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Sensitivity of Nematode Community Analysis to Agricultural Management Practices and Inoculation with Local Effective Microorganisms in the Southeastern United States

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Abstract: In order to ensure a soil system's sustained ability to carry out ecosystem services, indicators that assess soil health are needed. We examined the capacity of nematode maturity index (MI), structure index (SI), enrichment index (EI), and trophic groups as measures of soil health, by determining soil nematodes' sensitivity to cropping systems: rotation, perturbation, fertilization, and inoculation with local effective microorganisms (LEM). Plots were managed for two years under different rotations, annual ryegrass/fallow (ARF) and cereal rye/edamame soybean (CRS). In the third year of the study, all of the plots were managed exactly the same as a wheat/edamame rotation. Data were collected in both winter and summer of this year. In all three years, three inoculant treatments (LEM, False-LEM and No inoculate) were applied. In CRS plots, which received the most tillage and fertilization, there were greater SI values in soils that received LEM application. Nematode community structure described by each MI, SI, and EI were sensitive enough to reflect changes due to differences in soil management practices from previous years. Principal components analysis confirmed that nitrogen mineralization is an important measure to include when using nematode community analysis in the development of a soil health index.

Keywords: soil health; indicators; nematode; indices; local effective microorganisms; management; rotation

1. Introduction

Soils are an essential natural resource that provides not only food, fiber and building materials through agricultural production but also offers a host of other important ecosystem services. These services include capturing and storing water, decomposing organic matter, cycling nutrients, gas exchange, carbon sequestration, and suppression of disease and pests [1]. According to Daily (1997) [2], soils are an important natural capital which determine the economic status of nations. However, mankind's efforts to increase agricultural productivity to meet the world's ever-growing demands are threatening these valuable resources. Recently, the concepts of soil quality and soil health have evolved in an effort to recognize soil resources as dynamic, living systems that emerge through a

unique balance and interaction of soil's biological, chemical, and physical components [3]. There is ongoing debate over the differences between the two terms and their exact definitions. For the sake of clarity, we will be referring to soil health as the capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health [4].

A great challenge faced by scientists and conservationists is deciding on effective indicators to assess soil health. Soil health indicators, as with other ecological indicators, can help in assessing the condition of the soil, in monitoring trends, in providing an early warning signal of changes in the soil, or in diagnosing the cause of a problem [5]. Soils, however, are complex ecological systems that vary greatly over time and over space. Therefore, development of a system of soil health measurement that is sensitive to soil management, sensitive to changes in soil function, and easily measurable is not a simple task [6]. Because soil health/quality cannot be measured comprehensively with a single indicator, soil health and soil quality assessments often focus on determining a "minimum data set" (MDS) of soil characteristics with the greatest influence on soil health [7]. Physical and chemical measures such as, texture, density, and soil organic matter SOM are the some of the most prevalent indicators used in these datasets [8–11]. Considering that many of the functions and services that we associate with healthy soils such as decomposition of organic material, nutrient cycling, provisioning of plant-available nutrients, carbon sequestration and disease suppression are carried out by the biological fraction of the soil, there is a clear need for the determination of biological indicators that can be effectively incorporated into our soil health indices.

Soil faunal indices are one type of biological indicator that integrates large amounts of data helpful in determining ecological change [12]. Soil fauna influence soil biological processes, nutrient cycling and soil structure [13]. These influences are related to soil faunal functions in regulating the bacterial and fungal decomposition pathways in the soil food web and potentially providing regulation of microbial responses to changes in soil quality [14]. Of the soil fauna species, nematodes have demonstrated a particularly excellent potential as bioindicators for soil health. This is because nematodes have a wide range of functional diversity, abundance in soil ecosystems, positions as primary and intermediate consumers in soil food webs and differing sensitivity to abiotic and biotic stressors [15–17]. In a survey of 183 biological indicator candidates scored by experts and stakeholders against a wide range of scientific and technical criteria, nematode community analysis was found to be one of the most suitable biological indicators for monitoring the provisioning of ecosystem services [18]. Soil nematode communities can be analyzed in several ways: (1) through functional guilds, which are defined as assemblages of species with the same feeding habits, similar biological attributes and similar response to environmental conditions [19], (2) through trophic groups, which are defined according to specific feeding habits, or (3) through nematode indices which are calculated using functional guilds. These indices provide information as to the maturity, the structure or the enrichment of a soil ecosystem.

Early nematode indices consisted of simple proportions and ratios of nematode trophic groups [20]. Later, a Maturity Index (MI) was developed [21], which has been found to better "differentiate the ecological condition of soils on a regional scale than do individual or ratios of trophic groups" [22,23]. The MI is considered a measure of environmental disturbance, with low values indicating a disturbed or enriched environment. It is recommended that the MI be calculated using only free-living nematode taxa and that a separate value (PPI) be calculated with the plant parasitic nematode taxa, which have been proven to exhibit a response to disturbance and enrichment that counters that of the free-livers [24]. The development of a Structural Index (SI) and Enrichment Index (EI) followed [19]. The SI measures food web complexity, as affected by stress or disturbance and the EI reflects the flow of resources into the food web.

Nematodes were used in water quality assessment as early as the 1970s [25,26] and have been used since the 1980s in determining the remediation of soil ecosystems after environmental degradation [16,20,27–29]. Researchers have now begun using nematode community analysis to determine impacts of agricultural management practices such as conservation tillage [30,31], crop

rotation [32], phosphorus (P) and nitrogen (N) fertilization [33], and cover cropping [31] on soil health. It is evident from research [30–33] that nematode populations respond quickly and significantly to various forms of agricultural management practices, including different kinds of tillage, nutrient and organic matter inputs, crop rotations and cover cropping.

In addition to crop rotation, cover cropping, reduced tillage, and organic amendments, another way of impacting the soil microbial community is via the introduction of microbes into the soil through inoculation. Professor Teruo Higa, University of the Ryukyus, Okinawa, Japan, developed the concept of effective microorganisms (EM^{TM}) in 1982. EM is a mixed culture of beneficial microorganism. The purpose of these beneficial organisms is to improve crop growth and yield by increasing photosynthesis, producing bioactive substances (e.g., hormones and enzymes), controlling soil diseases, and accelerating decomposition of lignin materials in the soil [34]. Over time, multiple formulations of EM have been developed, and EM and modifications of EM products are now used in over 100 countries.

In parts of Latin America and Asia, Dr. Higa's theory of applying a mixed, concentrated solution of these groups of organisms to agricultural soils has led to the development of a locally-produced version of these effective microorganisms which we term local effective microorganisms (LEM). The microbes found in LEM are derived from yeast, raw milk and decomposing leaf litter in forested areas on or near the same farm to which the LEM is to be applied [35–37]. This home-made, locally-derived microbial inoculant is used to reestablish/increase biodiversity and soil function in disturbed soil ecosystems, such as agricultural soils, through inoculation with local, native soil microorganisms. Within the body of research that has been done on EM^{TM}, there have been positive results [38–40], negative results, [41,42] and mixed results [43,44]. Despite the widespread adoption of LEM by producers in some regions of the world and its promotion by various extension agencies [35,36], little research has been published on LEM's effect on crops and soils. The literature that does exist [37,45], however, indicates that LEM has the potential as a locally-available beneficial management tool.

One way of observing impacts of introducing a suite of microorganisms into a soil food web, is by observing changes in soil food-web indicators such as nematodes. According to Neher [20], nematodes are one or two steps higher in the food chain than microbes, are well-suited as integrators of physical, chemical, and biological properties related with their food resources. However, more information is needed, especially in the southeastern USA, on nematode community characteristics, in terms of maturity, structure, or enrichment, response to microbial inoculant applications such as LEM. We must also further understand whether nuances in tillage and fertility expressed in nematode community structure and, in turn, soil health, described in the literature, will persist when the agroecosystems are converted into the same management system.

