HIDDEN STEWARDS OF THE SOIL: FREE-LIVING NEMATODES AS SENTINELS OF ECOSYSTEM FUNCTIONING

By

Tvisha Martin

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ABSTRACT

Soil biodiversity is instrumental for ecosystem functions such as nutrient cycling and carbon (C) storage. However, as anthropogenic disturbance places soil biodiversity at risk, we may witness disruptions in these essential ecosystem services. Despite its significance, understanding and quantifying shifts in soil biodiversity and their impacts on ecosystem functioning remains challenging. My dissertation addresses three knowledge gaps regarding the use of free-living nematodes as bioindicators of soil biodiversity and function in agroecosystems. First, I assess the relationship between nematode community assemblage and soil C pools and assess how these dynamics shift through time in contrasting agroecosystems. Regenerative agriculture can enhance soil food web structure through improved soil health; however, we have yet to understand if this is true over a long-term period. In chapter 1, I assess the effects of long-term regenerative agriculture practices on soil food web structure through quantifying free-living nematodes in 1991 and 2021 at the W.K. Kellogg Biological Station Long-term Ecological Research site. I found that after 20-years, nematode communities shifted from bacterivore to fungivore dominance in perennial systems. Soil C accumulation was also four times greater after 20-years but only in the early successional and a mown grassland systems. This decadal study demonstrates that the long-term maintenance of perenniality and diversity alters soil food web structure and drives soil C accumulation in agricultural systems.

Second, I explore resistance and resilience of soil food webs to drought in a perennial vs. annual row crop. The impact that drought duration has on the soil food webs is seldom investigated, and even less is known regarding the role that agricultural management has on soil food web resistance and resilience to drought. In chapter 2, I aim to 1) understand how management intensity impacts the resistance of nematode communities to drought and 2) assess how the immediate alleviation of drought impacts soil food web resilience in contrasting agroecosystems**.** This study was conducted at the W.K. Kellogg Biological Station Long-term Ecological Research Site, where three rainfall manipulations (drought, variable, and control) were induced in two systems (early successional and no-till row-crop). Sampling for nematode communities was conducted before drought was imposed (pre-drought), six-weeks after drought was induced (peak-drought), and two days after rewetting (post-drought). I found that nematode communities in early successional systems were both resistant and resilient to drought. However, no-till systems were less resistant to drought stress, whereby fungivore *r* and *K* strategist

nematode abundances declined under increased drought stress. Additionally, the alleviation of drought indicated that while early successional systems remained resilient to drought, no-till systems were slow to recover post-drought. Overall, this chapter demonstrates that reduced management intensity within agroecosystems is a valuable option for fostering soil food webs that are resistant to drought.

Third, I assess how trophic level interactions within the soil food web influence N cycling. Bacterivore nematodes play a vital role in the nitrogen (N) cycle through their trophic interactions with bacterial communities, and their direct excretion of plant available ammonium. Here I 1) explore how the presence and absence of dominant bacterivore nematodes with different life-history strategies impact soil N pools and plant N use, and 2) assess how bacterial trophic channels interact with soil nitrogen use efficiency under the presence of varying bacterivore nematode species. This greenhouse microcosm experiment was conducted using soil collected from an organic farm that was defaunated. Microcosms were treated with four different nematode inoculums: *Acrobeloides nanus* (A.nanus), *Rhabditid intermedia (R,intermedia)*, a co-inoculation of both species, and no nematodes. *A.nanus* and *R.intermedia* vary in their life-history strategies. The results from this study demonstrate that nematode diversity through co-inoculation can significantly increase organic nitrogen pools and soil nitrate. Additionally, co-inoculum treatments drove significant relationships between total nematode abundance and root N, aboveground biomass, and root biomass. I also found that co-inoculations of bacterivore nematodes enhance nitrogen use efficiency (NUE) and impact α-diversity metrics of bacteria. Overall, results indicate that a diversity of bacterivore nematodes, which vary in life-history traits, is essential for overall N cycling and NUE.

Taken together, these results indicate that free-living nematodes are highly connected to sustained ecosystem functioning and serve as valuable bioindicators of climatic disturbance and shifts in agricultural management practices. Moreover, this work supplies evidence that the conservation of soil biodiversity is essential for maintaining soil health and ecological function

Dedicated to my mother, father, sister and brother- you have taught me to always be curious and fostered my love for the natural world

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CHAPTER 1: INTRODUCTION

OVERVIEW

Soil ecosystems are home to approximately 59 ± 15% of life on Earth (Anthony et al., 2023). Beyond its sheer biological diversity, soil biodiversity underpins critical ecosystem services that sustain life, including crop productivity, nutrient cycling, and carbon (C) sequestration (Bach et al., 2020). These functions are essential for maintaining soil fertility, regulating atmospheric carbon levels, and supporting global food production (Kopittke et al., 2019). However, anthropogenic pressures such as land-use change, pollution, and climate change are placing soil biodiversity at risk (Kopittke et al., 2019). As biodiversity declines, we may witness disruptions in these essential ecosystem services, with potentially severe consequences for global food production and climate regulation (Malhi et al., 2020). Despite its significance, understanding and quantifying shifts in soil biodiversity and their impacts on ecosystem functioning remains challenging.

Free-living nematodes pose as a valuable solution for the development of bioindicators of soil biological functioning (Martin & Sprunger, 2022; Martin et al., 2022). Nematodes span trophic levels in the soil food web and are specialists in their feeding preferences (Ferris et al., 2001). Specifically, bacterivores feed on bacteria, fungivores feed on fungi, predatory nematodes feed on other nematodes, plant parasites feed on plant tissue, and omnivores preferentially feed based on resource availability (Bongers, 1990). The abundances of varying nematode feeding groups can indicate the decomposition channel (fungi vs. bacteria), plant health (plant parasitic abundances), and structure of the soil food web (abundances of predator and omnivorous nematodes) (Bongers, 1990). Nematodes also range in their sensitivity to disturbance because they span the *r*-*K* strategist continuum (Yeates, 2003). In nematology, this continuum is referred to as the colonizer-persister (cp) continuum (Yeates et al., 1993). Nematode *r* strategists, or colonizers (c), are resistant to disturbance and reproduce rapidly, whereas nematode *K* strategists, or persisters (p), are sensitive to disturbance and reproduce and slower rates (Yeates et al., 1993). Thus, the ratio of colonizer and persister nematodes can indicate the disturbance of the soil food web or referred to as the basal index (BI) (Ferris et al., 2001). Other indices have also been calculated utilizing the cp continuum and nematode feeding groups. The Channel Index (CI) indicates the decomposition channel (bacteria vs. fungal), the Enrichment Index (EI) indicates the nitrogen (N) input in a system, the Maturity Index (MI) indicates the soil food web

structure of a particular system, and the Plant Parasitic Index (PPI) indicates plant predation (Ferris et al., 2001; Bongers, 1990).

Through utilizing nematode indices and the respective feeding groups as bioindicators we can start to infer shifts in soil food web structure and function. However, free-living nematodes still lack integration into frameworks that measure soil function (Martin et al., 2022). Particularly, there is a substantial lack of incorporation of soil bioindicators, such as nematodes, within the soil health framework (Sprunger & Martin, 2023), which is defined as a suite of indicators that can be used to assess the ability of soil to support plant, animal, and human life (Lehmann et al., 2020). We know little regarding how free-living nematode communities shift alongside other physical and chemical indicators of soil health, such as C and nitrogen (N), which can aid in our understanding of how soil functioning shifts through changes in the soil food web.

Increased disturbance from agricultural production and extreme weather events has led for the need to rapidly detect and predict shifts in soil functioning (Raza et al., 2019). Many indicators of soil functioning are not sensitive enough or are slow to respond to disturbance (Fierer et al., 2009). While soil health indicators such as permanganate oxidizable carbon (POXC), mineralizable C, and autoclave-citrate extractable (ACE) protein have been developed to rapidly indicate shifts in the stable C, labile C, and organic N pools, respectively (Culman et al., 2013; Hurisso et al., 2018), we still need indicators that can rapidly detect shifts in the soil food web to system disturbance from agriculture and climate (Omer et al., 2023). Thus, the development and vetting of free-living nematodes as bioindicators of the soil food web is needed for sustaining soil health.

DISSERTATION OBJECTIVES

The first two chapters of this dissertation aim to use free-living nematodes as bioindicators of soil food webs to answer two ecosystem science level questions: How do long-term regenerative agricultural practices conserve soil biodiversity and ecosystem functioning? Do systems with greater plant diversity and perenniality foster resistant and resilient soil food webs to drought? The last chapter of this dissertation aims to answer a foundational nematode community ecology question: Is the impact of bacterivore nematodes on nitrogen (N) cycling species specific? Do bacterivore life-history strategies drive N use or is it bacterivore species diversity. To address these questions, I use methods from soil science, nematology,

biogeochemistry, and statistical modeling.

