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The Soil Food Web Model as a Diagnostic Tool for Making Sense out of Messy Data: A Case of the Effects of Tillage, Cover Crop and Nitrogen Amendments on Nematodes and Soil Health

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Abstract: Tillage, cover crops (CC) and nutrient amendments are regenerative agricultural practices (RAPs) which enhance desirable ecosystem services (DESs), including the beneficial nematode community structure (BNCS), soil organic matter (SOM), pH, and available nitrogen, and the Ferris et al. soil food web (SFW) model relates changes in the BNCS to biophysicochemical conditions generating DESs. However, the SFW model's power to identify soil health conditions influencing DESs' outcomes has been limited. We tested how tillage, winter rye CC, and 0, 112, or 224 kg N/ha from inorganic and compost sources affected the DESs after four years of corn production. The SOM and NO_3 was much greater in the no-till than the tilled soil, and the SOM in the 224 kg organic source, compared with the rest of the N rates, was significantly increased. The N recovery was not proportional to what was applied. The variable effects of the RAPs on the DESs suggest either changing or continuing treatments until suitable outcomes are achieved, all without knowing the source(s) of variability. The SFW model revealed primarily resource-limited and structured (Quadrant C) conditions, suggesting that (1) nutrient cycling needs biological activities and (2) the presence of a process-limiting factor may have contributed to the variable results. The impacts of the SFW model as a diagnostic tool are outlined.

Keywords: cover crop; ecosystem services; decision making; nematodes; nutrient cycling; regenerative agricultural practices; soil health; tillage

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1. Introduction

1.1. The Challenges for Achieving Healthy Soils

It is well established that soil health degradation in agricultural and non-agricultural soils, including urban, recreational, and forest landscapes, is a global problem. Soil health, defined as "the capacity of a soil to function", has biological, physicochemical, nutritional, structural, and water-holding integrity components which need to be kept in balance [1–3], and healthy soil is soil which generates three sets of desirable ecosystem services (DESs) simultaneously. These are (1) improved soil structure, physicochemistry, nutrient cycling, and water holding capacity; (2) suppressing pests and diseases while ensuring increased beneficial organisms in the same environment; and (3) improved biological functioning and crop yield [4–16]. As recent extensive reviews have shown, a broad range of regenerative agricultural practices (RAPs) are a critical part of improving soil health [17–20]. Conservation tillage, cropping systems, cover crops, and organic soil amendments are examples of commonly used RAPs to improve soil health [5,6,20]. While there have been tremendous advances in the three sets of DESs using RAPs, developing integrated soil health management strategies remains difficult for several reasons. First, key soil health indicators which RAPs influence, such as changes in the soil organic matter (SOM) percentage, soil pH, nutrient cycling, and nematode community structure (NCS) as indicators of soil health, to mention a few, are in disciplinary silos [21–24]. There are no cross-disciplinary standards or quantitative ways for characterizing the different soil health indicators. Second, the effects of RAPs on components and indicators of soil health vary widely by soil type, cropping system, season, time, and space [22–24]. Under these circumstances, addressing soil health degradation conditions effectively becomes a fast-moving target and a puzzle of many dimensions. Third, there is a lack of integrated understanding of process-based outcomes which consider the overall environment where the interactions that generate the DESs take place. RAPs affect the components and indicators of soil health differently, making it difficult to quantify without integrated understanding of the belowground biophysicochemical environment. Fourth, there is the lack of a portfolio of practice- and outcome-based matrices which account for local biogeographic information. Fifth and finally, there is a lack of integration platforms which identify soil conditions and align the three sets of DESs simultaneously or step by step to achieve healthy soil [25]. In this case, the Ferris et al. [26] soil food web (SFW) model can serve as a diagnostic tool for identifying soil health conditions and outcomes as well as a platform for the step-by-step integration of DESs. This, in turn, could lead toward identifying soil health conditions from a single core of soil [21].

1.2. How the SFW Model Identifies Soil Health Conditions

Nematodes are the most abundant metazoa on the planet, and many indices of ecological and belowground biophysicochemical disturbances have been described based on changes in the abundance and diversity of soil-dwelling nematode communities [15,27–29]. The SFW model, based on analysis of nematode assemblages, is the most comprehensive tool for inferring belowground biophysicochemical changes. It uses changes in the abundance and function of the beneficial (bacterivore, fungivore, predator, and omnivore) nematode community structure (BNCS) relative to resources and reproduction (enrichment index (EI)) as well as life history and intolerance of disturbances (structure index (SI)) to describe the nutrient cycling potential and suitability of soil conditions for agroecosystems [22]. The effects of RAPs on one or more of the components or indicators of soil health could be desirable, undesirable, or variable, leading to decisions to continue, discontinue, or keep trying the treatments until the desirable outcome is achieved, respectively [21]. The critical knowledge gap here is that it is difficult to know if the variable outcomes are because the RAPs did not work or the soil conditions were not ideal for the RAPs to work. In simple terms, the SFW model applies biological, ecological, and mathematical principles to changes in the EI and SI. The model uses the relationship between the SI (x axis) and EI (y axis) to identify four best-to-worst case categories of soil conditions for nutrient cycling potential and agroecosystem fitness. These are enriched and unstructured (Quadrant A), enriched and structured (Quadrant B, best case), resource-limited and structured (Quadrant C), and resource-depleted and unstructured (Quadrant D, degraded and worst case). Enriched means N is available, limited requires carbon availability and biological activity for N to be released, and depleted biologically degraded. Simply put, knowing how soil conditions relate to variable soil health outcomes is the first step toward aligning DESs and developing suitable soil health management strategies, and the SFW model is the tool for it.

1.3. Why the Current Study Is Needed

The Ferris et al. [26] SFW model has been cited about 1800 times and applied to describe the effects of RAPs and related treatments on nutrient cycling and suitability for agroecosystem functioning in many ways. Examples range from considering separate changes in the EI and SI [30] to describing the outcome through the interaction of the EI and SI in quadrant format [30–38], to mention a few. While all are valid, they lead to different conclusions. Let us assume that an RAP results in values of 30% EI, 80% SI, and low available N. If the EI and SI are considered separately, then the results would suggest that the RAP is working for the SI and likely to be looked at favorably. Considering the SI in relation to N would suggest an inverse relationship. The shortcoming of describing changes in the EI or SI separately or linearly is that it is not easy to describe what the whole system is doing. The same EI and SI results visualized through the interaction of the EI and SI would fall in Quadrant C of the SFW model, reveal a resource-limited and structured system, support the low available N, and lead to a better decision as to what the next stages for finding a solution may be. The SFW model is a unique tool for assessing SFW functions and translating the variable outcomes into practical nutrient cycling and soil health management strategies.