The objectives of this study are a) to determine if application of local effective microorganisms (LEM) has an effect on nematode community structure or other soil health parameters by observing treatment effect of LEM application on nematode maturity index (MI) values, structure index (SI) values and enrichment index (EI) values, b) to determine if nematode MI, SI and EI values are sensitive enough to detect the effects of different cropping systems and associated management and c) to investigate the most beneficial ways to use nematode community structure analysis in the measurement of soil health in agricultural systems.

2. Materials and Methods

2.1. Site Description and Design

This study was conducted in the southeastern United States, classified as Acrisols in the World Reference Base soil classification system [46] on organically managed research plots at the J. Phil Campbell Research and Education Center in Watkinsville, Georgia, (33°52′ N, 83°27′ W). The soil at the site is a fine kaolinitic, thermic Typic Kanhapludults and had been managed under certified organic management since 2012. It is in a Cecil sandy loam series with a 2–6 percent slope [47]. The region has

a mean annual rainfall of 1230 mm and an average minimum and maximum annual temperature of 10.4 °C and 22.5 °C [48].

The treatments applied were local effective microorganisms (LEM), made by cultivating actively decomposing O-horizon biomes from well-established mixed hardwood pine forests with a growing media (unpasteurized milk, baker's yeast, semolina flour, charcoal and molasses) under anaerobic conditions; False-LEM (FLEM), made with the same growing media as LEM but without the O-horizon, under the same conditions; and a ground water control (CONT). After six weeks, the solid LEM and FLEM were extracted in a 1:16 sugar: water solution [35–37].

Twenty-four, 18 m^2 (3 m × 6 m) plots were arranged in a randomized complete block design. Rotations for two years (winter 2014 to summer 2016), were cereal rye (*Secale cereale* L.), planted in the winter and butterbean edamame soybean (*Glycine max* L.), planted in the summer (CRS, 12 plots). On the other twelve plots, annual ryegrass (*Lolium perenne* L. spp. *multiflorum* (Lam.)) was planted in the winter and were left fallow in the summer (ARF). The CRS systems were fertilized each summer with composted broiler litter (Table 1) and the ARF systems were fertilized twice each spring with liquid swine effluent (Table 1). For the third year of the study, four, 2 m × 3 m plots were added, which had been fallow and had received no fertilizer amendments since 2010 and represent our bare control plots (BC) and the state of the plots since 2010. These plots were dispersed throughout the organically managed area in places where there was little foot traffic or influence from treated plots. During the third year of the study (winter 2016 to summer 2017), all 28 plots were treated exactly the same. Third year agroecosystem management was red Turkey wheat (*Triticum aestivum* L.) in winter and edamame in summer with no fertilizer either season. It was during this third year that all of the data for this research was gathered. Throughout the three years, eight of the plots received treatments of LEM (four in ARF and four in CRS), eight received FLEM (four in ARF and four in CRS) and eight received an equal amount of water CONT, except for BC which only received water in the third year. For the first two years of management, inoculant treatments were incorporated with the fertilizer amendments applied plus an additional application of LEM, FLEM or water within the first week of plant emergence. The third year, the inoculants were applied only after crop emergence.

Table 1. Disturbance (number of times tilled) and enrichment (nitrogen applied) in each rotation system.

Number of Times Tilled			
	ARF System	CRS System	Bare Control
Year 1	1	2	1
Year 2	1	2	0
Year 3	2	2	2
Total	4	6	3
Nitrogen Applied (kg ha^{-1})			
	ARF System	CRS System	Bare Control
Year 1	50	62	0
Year 2	27	43	0
Year 3	0	0	0
Total	77	105	0

Annual ryegrass is abbreviated by ARF (nitrogen source = swine effluent). Cereal rye is abbreviated by CRS (nitrogen source = composted broiler litter).

2.2. Nematode Sampling, Extraction and Analysis

Soil samples for nematode analyses were taken four weeks after planting and application of amendments in both the winter and summer growing seasons. Soil cores for nematode analysis were taken from 0–10 cm [49–53] from each of the 24 plots. Plots were divided into two halves and 7 cores from points randomly dispersed throughout each of the two halves were composited, A and B (24 × 2). Soils from the A and B composites were extracted and counted separately then averaged together for

each plot, in order to help account for some of the spatial variability often encountered in soil nematode populations. After samples were taken from the field, the composited soils were kept refrigerated at 4 °C before extraction. One hundred cubic centimeters of soil were extracted at room temperature (20 °C) using the centrifugal flotation method as described by Southey (1970) [54]. Extracts were kept refrigerated (4 °C) before the nematode populations were counted. Extracts were brought up to 40 mL, gently and thoroughly shaken, before a 10 mL aliquot was pipetted into a dish for counting. Nematodes were counted live, on an inverted, compound microscope and identified to trophic group or family according to Goodey (1963) [55] and Yeates et al. (1993) [17]. In 2017, nematode communities were analyzed to family and assigned colonizer-persister values according to Bongers and Bongers (1998) [56]. Because free-living nematode communities frequently display opposite responses to that of plant parasitic nematode communities in N enriched environments [24], it is recommended that the maturity indices for these two populations of nematodes be calculated separately. Maturity Index for free-living nematodes (MI), Maturity Index for plant parasitic nematodes (PPI), Structure Index (SI) and Enrichment Index (EI) values were calculated according to methods described by Ferris [19].

2.3. CO_2 Burst Incubation and Analysis

Soil cores were taken from a depth of 0–15 cm, 3–5 days after emergence and application of amendments in both the winter and summer growing seasons. Sampling methodology as described in Section 2.2. Soils were dried at 55 °C for 72 h. Dried soils were brought to 50 percent water-filled pore space. Percent pore space was determined using the calculated bulk density of the soil and an estimated particle density of 2.65. Volume of water needed to reach 50 percent water-filled pore space was determined by multiplying the total volume of soil by percent pore space then dividing the total pore space in half. The soils were then incubated for 28 days at 25 °C. NaOH traps were removed and titrated at 3 days and at 28 days to determine the quantity of CO_2–C respired using the procedure described by Franzluebbers (2016) [57].

2.4. Nitrogen Mineralization Sampling, Incubations and Analysis

Samples for nitrogen mineralization analysis were taken at the same time and following the same methodology as CO_2 burst samples described above. Soils were sampled at a depth of 0–15 cm using 2.5 cm diameter soil augers. Soils were dried at 55 °C for 72 h. Dried soils were brought to 50% water-filled pore space and incubated for 28 days. Before sampling and at 28 days, 5 g sub-samples were taken and extracted for mineralized nitrogen using a 2M KCl solution [58]. NH_4^+ and NO_3^- were measured spectrophotometrically on a Tecan Infinite M200 Pro nanoquant using the salicylic acid method and the vanadium (III) chloride method respectively.

2.5. Harvesting and Yield

Edamame, from the inside four rows, were hand harvested from each plot and fresh bean + pod weight was recorded. Aboveground biomass for the harvested rows in each plot was weighed. The harvested biomass was mixed and a random hand-grab sample was taken to determine moisture content, used to calculate dry weight of the harvested biomass. Wheat was harvested using a Hege 125 plot combine. A hand-grab sample of plant biomass for both edamame and wheat harvests from each plot was collected, weighed and dried at 60 °C for 48 h. Stabilized dry weights were recorded and used in dry matter (DM) correction of fresh weights [59].

2.6. Statistics

All statistics were analyzed using Jmp Pro 13 [60]. Yield, mineralized N, CO_2 flush values, nematode index values and abundance of nematode trophic groups were compared using one-way ANOVA. Interaction effect of seasons on nematode index values was determined using two-way ANOVA. When P-values were ≤ 0.05 means were each compared post-hoc, between treatments using Student's T Test (illustrated with upper case letters in figures and tables indicate differences at $\alpha = 0.05$).

