 3 years] reduced N_2O emissions. These findings suggested that proper soil husbandry practices following winter cover cropping could mitigate N_2O emissions over time.

Keywords: canonical correspondence analysis; cereal rye; crimson clover; greenhouse gas emission; forage radish; soil health

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1. Introduction

Nitrous oxide (N_2O) released from agricultural lands is of concern as it is a greenhouse gas with a global warming potential of approximately 265 folds greater than that of carbon dioxide (CO_2) in a 100-year lifetime [1]. Globally, agricultural soils are responsible for 75% of N_2O emissions [2], and in 2014, roughly 79% of total N_2O emissions in the United States came from agricultural soils [3]. On croplands, N_2O emissions increase rapidly with

increased nitrogen (N) fertilizer input [4], especially when the N rate exceeds the demand of a crop [5]. The challenge of finding a balance of reduced N input without sacrificing yield, while mitigating N_2O emission has not been well investigated.

Nitrous oxide is formed in soils during the microbiological processes of nitrogen fertilizers through nitrification and denitrification. Whereas nitrification involves the aerobic conversion of ammonium (NH_4^+) to nitrate (NO_3^-), denitrification involves the anaerobic conversion of NO_3^- to N_2 [6]. Both processes can occur simultaneously in soils and produce N_2O as a byproduct. Nitrous oxide production is affected by several edaphic factors, including temperature, pH, water-holding capacity, fertilizer rate, soil type, oxygen concentration, and availability of organic carbon; and cultural practices including chemical use, irrigation methods, tillage practices, and plasticulture [7,8]. Many studies have been devoted to understanding factors affecting N_2O emission and how to mitigate N_2O emission on agricultural land [9–11].

The addition of cover crop residues to soil can introduce carbon (C) and N to crop lands which would have a positive impact on soil structure and fertility, as well as enhance soil microbial activities and soil nutrient cycling. Notwithstanding, adding organic hay to soil causes some soil fauna such as earthworms and enchytraeid worms to accelerate N_2O emission [9]. Garland et al. [12] reported that although growing cover crops caused 3.9 kg N ha^{-1} of N_2O emission within a year, it also made 47.1 kg ha^{-1} of N available, and only 0.62 kg N_2O-N ha^{-1} was emitted the subsequent year when no cover crop was planted in the same field. Through controlled microcosm study, Kuiper et al. [9] demonstrated that the role of the soil fauna in N_2O emissions or acceleration is time, substrate resources, and fauna density dependent, and that the soil fauna can suppress, increase, delay or accelerate N_2O emissions.

Reduced- and no-till systems can alter N_2O emissions by modifying soil N and C availability, soil structure, soil moisture, and microbial community structure and activity. In a single growing season, Garland et al. [13] observed that tillage has no effect on N_2O emissions in a vineyard. However, van Kessel et al. [14] found that no-till and reduced tillage practices increased N_2O emissions during the first 10 years after switching from conventional tillage, but decreased emissions once the practice was in place for longer than 10 years. Therefore, investigations on how practices impact N_2O emissions should be evaluated over an extended period.

Plasticulture refers to the use of plastic mulch for weed control, soil fumigation, anaerobic soil disinfestation, or other purposes. While one might perceive plasticulture as a non-chemical alternative to soil fumigants or herbicide application, Li et al. [15] found that plasticulture increased N_2O emissions within a short period after installation of a tomato raised bed production system. This was caused by accumulation of N_2O under the virtually impermeable film. A three-year study was conducted by Chen et al. [16] in a well-drained sandy loam field transitioning to organic vegetable production in Mid-Atlantic coastal plain of Maryland to investigate N_2O emissions as affected by four different practices following a winter cover crop: strip till (ST), no-till (NT), conventional till with black plastic (BP) and conventional till without black plastic i.e., bare-ground (BG). They found that nearly 80% of annual N_2O-nitrogen (N) emissions occurred during the vegetable crop after the cover crop was terminated. The study also found that accumulative annual N_2O-N emissions were greatest in BP and that terminating the cover crop by NT reduced cumulative annual N_2O-N emissions more than conventional till-BG [16]. The question that remained is would cultural practices that can improve soil food web structure responsible for the mitigation of N_2O emission in an agroecosystem.

While growing winter cover crops has been shown to improve soil health in Maryland [17], it is uncertain as to whether the benefits of winter cover cropping on soil health cease to occur or differ if the cover crop was followed by different tillage treatments. Free-living nematodes have proven to be good soil health indicators [18], as they respond quickly to tillage practice, fertilizer inputs, and CO_2 concentration in the soil [18–20]. Neher et al. [20] assessed the effects of elevated CO_2 in soil by calculating nematode

biomass and respiration rates. Ferris [21] further developed metabolic footprints of nematodes using standardized morphometric characteristics and estimated respiration rates of each nematode taxa. By calculating the metabolic footprint of nematode assemblages, one can assess the magnitudes of ecosystem functions and services performed by each nematode functional guild. The current study explored how nematode assemblages are responsive to cover crop tillage methods and how they would affect N_2O emission.

Although considerable findings on the effects of earthworms or enchytraeid worms on N_2O emissions have been reported [9,22], studies investigating nematode effects on N_2O emissions have been contradictory or limited. Kuiper et al. [9] found that adding fungivorous nematodes to a sandy soil did not increase N_2O emissions. In contrast, Tianxiang et al. [22] found that soil with greater populations of nematodes (natural population of nematodes dominated by bacterivorous nematodes), or nematodes co-existing with earthworms enhanced CO_2 and N_2O emission compared to soils in which nematodes were killed or soils with lower populations of nematodes. Both studies were conducted in microcosms and over a short period (15 days [22] or 2 months [9]). We hypothesized that bacterivorous nematodes would increase N_2O emission initially, but a more structured soil food web with higher counts of omnivorous or predatory nematodes would reduce N_2O emissions over time.

The current research is part of the Chen et al. [23] three-year investigation that studied the influence of conservation tillage practices on weeds, soil quality and crop yields. The goal of this investigation was to determine if nematode communities are influenced by a conventional tillage, strip tillage, no-tillage and plasticulture system differently and whether their presence could influence N_2O emission. Specific objectives of this study were to (1) examine if growing the winter cover crop followed by conventional tillage and/or establishment of a plasticulture system would have reduced soil health benefits compared to winter cover cropping followed by conservation tillage practices; (2) determine the relationships between nematode assemblage and N_2O emissions during the non-cash crop period (winter cover cropping cycle) in the winter, and eggplant (*Solanum melongena*) or sweetcorn (*Zea mays*) cropping cycle in the spring/summer. It was hypothesized that (1) there would be a reduction in soil health parameters in the plasticulture system compared to conservation tillage following a winter cover crop planting; and (2) that different nematode trophic groups would affect N_2O emissions varyingly.

2. Materials and Methods

2.1. Experimental Site and Design

A field experiment was conducted at the University of Maryland, Central Maryland Research and Education Center, Upper Marlboro Facility in Upper Marlboro, MD, USA (Lat. 38°86′ N; Long. 76°78′ W) and repeated over three growing seasons from September 2011 to April 2014. These are referred to as the 2012, 2013, and 2014 trials. The soils were Annapolis series (fine-loamy, glauconitic, mesic Typic Hapludults) with 2–5% slope, 79.6% sand, 11.1% silt, and 9.3% clay [24]; 0.78% soil organic carbon and 0.06% total N. In general, a rye (*Secale cereal* L.) cover crop was grown throughout the field site prior to the initiation of the experiment (late fall 2010 to spring 2011), followed by soybean in summer 2011, which was then terminated using no-till practice with a flail mower in August 2011.