CHAPTER ORGANIZATION

Regenerative agriculture is defined as encompassing principals of reduced agricultural disturbance, incorporation of living roots year-round, and greater plant diversity and may preserve soil biodiversity (Giller et al., 2021). Currently, the effect of regenerative agricultural practices on free-living nematode communities has taken place in isolated surveys (Natalio et al., 2024). Thus, there is an essential need for long-term monitoring of soil biodiversity within single locations under varying regenerative agricultural practices (Natalio et al., 2024). In chapter 2, I gauge the effect of the long-term implementation of a suite of regenerative agricultural practices through comparing free-living nematode communities in 1991 and 2021 at the Kellogg Biological Station Long-term Ecological Research (KBS-LTER) site. Additionally, I quantify how shifts in soil biodiversity over 30-years within varying agricultural management practices co-relates to shifts in ecosystem functioning, through the assessment of labile and stable carbon (C) pools.

Climate change is causing extreme weather events, with variable weather patterns such as drought and flooding only expected to get more severe in the coming years (Ford et al., 2021). We are currently faced with implementing climate-smart agriculture that is resistant and resilient to extreme weather events (Tunio et al., 2024). Moreover, management practices that sustain ecosystem functioning such as nutrient cycling are critical for continued agriculture production (Power, 2010, p. 201). The Midwest region of the USA has started to experience more frequent and variable droughts during the growing season, however, there has been seldom research conducted on the impact of drought on ecosystem functioning within agricultural landscapes (NOAA, 2023). Bioindicators such as free-living nematode communities are sensitive and rapid indicators of disturbance (de Vries et al., 2012). In chapter 3, I assess the resistance of free-living nematode communities to the effects of variable and six-week drought period within an early successional and annual no-till monoculture system at the KBS-LTER. In addition, I evaluate the effect of rewetting after drought to understand the recovery of the nematode community within systems that contrast in plant diversity.

Free-living nematodes, in particular bacteria feeding nematodes (bacterivores), both directly and indirectly affect nitrogen (N) cycling (Ingham et al., 1985). Bacterivores directly affect N cycling through the excretion of plant available ammonium from consumption of their

prey (Ferris et al., 1997). Bacterivores indirectly affect N cycling given that the predation of bacteria keeps the microbial population in an active growth phase thus increasing decomposition, and thus N mineralization rates (Schratzberger et al., 2019). Both these direct and indirect effects can substantially enhance plant growth (Trap et al., 2016). Systems such as organic agriculture that do not receive inorganic N additions, are dependent on biological N cycling (Breza et al., 2023). It is currently unknown whether species of bacterivore nematodes that differ in life-history strategies have a varying effect on N cycling. Given that soil biodiversity is predicted to rapidly decline (Phillips et al., 2024), it is imperative to understand if certain species or life-history strategies of nematodes are vital for sustained N cycling. In chapter 4, I investigate how the presence an absence of dominant bacterivore nematodes with different life-history strategies impact soil N pools and plant N use. Additionally, I will assess how the interaction between bacterivore and bacteria alters soil N use efficiency based on bacterivore nematode life-history strategy.

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CHAPTER 2: LONG-TERM MAINTENANCE OF SOIL HEALTH PROMOTING PRACTICES ENHANCES NEMATODE COMMUNITIES AND DRIVES SOIL CARBON DYNAMICS IN AGROECOSYSTEMS

ABSTRACT

There is an expectation that soil health promoting practices will enhance soil food web structure and increase soil carbon (C), yet this has rarely been tested over long-term periods. Here, I seek to understand how nematode communities and soil C indicators shift over a 30-year period across a range of agroecosystems within the W.K. Kellogg Biological Station Long-Term Ecological Research Site located in Michigan, USA. The study examines eight systems along a management intensity gradient with varying soil health practices. These include four annual row-crop rotations (corn-soybean-wheat) differing in tillage, fertilizer use, and cover crops; two perennial monocultures (Poplar, Switchgrass); and two unmanaged polycultures (early successional community, mown grassland). Soils were sampled in 1991 and 2021, and nematode communities were extracted and identified using identical techniques for each year. Soil health indicators of permanganate oxidizable carbon (POXC) and mineralizable carbon (MinC) were measured for each year and system. After 30 years, nematode communities shifted from bacterivore and plant parasitic dominance to fungivore dominance, in unmanaged successional systems. Both POXC and MinC values were significantly greater after 30 years, but only in the mown grassland systems. By 2021, MinC was significantly correlated with nematode communities in early successional and mown grasslands and POXC, was significantly correlated with nematode communities in early successional and poplar systems. Together, this decadal study demonstrates that the long-term maintenance of soil health promoting practices can alter soil food web structure and increase soil C indicators in agroecosystems.

INTRODUCTION

Agricultural intensification has led to widespread soil degradation (Squire et al., 2015) and is a primary driver of plant and animal biodiversity around the world (Emmerson et al., 2016). This is especially detrimental for complex soil food webs that support key ecosystem services including nutrient cycling, plant growth, and decomposition (Nielsen et al., 2015). Prior research has indicated that after two-years, agricultural intensification can reduce nematode, earthworm, and microarthropod abundances (Postma-Blaauw et al., 2010). That said, we have little understanding of how soil food web structure trends might shift under the implementation of long-term management practices that reduce agricultural intensification and incorporate soil

health promoting practices.

Soil health is defined as the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans (*Soil Health | Home*, 2025). Soil health is expected to increase soil carbon (C) storage, enhance soil biodiversity, and support numerous other ecosystem services. Soil health promoting practices are those that follow key principles of soil health which include a) reducing soil disturbance, b) maximizing plant biodiversity, c) extending year-round ground cover, and d) increasing the presence of living roots throughout the year (Doran & Parkin, 1994; Sela et al., 2016). In recent years, numerous studies have worked to assess how various soil health promoting practices impact key ecosystem services (McDaniel & Middleton, 2024; Romero et al., 2024; Sprunger et al., 2020). For instance, agroecosystems with greater diversity and perenniality were found to increase various indicators of soil C (Sprunger et al., 2020). Less is known regarding how the long-term (20+ years) maintenance of soil health promoting practices influences belowground soil biodiversity. Thus, there is still limited understanding on whether improved soil health can reverse the decline of soil biodiversity and ecosystem functioning over long periods of time.

Monitoring belowground soil food web structure and ecosystem functioning has remained a consistent challenge, however the identification of free-living nematode communities may serve as a viable bioindicator for measuring shifts in soil food web structure (Delgado-Baquerizo et al., 2020; Sprunger & Martin, 2023). Nematodes are specialists and can be sorted into various feeding groups; bacterivores feed on bacteria, fungivores feed solely on fungi, herbivores feed on plant roots, and predators and omnivores feed on other nematodes and microorganisms (Bongers, 1990). Moreover, nematodes span the *r*-*K* strategist life-history strategy continuum, where losses in *K*-strategist nematodes (predator/omnivores) can indicate a loss of structure within the soil food web (Cesarz et al., 2017). Nematodes are extremely sensitive to changes in the soil environment and can serve as rapid indicators of soil health and ecological functioning (Delgado-Baquerizo et al., 2017; Martin & Sprunger, 2022b; Wall et al., 2008). Ratios of nematode feeding groups can also be used to calculate indices that represent the state of the soil food web and overall ecosystem functioning (Ferris et al., 2001). For example, based on the ratios of fungivore to bacterivore nematodes we can infer the dominant decomposition pathway within a given system (Porazinska et al., 1999). Given that free-living nematodes span multiple trophic levels and impact major ecosystem processes such as nitrogen

(N) cycling, it is plausible that these micro-fauna may be able to indicate shifts in belowground biodiversity and functioning (Gebremikael et al., 2016).

While nematode communities are frequently linked to key N dynamics, less is known regarding how trophic level interactions influence soil C cycling, which could have important implications for soil C accrual. While nematode respiration and biomass contribute to C cycling (Ferris, 2010), we have a poor understanding of how nematode community composition is related to various indicators of soil C. The development of soil health indicators that reflect forms of processed and labile C has made it possible to understand the underlying mechanisms associated with soil C accrual and their relation to soil faunal metrics (Martin & Sprunger, 2022a). Mineralizable carbon (MinC) indicates a labile or active C pool which is strongly associated with nutrient mineralization (Adhikari et al., 2023).

Whereas permanganate oxidizable carbon (POXC) reflects more processed pools of C and has been shown to be an early indicator of C stabilization (Hurisso et al., 2016; Sprunger et al., 2020). Both MinC and POXC have become widely used within an agricultural context, due to the low cost and rapid measurement of these C pools (Culman et al., 2013). Nonetheless, we have little understanding of how nematode communities shift over time in relationship to these indicators. Understanding how nematode communities are related to soil C accrual will strengthen our understanding of how soil food webs influence important ecosystem processes related to climate change mitigation via C sequestration.