Data falling more than 1.5 times the interquartile range above or below the highest or lowest quartile of the outlier box plot were removed as outliers. Normality was then determined through the Shapiro–Wilk test with a confidence (interval of 0.95). To better understand the relationships between nematode community structure and variables related to soil health, we examined the correlations of both nematode indices and nematode trophic groups with several soil health variables. Soil health variables included CO_2 respired over three days from re-wetted soils, nitrogen mineralized over a one-month soil incubation and the yield and biomass produced from the wheat and edamame crops being grown. The yield and biomass were both represented as overall mass as well as mass divided by the amount of N present in the soils at the beginning of the season. Correlations are defined here as the strength of the linear association between variables, determined using the Pearson's correlation coefficient "r". We performed a principal component analysis to further interpret influences and relevancies of each of the soil health variables, rotations and inoculant treatments. Principal components analysis (PCA) was performed using Jmp Pro 13 statistical software. Because variable values were on highly variable scales, loadings and score plots were calculated using the correlation matrix.

3. Results and Discussion

3.1. Sensitivity of Nematode Community Structure Analysis to Previous Land Management Practices

In the winter, soils from plots previously managed in the bare control (BC) and ARF rotation systems had higher MI and SI values than the soils managed in the CRS rotation system (Table 2). As expected, the EI values were significantly higher in the two enriched systems (ARF and CRS) than in the BC soils. In the summer sampling, like in the winter sampling, the SI values were higher in the soils from plots previously managed in the BC and ARF rotation systems than soils managed in the CRS rotation system (Table 2).

Table 2. Nematode index values of rotation managements, annual ryegrass/fallow (ARF), and cereal rye/edamame soybean (CRS), and bare control (BC) in the winter (above) and summer (below).

Winter 2017 (Wheat)			
Index	ARF (Std. Err.)	CRS (Std. Err.)	BC (Std. Err.)
MI	2.19 A (0.07)	2.10 B (0.01)	2.20 A (0.10)
PPI	3.21 B (0.07)	3.71 A (0.11)	3.08 B (0.04)
SI	66.14 A (3.57)	14.4 B (1.38)	65.73 A (4.27)
EI	35.11A(4.87)	35.76 A (2.98)	20.12 B (2.82)
Summer 2017 (Edamame)			
Index	ARF (Std. Err.)	CRS (Std. Err.)	BC (Std. Err.)
MI	2.14 A (0.05)	2.04 B (0.05)	2.29 A (0.36)
PPI	3.25 B (0.03)	3.72 A (0.09)	3.32 B (0.18)
SI	55.44 A (2.67)	42.13 B (2.97)	65.55 A (4.17)
EI	27.60 B (2.35)	34.28 A (5.06)	20.34 B (9.43)

Nematode Indices abbreviated as MI (maturity index for free-living nematodes), PPI (maturity index for plant parasitic nematodes), SI (structure index), and EI (enrichment index). Different uppercase letters indicate significant differences in index values between inoculant treatments; $\alpha = 0.05$. No letters indicate no significant differences.

In both winter and summer samplings, the soils from the CRS system had the lowest MI and SI values and the greatest EI values and the CRS system experienced the most disturbance (in terms of tillage) and the most enrichment (in terms of nitrogen fertilization). The BC system soils, in both seasons, had the greatest SI and MI values and the lowest EI values and the BC system experienced the least disturbance and enrichment. Relating these results to Ferris [19] which plots EI and SI in quadrants related to high and low disturbance and enrichment: while there were differences in N fertilization and EI values, all our treatments and rotations should fall within the low enrichment quadrants (C and D), as minimal nutrient requirements were applied for crop production. Relating disturbance indicators

SI and MI: tillage was lower in the BC and ARF systems placing them in quadrant D and the other more disturbed cropping system, CRS, would fall toward the C quadrant. This illustrates that the nematode index values consistently and accurately reflected the disturbance and enrichment that the soil ecosystems had received in past years. We demonstrated that the nematode indices are sensitive to variations from the previous two-year rotations and associated management. It is also important to note that, while these trends remained the same from the winter to the summer sampling, there were differences in how sensitive the indices were to the cropping system in the each of the seasons. There was a significant effect of season on MI and SI values ($p = 0.013$ and $p = 0.028$, respectively), but there was no effect of season on PPI ($p = 0.27$) or EI ($p = 0.50$). When considering practical application of these indices, it is critical to keep in mind potential effects of season when soils are sampled. To ensure the greatest efficacy of nematode indices as measures of soil health, consistency in season should be maintained as much as possible.

3.2. Local Effective Microorganisms' Impact on Nematode Community Structure and Agronomic Measures

In this study, the most highly disturbed and enriched rotation (Table 1) was the CRS rotation system. In the winter sampling CRS system, both the LEM and FLEM plots had significantly higher SI values than CONT plots (Table 3).

Table 3. Nematode index values from inoculant treatments (LEM, FLEM and CONT) by Season (winter/summer) and rotation system—Annual ryegrass/fallow system (ARF), and cereal rye/edamame soybean system (CRS).

WINTER			
ARF System			
Index	LEM (Std. Err.)	FLEM (Std. Err.)	CONT (Std. Err.)
MI	2.19 (0.07)	2.10 (0.15)	2.20 (0.14)
PPI	3.11 b (0.03)	3.11 b (0.04)	3.41 a (0.19)
SI	62.44 (5.59)	65.42 (6.35)	71.07 (9.34)
EI	35.12 (13.05)	37.98 (6.74)	32.11 (5.68)
CRS System			
Index	LEM (Std. Err.)	FLEM (Std. Err.)	CONT (Std. Err.)
MI	2.02 (0.03)	2.01 (0.02)	2.00 (0.02)
PPI	3.50 b (0.20)	3.62 ab (0.20)	4.00 a (0.00)
SI	15.11 A (2.60)	24.72 A (1.93)	5.01 B (2.43)
EI	32.80 (4.89)	37.86 (3.94)	36.59 (1.81)
SUMMER			
ARF System			
Index	LEM (Std. Err.)	FLEM (Std. Err.)	CONT (Std. Err.)
MI	2.13 (0.10)	2.14 (0.10)	2.14 (0.09)
PPI	3.28 (0.06)	3.21 (0.06)	3.25 (0.06)
SI	53.62 (7.40)	53.35 (1.03)	59.35 (3.91)
EI	30.13 (3.11)	22.90 (2.87)	29.77 (5.64)
CRS System			
Index	LEM (Std. Err.)	FLEM (Std. Err.)	CONT (Std. Err.)
MI	2.12 (0.09)	1.98 (0.12)	2.01 (0.03)
PPI	3.54 b (0.19)	3.65 ab (0.15)	3.97 a (0.03)
SI	66.10 A (8.32)	37.74 B (0.82)	35.22 B (4.78)
EI	32.02 (13.24)	36.92 (4.74)	33.91 (4.36)

Nematode Indices abbreviated as MI (maturity index for free-living nematodes), PPI (maturity index for plant parasitic nematodes), SI (structure index), and EI (enrichment index). Systems abbreviated as ARF (annual ryegrass/fallow) and CRS (cereal rye/edamame soybean). Inoculation treatments abbreviated as CONT (control treatment of composted broiler litter, no inoculant), FLEM (Composted broiler litter + liquid FLEM) and, LEM (composted broiler litter + liquid LEM). Different uppercase letters indicate significant differences in index values between inoculant treatments; $\alpha = 0.05$ and different lowercase letters indicate significant differences in index values between inoculant treatments; $\alpha = 0.1$. No letters indicate no significant differences.

At the summer sampling CRS system, the LEM plots had higher SI values than FLEM plots and CONT plots (Table 3). This suggests a more complex or balanced community structure in LEM-treated soils. More highly structured nematode communities indicate recovery from stress and more links within the soil food web [19]. The greater SI values among the LEM, and sometimes FLEM, plots in this system suggest that the addition of LEM over the years may be able to help counteract the negative impact of disturbance and enrichment on soil food web complexity.