Before cash crop planting of each season (Fall of 2011, 2012, and 2013), the entire study sites were planted with a cover crop mix of forage radish (*Raphanus sativus* L. var. *longipinnatus* 'Daikon'), crimson clover (*Trifolium incarnatum* L.), and cereal rye (rye, *Secale cereale* L. 'Wheeler') using a no-till drill. While forage radish and crimson clover were seeded consistently at 4.5 and 12.3 kg seeds ha^{-1}, respectively, in all seasons, that of rye was 67.3 kg ha^{-1} in 2012, and 44.8 kg ha^{-1} in 2013 and 2014 based on learning experience from the 2012 Trial. All cover crops were terminated between late April and early May using John Deere 25A flail-mower (John Deere®, Moline, IL, USA). Cover crop biomass was subsampled from three representative quadrants prior to flail mowing to determine the dry matter, nitrogen content, and C: N ratio determination.

Following flail mowing, the four treatments installed were (1) conventional tillage without surface mulch [Bare Ground (BG)], (2) conventional tillage with black plastic mulch (BP), (3) strip-till (ST), and (4) no-till (NT). Treatments were arranged in randomized complete block design with four replications. A total of 16 field plots of 12 × 12 m^2 dimensions were separated by a minimum of 4.6 m distance between plots to allow maneuvering of equipment during field operations. For the BG and BP treatments, plots were rototilled using rototiller (HD30, Howard, Callington, Cornwall, UK) in 2012 but were chisel plowed (CPPR2-5, Brillion Farm Equipment, Marysville, KS, USA) in 2013 and 2014. For the ST treatment, a two-row strip-tiller (Bigham Brothers Inc, Lubbock, TX, USA) was used to till to 20 cm deep with a tilled width of approximately 25.5 cm. The cover crop residue outside the tilled zone was left undisturbed.

After installing the treatments, eggplant 'Dancer' seedlings were transplanted in each plot during the 2012 and 2014 Trials, whereas sweet corn 'Luscious' was direct seeded in the 2013 Trial. Twelve crop rows were planted for each plot. For BG, NT, and ST treatments, individual row spacing was 0.9 m. In the BP plot, due to the constraint from BP mulch, 6 double-crop rows of 0.8 m wide black plastic mulch were formed, and each double row was separated by 1.0 m of an alleyway. Double crop rows spaced 45 cm apart were centered on the bed. Eggplants were transplanted at 41 or 47 cm apart within rows on 24 and 29 May 2012 and 2014, respectively, whereas sweet corn was directly seeded at 23 cm plant spacing on 20 May 2013.

Crops were managed organically with a targeted nitrogen (N) rate of 134 kg N ha^{-1}. Subtracting plant-available nitrogen estimated from the cover crop of roughly 50 kg N ha^{-1} [25], an additional 84 kg N ha^{-1} of organic fertilizer was applied. In replication I and II, blood meal (12-0-0, Seven Springs Farm, Floyd, VA) and feather meal (microSTART60 Plus 7-2-2, Perdue AgriRecycle LLC, Seaford, DE, USA) were used to minimize phosphorus input because high soil phosphorus was detected in these plots from past farming activities. Poultry litter (microSTART60 3-2-3, Perdue AgriRecycle, LLC) was used in replications III and IV. Regardless of fertilizer types, organic fertilizers were applied prior to crop planting among crop rows at 45 kg N ha^{-1} in BG, NT, and ST, but at 84 kg N ha^{-1} in plots of BP treatment as the black plastic impeded side-dressing. Four weeks after planting, the remaining 39 kg N ha^{-1} of organic fertilizer was side-dressed in BG, NT, and ST treatment plots.

Crops were surface drip-irrigated to mitigate periods of low rainfall and to avoid excessive soil wetness. In 2012, 92 mm of irrigation water was supplied for all treatments. In 2013 and 2014, however, BP treatment plots were irrigated separately from the other three treatments based on the assumption that rainwater infiltration into the soil in the BP plots would be reduced by the plastic mulch. Total irrigation water was 47 and 106 mm in the BP and 20 and 64 mm in the other treatment plots in 2013 and 2014, respectively.

Eggplants were harvested two and three times per week from 8 to 13 week after transplanting (WAT) in 2012 and three times per week from 8 to 15 WAT in 2014. Eggplant fruits were sorted into marketable and unmarketable weights following US Standards for Grades of Eggplant [26]. Sweet corn ears were harvested, rated, and counted at 9, 12, and 13 WAP in 2013 using United States Standards for Grades of Sweet Corn [27].

2.2. Greenhouse Gas Emission from the Soil (N_2O-N)

In situ soil surface N_2O-N fluxes were determined using the vented, static flux chamber methodology standards set forth by the USDA-ARS GRACEnet Project [28]. Chambers were installed to a depth of 10 cm to ensure a gas-tight seal between the chamber walls and the soil. Data from the gas chambers installed between individual plants were used for this study to examine the relationship with nematode assayed from intra row root rhizosphere. Chambers in the BP plots were covered with the same black plastic mulch used for bed covering to mimic the BP mulching conditions. More detailed descriptions of the setup were described in Chen et al. [23]. Gas emissions from the soil were sampled 9, 17, and 9 times during the cover crop growing seasons of 2012, 2013, and 2014 trials, respectively, and 45, 46, and 45 times during the cash crop growing seasons of 2012, 2013, and 2014 trials,

respectively. Gas samples during cash crop seasons were taken daily for a period of 2–4 days after significant management events and rainfall events (daily total rainfall $\geq$ 5 mm), then reduced to once every 2–3 days and/or once per week during the late crop growth stages. The purpose was to capture N_2O-N emissions induced by management or rainfall events to quantify annual cumulative N_2O-N emissions more accurately [29]. Background emissions were measured the day before a management or rainfall event.

At the time of deployment, air samples (10 mL) were taken from the sampling port on top of the lid using polypropylene syringes at 0, 8, 16, and 24 min after chamber closure and injected into 12 mL Exetainers (Labco, Buckinghamshire, UK) flushed with highly purified nitrogen gas (N_2) (UHP300, Airgas USA LLC, Hyattsville, MD, USA) for 3 min at room temperature. Gas samples were analyzed with a Varian 450-GC gas chromatograph equipped with a Combi-PAL automatic sampling system and an electron capture detector (Varian BV, Middelburg, The Netherlands). Nitrous oxide concentration was computed using the ideal gas law to convert from ppm to μmol L^{-1}.