This study seeks to explore how the long-term maintenance of soil health promoting practices influences nematode community composition and soil C indicators. My objectives are to 1) assess how nematode community structure shifts over a 30-year period across agroecosystems with minimal to full integration of soil health practices, 2) explore how the relationship between nematode community structure and soil C indicators have shifted over time in contrasting agroecosystems. I hypothesize that systems with greater soil health promoting practices will 1) drive nematode communities to have enhanced structure through greater abundances of predator and fungivore nematodes relative to conventional-based agriculture; 2) have greater processed pools of C after 30 years of reduced management intensity. Lastly, I hypothesize that regardless of time point, less structured nematode communities will be more associated with mineralization processes while more structured nematode communities will be associated with C stabilization processes.

MATERIALS AND METHODS

Site Description

This study took place at the W.K. Kellogg Biological Station's Long-term Ecological Research Site (KBS-LTER) located at 42° 24'N, 85° 23'W in Southwest Michigan, USA. The soil type is a Kalamazoo series, Typic Hapludalfs, fine loamy, mixed and mesic. The average temperature during sampling in 2021 was 14.3° C, whereas the average temperature in 1991 was 9.2 ° C. Cumulative precipitation and soil moisture were monitored in both 1991 and 2021, indicating that 2021 was a considerably drier year due to reduced precipitation (Fig. S2.1 and Supplementary Table S2.1). The KBS-LTER was established in 1987. Prior to establishment, the agricultural land was conventionally managed. The KBS LTER is a completely randomized design with six replicates for all treatments, except the mown-grassland systems which has four replicates. The KBS-LTER is comprised of eight different systems that make up a management intensity and consist of varying levels of soil health management (Table 2.1). The systems consist of four annual row cropped systems: conventional (chisel plow, fertilizer inputs), no-till (same fertilizer inputs and rates as conventional), reduced input (chisel plow, reduced fertilizer, and cover crops), and biologically based (chisel plow, zero fertilizer inputs, and cover crops); two perennial monocultures: poplar (*Populus nigra x P.maximowiczii*) and switchgrass (*Panicum virgatum*); and two successional reference systems: an early succession and mown-grassland (mid-succession).

The conventional system is a corn-soybean-winter wheat rotation and is spring chisel plowed with a second pass for preparing the seed beds. Winter wheat is planted in the fall and only requires one secondary tillage. Herbicides and pesticides are applied as prescribed by Michigan integrated pest management. In 2021, nitrogen, phosphorus, potassium, and agricultural lime were applied at 228 kg ha-1 of 28% UAN, 135 kg ha-1 of 0-46-0 phosphorus, 168 kg ha-1 of 0-0-60 potassium, and 4 kg ha-1 of ammonium sulfate, respectively. The no-till system is managed like that of the conventional system but has not been tilled since establishment. A greater amount of herbicides are also applied to control for weeds. The reduced input system has a 33% reduction of nitrogen applied relative to the conventional system. Additionally, the reduced input system is planted with winter cover crops, which consists of a ryegrass *(Lolium multiflorum)* following corn and red clover (*Trifolium pratense)* following winter wheat. The biologically based systems receive no external nitrogen inputs and is entirely

dependent on the nitrogen added via cover crops, which are the same cover crops used within the reduced input system. The weeds in the biologically based system are controlled by rotary hoeing as there are no additional inputs from herbicides or pesticides.

The poplar system is harvested every ten years and weeds are controlled with herbicides. Nitrogen fertilizer is added, when necessary, based on annual soil test results. [1] The switchgrass system is on a 5-year rotation with a 1-year break crop. From 1989-2019, the system was planted with alfalfa. The early successional community reflects the natural process that occurs once a system is abandoned from row-crop agriculture. This system is left unmanaged except for a burn in the spring to control for woody species. The early successional systems are comprised of 20 different species of perennial forb, graminoid, and shrubs and are dominated by *Solidago canadensis, Poaceae* spp.*,* and *Hieracium* spp (Young et al., 2024.). The never-tilled mown-grassland was naturally established after it was abandoned from a 10-ha woodlot in 1960, this system is mowed to inhibit tree colonization. Additional details on management at the KBS-LTER can be found in Table 2.1 (Robertson & Hamilton, 2015).

Sample Collection

In 1991, soil samples were collected prior to tilling and planting during the soybean phase of the rotation. Specifically, six soil cores were taken at random sampling stations within each system and replicate at a 10 cm depth using a soil corer (2.54 cm diameter). Soil cores were homogenized and stored at 4 °C for further processing. In 2021, soil collection was carried out to the exact similarity as the sampling in 1991. To do so, soil samples were collected prior to tilling and during a soybean phase of the rotation in May 2021 at the KBS-LTER (Freckman & Ettema, 1993). This sampling time is exactly replicated to control for the seasonal variation of nematode communities that occurs within a growing season. To sample, three 2.54 cm soil cores were taken from each of the five sampling stations within each system at a 10 cm depth. All fifteen soil cores were taken from each replicate and system to make one core composite sample. Gravimetric soil moisture was assessed immediately after sampling (Table S2.1). Soil samples were sieved to 2mm and stored at 4° C for further processing.

[1] Although the poplar system is meant to be a perennial monoculture, this system is often considered a polyculture perennial system due to its thick and diverse understory (Sprunger & Philip Robertson, 2018)

Nematode Extraction and Identification

Nematodes were extracted from the soil (50 g) using the Baermann funnel extraction technique for 72 h (Flegg & Hooper, 1970) in both 1991 and 2021. Nematodes were then collected and fixed in a 4 % paraformaldehyde solution. Nematodes were counted using a dissecting scope. Afterwards, 100 nematodes were identified to genus at 40-100 x magnification (Bongers, 1990). I obtained the raw 1991 data (nematode taxa, relative abundance, and soil moisture) through correspondence with Dr. Diana Wall.

Soil C Metrics

I selected soil C metrics that could be carried out on air dried soils, since archived soil samples were a critical part of this study. Soil samples from the 1991 KBS-LTER soil archive were collected and ground to 2 mm for MinC and POXC analyses, which are indicators of labile and more processed C, respectively (Hurisso et al., 2016). KBS-LTER archived soils were collected during July of 1991. Samples collected from 2021 were dried at 65° C and ground to 2mm. Mineralizable C was measured using protocols adapted from (Franzluebbers et al., 2000; Hurisso et al., 2016). Briefly, soil (10 g) was weighed and placed into a falcon tube, rewetted to 50% water-holding capacity with deionized water, and incubated at 25° C for 24 h. Then, 1 mL of headspace air was extracted and injected into a LI-820 infrared gas analyzer (LI-COR, Biosciences, Lincoln, NE) to determine the concentration of carbon dioxide (CO_2). The POXC analysis was adapted from (Culman et al., 2012; Weil et al., 2003). Briefly, 20 mL of a 0.02 M potassium permanganate ($KMnO_4$) solution was added to 2.5 g of soil, the mixture was shaken for 2 min and afterwards settled vertically for 10 min. The supernatant was diluted to a 99:1 deionized water to supernatant ratio. Finally, the sample absorbance was read on a spectrophotometer at 550 nm. Although POXC is a widely used metric that has shown correlations with processed pools of C, a recent paper has found uncertainties with the oxidation-reduction reaction, which could lead to overestimations of soil C (Margenot et al., 2024). That said, POXC continues to be a useful indicator of soil C trajectories, especially when compared across different systems (Sprunger et al., 2020).