Developing balanced soil health management practices requires an integrated approach of accounting for as many components and indicators of soil health as possible, but there are logistical and financial limitations to measuring multiple DESs (e.g., pH, SOM, N, and nematodes) simultaneously. Although visualizing the data on the SFW's quadrant framework describes the status of the soil health conditions, the overarching question is why the SFW model is not broadly applied and adopted as a diagnostic tool. There are disciplinary and cross-disciplinary factors. Within nematology, there appears to be a lack of focus on exploiting what outcomes of the interactions of the EI and SI represent and how the outcomes relate to the totality of the soil health components and management implications [30–35]. The model applies biological, ecological, and mathematical principles to translate complex biophysicochemical processes into practical applications, which may present cross-disciplinary challenges to adopt. The long-term project goal is to develop integrated and sustainable soil health management strategies in cropping systems by advancing models which identify suitable and sustainable soil health outcomes across cropping systems. The objective of the study reported herein was to demonstrate utilization of the SFW model as a diagnostic tool for identifying soil health conditions pre- and post-application of RAPs. To test the objective, we superimposed CC and nutrient amendments over four years of continuous corn under long-term tillage treatment and measured the changes in the BNCS, SOM, available N (NO_3 and NH_4), EI, and SI. The working hypothesis is that the SFW model will identify pre- and post-treatment soil health conditions and inform the underlying potential sources for variable or unsuitable outcomes as well as ways to improve soil health management. We expect to find variable outcomes within and across parameters, treatments, and time which make it difficult to draw integrated conclusions. By identifying the soil conditions influencing the pre- and post-treatment application, the SFW model could lead to precise changes or adjustments which improve soil health management strategies.

2. Materials and Methods

2.1. Experimental Site and Design, Cover Crops, and Amendment Application

This study is part of the MSU Cropping Systems, Nematode and Soil Health Management Study located at the south campus (42°41′42.25″ N, 84°29′37.01″ W) of Michigan State University (MSU) in the USA. This site has sandy loam with 72.2% sand, 20.4% silt, and 7.4% clay textured soil [39]. Recognizing that generating consistency in belowground biophysicochemical changes takes time [40], a study on tillage consisting of no tilling and conventional tillage and a corn-soybean rotation was initiated in 2009 and maintained for 6 years. Conventional tillage was performed with a chisel plow every spring prior to planting [41]. In 2015, a combination of a winter rye cultivar (CV) Wheeler cover crop and five amendments designed to deliver either zero (control (C)), standard (IN), or high N (IH) from inorganic commercial-grade compost and standard (ON) and high N (OH) from cow manure-based compost ("Dairy Doo", Sears, MI) were superimposed in 80 plots. Each plot was 3.04 m wide and 6.08 m long, and seeds were drilled in four rows.

Usually, soil nutrient amendments are applied to correct deficiencies and improve crop yield. The standard and high rates were applied to deliver 112 kg and 224 kg N/ha, respectively. The 0, 112, and 224 kg/ha N rates were designed to simulate the effects of deficient, standard, and extremely high levels of amendments on soil biophysicochemical changes. Each treatment was replicated four times. The required nutrient amendments per plot were weighed, bagged, hand-scattered and mechanically raked before planting [33].

Corn was drilled at a rate of 31,000 viable seeds/ha using a four-row planter (White 5100, Luxemburg, WI, USA [41]), and Round-Up was applied across all plots as needed to control weeds. At the end of the season, corn stalks were removed. The cover crop was sown when about 50% of the corn leaves were dead (usually late September of the growing season) and killed with herbicide in the following spring. Among other reasons, cover crops are used to preserve soil structure and the nutrients therein. The rationale for using rye and corn, both N scavengers, was to maintain the applied N in the corn root zone and determine its influence on soil biology and nutrient cycling.

2.2. Soil Sampling, Analysis, and Nematode Extraction and Enumeration

Approximately 600 mL of soil was randomly collected using custom-made steel cones (2.5 cm in diameter) from the top and −15–20 mL from the center two rows of each plot at planting and midseason in both years, including at harvest in 2018. A grand total of 1600 nematode samples plus 80 soil parameter samples were analyzed, with 640 (80 plots × 4 reps × 2 times) in 2015 and 960 samples (80 plots × 4 reps × 3 times) in 2018 for nematodes. A sub-sample of 250 mL from the soil of each of the 2018 harvest samples was used for analysis of the soil pH, SOM, and available N (NH_4 + NO_3) by the MSU Soil and Plant Nutrient Laboratory using standard procedures [42–44]. These nutrients have an intricate relationship with soil biology and the nematode–microbe–soil interactions that drive the SFW, which is the foundation of nutrient cycling [45–47].

Nematodes were extracted from a sub-sample of 100 mL of soil with a semi-automatic elutriator with 60% extraction efficiency [48–50]. Briefly, a 1:1:3 ratio of soil, dish soap (non-phosphate), and tap water was run through a semi-automatic elutriator, passed through sieves (850 µm, 250 µm, and 20 µm), centrifuged (4000 rpm in 456 sugar/L tap water for four minutes), and fixed in double TAF solution (14 mL 40% formalin, 4 mL triethanolamine, and 91 mL distilled water) [49]. Herbivore (HV), bacterivore (BV), fungivore (FV), omnivore (OV), and predator (PR) trophic groups were identified to the genus or family level and enumerated using an inverted microscope (Motic Type 101 M, AE 2000) and assigned corresponding colonizer-persister (c-p) values from c-p 1 to c-p 5 [51–53]. Nematode abundance was expressed per 100 mL of soil.

The graph of the SFW structure and function was generated by calculating EI = (100[e/(e + b)]) and SI = (100[s/(s + b)]) based on the weighted abundance of nematode guilds representing the structure (s = $\Sigma K_s n_s$), enrichment (e = $\Sigma K_e n_e$), and base (b = $\Sigma K_b n_b$), where K is the specific weight of each guild and n is the abundance of nematodes in each functional guild in the sample [26]. The EI indicates changes in the nematode population's density relative to food resources and reproduction rate, and the SI indicates changes related to resistance to disturbances.

2.3. Statistical Analysis

This study investigated the main effects of tillage practices, cover cropping, and nutrient amendments, as well as their two- and three-way interaction effects on the HV, BV, FV, OV, PR, SI, and EI variables. We conducted the analysis using the PROC GLM procedure in SAS [54]. This procedure allowed for the fitting of generalized linear mixed models, accommodating the non-normal distribution of the response variables, and accounting for random effects. Fixed effects included tillage, cover crops, nutrient amendments, and their interactions, while random effects accounted for variability between the experimental units or blocks. The models were fitted using appropriate distributions and link functions tailored to the nature of each response variable, aiming to assess the significance and quantify the magnitude of the main effects and interactions.