2.3. Nematode Community Analysis

Soil samples were collected 2 weeks prior to cover crop tillage treatment (30 April 2012, 21 May 2013, and 14 May 2014) during the cash crop season (29 May 2012, 9 July 2013, 17 June and 14 July 2014) and at or soon after crop harvest (21 August 2012, 27 August 2013, and 26 August 2014). Six soil cores were collected with a 2.54 cm diameter soil probe, inserted 20 cm deep into the soil close to the root zone, composited into a bucket, homogenized by hand, put in Ziploc bags, and transferred to the laboratory for analysis. Nematodes were extracted from a subsample of 100 cm^3 of soil per sample by the sieving and centrifugal flotation method [30]. Nematodes were identified to the genus level wherever possible and counted under an inverted microscope. Each nematode was assigned to one of the trophic groups: algivores, bacterivores, fungivores, herbivores, omnivores, or predators according to Yeates et al. [31]. Nematode richness was calculated based on the total number of taxa recorded. Simpson's index of dominance was calculated as $\lambda = \sum (pi)^2$, where p*i* is the proportion of each of the *i*th taxon present [32], whereas diversity was calculated as $1/\lambda$. The fungivore to fungivore + bacterivore (F/F+B) ratio was calculated to characterize the dominant decomposition pathways [33]. Taxonomic families were assigned a colonizer–persister (c-p) rating according to the 1–5 c-p scale of Bongers and Bongers [34]. Free-living nematode maturity index (MI) was calculated as $\sum (pici)$, where p is the proportion, and c is the c-p value of taxon *i* [35]. Similarly, plant–parasitic nematode maturity index (PPI) was calculated for taxa that were categorized as plant-parasitic nematodes. In addition, enrichment index (EI) was calculated as $100 \times [e/(e + b)]$ to assess soil food web responses to available nutrient resources, structure index (SI) was calculated as $100 \times [s/(s + b)]$ to reflect the complexity of trophic connection in soil food webs, and channel index (CI) was calculated as $CI = 100 \times [0.8\ F2/e]$ to determine if a soil food web was dominated by fungal or bacteria decomposition where e, s, and b are enrichment, structure, and basal food web components, and F2 is the abundance of fungivores with a c-p value of 2 [35,36].

The nematode data were also subjected to metabolic footprint (F) calculation using $F = \sum(N_t(0.1(W_t/m_t) + 0.273(W_t{}^{0.75})))$ where N_t is the abundance, W_t is the fresh weight, and m_t is the c-p value of a t taxa [21]. The metabolic footprint for EI and SI of each treatment over time were then displayed in a trajectory graph: the *x*-axis coordinates of the metabolic footprint are calculated as $\pm SI \times 0.5F_s/k$ (F_s is the sum of standardized C utilization by structure indicator taxa), whereas the *y*-axis coordinates of the metabolic footprint are calculated as $\pm EI \times 0.5F_e/k$ (F_e is the sum of standardized C utilization by structure indicator taxa). The scalar (k), maintained constant for all footprints on a graph, is to adjust for acceptable visual representation in a graph.

2.4. Statistical Analysis

The nematode data were checked for normality using Proc Univariate in Statistical Analytical Software (SAS) version 9.4 (SAS Institute Inc., Cary, NC, USA). Wherever necessary, data were normalized using log10 (x + 1) or square root transformation prior to analysis of variance (ANOVA) of year × sampling date × treatment using Proc GLM in SAS. The main interest was to examine changes in nematode community analysis and functional metabolic footprint changes over time. Thus, regardless of interaction in year × sampling date × treatment in ANOVA, nematode community analysis would still be presented for each year separately. Means of each treatment from repeated measures over the sampling date for each year were presented and separated using the Waller–Duncan k-ratio (k = 100) t-test wherever appropriate, unless significant interaction was observed between treatment and sampling date, data would then be presented by sampling date. Only the true means were presented. N_2O emission corresponding to all nematode sampling date were subjected to Orthogonal Contrasts using Proc Mixed in SAS where (1) black plastic vs. no black plastic (BG, NT, ST), (2) bare ground vs. conservation tillage (NT/ST), and (3) no-till vs. strip-till (NT vs. ST) were compared. In each trial, canonical correspondence analysis (CCA) was performed using CANOCO™ 5.5 for Windows software (Microcomputer Power, Ithaca, New York, NY, USA) to visualize the relationships between the environmental variables (nematode community indices such as CI, EI, MI, and SI, N_2O emission and soil temperature) and abundance of nematode trophic groups from each sampling date.

3. Results

3.1. General Edaphic Data

Overall, cover crop biomass in 2012, 2013, and 2014 was 6708, 6162 and 6445 kg ha^{-1} and 23.3, 30.6, and 22.8 in terms of C: N ratio, respectively [23]. Average daily temperature and total precipitation during the crop growing season was 24.1, 22.3, and 22.1 °C and 282, 550, and 451 mm in 2012, 2013 and 2014, respectively. Detailed soil temperature data were reported in Chen et al. [23]. At the post plant time of the 2012 cash crop season, no difference in surface soil temperature (0–5 cm) was detected among treatments. However, during the 2013 and 2014 cash crop seasons when soil temperature was recorded hourly at 5–10 cm deep, a higher soil temperature was observed most often in BP, followed by BG, whereas NT and ST usually had a lower soil temperature during the initial five weeks of the vegetable growing season (May to end of June). However, later in the vegetable growing season, soil temperature did not differ among treatments [23].

3.2. Effects of Plasticulture and Conservation Tillage on Nematode Community Assemblage

A total of 95 genera of nematodes were reported at this experimental site throughout 2012 to 2014. In reference to Yeates et al. [31] and Nemaplex database [37], the guild (feeding group and colonizer-persister (c-p) value) of each nematode genus was assigned in Table 1.

Prior to cover crop planting in 2012, no difference in nematode abundance for each trophic group was detected after termination of a soybean planting in August of 2012 ($p > 0.05$, data not presented). The subsequent nematode data collected since the initiation of the experiment in 2012, 2013, and 2014 showed no significant interaction between year × sampling date × treatment in ANOVA. However, due to the interest to evaluate changes in nematode community over time in response to the pre-plant soil treatments, nematode community analysis was still presented for each year separately (Table 2).

Using BG as the control throughout this experiment, the effects of different treatments on soil health were compared. Since significant interaction between date×treatment for abundance of bacterivores, omnivores, and predatory nematodes as well as CI were detected in 2012 (Table 2), these parameters were analyzed by sampling dates. Although BP, NT, and ST all decreased abundance of bacterivorous nematode initially, these treatments were not different from BG thereafter (Table 3). Similarly, soil treatments affected abundance of omnivores transiently, whereby it was only increased by NT in the first sampling date, reduced by NT in the second date, but no difference among all treatments from BG

in the last sampling date. On the other hand, BP had a negative effect on abundance of predatory nematodes initially, as well as at the end of the 2012 season ($p \leq 0.05$), resulted in 41.4% reduction by BP compared to BG. NT and ST had higher CI than BG and BP initially ($p \leq 0.05$), but not at the two later sampling dates (Table 3). NT also increased F/(F+B) ratio compared to BG throughout the 2012 season (Table 2).

Table 1. Nematode genera found in the experimental site throughout 2012–2014.