In the ARF systems, we did not see significant differences in nematode indices between inoculant treatments in the summer. In the winter PPI was greater ($p < 0.10$) in the CONT which suggests that either inoculant can disrupt the establishment of Trichodoridae (Table S2, ring nematode) during the winter.

Although no significant differences in MI (free-living maturity index) values were found between inoculant treatments at $p < 0.05$ or 0.10, PPI (plant parasitic maturity index) values were greater in CONT plots than LEM plots in both the ARF and CRS system soils in the winter ($p < 0.10$; Table 3) and in the CRS system soils in the summer ($p < 0.10$; Table 3). Higher PPI values are associated with more highly disturbed and enriched systems [24,49]. Because inoculant treatments received the same amount of tillage and fertilization within systems, it is unclear why LEM inoculant reduced the PPI. While it is beyond the scope of this research, further research is needed to understand influences of different bio inoculants on nutrient flows within the soil biome which could have significant impact on pest and disease pressures.

There was a difference in wheat yield within the annual ryegrass/fallow (ARF) cropping system plots in which CONT plots yielded more wheat than LEM plots (Figure 1). Within the ARF rotation, no treatment differences were found in amount of nitrogen mineralized (Figure 2), nematode MI, EI or SI values (Table 2), nor plant parasitic nematode (PPN) populations ($p = 0.506$). There were, however, differences between treatments in the populations of individual PPN families (Figure 3).

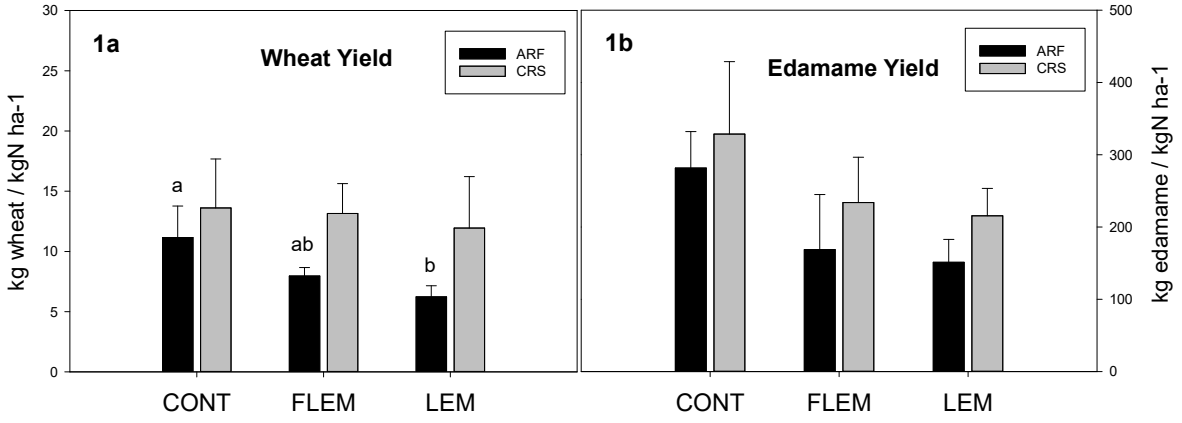

Figure 1. Amount of crop per kg N in the soil after treatment application: wheat (winter, 2017) and edamame soybean (summer, 2017) harvested from plots managed previously under an annual ryegrass/fallow rotation (ARF) and under a cereal rye/edamame soybean rotation (CRS). Different letters indicate differences between inoculant treatments. No letters indicate no significant differences between treatments; $\alpha = 0.1$.

The families of PPN identified from the ARF soils include several common agricultural pests including ring (*Criconemella*), spiral (*Helicotylenchus)*, lesion (*Pratylenchus*), lance (*Hoplolaimus*) and stubby root (*Paratrichodorus*) nematodes. According to a range of action thresholds compiled by the University of Missouri Extension Services (http://extension.missouri.edu/scott/documents/Ag/Agronomy/Nematode-Thresholds-Summary.pdf) and recommendations by the University of Georgia cooperative extension [61], the only nematode pests present at numbers great enough to potentially cause significant crop loss are lesion and lance nematodes. Pratylenchidae, the family which contains

lesion nematodes, are present at numbers entering the ranges of the action thresholds reported among all treatments. Hoplolaimidae, the family which contains lance nematodes, however, are only high enough to cause damage among the LEM-treated plots. Lower yields in the LEM ARF winter plots could be attributed to this. These findings suggest that under certain situations, the application of LEM could increase PPN populations and may induce damage significant enough to cause yield loss. Since not all PPN populations seem to be affected in the same way and the PPN populations were not influenced equally within different management systems or seasons, it would be valuable to study LEM's effect on specific genera of PPN. Further, this work may need to be conducted at a range of soil temperatures, since previous research has shown LEM to have less influence when soil temperatures were below 15 °C [37].

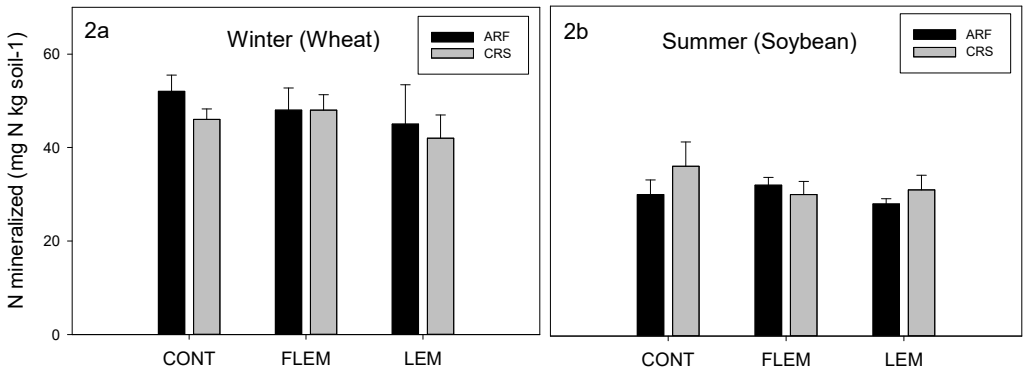

Figure 2. Amount of N mineralized (minus the amount of mineralized N in initial soils sampled after treatment application and analyzed before incubation) from plots managed previously under an annual ryegrass/fallow rotation (ARF) and under a cereal rye/edamame soybean rotation (CRS); $\alpha = 0.05$. No letters indicate no significant differences.

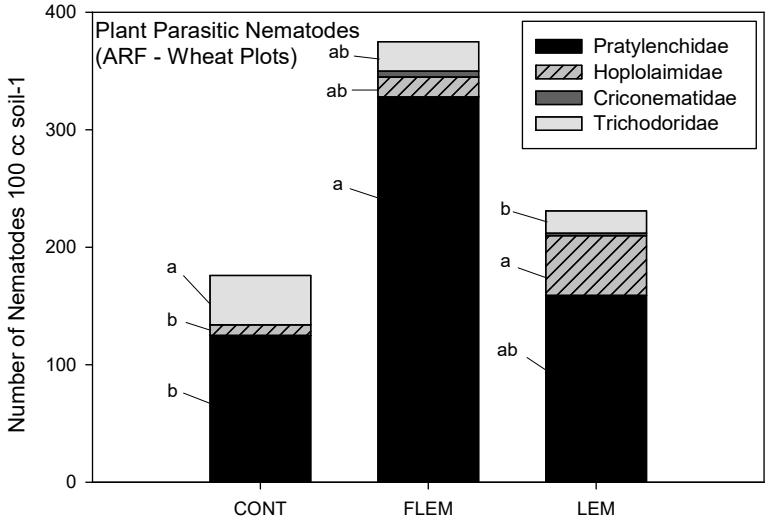

Figure 3. Comparison of plant parasitic nematode families sampled in the annual ryegrass/fallow rotation system (ARF) during the winter (wheat season). Different letters indicate differences between treatments. No letters indicate no significant differences between treatments; $\alpha = 0.1$.