An SFW model was developed to assess the impacts of tillage practices, cover crop types, and nutrient amendments across two distinct years: 2015 and 2018. The model aimed to elucidate how these agricultural management practices influence the dynamics and biodiversity of the soil ecosystem over time. To quantify the effects, the least squares means (LSMEANS) derived from tillage practices, cover crops, and nutrient amendments were analyzed using the PROC GLIMMIX procedure in SAS [54]. This approach allowed for the fitting of generalized linear mixed models, considering both fixed effects (tillage, cover crops, and nutrient amendments) and random effects to account for variability between the experimental units or blocks. To visualize the SFW structure and function through the intersection of the EI (y axis) and SI (x axis) by tillage, graphs were generated in SAS [54].

To analyze the agricultural data, we utilized the dplyr package in R for data manipulation and summarization [55]. First, the dataset containing the HV, BA, FV, OV, PR, SI, and EI variables, alongside the factors of tillage practices, nutrient rates, and cover crops, was loaded and prepared. Using the *group by ()* and *summarize ()* functions, we computed the means and standard errors (SEs) of each response variable grouped by combinations of tillage, nutrient rates, and cover crops. Subsequently, for visualization, we employed the *ggplot2* package to create dot plots [56]. Error bars corresponding to the previously computed standard errors were added using *geom_errorbar()*, providing visual comparisons between agricultural practices.

Correlation plots were generated using the corrplot package in R [55] to examine the relationships between the nutrient variables (pH, SOM, NO_3, and NH_4) and SFW indicators (EI and SI). Spearman correlation coefficients were calculated due to the non-parametric nature of the data. Each plot included a title indicating the treatment or condition under investigation. Within these plots, correlations which were not statistically significant ($p \geq 0.05$) were visually marked or crossed out, while significant correlations ($p < 0.05$) were unmarked. This approach allowed for the clear visualization and interpretation of significant relationships between variables across different experimental conditions or treatments, facilitating insights into potential dependencies and associations in the dataset.

3. Results

3.1. Data Organization

The main effects of tillage, cover crops, and nutrient amendments on the measured soil parameters, beneficial nematode abundance, EI and SI, correlations of the EI and SI with the soil parameters, and EI and SI fitted to the SFW model are presented in Sections 3.2, 3.3, 3.4, 3.5, and 3.6, respectively. The interaction effects of tillage, cover crops, and nutrient amendments on the abundance of beneficial nematodes and the EI and SI, which were highly variable, are presented in Supplementary Figures S1 and S2. Since herbivore nematodes were not part of the EI- and SI-based SFW calculations, they are not included.

3.2. Effects of Tillage, Cover Crops, and Soil Amendments on Soil Parameters

The measured soil parameters at the end of the study were the soil pH, SOM, NO_3, and NH_4, and the cover crop treatment had no effect on any of them (Table 1). The effects of tillage on NH_4 and tillage and nutrient amendments on the soil pH were not significant. The contents of SOM and NO_3 were significantly higher in the no-till soil than with conventional till treatment ($p \leq 0.05$). The SOM was the highest for the high rate of organic amendment followed by standard organic and inorganic amendment, and the lowest SOM ($p \leq 0.05$) was found for the check and high inorganic amendment. NH_4 was the highest for the high inorganic amendment, followed by the standard inorganic and high organic amendment and the standard organic treatment and check. However, the amount of N recovered was not proportional to what was applied. There was no significant two- or three-way interaction effect on any of the measured soil parameters.

Table 1. LS means of the main effects of tillage (no tilling or tilled) without (−) and with (+) winter rye cover crop and nutrient amendments delivering either zero or check (CK), organic standard (ON) or high amount (OH), and inorganic standard (IN) or high (IH) amount of nitrogen on soil pH, percent soil organic matter (SOM), and available nitrogen in the form of nitrate (NO_3) and ammonium (NH_4) in 2018.

	Tillage [x]		Cover Crop		Nutrient Amendments [x]				
Item	**No-Till**	**Tilled**	−	+	**Check**	**IH**	**IN**	**OH**	**ON**
pH	6.49	6.61	6.59	6.51	6.66	6.31	6.13	6.92	6.74
SOM	2.62 a	2.12 b	2.30	2.44	2.06 c	2.18 c	2.41 b	2.69 a	2.49 b
NO_3 [y]	72.90 a	53.99 b	62.63	64.26	58.04	60.50	64.83	65.83	68.01
NH_4 [y]	3.51	3.30	3.15	3.67	2.52 d	4.24 a	3.61 b	3.49 b	3.18 c

[x] Means followed by the same or no letters with tillage and nutrient amendment treatments are not statistically different ($p \leq 0.05$). [y] Values are in ppm. Tillage, cover crops, and nutrient amendment applications are as described in Section 2.

3.3. Effects of Tillage, Cover Crops, and Soil Amendments on Nematodes

The bacterivore, fungivore, predator, and omnivore genera present in 2015 and in 2018 as well as their c-p values are presented in Table 2. The system was dominated by fast-reproducing bacterivores.

While tillage and cover crop treatments had little effect on the abundance of bacterivore, fungivore, omnivore, or predatory nematodes within a year, there were varying significant differences ($p \leq 0.05$) between the years (Figure 1A–D). For example, the abundance of bacterivore nematodes was significantly higher ($p \leq 0.05$) in the no-till soil than in the conventional tilled plots in 2018 compared with 2015 (Figure 1A). Fungivores and omnivores were more abundant in both tillages in 2018 than in the no-till plots in 2015

(Figure 1B,C), and predators were more abundant in both tillages in 2018 than in 2015 (Figure 1D).

Table 2. Genera and c-p values of bacterivore, fungivore, predator, and omnivore nematodes present in 2015 and in 2018.