Nematode	Guild [z]	Nematode	Guild	Nematode	Guild
Achromadora	[z] A-3	*Zeldia*	B-2	*Rotylenchulus*	H-3
Prochromadora	A-3	*Prismatolaimus*	B-3	*Tylenchorhynchus*	H-3
Monochromadora	A-3	*Plectus*	B-2	*Paratrichodorus*	H-4
Alirhabditis	B-1	*Rhabdolaimus*	B-3	*Trichodorus*	H-4
Anguilloides	B-1	*Teratocephalus*	B-3	*Longidorus*	H-5
Bunonema	B-1	*Alaimus*	B-4	*Xiphinema*	H-5
Diplogasteridae	B-1	*Aphelenchoides*	F-2	*Californicus*	O-4
Diploscapter	B-1	*Aphelenchus*	F-2	*Dorylaimoides*	O-4
Halicephalobus	B-1	*Deladenus*	F-2	*Ecumenicus*	O-4
Panagrobelium	B-1	*Ditylenchus*	F-2	*Enchodelus*	O-4
Panagrolaimus	B-1	*Ecphyadophora*	F-2	*Epidorylaimus*	O-4
Plectonchus	B-1	*Filenchus*	F-2	*Eudorylaimus*	O-4
Rhabditidae	B-1	*Neotylenchus*	F-2	*Labronema*	O-4
Tricephalobus	B-1	*Nothotylenchus*	F-2	*Mesodorylaimus*	O-4
Acrobeles	B-2	*Paraphelenchus*	F-2	*Pachydorylaimus*	O-4
Acrobeloides	B-2	*Paurodontidae*	F-2	*Pungentus*	O-4
Cephalobus	B-2	*Pseudohalenchus*	F-2	*Aporcelaimellus*	O-5
Cervidellus	B-2	*Tylenchus*	F-2	*Aporcelaimus*	O-5
Chronogaster	B-2	*Psilenchus*	F-2	*Belondira*	O-5
Drilocephalobus	B-2	*Diphtherophora*	F-3	*Indodorylaimus*	O-5
Eucephalobus	B-2	*Triplonchium*	F-3	*Paraxonchium*	O-5
Heterocephalobus	B-2	*Leptonchus*	F-4	*Seinura*	P-2
Monhystera	B-2	*Tylencholaimellus*	F-4	*Tobrillus*	P-3
Monhysterella	B-2	*Tylencholaimus*	F-4	*Tripyla*	P-3
Panagrocephalus	B-2	*Psilenchus*	H-2	*Cryptonchus*	P-4
Paracrobeles	B-2	*Helicotylenchus*	H-3	*Mononchus*	P-4
Paraplectonema	B-2	*Heterodera*	H-3	*Mylonchulus*	P-4
Plectus	B-2	*Hoplolaimus*	H-3	*Prionchulus*	P-4
Pseudoacrobeles	B-2	*Meloidogyne*	H-3	*Discolaimus*	P-5
Stegelletina	B-2	*Mesocriconema*	H-3	*Nygolaimus*	P-5
Tylocephalus	B-2	*Paratylenchus*	H-3	*Paravulvus*	P-5
Wilsonema	B-2	*Pratylenchus*	H-3		

[z] A = algivore, B = bacterivore, F = fungivore, H = herbivore, O = omnivore, p = predator. Number following the feeding group are c-p values assigned according to Ferris et al. [36] and Nemaplex database [37].

For 2013 and 2014 ANOVA, no significant interaction between treatment × date was observed among all the nematode parameters; thus, means from all sampling dates for each year were presented (Table 2). In 2013, no differences in the abundance of bacterivores and omnivores were detected between BG and other treatments ($p \leq 0.05$, Table 2). However, fungivores were lower in BP ($p \leq 0.05$) and predatory nematodes were greater in NT ($p \leq 0.05$) compared to BG. Similarly, MI and CI were lower in BP compared to BG ($p \leq 0.05$). The abundance of herbivorous nematodes was higher in ST and BP compared to BG ($p \leq 0.05$). In 2014, NT and ST had the lowest abundance of omnivores, and BP had a lower number of predatory nematodes than the other treatments ($p \leq 0.05$, Table 2). Similar to 2013, ST had higher abundance of herbivorous nematodes than BG.

Table 2. The effect of plasticulture and tillage treatments on nematode community in 2012, 2013, and 2014.

Parameter	Treatments				Treatments		Treatment × Date	
	[z] BG	BP	NT	ST	F-Value	Pr > F	F-Value	Pr > F
			2012					
Abundance			nematodes/100 cm^3 soil					
Bacterivore	798 ± 184 a [y]	529 ± 57 ab	408 ± 68 c	463 ± 53 bc	5.11	0.0052	8.47	<0.0001
Fungivore	1003 ± 230 a	835 ± 190 a	1169 ± 207 a	1071 ± 209 a	1.06	0.38	1.19	0.338
Herbivore	505 ± 82 a	733 ± 420 a	520 ± 161 a	469 ± 176 a	0.71	0.5556	0.32	0.9209
Omnivore	120 ± 13 a	114 ± 10 a	120 ± 21 a	131 ± 17 a	0.41	0.7438	3.08	0.0167
Predatory	41 ± 8 a	24 ± 5 b	33 ± 7 ab	34 ± 9 ab	3.85	0.0182	5.47	0.0005
Indices [x]								
F/F+B	0.50 ± 0.22 b	0.53 ± 0.24 b	0.68 ± 0.19 a	0.63 ± 0.2 ab	3.72	0.0209	1.95	0.1025
Richness	24 ± 1 a	24 ± 1 a	24 ± 1 a	24 ± 1 a	0.31	0.8177	2.49	0.0424
MI	2.26 ± 0.09 ab	2.16 ± 0.04 b	2.28 ± 0.07 ab	2.28 ± 0.04 a	2.41	0.0842	5.09	0.0009
EI	42.01 ± 4.93 a	46.76 ± 4.77 a	41.78 ± 3.24 a	40.07 ± 3.59 a	1.54	0.2222	2	0.0937
SI	40.19 ± 6.1 a	39.37 ± 3.51 a	37.24 ± 5.26 a	40.26 ± 3.51 a	0.27	0.8642	3.43	0.0097
CI	70.65 ± 8.68 b	61.41 ± 8.63 b	82.46 ± 4.63 a	82.89 ± 4 a	6.35	0.0016	5.49	0.0005
			2013					
Abundance			nematodes/100 cm^3 soil					
Bacterivore	664 ± 115 ab	766 ± 79 a	478 ± 80 b	630 ± 131 ab	2.84	0.0526	2.16	0.073
Fungivore	604 ± 98 a	447 ± 85 b	710 ± 127 a	570 ± 77 ab	3.29	0.0326	2.36	0.0527
Herbivore	261 ± 71 b	770 ± 253 a	339 ± 72 ab	622 ± 160 a	3.9	0.0172	1.1	0.3809
Omnivore	46 ± 8 a	94 ± 21 a	44 ± 8 a	53 ±9 a	1.19	0.3285	0.92	0.4902
Predatory	16 ± 6 b	8 ± 2 b	29 ± 5 a	17 ± 2 ab	5.25	0.0045	1.18	0.339
Indices								
F/F+B	0.48 ± 0.04 a	0.35 ± 0.04 a	0.58 0.06 a	0.49 ± 0.04 a	6.11	0.002	1.73	0.1451
Richness	25 ± 2 a	26 ± 1 a	28 ± 1 a	28 ± 1 a	1.28	0.2958	0.39	0.8830
MI	1.85 ± 0.06 a	1.69 ± 0.03 b	1.90 ± 0.04 a	1.79 ± 0.02 ab	5.11	0.0052	0.27	0.9487
EI	63.15 ± 1.89 a	66.85 ± 1.75 a	66.12 ± 3.12 a	67.47 ± 2.25 a	0.83	0.4893	0.99	0.4476
SI	33.49 ± 5 a	32.84 ± 5.05 a	42.47 ± 3.44 a	36.05 ± 4.58 a	1.8	0.1667	0.55	0.7643
CI	37.63 ± 3.55 a	24.1 ± 2.99 b	44.13 ± 7.55 a	33.9 ± 4.5 ab	3.73	0.0206	1.56	0.1906
			2014					
Abundance			nematodes/100 cm^3 soil					
Bacterivore	486 ± 154 a	418 ± 66 a	292 ± 32 a	387 ± 70 a	0.28	0.8418	0.2	0.9933
Fungivore	384 ± 39 a	415 ± 102 a	364 ± 64 a	485 ± 135 a	0.45	0.7211	0.93	0.5059
Herbivore	135 ± 46 b	234 ± 136 b	239 ± 99 ab	209 ± 57 a	2.88	0.0461	0.63	0.7621
Omnivore	40 ± 6 a	31 ± 4 ab	25 ± 4 b	27 ± 6 b	3.67	0.0189	2.36	0.0579
Predatory	12 ± 3 a	6 ± 2 b	12 ± 3 a	18 ± 4 a	3.48	0.0233	0.94	0.4976
Indices								
F/F+B	0.53 ± 0.05 a	0.49 ± 0.04 a	0.53 ± 0.05 a	0.52 ± 0.05 a	0.51	0.6786	0.97	0.4735
Richness	22 ± 2 a	20 ± 1 a	23 ± 1 a	23 ± 1 a	1.41	0.2514	0.78	0.6349
MI	1.72 ± 0.07 a	1.66 ± 0.11 a	1.6 ± 0.1 a	1.57 ± 0.07 a	0.73	0.5375	0.32	0.9647
EI	65.55 ± 1.96 a	68.02 ± 3.15 a	66.87 ± 2.46 a	68.16 ± 2.55 a	0.31	0.8179	0.56	0.8211
SI	33.47 ± 2.99 a	33.27 ± 3.74 a	39.83 ± 3.27 a	35.9 ± 3.62 a	1.54	0.2172	1.98	0.0648
CI	39.64 ± 4.94 a	37.38 ± 6.53 a	38.71 ± 5.33 a	37.17 ± 5.27 a	0.07	0.9752	0.59	0.7975