Calculations and Statistical Analyses

Nematode indices can be used to indicate soil food web function and are calculated through utilizing the ratios of nematode feeding groups. The maturity index (MI) is used to indicate soil food web structure, the channel index (CI) indicates the dominant decomposition

channel, and the enrichment index (EI) can reflect the level of N enrichment within a system. [2]Nematode indices were calculated according to (Ferris et al., 2001) using the Nematode Indicator Joint Analysis (NINJA) platform (Sieriebriennikov et al., 2014). I used a linear regression model to assess which systems are dominated by mineralization (MinC) or stabilization (POXC) processes, where MinC is the predictor variable and POXC is the response variable, and then extracted the residuals (Hurisso et al., 2016). Analysis of variance was used to assess the effect of timepoint and system on nematode community feeding groups, indices, and soil C metrics. System, year, and the interaction between system and year were treated as fixed factors, while replicate was treated as a random factor. The *lme* package in R was used to assess the effect of fixed factors on nematode communities and soil C metrics (Bates et al., 2015; R Core Team, 2021). Normality was assessed using studentized residuals with *Mass* in R (Venables & Ripley, 2002). Unequal variance was assessed using Levene's test. Paired t-tests were utilized to compare between year within each treatment for each independent variable. The Bonferroni adjustment was utilized to control for family-wise error rate. Sample number (n) for each dependent variable was recorded. To assess if nematode communities were significantly affected by timepoint or system permutational analysis of variance (PERMANOVA) was conducted with parameters for Bray Curtis distance using 100 permutations. Additionally, to understand how nematode communities differed between timepoint and system I conducted a non-metric dimensional scaling analysis (NMDS) with Bray-Curtis distance measures using the metaMDS function in R with the *vegan* package (Oksanen et al., 2022). Two NMDS's were performed one for 1991 data that consisted of 48 taxa and 2021 which utilized 53 taxa. I utilized two separate NMDS's to accurately assess differences of nematode community structure between the treatments within each year. An outlier fraction test was conducted on both 1991 and 2021 community datasets using the *ICIKendallTau* and *visualizationQualityControl* packages, where outliers were identified based on a fraction differences and investigation of the raw data (Flight & Moseley 2024). Default parameters were used, and the final stress values were 0.20 for 1991 and 0.23 for 2021. Correlations between the nematode communities and soil C metrics were analyzed using a vector analysis with 1000 permutations. Vector analyses were

[2] I chose to represent soil food web structure through the MI, as the MI is a more sensitive and comprehensive indicator of soil food web structure as compared to the structure index (SI) ((Du Preez et al., 2022; Martin & Sprunger, 2022a).

performed using the scores function in the *vegan* package in R.

RESULTS

Nematode community structure

Comparing nematode feeding group abundance between 1991 and 2021 allowed for an assessment of nematode community structure over a 30-year period. This analysis revealed substantial shifts across each feeding group, except for predators and omnivores (Table S2.3). However, the diversity of predator and omnivore genera identified appeared to decline in all systems after 30 years (Table 2.2). For example, between 1991 and 2021, bacterivore nematode abundances on average declined by 50% in biologically based and early successional systems (Fig. 2.1). Additionally, bacterivore genera *Cervidellus* and *Diplogasteridae* were not found in any of the systems in 2021 when compared to 1991. This result indicates a substantial shift in decomposition patterns especially within these two systems over the 30-year period. Moreover, this speculation is further supported by an increase in the relative abundances of fungivores, in unmanaged successional systems and systems managed with cover crop rotations (reduced input, biologically based, poplar, early successional, and mown grassland). Most notable, fungivores significantly increased by 66% within the early successional systems between 1991 and 2021 (Fig. 2.1). Abundances of the fungivore nematode *Tylencholaimellus* dominated the fungivore population in successional systems by 2021 (Table 2.2). Another prominent trend in nematode feeding group abundances was the 59% decrease in plant parasitic abundances between 1991 and 2021 across all systems. Moreover, the decreases in plant parasitic abundances were most pronounced in poplar, switchgrass, and early successional systems (Fig. 2.1). Additionally, plant parasitic taxa of *Dityelnchus* and *Tylenchorynchus* were not recovered again in 2021, however, *Helicotylenchus* and *Miculenchus* were found in all systems in 2021 but not 1991 (Table 2.2).

Nematode indices

The assessment of nematode indices can aid in understanding how soil food web complexity and functioning have shifted over the course of 30 years. The maturity index (MI), a measure of soil food web structure and energy flow to higher trophic levels, increased by 15% over the 30-year period across all systems, except for the no-till system, where the MI decreased (Table 2.3). The early successional system increased the channel index (CI) threefold over the 30-year period (Table 2.3). This result indicates that the early successional system was dominated by fungivores rather than bacterivores after a 30-year period.

Mineralizable Carbon and Permanganate Oxidizable Carbon

I measured soil C metrics in 1991 and 2021, to quantify how labile and more processed pools of C shift over time. Quantifying MinC across all systems in 1991 and 2021 revealed that poplar, early successional, and mown grassland systems were found to, on average, have 4 times greater MinC after 30 years of management. Whereas MinC decreased by 70% in conventional systems over the 30-year period (Fig. 2.2). These results clearly reflect the ability of systems with reduced management intensity to increase labile C pools and that long-term conventional management leads to overall declines in labile C pools. I also measured POXC in 1991 and 2021, to gauge the effect of management on more processed C pools. My results indicate that poplar, early successional, and mown grassland systems had 18% greater POXC when compared to all other systems in 2021. Poplar and mown grassland systems had the highest increases of POXC values, with poplar increasing by 22% and mown grasslands increasing by 23% over 30 years (Fig. 2.2). I also used residuals from a linear regression model between MinC and POXC to assess which systems are dominated by mineralization (MinC) or stabilization (POXC) processes (Hurisso et al., 2016). Given that positive residuals indicate greater than predicted POXC values and negative residuals indicate greater than predicted MinC values, we can interpret positive residuals as trending toward stabilization processes and negative values as trending towards mineralization processes. In 1991, reduced input, no-till, and biologically based systems were trending towards mineralization processes, while all other systems were trending towards stabilization (Table 2.4). However, after 30 years, trends indicate that the conventional system was dominated by mineralization, and no-till, poplar, switchgrass, early successional, and mown grassland systems were dominated by stabilization mechanisms (Table 2.4). These findings are consistent with soil C trajectories in these systems, where the conventional system is likely losing C, while the other systems are either maintaining or increasing soil C stocks (Martin & Sprunger, 2022a; Syswerda et al., 2011).

NMDS and Vector analyses

I conducted a NMDS analysis to understand shifts in nematode community composition between systems over a 30-year period (Figure 2.3). Results from the PERMANOVA indicate that factors of system had a significant effect on nematode community structure in 1991 and a marginal significant effect in 2021 (Table 2.S2). NMDS results showed that in 1991, nematode communities within reduced input and biologically based were similar to each other and

communities in no-till and conventional systems were similar, given the clustering of systems. However, in 2021, systems became more differentiated, whereby the NMDS axis 1 represents a system effect with reduced input, poplar, early successional, and mown grassland systems clustered together, and no-till, conventional, and switchgrass systems dispersed from this cluster on NMDS axis 1 (Fig. 2.3). Then, I overlayed a vector analysis to correlate the relationship between soil C pools and nematode community structure over time. The vector analyses indicate that in 1991 and 2021 POXC and MinC were significantly related to nematode community composition (Table S2.5). In 1991, MinC had a significant and strong relationships with communities in reduced input and biologically based systems whereas POXC had strong and significant relationships with communities in early successional and poplar systems (Fig. 2.3). In 2021, MinC was significantly correlated with nematode communities in early successional and mown grasslands and POXC was significantly correlated with nematode communities in early successional and poplar systems (Fig. 2.3). These results indicate that varying management practices can alter nematode community composition and their relationship with indicators of soil C over time.

DISCUSSION

My findings demonstrate that implementing varying levels of soil health management can enhance nematode community structure and function relative to conventional agriculture. This has important implications for soil biodiversity because nematodes play a vital role in sustaining soil food webs and numerous ecosystem functions, including soil C processes (Martin & Sprunger, 2021). Contrary to my hypothesis, not all systems that integrate soil health promoting practices have similar effects on nematode community structure and function. For example, the implementation of solely no-till practices had no significant differences on soil food web structure and soil C when compared to conventional systems. However, when annual agricultural systems integrate cover crops and reduce external inputs (reduced input and biologically based systems), the MI substantially increased. Thus, diversified crop rotations through cover crop implementation appeared to be a key driver of sustained food web functioning within annual systems. These results are not surprising given that both the reduced input and biologically based systems have little to no synthetic fertilizer input and have depended mostly on organic matter inputs over the past 30 years (Naasko et al., 2024; Robertson & Hamilton, 2015).

My results indicate that unmanaged successional and poplar systems are the most effective at enhancing nematode community structure and function. For instance, the substantial increase in fungal dominance after 30 years in poplar and early successional systems may reflect a shift in the soil food web decomposition pathway, in which systems dominated by fungivores are often associated with increased C and N cycling and enhance food web structure (Ferris & Matute, 2003; Kane et al., 2023; Porazinska et al., 1999). The MI, which is indicative of the overall complexity of the soil food web, was greater in early successional systems during both timepoints of this study. The greater MI in these systems may be caused by an increase of fungivore *K*-strategists nematode abundances (Dietrich et al., 2021; Ikoyi et al., 2023). However, contrary to my hypothesis, predator and omnivore nematode abundance did not change over 30 years. This suggests that *r*-strategist nematodes that reflect the predominant decomposition pathway, are more responsive to shifts in management relative to predator and omnivore nematodes. The decline in plant parasitic nematode abundances in all systems over 30 years may be caused by climatic or seasonal differences. Specifically, precipitation and soil moisture were significantly lower in 2021 when compared to 1991, where reduced soil moisture may cause declines in abundances of plant parasitic nematodes. Another reason for the substantial decrease in plant parasitic nematodes could be longevity a diversified corn-soybean-wheat rotation. The use of crop rotations has been shown to disrupt plant parasitic nematode life cycles through the introduction of non-host specific plants (Afzal & Mukhtar, 2024). Prior to 1989, the land that comprised all of the KBS-LTER was managed as continuous corn.