Bacterivores	cp	Bacterivores	cp	Predators	cp
Rhabditis	1	*Leptolaimus*	3	*Clarkus*	4
Rhabditella	1	*Paraplectonema*	3	*Miconchus*	4
Rhabditophanes	1	*Chromadorina*	3	*Mononchus*	4
Pellioditis	1	*Prismatolaimus*	3	*Mylonchulus*	4
Diploscapter	1	*Leptonchus*	4	*Discolaimus*	5
Pristionchus	1	*Bathyodontus*	4	*Discolaimium*	5
Chiloplacus	2	*Alaimus*	4	*Nygolaimus*	5
Acrobeles	2				
Acrobeloides	2	**Fungivores**	**cp**	**Omnivores**	**cp**
Acrolobus	2	*Aphelenchoides*	2	*Dorylaimus*	4
Cephalobus	2	*Aprutides*	2	*Dorydorella*	4
Cervidellus	2	*Aphelenchus*	2	*Dorylaimoides*	4
Bunonema	2	*Filenchus*	2	*Epidorylaimus*	4
Eucephalobus	2	*Tylencholaimus*	4	*Eudorylaimus*	4
Monhystera	2	*Doryllium*	4	*Microdorylaimus*	4
Heterocephalobus	2			*Mesodorylaimus*	4
Anaplectus	2			*Prodorylaimus*	4
Panagrolaimus	2			*Pungentus*	4
Plectus	2			*Aporcelaimus*	5
Aulolaimus	3			*Aporcelaimellus*	5
Microlaimus	3			*Oxydirus*	5

Not included in the list above and only present in 2015 were *Achromadora*, *Tripyla*, *Boleodorus* (bacterivores), and *Tylopharynx* (fungivore).

Nutrient treatments showed varying effects on nematodes. With the exception of higher omnivore nematodes in 2018 than in 2015 with the standard organic amendment (Figure 1C), nutrient amendments did not affect the bacterivore, fungivore, or predator nematode population densities between years (Figure 1A,B,D). However, the bacterivore population densities in the standard inorganic amendment in both years and the high-rate organic and standard organic amendments in 2015 were significantly lower than those for the check, high inorganic, and standard organic amendments in 2018.

The check, standard inorganic, and both organic amendments in 2018 had higher fungivore nematode densities than that for the inorganic high nutrient amendment in 2015. There were more omnivore nematodes in the inorganic standard rate in 2018 than in all treatments, but not for the check in 2018 and standard inorganic and high organic amendments in 2015. The high organic amendment in both years had significantly more omnivores in both years than the 2015 standard organic amendment. The abundance of predator nematodes was significantly lower in the high inorganic amendment in both years than in the check in 2018, high-rate organic amendment in both years, and standard-rate organic amendment in 2018.

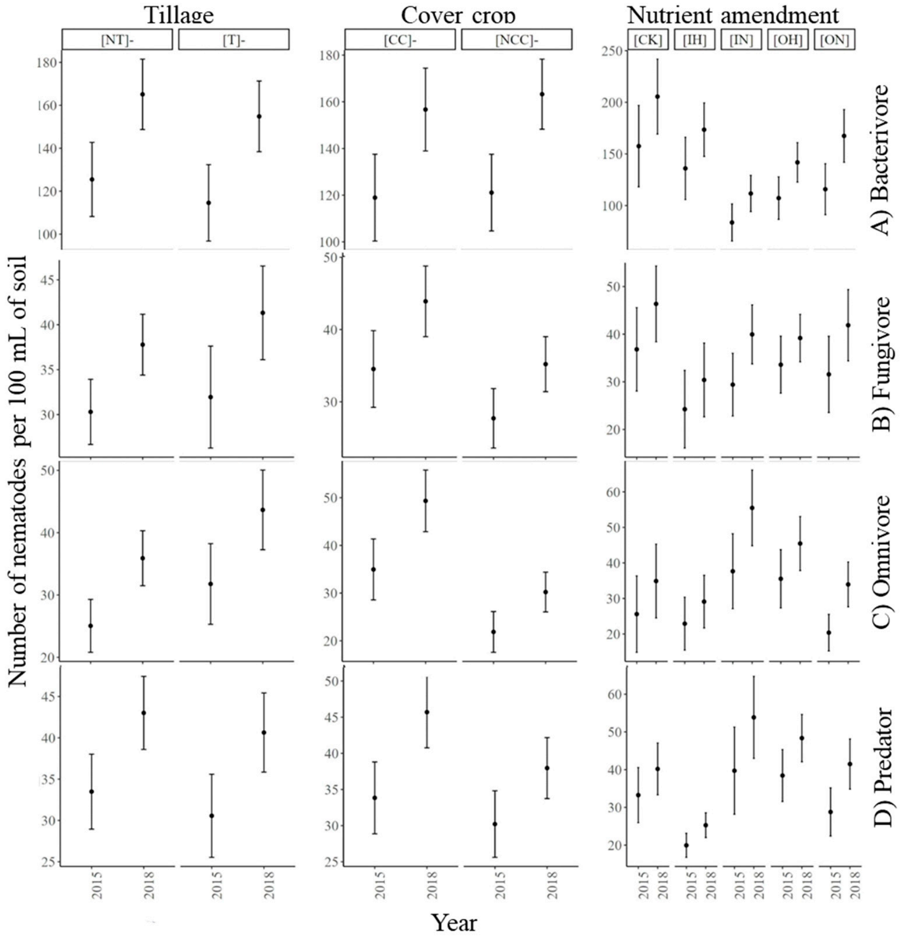

Figure 1. The main effects of no tilling (NT) or tilling (T) with (CC) and without (NCC) winter rye cover crop and nutrient amendments delivering either zero or check (CK), inorganic high (IH) or standard amounts (IN), or organic high (OH) or standard amounts (ON) of nitrogen treatments on the LS means of the numbers of nematodes per 100 mL of soil for (**A**) bacterivore, (**B**) fungivore, (**C**) omnivore, and (**D**) predator nematodes in 2015 and in 2018. Error bars within tillage, cover crop, and nutrient amendment categories between years and across treatments which do not overlap are significantly different ($p \leq 0.05$). Tillage, cover crop, and nutrient amendment applications are as described in Section 2.

3.4. Effects of Tillage, Cover Crops, and Soil Amendments on EI and SI

The EI tended to decrease with time in both tillage treatments and significantly ($p \leq 0.05$) so between the no-till plots in 2015 and the tilled plots in 2018 (Figure 2, top). With or without cover crops, the EI tended to decrease with time and significantly ($p \leq 0.05$) so between the cover crop group in 2018 and no cover crop group in 2015 (Figure 2, top). While the trend was a decreasing EI over time, there was no treatment effect between years within an amendment (Figure 2, top). The EI in the higher-rate organic amendment in 2015 was significantly higher than both inorganic amendments in both years and the standard organic amendment in 2018 ($p \leq 0.05$). In the standard organic amendment in 2015, the EI was significantly higher than that for the standard inorganic amendment in 2018.