[z] BG = Bare ground (Conventional tillage without mulch), BP = Conventional tillage with black plastic, NT = no-till, ST = strip-till. [y] Values are means from four replicated plots repeated measured over 3 times per trial (n = 12). Means followed by the same letter(s) in a row are not different according to Waller-Duncan k-ratio (k = *100*) t-test. [x] F/(F + B) = ratio of fungivores/(fungivores + bacterivores), MI = maturity index, EI = Enrichment index, SI = Structure index, CI = Channel index.

Table 3. The effect of plasticulture and tillage treatments on abundance of bacterivorous, omnivorous and predatory nematodes at 2 weeks before cover crop termination (4/30), mid (5/29) and end (8/31) of eggplant crop in 2012.

Parameter	Treatments					
	[z] BG	BP	NT	ST	F-Value	Pr > F
			04/30/2012			
Abundance			nematodes/100 cm^3 soil			
Bacterivore	1640 ± 98 a [y]	563 ± 118 b	363 ± 98 b	464 ± 113 b	16.18	0.0006
Omnivore	89 ± 16 b	102 ± 17 ab	183 ± 46 a	157 ± 43 ab	3.61	0.0587
Predatory	58 ± 13 a	24 ± 5 b	62 ± 2 a	70 ± 16 a	4.26	0.0395
CI	34 ± 7 b	38 ± 8 b	74 ± 8 a	80 ± 1 a	16.10	0.0006
			05/29/2012			
Abundance			nematodes/100 cm^3 soil			
Bacterivore	424 ± 59 a	484 ± 66 a	654 ± 79 a	566 ± 98 a	1.46	0.2896
Omnivore	132 ± 17 a	111 ± 14 ab	99 ± 11 b	141 ± 22 a	4.08	0.0438
Predatory	51 ± 11 a	42 ± 6 a	20 ± 4 b	15 ± 3 b	10.92	0.0023
CI	78 ± 6 a	80 ± 5 a	73 ± 3 a	68 ± 2 a	1.28	0.3381
			08/31/2012			
Abundance			nematodes/100 cm^3 soil			
Bacterivore	329 ± 49 ab	540 ± 127 a	207 ± 23 b	360 ± 42 ab	4.58	0.0329
Omnivore	140 ± 27 a	131 ± 20 a	79 ± 23 a	97 ± 9 a	1.94	0.1943
Predatory	16 ± 3 a	7 ± 2 b	17 ± 5 ab	18 ± 5 a	3.12	0.0810
CI	100 ± 0 a	66 ± 21 a	100 ± 0 a	100 ± 0 a	2.62	0.1153

[z] BG = Bare ground (Conventional tillage without mulch), BP = Conventional tillage with black plastic, NT = no-till, ST = strip-till. [y] Means (n = 4) followed by the same letter(s) in a row are not different according to Waller-Duncan *k*-ratio (*k* = 100) *t*-test.

3.3. Effects of Cover Crop Treatments on Nematode Functional Metabolic Footprint over Time

A graphic display of the metabolic footprint for enrichment and structure indicators to depict the functional characteristics of the soil food web condition is shown in Figure 1 where the trajectory of SI-EI was surrounded by sequentially joining points of the functional metabolic footprint calculation for SI (Fs) and EI (Fe): SI—0.5Fs/k, EI; SI, EI + 0.5Fe/k; SI + 0.5Fs/k, EI; SI, EI—0.5Fe/k; and SI–0.5Fs/k, EI. The metabolic footprint or standardized Carbon utilization by the indicator taxa (Fe or Fs) were adjusted by k = 10 for acceptable visual representation and comparison of footprints of different treatments, as suggested by Ferris [21].

While EI and SI did not reveal differences among treatments (Table 2), metabolic footprint showed a successional transition of the soil food web from relatively nutrient enriched but disturbed (EI $\geq$ 50%, SI $\leq$ 50%) towards relatively nutrient depleted but stable (EI $\leq$ 50%, SI $\geq$ 50%) conditions in 2012 (Figure 1A–C). The cycle of nutrient enrichment by the planting of cover crop mix repeated in 2013 and 2014 at the beginning of each season (Figure 1D,G) with NT and ST slightly ahead of the other treatments in SI in 2013 and 2014, respectively. It is encouraging to document that all treatments no longer become nutrient depleted towards the end of the 2013 and 2014 seasons (Figure 1F,I), as observed in 2012 (Figure 1C), but the succession of SI were not as apparent as that observed in 2012. While NT tended to be ahead of the game in terms of increasing SI during the mid seasons of 2013 and 2014 (Figure 1 E,H), BG treatment was able to match up with NT towards the end of both seasons (Figure 1 F,I). In fact, even BP had a similar SI-EI metabolic footprint compared to all other treatments at the end of 2013 (Figure 1H).