3.3. Using Nematodes in the Measurement of Soil Health in Agricultural Systems

While recent literature has consistently found agricultural management practices to have an effect on nematode community structure [29–32], more research is needed to further our understanding of how to best use nematode community analyses in conjunction with other measures of soil function and health to inform land management and policy decisions. When looking at the correlations

between variables, we found that the MI and SI values were negatively correlated with yield and biomass. In contrast, the EI values were positively correlated with yield and biomass. The strength of correlations between nematode indices and yield and/or biomass were stronger in the winter than in the summer (Table 4).

Table 4. Winter (wheat): Correlations between nematode indices and measures of soil health.

	MI	**PPI**	**SI**	**EI**	**CO_2 3 days**	**CO_2 28 days**	**N Min.**	**Yield**	**Biomass**
MI	-	**0.63**	**0.79**	**−0.54**	0.19	0.02	0.05	**−0.49**	−0.38
PPI	**−0.63**	-	**−0.64**	0.24	0.03	0.27	0.09	**0.55**	**0.42**
SI	**0.79**	**−0.64**	-	−0.16	0.26	0.20	0.21	**−0.47**	**−0.41**
EI	**−0.54**	0.24	−0.16	-	−0.05	0.05	−0.10	0.33	**0.51**
Summer (edamame): Correlations between nematode indices and measures of soil health.									
	MI	**PPI**	**SI**	**EI**	**CO_2 3 days**	**CO_2 28 days**	**N Min.**	**Yield**	**Biomass**
MI	-	0.07	**0.62**	**−0.67**	−0.28	−0.30	0.01	0.08	**−0.46**
PPI	0.07	-	−0.35	−0.00	−0.21	−0.02	**0.40**	0.00	−0.23
SI	**0.62**	−0.35	-	**−0.44**	−0.29	−0.30	−0.05	−0.37	**−0.40**
EI	**−0.67**	−0.00	**−0.44**	-	0.24	0.03	−0.00	0.12	**0.31**

Nematode Indices abbreviated as MI (maturity index), SI (structure index), and EI (enrichment index). CO_2 values represent the amount of CO_2 respired from soils during either 3 or 28 days of incubation (mg C kg soil^{-1}). N Mineralized values indicate amount of N mineralized in soils after 28 days of incubation (mg N kg soil $^{-1}$). Yield reported as kg fresh edamame (beans + pods). Biomass reported as kg aboveground biomass (after harvesting), on a dry matter basis. Values in bold indicate significant correlations; $\alpha = 0.05$.

One difficulty of incorporating nematode community structure indices into measures of soil health is how to translate the nematode index values into a soil health value. If soil health is defined as the capacity of soil to provide numerous and varied ecosystem services, such as sustaining plant and animal productivity, enhancing water and air quality, and promoting plant and animal health, then, especially in the context of an agroecosystem, it must be measured with regards to a balance between multiple, sometimes opposing, components. In Ferris et al. [19], the authors provide an example of how a multi-dimensional measure of soil health can be created using a quadrant system of EI and SI indicators. We believe that, like in the quadrant system proposed [19], it is important to choose multiple soil health indicators that are complementary to each other in order to provide a multi-dimensional and balanced measure of soil health.

An example from our study for why a diverse range of indicators should be considered when measuring soil health, are the negative correlations between MI and SI values and crop production values. Large MI and SI values are associated with healthy soil because they indicate greater complexity and maturity of the soil food web [20,21] which, in turn, indicates a more efficient, more resilient soil ecosystem. In both seasons of our study, the MI and SI had a negative correlation with crop production. This low-input case emphasizes the importance of using multiple nematode community metrics to provide a balanced soil health measure for agroecosystems. Because the EI is calculated based on the numbers of nematodes that quickly take advantage of nutrient inputs (enrichment) [62], this index tends to be positively correlated with crop production. These advantageous nematodes are also the ones that have low c-p values. When these enrichment opportunists [62] increased in our samples, the MI and SI tended to decrease, resulting in an inverse relationship between the indices. Because soil health in agroecosystems should be defined by both its ability to carry out critical soil functions provided by a mature and complex microbiome as well as its ability to maintain high agricultural yields, we feel that it is important to combine indicators that reflect these highly varied components of soil health, much like Ferris et al., 2001 [19].

When comparing abundance of nematodes in each trophic group and measured soil health variables, the most consistent correlations were again related to yield (Table 5). Both fungal-feeding nematodes (FF) and Tylenchidae (TYL) were positively correlated with yield and biomass in the winter. In the case of the nematode trophic groups, the yield relationships were all stronger in the winter where differences in yield between inoculant treatments were significant. The plant parasitic nematodes (PPN) had an inverse correlation with yield and biomass in the winter (Table 4).

Table 5. Correlations between nematode trophic groups and soil health indicators in the winter (wheat) and summer (edamame) sampling seasons.

	Winter					Summer			
	CO_2 3 Days	**N Min.**	**Yield**	**Biomass**		**CO_2 3 Days**	**N Min.**	**Yield**	**Biomass**
BF	−0.15	0.05	0.36	0.27	BF	0.34	0.06	0.27	0.38
FF	**−0.46**	−0.28	**0.50**	**0.44**	FF	0.26	−0.25	0.17	0.30
PPN	−0.16	−0.05	**−0.48**	**−0.51**	PPN	0.25	−0.15	0.23	0.30
TYL	−0.34	−0.04	**0.51**	**0.43**	TYL	0.27	−0.36	0.36	−0.01
DOR	0.33	0.08	−0.18	−0.16	DOR	−0.09	0.16	−0.09	−0.23
MON	0.21	−0.31	−0.13	0.17	MON	−0.04	0.22	−0.04	−0.33

Nematode classifications BF, FF, PPN, TYL, DOR and MON stand for bacterial-feeding, fungal-feeding, plant parasitic, Tylenchidae, Dorylaimidae, and Mononchidae, respectively. CO_2 values represent the amount of CO_2 respired from soils during either 3 or 28 days of incubation (mg C kg^{-1} soil). N Mineralized values indicate amount of N mineralized in soils after 28 days of incubation (mg N kg^{-1} soil). Yield reported as kg fresh edamame (beans + pods). Biomass reported as kg aboveground biomass (after harvesting), on a dry matter basis. Values in bold indicate significant correlations; $\alpha = 0.05$.

Because BF and FF nematodes are intricately linked to decomposition pathways through grazing on primary decomposers and re-releasing nutrients into the soil solution [20], it makes sense for BF and FF nematodes to have a positive correlation with enriched systems and higher yields. Because of the Tyelenchidae family's ambiguous position in the soil food web [62], it was interesting to find that, in both seasons, nematodes from the Tylenchidae family showed a similar trend in correlations with CO_2, N min and yield as the FF nematodes. In nematode trophic analysis it is unclear whether to place Tylenchidae among the plant parasitic nematodes or the fungal-feeders [63–67]. These findings suggest that, in our case, Tylenchidae are more closely associated with fungal-feeders. Preforming an expanded study analyzing correlations between trophic groups and various plant and soil responses, in addition to direct observation of Tylenchidae trophic-habits, could strengthen these findings and provide important insight into how to place Tylenchidae in future nematode trophic analyses.

When principal components analysis was performed, we found that in the winter score plot, the rotation treatments are divided clearly by component 1 (western and eastern hemispheres) (Figure 4), where CRS rotations are primarily in the eastern hemisphere. In summer score plot, the ARF rotation differentiates from the CRS and BC rotations along component 2 (northern and southern hemispheres) and the BC rotation separates from the CRS rotation along component 1 (Figure 4). There was no clear grouping or differentiation of soils receiving inoculant treatments in relation to the principal components.