My findings also elucidate the impact of long-term soil health management on indicators of soil C. Synonymous with my hypothesis, I found that after a 30-year period, unmanaged successional systems and monoculture perennial systems had on average, the greatest MinC and POXC values. Residuals also indicate that perennial based systems were more closely associated with stabilization processes than annual systems, which were dominated by mineralization processes. The enhanced stabilization mechanisms in unmanaged successional and perennial systems suggests that certain biological processes may have been altered within these systems after 30 years (Córdova et al., 2025; van der Heijden et al., 2016). These findings are consistent with soil C trajectories in these same systems, where the conventional system is likely losing carbon, while the other systems are either maintaining or increasing soil C pools (Syswerda et al., 2011). My results further support this notion given that nematode communities in poplar,

early successional, and mown grassland systems had a positive relationship with indicators of more processed C pools. Contrary to my hypotheses, sustainably managed annual systems did not lead to greater C accrual; demonstrating the importance of management that utilizes perenniality to achieve greater ecosystem functioning.

Perennial crops contribute large belowground organic matter inputs and bolster soil aggregate formation due to extensive root systems (Cates et al., 2016; Nunes et al., 2020), however my study shows that the combination of plant diversity and perenniality is the most powerful management for enhancing soil biodiversity and ecosystem function. Given that the early successional systems is comprised of 20 different perennial shrub graminoids, and forb species within one growing season, we can argue that this system represents both perennilaity and diversity (Young et al., 2024.). I found that monoculture perennials, such as switchgrass, led to bacterivore dominated food webs and had on average lower levels of MinC when compared to the early successional system, thus indicating that the combination of plant diversity and perenniality is essential for enhanced labile C pools and fungal dominated (slowed) decomposition pathways. Increased plant diversity within perennial systems is often a driver of ecosystem functions as greater plant diversity has been found to 1) enhance the resiliency of systems due to functional redundancy and species complementarity (Fornara & Tilman, 2008; Hooper & Vitousek, 1997; Sprunger et al., 2017) and 2) provide a diversity of belowground inputs, both of which are essential for maintaining soil biodiversity and soil C (Chen et al., 2019; Picasso et al., 2011; Weise et al., 2020).

This study shows that increasing year-round ground cover, and increasing the diversity of residue return can reverse the decline of soil food web structure (Bender et al., 2016). In previous literature, these results were not as apparent given that the longevity of most regenerative agriculture and soil health studies are 3-5 years (Kladivko, 2001; Sprunger et al., 2019). My findings demonstrate that the long-term maintenance of soil health promoting practices is critical for nematode communities to mature and contribute to more structured soil food webs (Bokhorst et al., 2017; Dietrich et al., 2021).

One limitation of this study is that I only captured nematode communities at two timepoints over the span of a 30-year period. Free-living nematode communities shift temporally, where I have found that in 2021 the distribution of nematode feeding groups within early successional systems shifted in their distribution from fungivore dominated in March to

plant-parasitic and fungivore dominated in June and an inverse effect was indicated in no-till systems (Supplementary Table 1.S7). Thus, I recognize that the broad shifts in nematode feeding groups across the 30-year period should be interpreted with caution. However, the sampling timepoints within each year during 1991 and 2021 took place during the same time of the growing season and during the same crop rotation to mitigate temporal variations of the free-living nematode community. Moreover, long-term shifts in soil C dynamics have been well-documented in the KBS LTER (Córdova et al., 2025; Syswerda et al., 2011). My soil C metrics follow similar trends, whereby the conventional system appears to be losing C and most soil health promoting practices appear to be gaining C, even if not statistically significant. Taken together, the long-term maintenance of soil health promoting practices has a positive impact on ecosystem processing within agroecosystems.

Despite the limitations our study has critical implications for policy. For instance, perennial grasses and polyculture systems have an important role globally for both climate change mitigation, adaptation, and biodiversity (Milazzo et al., 2023). For this reason, various programs have been implemented to either require or encourage farmers to maintain grasslands. The Conservation Reserve Program (CRP) in the U.S. encourages farmers to convert row-crop agriculture to systems with year-round ground cover and enhanced plant diversity. Farmers can incorporate native grasses, riparian buffers, restored prairies, and numerous other types of perennial vegetative cover and are incentivized through a variety of payments. The CRP program has long been associated with enhanced ecosystem services and improved soil health in the U.S.(Staben et al., 1997).

CONCLUSION

Past research has worked to understand how soil health promoting practices influence soil biodiversity and ecosystem processes. My study demonstrates that when varying levels of soil health management are implemented and maintained for decades, positive shifts occur for both soil biodiversity and soil food web functioning. Moreover, these positive shifts appear to have significant impacts on soil C dynamics. These findings highlight the need for the implementation of regenerative agriculture to maintain soil biodiversity and enhance soil C sequestration.

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APPENDIX A: CHAPTER TWO TABLES AND FIGURES

Table 2.1. System and crop classifications for each treatment in the KBS-LTER based on the management specifications.

Treatment	System	Crop	Principle of Soil Health	Management Specifications
Conventional	High Input	Annual	• Not applicable	• Corn-soybean-winter wheat rotation • Spring chisel plowed with a second pass for preparing the seed beds • Herbicides and pesticides applied • Receives nitrogen, phosphors, potassium, and lime
No-till	High Input	Annual	• Reduced soil disturbance	• Corn-soybean-winter wheat rotation • Not tilled since establishment • Herbicides and pesticides applied • Receives nitrogen, phosphors, potassium, and lime
Reduced Input	Organic	Annual	• Maximizing plant diversity	• Corn-ryegrass *(Lolium multiflorum*) -soy-winter wheat-red clover *Trifolium pratense)* rotation • 33% reduction of nitrogen applied relative to the conventional system

Table 2.1 (cont'd)

Biologically Based	Organic	Annual	• Maximizing plant diversity	• Corn-ryegrass *(Lolium multiflorum*) -soy-winter wheat-red clover *Trifolium pratense)* rotation • No external nitrogen inputs
Poplar	Perennial	Perennial	• Reduced soil disturbance • Living roots • Year-round ground cover • Maximizing plant diversity	• Harvested every ten years • Weeds are controlled with herbicides. • Vigorous understory making this system a polyculture
Switchgrass	Perennial	Perennial	• Living Roots • Year-Round Ground Cover	• 5-year rotation with a 1-year break crop. • From 1989-2019, the system was planted with alfalfa.
Early Successional	Successional	Perennial	• Reduced soil disturbance • Living Roots • Year-round ground cover • Maximizing plant diversity	• 20 different species of perennial forb, graminoid, and shrubs and are dominated by *Solidago candensis, Poaceae* spp., and *Hieracium* spp • Burned every spring to control for woody species
Mown Grassland	Successional	Perennial	• Reduced soil disturbance • Living Roots • Year-round ground cover	• Naturally established after it was abandoned from a 10-ha woodlot in 1960

Table 2.1 (cont'd)

	• Maximizing plant diversity	• Mowed to inhibit tree colonization.

Table 2.2. Means and (standard error) of nematode genus and family abundances identified in 1991 and 2021 among all eight treatments. Standard errors depict one standard deviation from the mean.

	Conventional		No-till		Reduced Input		Biologically Based		Poplar		Switchgrass		Early Successional		Mown Grassland	
	1991	2021	1991	2021	1991	2021	1991	2021	1991	2021	1991	2021	1991	2021	1991	2021
								Plant Parasitic								
Criconematidae	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0.1(0.1)	0.3(0.3)
Helicotylenchus	0(0)	0.7(0.7)	0(0)	1.6(1.6)	0(0)	1.2(0.7)	0(0)	1.4(0.5)	0(0)	1(1)	0(0)	0.2(0.2)	0(0)	1.3(0.7)	1.5(1.2)	0.7(0.7)
Paratylenchus	1.3(0.6)	0.9(0.9)	0.3(0.2)	0(0)	1.2(1)	0(0)	0.3(0.2)	0(0)	1.9(0.9)	2.1(1.6)	6.5(3.7)	2.2(1.3)	1.3(0.6)	0.4(0.4)	39(1.7)	0.34 (0.3)
Pratylenchus	15(3.1)	2.2(2.2)	19.9(3.3)	9.7(3.3)	9.8(1.0)	3.9(1.6)	9(1.8)	4.1(1.6)	4(1.3)	2.1(1.5)	3.1(0.7)	1.9(0.8)	8.9(1.8)	2.8(0.7)	4.4(1.4)	6.06 (1.5)
Psilenchus	0(0)	0(0)	0(0)	1.61 (1.61)	0(0)	0(0)	0.1(0.1)	0.2(0.2)	0.3(0.1)	0.3(0.2)	0(0)	0(0)	0.17(0.2)	0(0)	0.13 (0.13)	0.34 (0.34)
Tylenchidae	11(2.5)	6.7(39)	10.9(2.3)	9.6(6.6)	11.2(26)	2.9(1.4)	11.7(2.2)	5.6(2.4)	20(2.1)	1.8(0.7)	23.5 (6.4)	3.7(1.6)	19.7(4.0)	7.9(3.4)	10.1 (0.9)	3.4(0.3)