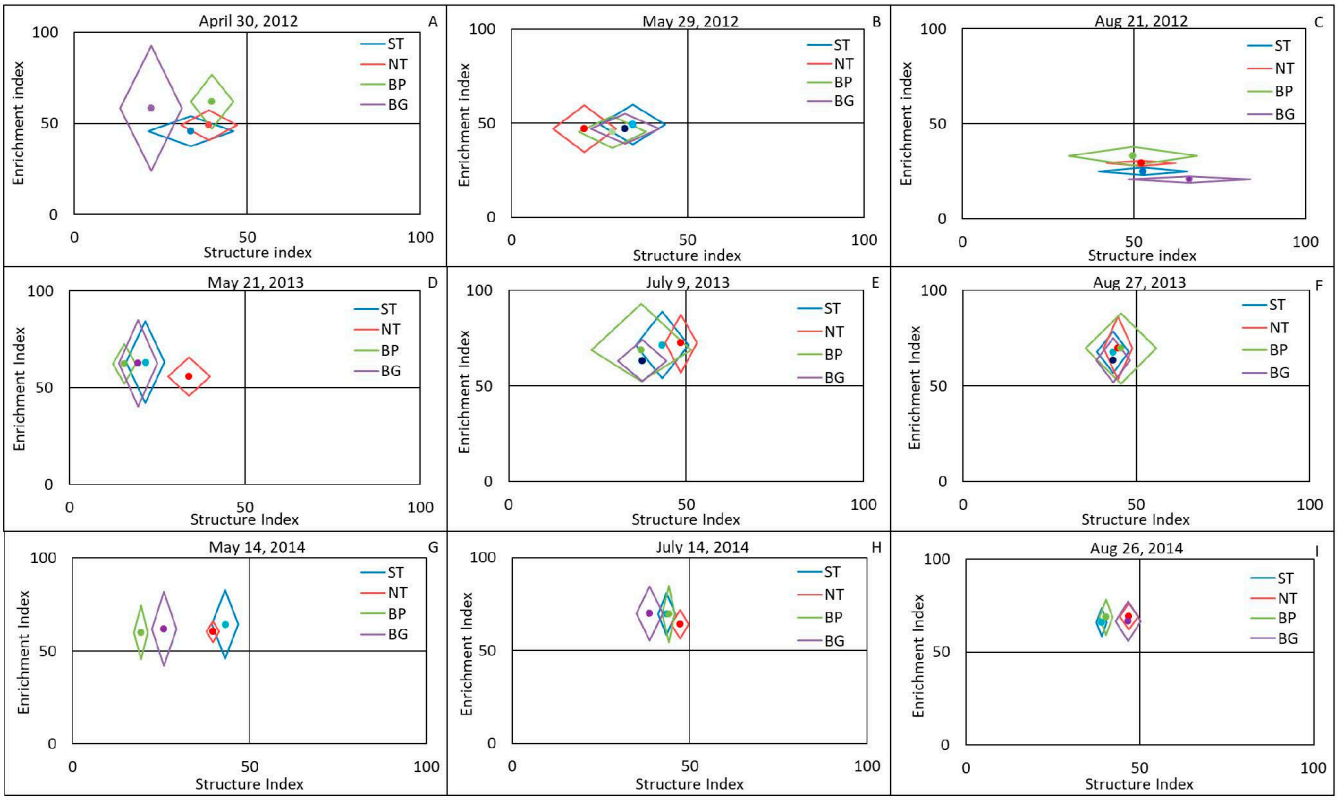

Figure 1. The functional metabolic footprints of soil food webs from each sampling date in 2012 (**A**–**C**), 2013 (**D**–**F**), and 2014 (**G**–**I**) field trials. The vertical axis of each footprint represents the enrichment footprint and horizontal axis represents the structure footprint. BG = Bare ground (Conventional till without mulch), BP = Conventional till with black plastic, NT = no-till, ST = strip-till.

3.4. N_2O Emission Corresponding to Nematode Sampling Events

Detailed N_2O emission and N_2O-N flux were reported by Chen et al. [16]. As such, only N_2O emission corresponding to sampling dates for nematode community analysis are presented here (Table 4). Significant differences were detected between black plastic (BP) vs. no black plastic (BG, NT, ST) in all three trials, whereas no differences were observed between BG vs. conservation tillage (NT, ST) or NT vs. ST in all trials (Table 4). In general BP consistently had higher N_2O emissions than the other three treatments. We used these data to perform the CCA in the next section.

Table 4. The effect of plasticulture or tillage treatments on nitrous oxide emission in 2012, 2013 and 2014.

	Treatments				Contrast (*p* Values) [x]		
Year	**[z] BG**	**BP**	**NT**	**ST**	**BP vs. no BP**	**BG vs. CT**	**NT vs. ST**
		N_2O g ha^{-1}					
2012	1.97 [y]	5.60	3.80	2.94	0.040 *	NS	NS
2013	3.28	7.10	1.33	4.21	0.019 *	NS	NS
2014	24.58	84.25	28.80	16.01	0.005 **	NS	NS

[z] BG = Bare ground (Conventional tillage without mulch), BP = Conventional tillage with black plastic, NT = no-till, ST = strip-till. [y] Values are means from four replicated plots repeated measured over 3 times per trial (n = 12). [x] Orthogonal contrasts between black plastic vs. no black plastic (BP vs. no BP), bare ground vs. conservation tillage or NT/ST (BG vs. CT), and no-till vs. strip-till (NT vs. ST). * and ** signify contrast significant at $p \leq 0.05$ and 0.01, respectively.

3.5. Relationships between Nematode Assemblage with N_2O Emissions

The relationships between nematode assemblages and N_2O emissions for each trial were depicted in ordination diagrams (Figure 2). The first two canonical axes in the ordination diagram explained 91.73%, 89.85%, and 85.59% of the variance between the environmental variables (nematode community indices, N_2O emission and soil temperature) and abundance of nematode trophic groups in 2012, 2013, and 2014 trials, respectively (Figure 2A–C). These ordinations demonstrate a close relationship between the environmental variables and nematode abundance in all trials.

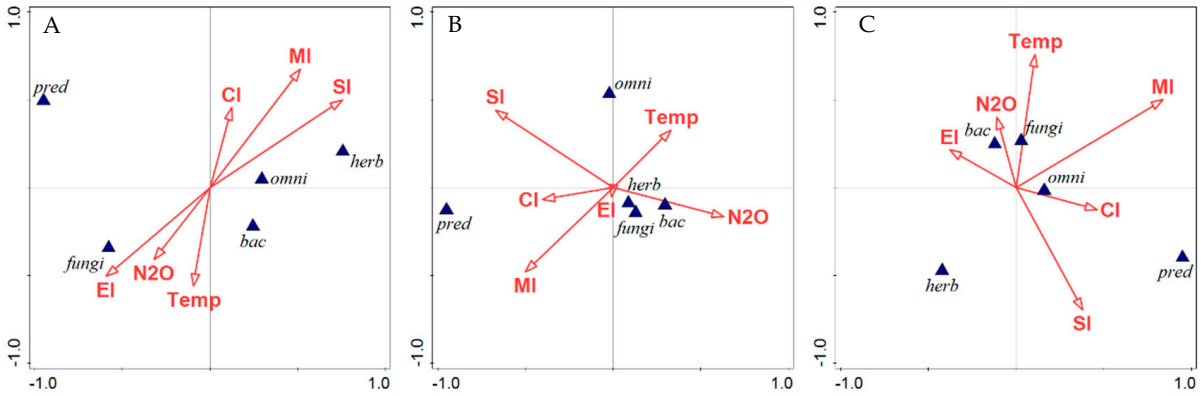

Figure 2. A canonical correspondence analysis biplot of environmental variables (red arrows) with nematode abundance variables (triangles) in (**A**) 2012 eggplant, (**B**) 2013 corn and (**C**) 2014 eggplant cropping systems following four soil treatments. Bac = bacterivores, Fungi = fungivores, Herb = herbivores, Omni = omnivores, Pred = predators; CI = channel index, EI = enrichment index, MI = maturity index, SI = structure index, Temp = soil temperature, and N_2O = N_2O emissions corresponding to nematode sampling time.

The N_2O emission was negatively related to SI and CI, but positively related to abundance of fungivorous nematodes consistently in 2012, 2013 and 2014. N_2O emission was also consistently positively related to EI in years eggplant was planted or when cover crop residues had C: N ratio of $\leq$ 30 (2012 and 2014). While the N_2O emission was not related to the abundance of bacterivorous and predatory nematodes in 2012, it was positively related to abundance of bacterivores and negatively related to abundance of predatory nematodes thereafter (2013 and 2014). In contrast, the abundance of omnivorous nematodes was negatively related to the N_2O emission in 2012, but not in 2013 and 2014. The relationship between N_2O emission with an abundance of herbivorous nematodes varied from negative in 2012, positive in 2013, to non-related in 2014. Soil temperature was positively related to the N_2O emission in 2012 and 2014 but was weakly related in 2013.