According to the PCA loading plots, it is evident that nematode indices and trophic group measurements strongly influenced the first principal component in both seasons. The second principal component in both seasons was governed primarily by mineralized N. Principal components analysis demonstrated that enough variability in the data was represented by components 1 and 2 to differentiate between rotational management and the influences of the rotations on nematode community structure. This supports hypothesis that nematode community indices reflect variations in cropping systems. Generally, our PCA indicates stronger associations between nematode indices and N mineralization in summer and less association in the winter. The influence of N mineralization values on principal component 2, seen in the loading plots, suggests that N mineralization reflects variability not otherwise accounted for by nematode indices.

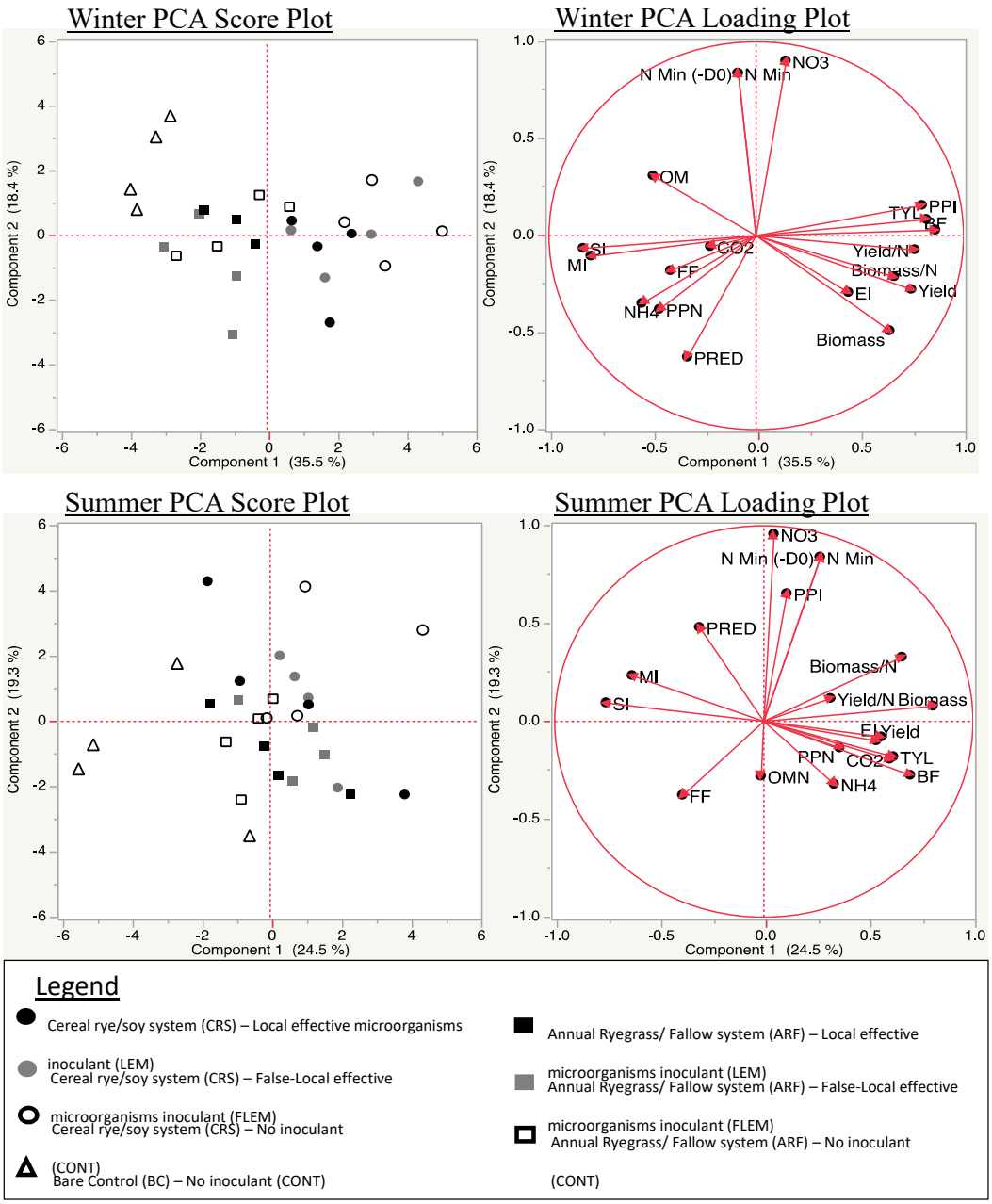

Figure 4. Triangles, squares and circles represent BC, ARF and CRS rotations, respectively. Black, gray and open symbols represent LEM, FLEM and CONT treatments, respectively. Variable abbreviations: NO^3 (nitrate present after 1 month incubation), NH_4 (ammonium present after 1 month incubation), N Min (inorganic N present after 1 month incubation), N Min (-D0) (Nitrogen mineralized during 1 month incubation), CO_2 (CO_2–C respired 3 days after rewetting dried soils), Biomass/N and Yield/N (biomass and yield reported as a proportion of N present in soil after treatment application), BF (bacterial-feeding nematodes), FF (fungal-feeding nematodes), PPN (plant parasitic nemaotdes), TYL (nematodes in the Tylenchidae family), OMN (omnivorous nematodes), PRED (nematodes that predate on mesofauna, including other nematodes). Shown above are principal component analysis score plot and loading plot for the soil health indicator values measured in the winter.

4. Summary and Conclusions

Soil health is a complex measure, best evaluated using a combination of indicators. Nematode indices and community analysis are excellent candidates to include in the creation of soil health minimum data sets (MDS). Nematodes provided important information about the complexity and

maturity of the soil food web through their demonstrated sensitivity to disturbance and enrichment. Through nematode community analysis we found that there were strong influences on the soil food web caused from cropping system management that included heavier tilling and N fertilization. It was also determined that in the more disturbed and enriched soils, application of LEM inoculant improved structure index values, indicating that the complexity of the food web was better maintained in those soils.

A soil's health is quantified by its ability to provide a variety of ecosystem services, which includes but is not limited to provisioning of agricultural resources. The inverse correlation found between the MI and SI indices and variables strongly correlated with food provision (crop yield) suggests that these measures should be used together to represent the balance of various functions sustained by a healthy soil. Principal component analysis further indicated that measures of N mineralization reveal unique information not reflected by the other indicators measured. When we examined the correlations of both nematode indices and nematode trophic groups with other soil health variables, such as CO_2 respiration, nitrogen mineralization and yield, we found that fungal-feeding nematodes, nematodes from the Tylenchidae family and EI values were positively correlated with crop productivity while MI and SI values were negatively correlated with crop productivity. Because nematode community structure reflected ecosystem disturbance and enrichment, nematode indicators should be used to complement N mineralization indicators to form a more robust index of soil health.

Supplementary Materials: The following are available online at http://www.mdpi.com/2571-8789/3/2/41/s1, Table S1: Average number of nematode families identified in samples from each cropping system, Table S2: Average number of nematode families identified in winter samples from each inoculant treatment, Table S3: Average number of nematode families identified in summer samples from each inoculant treatment.