The SI was significantly ($p \leq 0.05$) lower in 2018 than in 2015 in both tillage and amendment treatments (Figure 2, bottom). The SI was significantly lower in 2018 than in 2015 in the check, higher-rate inorganic, and standard-rate organic amendments compared with the rest of the treatments ($p \leq 0.05$). The standard inorganic and high-rate organic amendment in 2015 had significantly higher ($p \leq 0.05$) SIs than the standard organic amendment in both years.

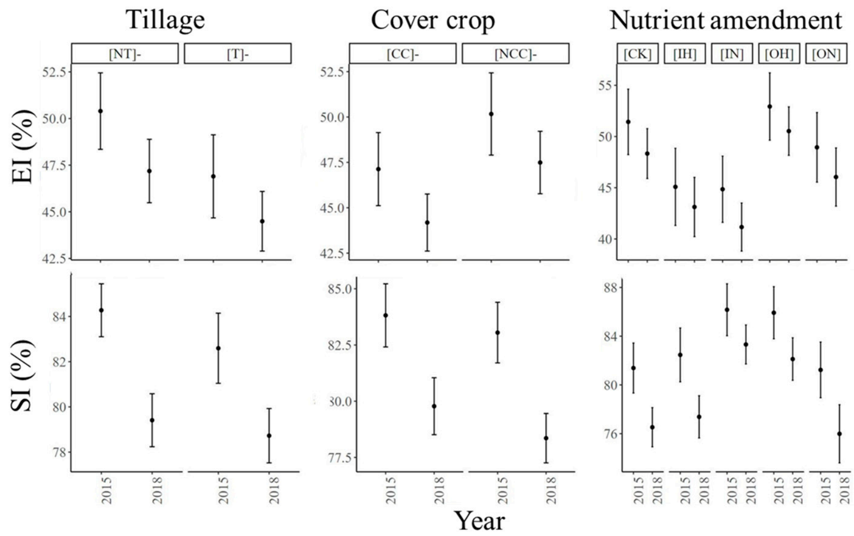

Figure 2. The main effects of no tilling (NT) or tilling (T) with (CC) and without (NCC) winter rye cover crop and the nutrient amendments delivering either zero or check (CK), inorganic high (IH) or standard amounts (IN) or organic high (OH) or standard (ON) amounts of nitrogen treatments on enrichment (EI) and structure (SI) indices in 2015 and in 2018. Error bars within tillage, cover crop, and nutrient amendment categories between years and treatments which do not overlap are significantly different ($p \leq 0.05$). Tillage, cover crop, and nutrient amendment applications are as described in Section 2.

3.5. Effects of Tillage, Cover Crops, and Soil Amendments on Multi-Factor Correlations

Figure 3 shows the degree of correlations (or lack thereof) among the SI, EI, soil pH, NO_3, NH_4, and SOM by tillage, cover crop, and nutrient amendment. Blue circles are positive correlations, and red circles are negative correlations, while the size of the circle indicates the intensity of the correlations. The SI was negatively correlated ($p \leq 0.05$) with NH_4 in both tillage and cover crop treatments as well as in all nutrient amendments, but not the check group. The soil pH and SOM were negatively correlated ($p \leq 0.05$) in the conventional tillage, both cover crop treatments, and the check as well as both inorganic soil amendments. The positive and negative correlations among the parameters were not statistically different (Figure 3).

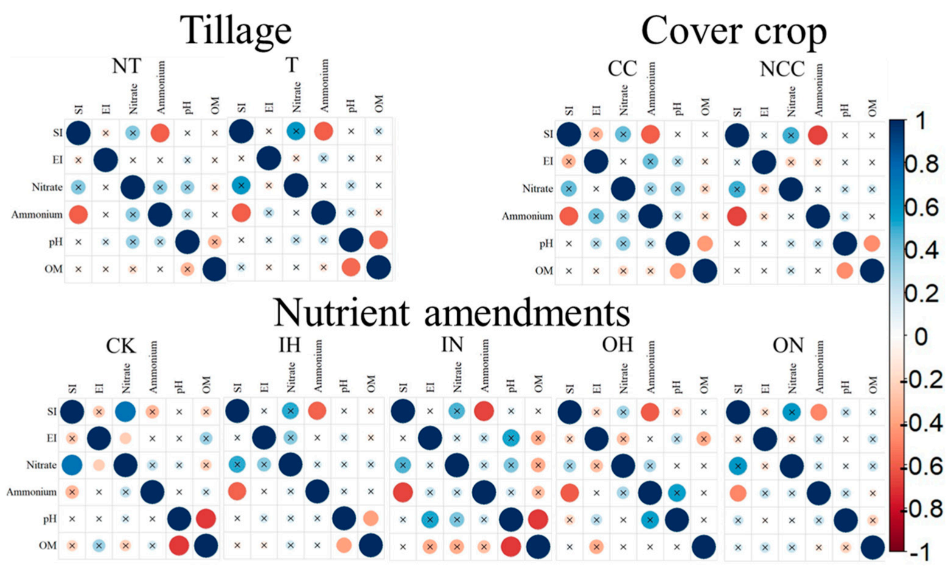

Figure 3. Spearman correlations among SI, EI, NO_3 (nitrate), NH_4 (ammonium), pH, and SOM (OM) in no-till (NT) or tilled (T) plots with (CC) and without (NCC) winter rye cover crop and nutrient

amendments delivering either zero (check), inorganic high (IH) or standard (IN) amounts, or organic high (OH) or standard (ON) amounts of nitrogen treatments in 2018. On the scale, white (near zero) indicates a lack of correlation, blue positive correlation and red negative correlation. Intensify of colors aways from zero indicate an increasing correlation coefficient. Dot sizes directly correspond to correlation coefficient, with larger dots indicating higher coefficient values and smaller dots indicating lower coefficient values. Squares marked with X mean that the correlations were not statistically significant ($p \leq 0.05$).

3.6. Visualizing the Effects of Tillage, Cover Crops, and Soil Amendments in the SFW Model

The intersection of the SI (*x* axis) and EI (*y* axis) is the quadrant which describes the functional outcome of the system (Figure 4). With the exception of the no-till area with cover crops in the standard organic amendment, there was no statistical difference between the years in the positions of the intersection data points among the nutrient treatments in either tillage treatment (Figure 4, left). The data points of the plots without cover crops of the standard organic amendment in no-till plots and the high-rate organic amendment in tilled plots were higher than those without cover crops. In the plots with cover crops, the data points were either on the border line of Quadrants B and C or in Quadrant C for both tillage treatments (Figure 4, left). In the tilled plots without cover crops, the data points of the high-rate organic amendment fell into Quadrant B (Figure 4, right). The data points for the rest of the treatments fell into Quadrant C. In the no-till plots without cover crops, the data points of both organic amendments and the check were mostly in Quadrant B. The data points of the high-rate inorganic amendment were in Quadrant C, and those of the standard rate were on the border line of Quadrant B (Figure 4, right).