4. Discussion

4.1. Effects of Plasticulture and Conservation Tillage on Nematode Community Assemblage

This study indicates that growing winter cover crops followed by conventional tillage and plasticulture (BP) during the vegetable cropping cycle will negate the soil health benefits compared to growing a winter cover crop followed by conventional tillage without plasticulture (BG) or conservation tillage practices [no tillage (NT) or strip tillage (ST)]. In 2012, the abundance of predatory nematodes was reduced in BP compared to BG despite similar tillage treatment between BP and BG. This indicated that the soil experienced greater disturbances in BP possibly due to warmer soil in BP [23] or creation of an anaerobic condition under the black plastic [38] and subsequently resulted in a less structured soil food web. Predatory nematodes have a longer life cycle and persist longer in the soil. However, if disturbed, they reproduce slowly [31]. In 2013, the abundance of fungivorous nematodes, maturity index (MI), and channel index (CI) were reduced in BP compared to BG, while the abundance of herbivorous nematodes increased. These reductions signify a

less healthy soil environment in BP than BG. Moreover, soils in the BP showed reduced fungal decomposition (low CI, low fungivores) and were populated by nematodes with lower maturity (low MI), suggesting a soil dominated by bacterial decomposition and more disturbed soil food web in BP [34]. Similarly, in 2014, BP contained the lowest number of predatory nematodes, which is indicative of a continuous disturbance to the soil food web.

Contrary to our hypothesis, conservation tillage (NT, ST) did not improve soil health parameters compared to the BG system, based on the nematode soil health indicators measured. During the three-year study of continuous practices, NT and ST had an increased number of predatory nematodes compared to BG in 2013. An increase in predatory nematodes suggests that soils are less disturbed and more structured. In 2012, there was a reduced abundance of bacterivorous nematodes in NT and ST which led to an increase in CI compared to BG indicating that the soil food web was depleted of nutrients and dominated by fungal decomposition. In 2014, there was a reduced abundance of omnivorous nematodes in NT and ST compared to BG. This suggests that these conservation tillage practices did not significantly improve soil food web structure despite three years of continuous conservation practice compared to the BG treatment. It is possible that the benefits of soil amendment from winter cover cropping of forage radish, crimson clover, and cereal rye in BG were more influential than the conservation tillage practices. Although BG had the same cover crop densities as NT and ST, the cover crop was incorporated into the soil, and as such, could be broken down faster than residue that remained on the surface in NT and ST, making nutrients more available to initiate bacteria decomposition. This is evident by the higher bacterivores in BG than in other treatments in 2012. Based on nematode community indices taken at the experimental site prior to treatment application, soil health conditions were already in relatively good conditions (EI > 50%, SI around 30–40%). This could be due to a history of growing a rye cover crop and practicing no-tillage throughout the field site prior to the experiment initiation (late fall 2010 to spring 2011). Further, a soybean crop was terminated late summer in 2011 prior to initiation of the current experiment when the winter cover crop was no-till planted.

The cover crops such as the mixture used in the current study are planted during late summer or early fall to protect the Chesapeake watershed in Maryland [39]. Forage radish has been widely adopted as a winter cover crop by farmers for its ability to alleviate soil compaction, capture excess N, and suppress weeds [40,41]. Crimson clover has been shown to enrich the soil due to its nitrogen fixing efficiency and thus can enhance crop growth [42]. Rye is commonly grown in the mid-Atlantic region to scavenge nutrients so as to reduce postharvest nutrient loss in the Chesapeake watershed [39]. We anticipated that continuous practice of conservation tillage such as NT and ST with this cover crop mixture would increase EI or SI, as had been reported previously [43]. In that study, when a rye and crimson clover cover crop mixture were terminated by a roller crimper followed by ST, this resulted in a higher enrichment index (more opportunistic bacterivorous nematodes) and improved soil food web structure (higher structure index) through the mid-season or the entire duration of the subsequent cash crop cycle.

When SI and EI were subjected to functional metabolic footprint calculation, changes among treatments were observed. Using the metabolic footprints based on C utilization for nematode production (body mass) and respiration rates and displayed them by structure and enrichment indicators in a trajectory graph, Ferris [21] was able to separate soil food web structures that were not separable using nematode weight abundance calculated EI and SI. In the 2012 trial, overall soil food web at the beginning of the eggplant crop was nutrient enriched but disturbed and slowly transitioned into nutrient depleted but more stable conditions (higher in SI) towards the end of the eggplant crop. This was much expected, as the soil was allowed to recover from the planting disturbance over a 4-month period, but the organic nutrients from the cover crop residues could be depleted at the end of the eggplant crop. What was unexpected was a higher SI metabolic footprint of BP than BG initially, and a higher EI metabolic footprint of BP than BG at the end of the 2012 trial. This could be a sudden boost in anaerobic bacteria in BP that continued throughout the season

as the black plastic mulch stayed throughout the eggplant crop. Another fact revealed in the functional metabolic footprint (Figure 1C) was that the functional metabolic footprint at the end of 2012 was minimized, as the rhomboid shape became flatten indicating that the productivity and turnover rates of the enrichment indicators, representative of nematode prey abundance, were not sufficient to maintain the needs of the predators (the structure indicators), i.e., the system was in a metabolically imbalanced state [21]. The overall depletion in nutrient enrichment of soil food web regardless of soil treatments in 2012 was not healthy for the soil ecosystem. However, continued cover cropping with the same soil treatments in 2013 changed the rhomboid shape toward square shape for BP indicated a balance prey and predatory nematodes ratio, or elongated shape for NT indicated good support of prey to predatory nematode ratio at the end of the 2013 season (Figure 1F). It was rather consistent that NT improved the functional metabolic footprint of SI at the initial sampling dates of 2013 and 2014, though the effect dissipated towards the end of the seasons (not different from the BG control). Perhaps the planting of cover crops is a stronger drive to improve the soil health condition than the soil treatments tested if sufficient time is allowed for the soil food web to recover. In both 2013 and 2014, the soil food web continued to maintain high nutrient enrichment (EI > 50%) by all treatments without showing nutrient depletion as was shown in 2012. While ST and NT increased initial SI metabolic footprint, BG was able to increase the SI metabolic footprint matching that of NT at the end of 2014, which was higher than ST and BP (Figure 1I).

Soil health improvement by conservation tillage might require longer observation than 3 years, but differences in soil health conditions (Tables 2 and 3 and Figure 1) and other soil microclimate measurements [23] that can influence nematode communities were detected among treatments that warrant a multivariate analysis to examine relationships between nematode assemblage with N_2O emissions.

4.2. Relationships between Nematode Assemblage with N_2O Emissions

The canonical correspondence analysis (CCA) conducted for the 2012, 2013, and 2014 trials demonstrated that different trophic groups of nematodes responded to N_2O emissions varyingly, but SI and CI were always negatively related to N_2O emissions. While an abundance of fungivorous nematode was always positively related to N_2O emissions, the relationship between N_2O with abundance of other nematode trophic groups tended to transition over time. Initially, in 2012, an abundance of bacterivores was only weakly positively related to N_2O emissions but rather strongly and negatively related to abundance of omnivorous nematodes. This is unexpected, as nitrification and denitrification are bacterial driven processes and are the main mechanisms of N_2O emission in soils [44,45]. This could be due to succession of nematode trophic group where the abundance of bacterivorous nematodes led to more abundant omnivorous nematodes, and more abundant omnivorous nematodes affected the relationship between the abundance of bacterivores with N_2O emission. However, in the 2013 and 2014 trials, N_2O emissions were positively related to the abundance of bacterivorous nematodes, much like what is expected [44,45] and negatively related to abundance of predatory nematodes. A higher abundance of predatory nematodes could have top-down regulation of bacterivores and thus reduced N_2O emission. These relationships between nematode abundance and N_2O emissions could further be influenced by soil temperature, C: N ratio of cover crops, or soil aeration, as discussed below.