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References

1. Dominati, E.; Patterson, M.; Mackay, A. A framework for classifying and quantifying the natural capital and ecosystem services of soils. *Ecol. Econ.* **2010**, *69*, 1858–1868. [CrossRef]
2. Daily, G.C. *Nature's Services*; Island Press: Washington, DC, USA, 1997; Volume 19971.
3. Karlen, D.L.; Mausbach, M.J.; Doran, J.W.; Cline, R.G.; Harris, R.F.; Schuman, G.E. Soil quality: A concept, definition, and framework for evaluation (a guest editorial). *Soil Sci. Soc. Am. J.* **1997**, *61*, 4–10. [CrossRef]
4. Doran, J.W.; Zeiss, M.R. Soil health and sustainability: Managing the biotic component of soil quality. *Appl. Soil Ecol.* **2000**, *15*, 3–11. [CrossRef]
5. Cairns, J.; McCormick, P.V.; Niederlehner, B.R. A proposed framework for developing indicators of ecosystem health. *Hydrobiologia* **1993**, *263*, 1–44. [CrossRef]
6. Armenise, E.; Redmile-Gordon, M.A.; Stellacci, A.M.; Ciccarese, A.; Rubino, P. Developing a soil quality index to compare soil fitness for agricultural use under different managements in the Mediterranean environment. *Soil Tillage Res.* **2013**, *130*, 91–98. [CrossRef]
7. Garrigues, E.; Corson, M.S.; Angers, D.A.; van der Werf, H.M.; Walter, C. Soil quality in Life Cycle Assessment: Towards development of an indicator. *Ecol. Indic.* **2012**, *18*, 434–442. [CrossRef]

8. Kelting, D.L.; Burger, J.A.; Patterson, S.C.; Aust, W.M.; Miwa, M.; Trettin, C.C. Soil quality assessment in domesticated forests—A southern pine example. *For. Ecol. Manag.* **1999**, *122*, 167–185. [CrossRef]
9. Wienhold, B.J.; Andrews, S.S.; Karlen, D.L. Soil quality: A review of the science and experiences in the USA. *Environ. Geochem. Health* **2004**, *26*, 89–95. [CrossRef] [PubMed]
10. Masto, R.E.; Chhonkar, P.K.; Purakayastha, T.J.; Patra, A.K.; Singh, D. Soil quality indices for evaluation of long-term land use and soil management practices in semi-arid sub-tropical India. *Land Degrad. Dev.* **2008**, *19*, 516–529. [CrossRef]
11. Bone, J.; Barraclough, D.; Eggleton, P.; Head, M.; Jones, D.T.; Voulvoulis, N. Prioritising soil quality assessment through the screening of sites: The use of publicly collected data. *Land Degead. Dev.* **2014**, *25*, 251–266. [CrossRef]
12. Yan, S.; Singh, A.N.; Fu, S.; Liao, C.; Wang, S.; Li, Y.; Cui, Y.; Hu, L. A soil fauna index for assessing soil quality. *Soil Biol. Biochem.* **2012**, *47*, 158–165. [CrossRef]
13. Knoepp, J.D.; Coleman, D.C.; Crossley, D.A., Jr.; Clark, J.S. Biological indices of soil quality: An ecosystem case study of their use. *For. Ecol. Manag.* **2000**, *138*, 357–368. [CrossRef]
14. Paz-Ferreiro, J.; Fu, S. Biological indices for soil quality evaluation: Perspectives and limitations. *Land Degrad. Dev.* **2016**, *27*, 14–25. [CrossRef]
15. Pattison, A.B.; Moody, P.W.; Badcock, K.A.; Smith, L.J.; Armour, J.A.; Rasiah, V.; Cobon, J.A.; Gulino, L.M.; Mayer, R. Development of key soil health indicators for the Australian banana industry. *Appl. Soil Ecol.* **2008**, *40*, 155–164. [CrossRef]
16. Bongers, T.; Ferris, H. Nematode community structure as a bioindicator in environmental monitoring. *Trends Ecol. Evol.* **1999**, *14*, 224–228. [CrossRef]
17. Yeates, G.W.; Bongers, T.D.; De Goede, R.G.M.; Freckman, D.W.; Georgieva, S.S. Feeding habits in soil nematode families and genera—An outline for soil ecologists. *J. Nematol.* **1993**, *25*, 315. [PubMed]
18. Ritz, K.; Black, H.I.; Campbell, C.D.; Harris, J.A.; Wood, C. Selecting biological indicators for monitoring soils: A framework for balancing scientific and technical opinion to assist policy development. *Ecol. Indic.* **2009**, *9*, 1212–1221. [CrossRef]
19. Ferris, H.; Bongers, T.; De Goede, R.G.M. A framework for soil food web diagnostics: Extension of the nematode faunal analysis concept. *Appl. Soil Ecol.* **2001**, *18*, 13–29. [CrossRef]
20. Neher, D.A. Role of nematodes in soil health and their use as indicators. *J. Nematol.* **2001**, *33*, 161.
21. Bongers, T. The maturity index: An ecological measure of environmental disturbance based on nematode species composition. *Oecologia* **1990**, *83*, 14–19. [CrossRef]
22. Neher, D.A.; Campbell, C.L. Sampling for regional monitoring of nematode communities in agricultural soils. *J. Nematol.* **1996**, *28*, 196. [PubMed]
23. Neher, D.A.; Peck, S.L.; Rawlings, J.O.; Campbell, C.L. Measures of nematode community structure and sources of variability among and within agricultural fields. *Plant Soil* **1995**, *170*, 167–181. [CrossRef]
24. Bongers, T.; van der Meulen, H.; Korthals, G. Inverse relationship between the nematode maturity index and plant parasite index under enriched nutrient conditions. *Appl. Soil Ecol.* **1997**, *6*, 195–199. [CrossRef]
25. Zullini, A. Nematodes as indicators of river pollution. *Nematol. Mediterr.* **1976**, *4*, 13–22.
26. Prejs, K. The littoral and profundal benthic nematodes of lakes with different trophy. *Ekologia Polska* **1977**, *25*, 21–30.
27. Yeates, G.W. Nematodes as soil indicators: Functional and biodiversity aspects. *Biol. Fertil. Soils* **2003**, *37*, 199–210.
28. Kozlowska, J. The effect of sewage sediments on communities of soil nematodes. *Pol. Ecol. Stud.* **1989**, *15*, 27–39.
29. Zullini, A.; Peretti, E. Lead pollution and moss-inhabiting nematodes of an industrial area. *Water Air Soil Pollut.* **1986**, *27*, 403–410. [CrossRef]
30. Zhang, X.; Li, Q.; Zhu, A.; Liang, W.; Zhang, J.; Steinberger, Y. Effects of tillage and residue management on soil nematode communities in North China. *Ecol. Indic.* **2012**, *13*, 75–81. [CrossRef]
31. Ito, T.; Araki, M.; Komatsuzaki, M.; Kaneko, N.; Ohta, H. Soil nematode community structure affected by tillage systems and cover crop managements in organic soybean production. *Appl. Soil Ecol.* **2015**, *86*, 137–147. [CrossRef]
32. Grabau, Z.J.; Chen, S. Influence of long-term corn–soybean crop sequences on soil ecology as indicated by the nematode community. *Appl. Soil Ecol.* **2016**, *100*, 172–185. [CrossRef]