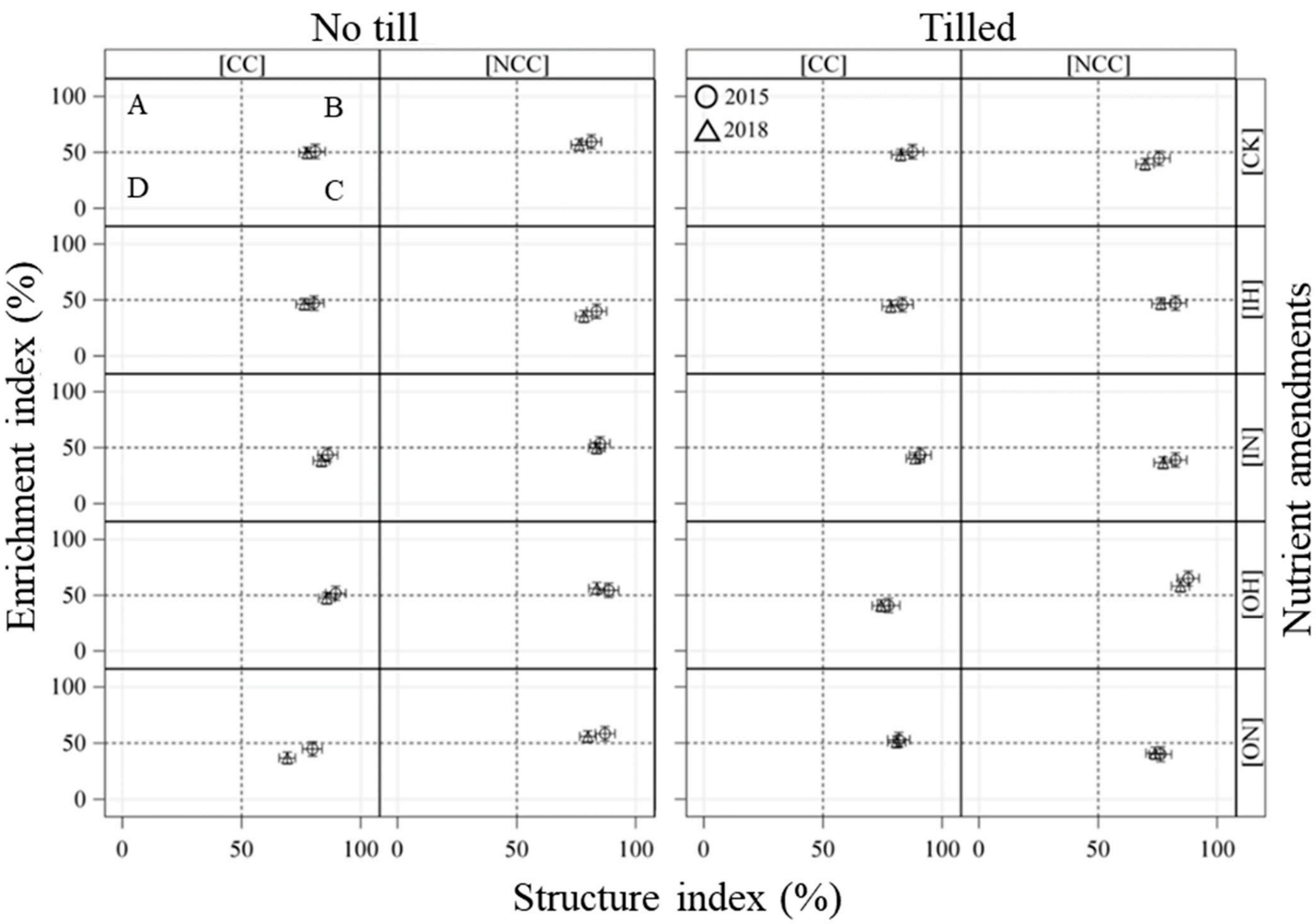

Figure 4. Visualizing the effects of no-till (left) or tilled (right) plots with (CC) and without (NCC) winter rye cover crop and nutrient amendments delivering either zero or check (CK), inorganic high

(IH) or standard (IN) amounts, or organic high (OH) or standard (ON) amounts of nitrogen treatments on the structure (*x* axis) and enrichment (*y* axis) indices in 2015 (○) and in 2018 (△), fitted into the SFW model. The quadrants represent disturbed and nutrient-enriched (A), structured and nutrient-enriched (B, best case), structured but nutrient-depleted (C), and degraded and nutrient-depleted (D, worst case) cases. The error bars within the tillage, cover crop, and nutrient amendment categories between years and across treatments which do not overlap are significantly different ($p \leq 0.05$). The tillage, cover crop, and nutrient amendment applications are as described in Section 2.

4. Discussion

4.1. Overview

The RAP values for improving soil health through direct or indirect influence on the SFW structure and functions [47,57,58] at which healthy soil has optimum biological, physicochemical, nutritional, structural, and water-holding components [1–3,21] and the SFW model is central to understanding and quantifying the conditions that generate the DESs are well established [59–65]. The major limitations to achieving healthy soil, however, include variable DESs and soil health outcomes, a lack of integrated understanding of the processes generating the DESs, and a lack of models which identify suitable soil health outcomes and align the DES either simultaneously or step by step for a given production system. As expected, and similar to many previous reports [57–65], the results from this study represent common examples of variable DES outcomes. These include RAPs resulting in (1) a higher soil parameter(s) (Table 1) against treatments which indicate negative or positive correlation with the EI and SI values (Figure 3) and variable effects on (b) nematode abundance (Figures 1 and S1) and (c) the EI and SI values (Figures 2 and S2) over time. Within the context of developing integrated soil health management strategies, these data are difficult to interpret, much less align with DESs to identify suitable soil heath conditions. Moreover, it is difficult to tell why the results were so variable until one looks through the lens of the SFW structure. The SFW model identified the soil conditions across RAPs and over time as primarily resource-limited and structured (Quadrant C; Figure 4) or borderline enriched and structured (Quadrant B; Figure 4), with little change between years, suggesting that there are process-limiting factors affecting nutrient cycling and possibly contributing to the variable outcomes. Identifying the presence of process-limiting factors opens the road to considering approaches for addressing the cause-and-effect relationships influencing soil health outcomes. Thus, the hypothesis that the SFW model will identify pre- and post-treatment soil health conditions and inform the underlying sources for variable or unsuitable outcomes as well as ways to improve soil health management is supported.