Table 2.2 (cont'd)

Tylenchorynchus	0(0)	0(0)	0.2(0.2)	0(0)	0(0)	0(0)	0(0)	0(0)	0.1(0.1)	0(0)	0(0)	0(0)	0(0)	0(0)	0.5(0)	0(0)
Xiphinema	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0.3(0.2)	0(0)	0(0)	0(0)	0.2(0.2)	0.4(0.4)	0.2(0.2)	0(0)
Bastianidae	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0.2(0.1)	0(0)
Ditylenchus	0(0)	2.6(2.6)	0(0)	0(0)	0(0)	1.2(0.8)	0(0)	0.4(0.3)	0(0)	0.8(0.7)	0(0)	2.1(1.2)	0(0)	7.1(5.7)	0(0)	0.6(0.3)
Miculenchus	0(0)	1.5(1.5)	0(0)	0(0)	0(0)	0(0)	0(0)	0.2(0.2)	0(0)	0.2(0.2)	0(0)	0(0)	0(0)	0.5(0.3)	0(0)	0(0)
								Bacterivore								
Acrobeles	0.3(0.3)	4.9(2.5)	0(0)	0(0)	0.2(0.1)	10.6(3.1)	0(0)	1.6(1.2)	0.1(0.1)	11.2(5.3)	0(0)	5.8(2.7)	0(0)	2.5(1.6)	0.2(0.1)	8.7(8.7)
Acrobeloides	12.6(2.5)	2.2(2.2)	11.5(1.2)	9.5(2.9)	12.8(1.7)	3.6(3.3)	11.5(1.7)	5.9(2.5)	14.6(1.2)	0.7(0.4)	11(1.1)	3.6(1.8)	7.1(1.3)	1.2(0.7)	11.9(2.9)	5.4(4.4)
Alaimus	0.1(0.1)	0(0)	0.2(0.2)	0.5(0.5)	0(0)	0.2(0.2)	0.1(0.1)	0.2(0.2)	0.1(0.1)	0.5(0.5)	0.1(0.1)	0(0)	0.1(0.1)	0.2(0.2)	0.1(0.1)	0.7(0.7)
Cervidellus	0(0)	0(0)	0.4(0.3)	0(0)	0.4(0.3)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0.7(0.3)	0(0)

Table 2.2 (cont'd)

Chiloplacus	0.4(0.3)	0.4(0.4)	0.7(0.6)	0(0)	0.2(0.2)	1.2(1.2)	0.4(0.2)	0.2(0.2)	0.6(0.5)	0(0)	1.4(0.9)	0.9(0.5)	2.5(0.4)	0(0)	0.1(0.1)	2.7(2.7)
Diplogast.	5.7(2.8)	0(0)	3.6(1.6)	0(0)	0.6(0.5)	0(0)	0.75(0.3)	0(0)	0.9(0.3)	0(0)	0.67(0.2)	0(0)	1.1(0.3)	0(0)	0.2(0.1)	0(0)
Eucephalobus	2.3(0.4)	0.7(0.7)	2.2(0.5)	7(7)	1.4(0.4)	1.0(0.7)	2.4(0.6)	1.5(0.4)	2.5(0.4)	1.4(0.8)	3.9(0.7)	0.3(0.3)	6.4(0.8)	2.8(1.2)	2.1(0.7)	3.4(2.4)
Monhysteridae	1.3(0.2)	0(0)	3.9(0.7)	0.5(0.5)	1.2(0.4)	0.9(0.7)	2(0.4)	0(0)	4.0(1.6)	0.2(0.2)	2.9(0.4)	0(0)	1.7(0.7)	0.4(0.4)	1.9(0.4)	0.3(0.3)
Panagrolaimellus	0.6(0.5)	0.9(0.9)	0.3(0.)	4(4)	6.2(2.3)	2.3(2.3)	8(4.4)	0.9(0.4)	0.6(0.4)	0.8(0.6)	2.1(1.5)	2(1.21)	1.8(0.6)	0.2(0.2)	1.2(0.3)	0(0)
Plectus	1.7(0.4)	2.1(0.6)	1.8(0.5)	3.6(0.4)	1.9(0.6)	2.0(0.6)	2.1(0.7)	1.8(1.1)	1.2(0.5)	1.7(0.7)	2.6(0.7)	2.1(1.0)	1.3(0.6)	2.7(1.5)	8.2(1.0)	1.3(0.7)
Prismatolaim	0(0)	0(0)	0.1(0.1)	0(0)	0.2(0.11)	0.3(0.3)	0.1(0.1)	0(0)	1.6(0.4)	0.2(0.2)	0.4(0.1)	0(0)	0.2(0.10)	0(0)	3.7(1.0)	0(0)
Rhabditidae	16.8(3.1)	30.6(13.4)	14.6(1.9)	7.6(4.4)	23.3(3.8)	11.5(2.1)	25(3.1)	19.3(10.4)	17.5(2.2)	10(5.3)	14.2(2.8)	20.7(7.3)	12.7(2.1)	2.9(0.6)	10.6(0.9)	5.4(0.9)
Wilsonema	0(0)	0(0)	0.1(0.1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0.2(0.2)	0(0)	0(0)	0(0)	0.2(0.2)	0.38(0.24)	0(0)

Table 2.2 (cont'd)

Hetercephalobus	0(0)	1.3(1.3)	0(0)	0(0)	0(0)	11.7(6.1)	0(0)	0.6(0.4)	0(0)	5.0(3.0)	0(0)	4.5(3.3)	0(0)	0(0)	0(0)	1.7(1.21)
							Fungivore									
Aphelenchoides	9.4(0.6)	13.4(5.5)	6.2(0.8)	19.7(0.)	4.2(1.2)	27.4(5.4)	3.8(0.6)	31.2(9)	8.6(1.4)	35.9(6)	3.4(1.4)	19.6(5.5)	6.9(1.1)	37.9(8.0)	23.7(2.4)	40.1(8.5)
Aphelenchus	16(1.6)	5.5(4.7)	16.9(1.8)	3.5(3.5)	18.3(1.2)	9.1(3.5)	17.1(1.6)	14.6(4)	13.5(2.4)	16.0(3.7)	13.9(2.2)	11.7(3.8)	16.3(2)	18.3(9.5)	4.7(0.6)	5.0(3.6)
Diphterophora	0.4(0.4)	0(0)	0.7(0.4)	1.6(1.6)	0.3(0.2)	0(0)	0.3(0.1)	0(0)	0.5(0.5)	0.7(0.7)	0.6(0.2)	0(0)	0.5(0.2)	0(0)	0(0)	0.3(0.3)
Tylenchol aimus/ellus.	0(0)	9.4(6.6)	0(0)	5.2(1.2)	0.1(0.1)	1.9(0.5)	0.2(0.12)	4.1(2.)	0.2(0.1)	1.7(0.8)	0.2(0.1)	2.9(1)	0.3(0.2)	6.6(1.6)	0.1(0.1)	6.7(3.7)
Achromadora	1(0.47)	0(0)	1.3(0.6)	0.5(0.5)	1.7(0.7)	0.2(0.2)	0.8(0.3)	0(0)	2.9(0.9)	0.3(0.3)	1.5(0.7)	0(0)	2.6(0.6)	0.8(0.5)	3.1(1)	0.7(0.3)
							Predator									
Clarkus	1.7(0.5)	1.7(1.7)	0.4(0.2)	0.5(0.5)	0.7(0.2)	0(0)	0.7(0.2)	0.3(0.3)	0.5(0.3)	0(0)	0.9(0.5)	1.4(0.7)	1.3(0.4)	0.3(0.28)	0.9(0.)	0.3(0.3)

Table 2.2 (cont'd)