33. Zhao, J.; Wang, F.; Li, J.; Zou, B.; Wang, X.; Li, Z.; Fu, S. Effects of experimental nitrogen and/or phosphorus additions on soil nematode communities in a secondary tropical forest. *Soil Biol. Biochem.* **2014**, *75*, 1–10. [CrossRef]
34. Higa, T.; Parr, J.F. *Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment*; International Nature Farming Research Center: Atami, Japan, 1994; Volume 1.
35. Suchini Ramirez, J.G. *Innovaciones Agroecologicas para una Producciones Agropecuaria Sostenible en la Region del Trifinio*, 1st ed.; CATIE: Turrialba, Costa Rica, 2012.
36. *Ministerio de Agricultura y Ganaderia Guia Tecnicia para la Difusion de Tecnologias de Produccion Agropecuaria Sostenible*, 1st ed.; MAG: San Jose, Costa Rica, 2010.
37. Ney, L.; Franklin, D.; Mahmud, K.; Cabrera, M.; Hancock, D.; Habteselassie, M.; Newcomer, Q. Examining trophic-level nematode community structure and nitrogen mineralization to assess local effective microorganisms' role in nitrogen availability of swine effluent to forage crops. *Appl. Soil Ecol.* **2018**, *130*, 209–218. [CrossRef]
38. Hu, C.; Qi, Y. Long-term effective microorganisms application promote growth and increase yields and nutrition of wheat in China. *Eur. J. Agron.* **2013**, *46*, 63–67. [CrossRef]
39. Khaliq, A.M.; Abbasi, K.; Hussain, T. Effects of integrated use of organic and inorganic nutrient sources with effective microorganisms (EM) on seed cotton yield in Pakistan. *Bioresour. Technol.* **2006**, *97*, 967–972. [CrossRef] [PubMed]
40. Daly, M.J.; Stewart, D.P.C. Influence of "effective microorganisms" (EM) on vegetable production and carbon mineralization—A preliminary investigation. *J. Sustain. Agric.* **1999**, *14*, 15–25. [CrossRef]
41. Mayer, J.; Scheid, S.; Widmer, F.; Fließbach, A.; Oberholzer, H. How effective are 'Effective microorganisms® (EM)'? Results from a field study in temperate climate. *Appl. Soil Ecol.* **2010**, *46*, 230–239. [CrossRef]
42. Van Vliet, P.C.J.; Bloem, J.; De Goede, R.G.M. Microbial diversity, nitrogen loss and grass production after addition of Effective Micro-organisms® (EM) to slurry manure. *Appl. Soil Ecol.* **2006**, *32*, 188–198. [CrossRef]
43. Sharif, M.; Khan, M.; Khan, M.A.; Wahid, F.; Marwat, K.B.; Khattak, A.M.; Naseer, M. Effect of rock phosphate and farmyard manure applied with effective microorganisms on the yield and nutrient uptake of wheat and sunflower crops. *Pak. J. Bot.* **2015**, *47*, 219–226.
44. Javaid, A.; Bajwa, R. Field evaluation of effective microorganisms (EM) application for growth, nodulation, and nutrition of mung bean. *Turk. J. Agric. For.* **2011**, *35*, 443–452.
45. Martinez, A.D.P.C.; Acosta Sanchez, R.L.; Morales Velasco, S.; Prado, F.A. Evaluación de microorganismos de montaña (mm) en la producción de acelga en la meseta de Popayán. *Biotecnología en el Sector Agropecuario y Agroindustrial BSAA* **2014**, *12*, 79–87.
46. *World Reference Base for Soil Resources 2014, Update 2015 International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*; World Soil Resources Reports No. 106; FAO: Rome, Italy, 2014.
47. Soil Survey Staff. Keys to Soil Taxonomy, 10th ed.; U.S. Department of Agriculture, Natural Resources Conservation Service. 2006. Available online: https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm (accessed on 28 February 2019).
48. Georgia Weather "Past Data". Available online: http://weather.uga.edu/?variable=HI&site=GAWU (accessed on 28 February 2019).
49. Freckman, D.W.; Ettema, C.H. Assessing nematode communities in agroecosystems of varying human intervention. *Agric. Ecosyst. Environ.* **1993**, *45*, 239–261. [CrossRef]
50. Yeates, G.W.; Bardgett, R.D.; Cook, R.; Hobbs, P.J.; Bowling, P.J.; Potter, J.F. Faunal and microbial diversity in three Welsh grassland soils under conventional and organic management regimes. *J. Appl. Ecol.* **1997**, *34*, 453–470. [CrossRef]
51. Forge, T.A.; Simard, S.W. Trophic structure of nematode communities, microbial biomass, and nitrogen mineralization in soils of forests and clearcuts in the southern interior of British Columbia. *Can. J. Soil Sci.* **2000**, *80*, 401–410. [CrossRef]
52. Cesarz, S.; Reich, P.B.; Scheu, S.; Ruess, L.; Schaefer, M.; Eisenhauer, N. Nematode functional guilds, not trophic groups, reflect shifts in soil food webs and processes in response to interacting global change factors. *Pedobiologia* **2015**, *58*, 23–32. [CrossRef]
53. Nivelle, E.; Verzeaux, J.; Habbib, H.; Kuzyakov, Y.; Decocq, G.; Roger, D.; Lacoux, J.; Duclercq, J.; Spicher, F.; Nava-Saucedo, J.E.; et al. Functional response of soil microbial communities to tillage, cover crops and nitrogen fertilization. *Appl. Soil Ecol.* **2016**, *108*, 147–155. [CrossRef]

54. Southey, J.F. *Laboratory Methods for Work with Plant and Soil Nematodes*, 1st ed.; Her Majesty's Stationery Office: London, UK, 1970.
55. Goodey, T. *Soil and Freshwater Nematodes. A Monograph*, 2nd ed.; John Wiley: London, UK; New York, NY, USA, 1963.
56. Bongers, T.; Bongers, M. Functional diversity of nematodes. *Appl. Soil Ecol.* **1998**, *10*, 239–251. [CrossRef]
57. Franzluebbers, A.J. Should soil testing services measure soil biological activity? *Agric. Environ. Lett.* **2016**, *1*. [CrossRef]
58. Villar, N.; Aizpurua, A.; Castellón, A.; Ortuzar, M.A.; Moro, M.B.G.; Besga, G. Laboratory methods for the estimation of soil apparent N mineralization and wheat N uptake in calcareous soils. *Soil Sci.* **2014**, *179*, 84–94. [CrossRef]
59. Marsalis, M.A.; Angadi, S.V.; Contreras-Govea, F.E. Dry matter yield and nutritive value of corn, forage sorghum, and BMR forage sorghum at different plant populations and nitrogen rates. *Field Crop Res.* **2010**, *116*, 52–57. [CrossRef]
60. SAS Institute Inc. *JMP (Version 13.0)*; SAS Institute Inc.: Cary, NC, USA, 2013.
61. Davis, R.F.; Bertrand, P.; Gay, D.; Baird, R.; Padgett, B.; Brown, E.; Balsdon, J. Guide for Interpreting Nematode Assay Results. 2013. Available online: http://extension.uga.edu/publications/detail.html?number=C834&title=GuideforInterpretingNematodeAssayResults#Action (accessed on 22 March 2019).
62. Ferris, H.; Bongers, T. Nematode indicators of organic enrichment. *J. Nematol.* **2006**, *38*, 3. [PubMed]
63. Dong, D.; Chen, Y.F.; Steinberger, Y.; Cao, Z.P. Effects of different soil management practices on soil free-living nematode community structure, Eastern China. *Can. J. Soil Sci.* **2008**, *88*, 115–127. [CrossRef]
64. Jaffee, B.A.; Ferris, H.; Scow, K.M. Nematode-trapping fungi in organic and conventional cropping systems. *Phytopathology* **1998**, *88*, 344–350. [CrossRef] [PubMed]
65. Sohlenius, B.; Persson, H.; Magnusson, C. Distribution of roots and nematodes in a young Scots pine stand in Central Sweden. *Ecol. Bull.* **1977**, *25*, 340–347.
66. Parmelee, R.W.; Bohlen, P.J.; Edwards, C.A. Analysis of nematode trophic structure in agroecosystems: Functional groups versus high resolution taxonomy. In *The Significance and Regulation of Soil Biodiversity. Developments in Plant and Soil Sciences*; Collins, H.P., Robertson, G.P., Klug, M.J., Eds.; Springer: Dordrecht, The Netherlands, 1995; Volume 63, pp. 203–207.
67. Carrascosa, M.; Sánchez-Moreno, S.; Alonso-Prados, J.L. Effects of organic and conventional pesticides on plant biomass, nematode diversity and the structure of the soil food web. *Nematology* **2015**, *17*, 11–26. [CrossRef]