4.2.1. Effects of Soil Temperature

Soil temperature can affect the magnitude of N_2O fluxes, as it can inhibit or enhance the enzymatic steps in nitrification and denitrification [45,46]. In 2012, since there were no obvious soil temperature differences among soil treatments, no clear relationship between abundance of bacterivorous nematodes with N_2O was observed (Figure 1). In 2013 and 2014, the highest soil temperature was recorded in BP, followed by BG from the early to the middle of the vegetable growing season (late May to end of June) [23]. Nitrification and

denitrification processes can be catalyzed by warmer temperatures and thus increase the abundance of soil bacteria involved in these processes and thus lead to higher abundance of bacterivorous nematodes. Lower soil temperature is a common phenomenon observed in conservation tillage systems where surface mulch generated from cover crop residues in a no-till system maintained a cooler soil temperature than conventional till especially during the initial five weeks after cover crop termination [47]. Therefore, N_2O emission was consistently higher in BP than the other treatments (Table 3), which coincided with the greatest N_2O-N fluxes in BP and the lowest in NT during each study year [23].

4.2.2. Effects of Different Nematode Trophic Groups and C: N of Cover Crops

The positive relationship between N_2O emissions and the abundance of bacterivorous nematodes in 2013 and 2014 supported other studies investigating the roles of bacterivorous nematodes on N_2O emission [22]. Zhu et al. [48] conducted a 50-day microcosm experiment and found that the addition of bacterivorous nematode *Protorhabditis oxyuroides* promoted soil N_2O, as well as CO_2 emissions. Zhu et al. [48] determined that the stimulation of N_2O was not related to changes in the abundance of bacteria or ammonia-oxidizing bacteria but rather the abundance of bacterivorous nematodes. This is because *P. oxyuroides* stimulated rates of mineralization of organic N to NH_4^+, oxidation of NH_4^+ to NO_3^-, immobilization of NO_3^- to organic N and dissimilatory of NO_3^- to NH_4^+. However, not all bacterivorous nematodes behave similarly as no effect on N_2O emission was found when another bacterivorous nematode (*Rhabditis intermedia*) was introduced. Chen et al. [23] also provided evidence that conventional tillage (BP and BG) promoted more N mineralization, and, as such, provided more substrates to generate N_2O. This coincided with higher bacterivorous nematode abundance in BP and BG than in NT in 2012 and 2013.

Enrichment index, which is a measure of weight abundance of opportunistic bacterivores, often indicates more nitrogen mineralization or nutrient enrichment [36] was positively related to N_2O emissions in 2012 and 2014, the years when the cover crop residues had C: N < 30. In 2013, when C: N of cover crop residues was 30.6, it was difficult for bacterial decomposition to take place [49]; thus, no clear relationship between EI and N_2O emissions was observed.

While Kuiper et al. [9] found that adding fungivorous nematodes (*Aphelenchoides subtenuis*) to sandy soil did not increase N_2O emissions during a 2-month microcosm study, the current study reported consistent positive relationship between the fungivorous nematode abundance to N_2O emission in all three years. Fungivorous nematodes did not stimulate N_2O emissions when Kuiper et al. [9] used hay with a C: N ratio of 13.7 whereas the C: N ratio of cover crop residues in the current experiment were all >20 (23.3, 30.6 and 22.8 in 2012, 2013 and 2014, respectively). Further studies are needed to investigate the roles of fungivores on N_2O emissions when using cover crop residues with different C: N ratios.

The current study provided an initial insight on the relationship between the abundance of omnivorous or predatory nematodes and N_2O emissions. It is encouraging to see that the consistent trend of a more structured soil food web indicated by higher SI or higher abundance of omnivorous and predatory nematodes was related to lower N_2O emissions in the CCAs. This is not surprising, as omnivorous and predatory nematodes could regulate the abundance of bacterivorous and fungivorous nematodes [50] as shown in Figure 1. If omnivores and predatory nematodes reduce the abundance of bacterivorous nematodes, N mineralization rates of cover crop residues would be reduced [51], and, as such, provide less substrates for N_2O emissions.

4.2.3. Soil Aeration

While nitrification is an aerobic process, denitrification occurs under anaerobic conditions where soil nitrates or nitrites are converted to N_2O or N_2 by denitrifying bacteria [6,52]. Although soil redox potential was not monitored in this experiment, soil oxygen is expected to be limited under BP compared to no plastic [38]. We speculated that soil aeration could also be another factor affecting the relationship between nematode assemblages with N_2O

emissions. Under extreme anaerobic conditions such as that in an anaerobic soil disinfestation (ASD) field where a readily degradable carbon source is soil incorporated, followed by irrigation to saturate the soil, then covering the soil with totally impermeable film for about 3 weeks [53], Guo et al. [54] and Li et al. [15] found that cumulative N_2O emissions were higher than those from the chloropicrin + 1,3-dichloropropene fumigated or untreated control soil especially in the warmer area.

5. Conclusions

Changes in soil food web structure from different soil treatments following cover cropping during the 3-year study were inconsistent or insignificant based on nematode abundance community analysis but showing a progressing trend of improvement with the nematode functional metabolic footprint analysis. However, the CCA showed a clear result of improving the soil food web structure through an increase in omnivorous and predatory nematodes and SI would lead to less N_2O emissions. The implications of this finding can be summarized here. Firstly, the mitigation of N_2O emissions through conservation tillage could help reduce greenhouse gas emissions contributed to agriculture [3]. Secondly, farmers could increase their N fertilizer efficiency by practicing conservation tillage after cover cropping. It is known that in conventional tillage systems, every 1000 kg of N fertilizers applied leads to an approximate 10–50 kg N loss as N_2O from soil, and N_2O emissions increase exponentially relative to increasing nitrogen inputs [55]. Chen et al. [23] calculated yield-scaled N_2O-N emissions (kg N_2O-N Mg^{-1} of eggplant or kg N_2O-N 10^{-3} ears of sweet corn) from the current experiment and determined that BP had the highest N_2O-N loss whereas NT had the lowest during all three years. Another outcome that can be predicted as a result of this study is that establishing a vegetable plasticulture system following a winter cover crop and conventional tillage will disturb the soil food web structure more as well as contribute to higher N_2O emissions than practicing conventional or conservation tillage without black plastic. Moreover, covering the soil with black plastic also disables organic farmers from applying split fertilizer applications. This can further exacerbate N_2O emissions.

Although growing cover crops is generally considered a good practice for improving soil health, as this study indicates, its efficacy is dependent on subsequent soil husbandry practices. Conventional tillage following cover cropping can increase the abundance of bacterivorous nematodes, and this is generally an indicator of improved soil nutrient cycling and an enriched soil food web. However, this practice may also cause a temporary increase in N_2O emission. In contrast, conservation tillage practice can be used to synergize the soil health benefits of cover cropping while mitigating N_2O emission by improving soil food web structure over time. In some instances, this process may take some time. For example van Kessel et al., [14] reported that conservation tillage reduced N_2O emissions significantly after 10 years of continuous practice. Nonetheless, by monitoring nematode community structure over an entire cropping season, this investigation provided a more extensive picture of the role that different trophic groups of free-living nematodes play in N_2O emission.

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