Miconchus	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0.1(0.1)	0(0)
Mylonchulus	0.3(0.3)	0.7(0.7)	0.2(0.2)	0(0)	0.2(0.1)	0(0)	0.3(0.2)	0.4(03)	0.6(0.2)	0(0)	0.8(0.3)	0(0)	1.2(0.2)	0.3(0.3)	0.2(0.1)	0(0)
Nygolaimus	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0.1(0.1)	0(0)	0(0)	0(0)	0(0)	0(0)	0.1(0.1)	0(0)	0(0)	0(0)
Paravulvus	0(0)	0(0)	0(0)	0(0)	0.3(0.2)	0(0)	0.2(0.2)	0(0)	1.1(0.4)	0(0)	1.6(1.1)	0(0)	1.2(0.2)	0(0)	0(0)	0(0)
Prionchulus	0(0)	0.4(0.4)	0(0)	3.2(3.2)	0(0)	0.5(0.5)	0(0)	0.2(0.2)	0(0)	0.8(0.3)	0(0)	0.3(0.3)	0(0)	0.2(0.2)	0(0)	0(0)
							Omnivore									
Discolaimus	0.3(0.1)	0(0)	0(0)	0(0)	0.2(0.1)	0(0)	0(0)	0(0)	0.1(0.1)	0.3(0.2)	0.2 (0.1)	0(0)	0(0)	0(0)	0(0)	0(0)
Qudsianematidae	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0.1(0.1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Aporcelaimus	0.1(0.1)	2.6(2.1)	0.3(0.2)	3.1(0.1)	0.2(0.1)	0.8(0.6)	0.1(0.1)	1.1(0.6)	0(0)	0.3(0.3)	0.1(0.1)	1.1(0.5)	0(0)	0.2(0.2)	0(0)	0.7(0.3)
Belondiridae	0(0)	0(0)	0.1(0.1)	0(0)	0(0)	0(0)	0.1(0.1)	0(0)	0(0)	0(0)	0(0)	0(0)	0.7(0.4)	0(0)	0.6(0.3)	0(0)
Dorylaimellus	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0.2(0.2)	0(0)	0(0)	0(0)	0.2(0.1)	0(0)	0(0)	0(0)	0.7(0.1)	0(0)

Table 2.2 (cont'd)

Ecumenicus	0.1(0.1)	0(0)	0(0)	0(0)	0.1(0.1)	0(0)	0.1(0.1)	0(0)	0.1(0.1)	0(0)	0.3(0.1)	0(0)	0.2(0.1)	0(0)	0(0)	0(0)
Epidorylaimus	0(0)	5.7(3.9)	0(0)	3.7(2.7)	0.1(0.1)	3.7(1.1)	0.1(0.1)	2.4(0.8)	0.1(0.1)	3.3(1.1)	0(0)	9.6(2.9)	0.1(0.1)	1.5(0.2)	0.4(0.1)	2.0(1.0)
Eudorylaimus	0.1(0.1)	0(0)	0.1(0.1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0.2(0.1)	0(0)	0.1(0.1)	0(0)
Laimydorus	0.2(0.2)	1.2(0.6)	0.1(0.1)	3.2(3.2)	0.1(0.1)	0.5(0.3)	0(0)	0.2(0.2)	0(0)	0(0)	0.1(0.1)	3.19(1.91)	0.3(0.2)	0.2(0.2)	0.1(0.1)	0.3(0.3)
Mesodorylaiums	0.4(0.3)	0(0)	0.3(0.2)	0(0)	0.9(0.3)	0(0)	1(0.4)	0.2(0.2)	0.8(0.4)	0(0)	2.5(0.6)	0(0)	0.6(0.1)	0(0)	0(0)	0(0)
Microdorylaimus	0.2(0.1)	0(0)	0.2(0.1)	0(0)	0.5(0.3)	0.2(0.2)	0.6(0.3)	0.4(0.4)	0.5(0.3)	0.3(0.3)	0.3(0.2)	0(0)	0.7(0.2)	0(0)	0.6(0.4)	0(0)
Paraxonchium	0(0)	0(0)	0(0)	0(0)	0.1(0.1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Prodorylaimus	0(0)	0(0)	0.1(0.1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0.1(0.1)	0.2(0.2)	0(0)	0(0)	0(0)	0.3(0.3)
Thonus	0(0)	1.1(1.1)	0.3(0.2)	0(0)	0.1(0.1)	1.0(1.0)	0.1(0.1)	0.6(0.4)	0.2(0.1)	0.2(0.2)	0(0)	0(0)	0.7(0.2)	0(0)	0(0)	0.3(0.3)
Thornia	0.2(0.1)	0(0)	0.1(0.1)	0(0)	0.2(0.2)	0(0)	0.2(0.1)	0(0)	0(0)	0(0)	0.1(0.1)	0(0)	0(0)	0(0)	0.1(0.1)	0(0)

Table 2.2 (cont'd)

Unknown Dorylaimus	0.5(0.2)	0(0)	1.1(0.3)	0(0)	0.8(0.4)	0(0)	0.5(0.2)	0(0)	0.3(0.)	0(0)	0.7(0.2)	0(0)	0.8(0.3)	0(0)	1.5(0.5)	0(0)
Pungentus	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0.2(0.2)	0(0)	1.7(1.7)
								Other								
InsectParasite	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0.1(0.1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

Table 2.3. Average and (standard error) of maturity, channel, and enrichment indices of all eight systems within the KBS-LTER. Standard errors depict one standard deviation from the mean. Different letters represent significant differences between system year combinations after Tukey's adjustment (n=88).

	Maturity Index		Channel Index		Enrichment Index	
	1991	2021	1991	2021	1991	2021
Conventional	1.78 (0.06) ab	2.2 (0.18) ab	21.76 (1.8) a	41.72 (19.26) abcd	73.02 (2.61) bcd	69.45 (9.38) abcd
No-till	1.84 (0.04) ab	1.78 (0.37) ab	25.85 (4.65) a	26.54 (16.56) a	68.18 (3.48) abcd	78.87 (11.84) bcd
Reduced Input	1.74 (0.05) a	2.06 (0.05) ab	16.4 (1.77) a	43.17 (7.07) ab	77.58 (1.59) cd	57.04 (2.20) abc
Biologically Based	1.69 (0.06) a	2.08 (0.11) ab	14.21 (1.58) a	51.80 (10.32) bc	78.87 (2.26) d	62.81 (6.11) abcd
Poplar	1.95 (0.04) ab	2.06 (0.06) ab	23.22 (2.89) ab	60.95 (9.47) cd	67.57 (1.79) abcd	54.45 (5.41) ab
Switchgrass	2.04 (0.1) ab	2.17 (0.15) ab	21.82 (3.17) ab	32.57 (6.93) ab	67.12 (2.99) abcd	68.05 (5.29) abcd
Early Successional	2.04 (0.05) ab	2.27 (0.10) b	29.91 (6.98) ab	74.36 (12.76) d	66.21 (2.79) abcd	51.61 (1.39) a
Mowed grassland	2.01 (0.02) ab	2.24 (0.07) ab	37.24 (4.06) ab	53.73 (15.68) cd	58.73 (0.81) abcd	54.49 (5.51) abc

Table 2.4. Average and (standard error) of residuals of all eight systems within the KBS-LTER. Negative numbers represent mineralization while positive numbers represent stabilization mechanisms. Standard errors depict one standard deviation from the mean. Different letters represent significant differences between year within each system (n=92).

Systems	Residuals	
	1991	2021
Conventional	35.15 (15.61) a	-138.73 (13.43) b
No-till	-126.12 (66.55) a	126.91 (109.21) b
Reduced Input	-152.18 (53.03) a	-66.28 (23.27) b
Biologically Based	-167.46 (50.04) a	-48.34 (8.67) b
Poplar	88.14 (24.05)	75.49 (44.01)
Switchgrass	103.68 (31.02) a	41.01 (77.54) b
Early Successional	25.54 (14.76) a	14.99 (60.78) b
Mowed Grassland	211.29 (27.51)	65.44 (51.10)