4.2. How the SFW Model Identified the Soil Health Conditions

It is well established that the SFW is the foundation for all of the belowground processes which generate DESs, including biodiversity, SOM, nutrient availability, soil pH, suitable soil and environmental conditions, and improved crop yield [53,55,57,59]. In simple terms, the Ferris et al. [26] SFW model applies biological, ecological, and mathematical principles to changes in the SI (*x* axis) and EI (*y* axis) and identifies four best-to-worst case quadrants of soil conditions for the nutrient cycling potential and agroecosystem fitness. These are enriched and unstructured (Quadrant A), enriched and structured (Quadrant B, best case), resource-limited and structured (Quadrant C), and resource-depleted and unstructured (Quadrant D, degraded and worst case). Enriched means N is available, limited requires biological activity for N to be released, and depleted means biologically degraded. By describing the soil across RAPs as resource-limited and structured (Quadrant C, Figure 4), the SFW model is identifying that the soil's biophysicochemical conditions are out of balance. Under resource-limited and structured conditions, N is limited and requires biological activities to be released.

Changes in the BNCS, EI, SI, SOM, N, and soil pH in response to RAPs are among the most studied DESs and soil health parameters, albeit in highly variable conditions [63,66]. Identifying the presence of process-limiting conditions with the SFW is highly significant. If the soil conditions are not suitable for generating DESs, then it is difficult to achieve healthy soils regardless of which RAP or how much of each RAP is applied. In order to appreciate the value of the SFW model as a diagnostic tool, it is worth considering the effects of RAPs on the groups' measured DESs (soil pH, SOM, N, BNCS, EI, and SI) individually and collectively.

4.3. Effects of Tillage, Cover Crops, and Soil Amendments on Soil pH, SOM, and N

Changes in the SOM, N, and soil pH in response to RAPs are among the most studied nutrient cycling and soil health parameters, albeit in highly variable conditions [59,63,64,66]. RAPs which result in suitable outcomes for these parameters are likely to be favored over those that do not. If the goal is to achieve sustainable soil health management, however, a focus on single or unintegrated multiple parameters has many shortcomings which need to be recognized. For example, the soil pH across RAPs was within the optimum range for corn production. The significantly higher SOM in the no-till plots than in the tilled plots and the highest SOM in the high-rate organic treatment would suggest promoting the two RAPs as suitable practices over CCs and the other nutrient amendments (Table 1). However, achieving balanced soil health based on the optimum soil pH and increasing the SOM without adequate available N and other components of nutrient cycling is difficult.

The rationale for applying the different rates of N was to show the maximum impact on the biophysicochemical components of soil health. Although the nutrient amendments resulted in higher NH_4 values than in the check, the amount of available N (NO_3 and NH_4) detected in the top 20 cm of soil (Table 1) was not proportional to what was applied. This suggests the presence of N availability-limiting factors [61]. It is logical to think that leaching, volatilization, time, and other unidentified process-limiting factors may have contributed to the low availability of N. Because of financial limitations, we did not perform stratified N analysis below the 20 cm sampling depth to rule out potential leaching. Assuming that leaching affected all treatments similarly, however, this would have resulted in differences among the treatments. If volatilization were a factor, then this would have resulted in less N detected in the inorganic forms than in the organic forms of the amendments because it takes time to break down the compost and release the N [38]. Time seemed to be less of a factor because four years was enough for decomposition of the compost to release N and repeated amendment applications to result in higher available N accumulations than the current results. A combination of the available N results and the resource-limited and structured conditions the SFW model identified (Figure 4) points toward yet to be identified process-limiting factors possibly confounding the overall soil health outcome. Nonetheless, knowing the favorable outcomes for the SOM and soil pH and the factors limiting N availability opens the road toward understanding the system and potentially designing soil health management strategies in a step-by-step alignment of these and other DESs [25].

4.4. Effects of Tillage, Cover Crops, and Soil Amendments on Nematodes, EI, and SI

Although there are no standardized quantitative values for cropping systems or soil types, changes in the abundance of BNCS, EI, and SI as indicators of soil health have been extensively studied, and many variable outcomes have been found [47,65,67,68]. In this study, bacterivore nematodes were two to three times more abundant than fungivores, predators, and omnivores, with notable changes over time rather than with RAPs (Figure 1). While suggesting that the system favored bacterivore nematodes, this makes it difficult to draw definitive conclusions on the effects of the RAPs on the BNCS. The relatively low EI and high SI values reflect the proportion of low and high c-p value nematodes present (Table 2). Similar to the BNCS, the effect of time on the EI and SI was greater than the effects of the RAPs (Figure 2).

Identifying promising DES results is an important component of developing integrated soil health management strategies by aligning the DESs in time and space. In this case, the relatively high abundance of bacterivore nematodes and SI values, along with the SOM and optimum soil pH (discussed above), will stand out. However, the general increase in abundance of bacterivore nematodes, decrease in the SI with time, and low N concentrations (discussed above) present conflicting trends which require understanding the processes driving the correlations or interactions influencing the DESs in time and space.

4.5. Interaction Effects of Tillage, Cover Crops, and Soil Amendments

Tests on the correlations and interaction effects of RAPs on DESs are critical components of establishing cause-and-effect relationships and identifying frameworks for developing integrated soil health management strategies [46,47,49]. In this study, the negative correlations between the SI and ammonium in terms of both tillage and CC, inorganic and high-rate organic N treatments, the SOM and pH in the tilled and no-CC categories, and in the check and high inorganic N treatments were the only statistically significant parameters (Figure 3). Since the soil pH was in the optimum range and the SOM was increasing, the negative correlation suggests that there may have been other confounding factors. The inverse relationship between the SI and ammonium was consistent with the low N and that the SFW conditions were resource-limited and structured (Quadrant C; Figure 4).

The two- and three-way interaction effects of the tillage, CC, and nutrient amendment treatments on the soil pH, SOM, and N were not significant, suggesting that the effects of the RAPs were independent of one another. Rather few of the interaction effects of the RAPs on the BNCS (Figure S1), EI, and SI (Figure S2) were significant and highly variable over time, which is not uncommon. With a combination of the inverse relationships between the SI and ammonium and the SOM and soil pH (discussed above) and no consistent interaction effects by the RAP over time, the trends in the SI, bacterivores, and SOM under optimum soil pH ranges for corn point to the complexity of the process-limiting factors under resource-limited and structured (Quadrant C; Figure 4) soil conditions. It is the use of the SFW model as a diagnostic tool which has enabled identifying the presence of process-limiting factors in the field.

4.6. Potential Broad Impacts of the SFW Model

In order to understand the broad potential impacts of the SFW model when used as a soil health diagnostic tool, one needs to answer the following questions. First, what would the conclusions likely have been for the soil pH, SOM, N, BNCS, EI, and SI without using the SFW model's diagnostic power? As discussed above, and as many of us have published before, there would have been an emphasis on interpretations of the positive and negative correlation and interaction effect results on the selected parameters, including identifying in which SFW quadrant the EI and SI interaction points fell [33,37,47]. All logical interpretations and conclusions drawn, including referencing in which SFW quadrant the EI and SI interactions fell, would be correct. The problem is that referencing in which SFW quadrant the data fell is only a partial interpretation of the data, which leads to the second question: How does the SFW model lead to expanded interpretation of the data? In addition to the descriptions herein [26], what data falling in the four SFW quadrants means was summarized by Melakeberhan et al. [68]. At minimum, application of the SFW model as a diagnostic tool and extensive interpretation of the data includes (1) identifying in which SFW quadrant the EI and SI interaction data points fall, (2) what the quadrant where the data points fell describes, and (3) how the description of the quadrant relates to other measured parameters. Third, what is the benefit of using the SFW model as a diagnostic tool? DESs are outcomes of functions of many complex, process-driven biophysicochemical interactions with countless limitations in terms of space and time [23–25,34,35,40,45,63,69–71]. When process-limiting factors are present under field or semi-controlled conditions, more often than not, the DESs outcomes will be variable, and the results of this study are an example of that. A major benefit of the SFW model is that it identifies the soil conditions where the interactions which generate the DESs take place. As described herein, knowing the soil conditions leads to a holistic understanding of confounding factors. This in turn leads to potentially designing informed solutions toward developing integrated and sustainable soil health management strategies instead of continuing or changing RAPs or treatments based on either suitable or unsuitable outcomes. Fourth, could application of the SFW model as a diagnostic tool be scaled up across ecoregions? Biogeographic data can be incorporated absolutely in at least two ways. One way is establishing cause-and-effect relationships of host–parasite interactions. For example, for a long time, it was known that the soil type was a factor in *M. hapla*'s parasitic variability (PV) (i.e., the same populations reproducing at different rates). Application of the SFW model as a diagnostic tool established connections among *M. hapla* distribution, PV, soil type, soil health, and the indicator soil microbiome [72]. Another way is relating established soil degradation and fertility management practices to specific soil health conditions across cropping systems. For example, most soil fertility management in a corn-soybean production system with high-yielding (46%), stable but low-yielding (26%), and variable (unstable) yielding (28%) landscapes in the US Midwest [73] is based on the 4R concept: right source, rate, time, and place [74]. Over-fertilization in the low-yielding areas and variable yielding contributed 44% and 31% of the total N loss, respectively, costing growers USD ~485 million and causing 6.8 MMT of CO_2 equivalents in greenhouse gas emissions in the environment [73]. Adding fertilizer to increase yields in low- and variable yielding areas or to improve yields in steady yielding areas without decision-making tools such as the SFW model, which identifies which application generates suitable outcomes that lead to sustainable soil health, is likely to contribute to an unsustainable soil fertility footprint. Determining how the biogeographic data in the high-, low-, and variable yielding zones relate to or corelate with the SFW model's description of soil health conditions as suitable, unsuitable, or variable could potentially minimize unsustainable practices. The results from the relationships between the yield zones and the SFW model descriptions of the zones will inform what type of one-size-fits-all or zone-specific approach is needed to design suitable and scalable soil health management strategies across the yield zones. This, however, will require greater cross- and multi-disciplinary integration and resources than currently exist. Otherwise, the cycle of soil health and agroecosystem degradation will continue.

5. Conclusions

When applying RAPs and related practices to improve soil health, variable outcomes in DESs such as the SOM, pH, available N (NO_3 and NH_4), beneficial nematodes, EI and SI are inevitable. Decisions to accept or not accept any outcomes without understanding the underlying confounding factors influencing cause-and-effect relationships could potentially lead to negative consequences for the use of RAPs, DESs, the environment, and overall soil health. This study measured changes in the DESs in response to four years of treatment of tillage, winter rye cover crops, and organic and inorganic N sources applied at standard and high rates and found results attributable to RAPs, time, or inconsistent interactions or correlations. For example, no tilling significantly increased the SOM and NO_3 compared with the tilled plots, yielded a higher SOM in the high-rate organic source than in the rest of the nutrient amendments, higher NH_4 amounts in the nutrient treatments than in the control, high bacterivore nematode counts, an SI above 75% over time, an optimum soil pH across RAPs, and available N not proportional to what was applied. Collectively, the data are too messy to make sense out of, and cherry-picking to accept, not accept or keep trying until suitable outcomes are achieved without knowing potential source(s) of variability is more or less a gamble. When the SFW model was applied as a diagnostic tool for identifying soil health conditions before and after RAP application, it was revealed that the soil health conditions were primarily resource-limited (Quadrant C) over time. Thus, this indicates the need for biological activity for N to be released and suggests that less than ideal conditions for generating DESs are likely to lead to variable outcomes. This study demonstrates the value of the SFW model as a foundation for a step-by-step basis of alignment of the DESs when designing integrated soil health management strategies.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/soilsystems9010005/s1. Figure S1. Three-way interaction effects of nutrient amendments delivering either zero or check (CK), inorganic high (IH) or standard amount (IN), organic high (OH) or standard amount (ON) of nitrogen treatments and no tillage with cover crop (NT + CC) or without (NT − CC) winter rye cover crop or tilled with (T + CC) or without (T − CC) cover crop on (A) bacterivore, (B) fungivore, (C) omnivore, and (D) predator nematodes in 2015 and in 2018. Non-overlapping error bars within tillage, cover crop, and nutrient amendment categories between years and treatments are significantly different ($p \leq 0.05$). Figure S2. Three-way interaction effects of nutrient amendments delivering either zero or check (CK), inorganic high (IH) or standard amount (IN), or organic high (OH) or standard amount (ON) of nitrogen treatments with no tillage with cover crop (NT + CC) or without (NT − CC) winter rye cover crop or tilled with (T + CC) or without (T − CC) cover crop on enrichment (A) and structure (B) indices in 2015 and in 2018. Non-overlapping error bars within tillage, cover crop, and nutrient amendment categories between years and treatments are significantly different ($p \leq 0.05$).

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