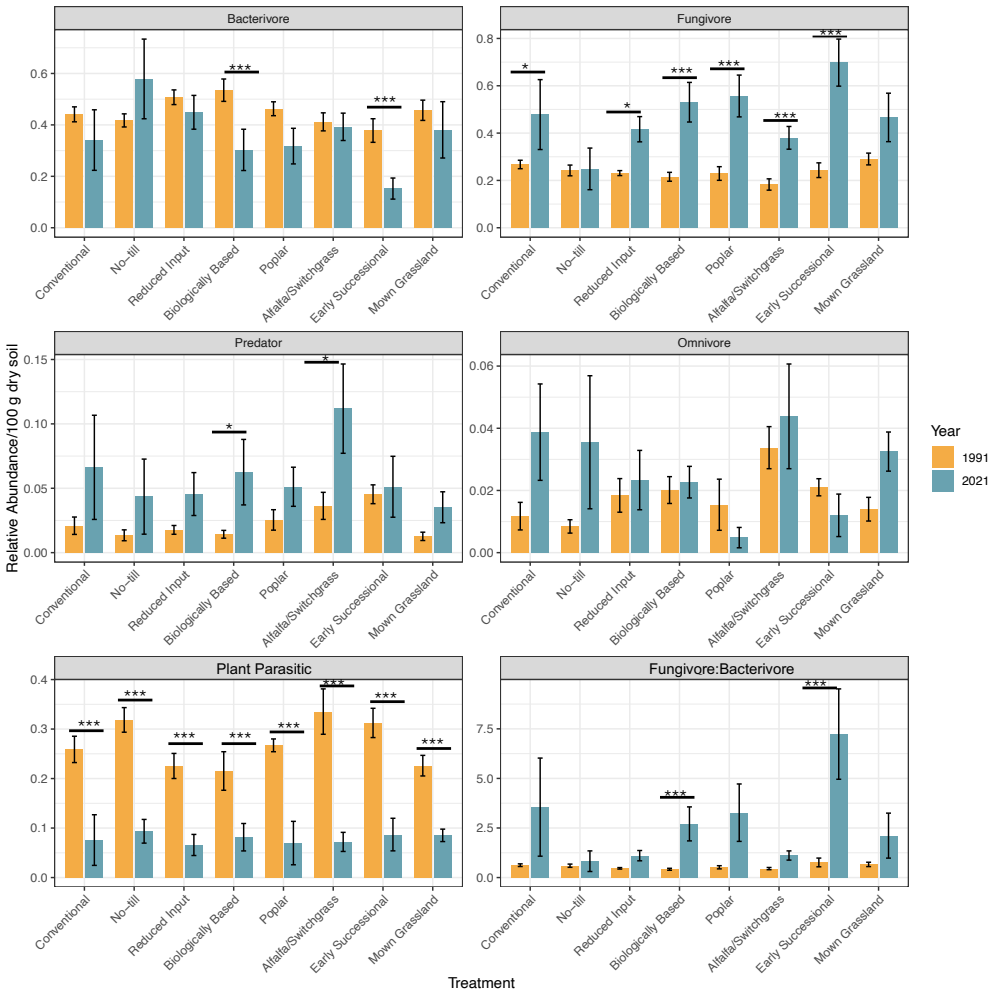

Figure 2.1 Mean relative abundance of bacterivore, fungivore, predator, omnivore, plant parasitic, and fungivore nematodes within all eight systems of the KBS-LTER. Standard error bars represent one standard deviation from the mean. Lines over a specific treatment within a feeding group corresponds to a significant pair-wise comparisons within that treatment between year (n=88).

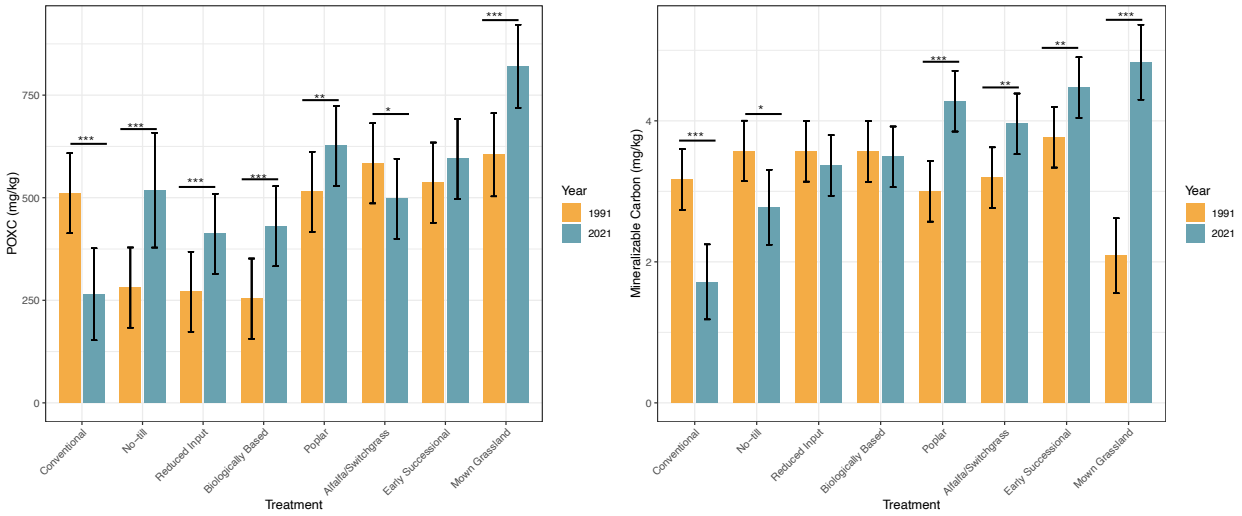

Figure 2.2. Average A) POXC and B) MinC values for all eight systems. Color depicts year 1991 (yellow) and 2021 (blue). Standard error bars represent one standard deviation from the mean. An unequal variance model was utilized for POXC and a log transformed model was utilized for mineralizable C. Lines over a specific treatment within the respective carbon indicator corresponds to a significant pair-wise comparisons within treatment between year (n=92).

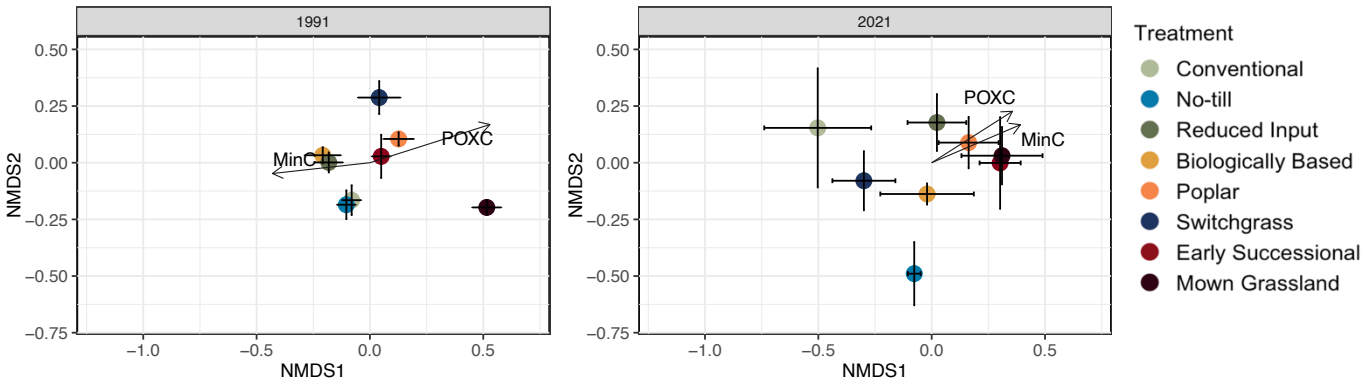

Figure 2.3. Non-metric dimensional scaling of nematode communities within all systems (color) of the KBS-LTER in 1991 and 2021. Points represent the average of all communities within the system and replicate. Standard error bars represent one standard deviation from the mean. Vectors are graphed over the NMDS, with length representing the strength of the relationship, and direction representing the system MinC and POXC had the strongest relationship (n=82).

APPENDIX B: CHAPTER TWO SUPPLEMENTAL

Table S2.1. Percent soil moisture of all treatments within the KBS-LTER during the years 1991 and 2021. Data depict means (SE) of all six replicated of the eight treatments. Different letters depict a significant difference at one standard deviation from the mean after Tukey's adjustment.

	1991	2021
Conventional	19.08 (0.58) d	12.53 (0.94) ab
No-till	18.45 (1.02) d	14.47 (2.07) abc
Reduced Input	17.74 (0.53) cd	11.65 (0.52) a
Biologically Based	19.07 (0.53) d	11.78 (1.33) a
Poplar	18.17 (0.5) d	10.6 (0.48) a
Switchgrass	19.59 (0.81) de	11.73 (0.64) a
Early Successional	18.84 (0.75) d	10.44 (0.26) a
Mowed grassland	23.54 (0.93) e	15.87 (0.59) bcd

Table S2.2**.** Permanova of nematode communities assessing the effect of treatment in 1991 and 2021 on nematode community composition.

Factor	F	P
	1991	
Treatment	4.33	0.001
	2021	
Treatment	1.32	0.09

Table S2.3**.** F statistics and P-values for all nematode feeding groups for the linear mixed effect models used to test significant effects of treatment, timepoint, and the interaction between treatment and timepoint. Linear mixed effect models for fungivore, predator, omnivore, and plant parasitic feeding groups utilized a square root transformation. Fungivore:Bacterivore ratios utilized an unequal variance model.

Factor	Bacterivore		Fungivore		Predator		Omnivore		Plant Parasite		Fungivore:Bacterivore	
	F	P	F	P	F	P	F	P	F	P	F	P
Treatment	2.74	0.01	3.59	<0.001	1.83	0.09	2.36	0.03	1.07	0.39	15.12	<0.001
Timepoint	7.92	0.006	15.02	<0.001	13.81	<0.001	0.54	0.46	137.05	<0.001	48.02	<0.001
Treatment*Timepoint	2.07	0.06	2.44	0.03	0.65	0.71	1.62	0.14	0.51	0.83	13.33	<0.001

Table S2.4. F statistics and P-values for nematode indices for the linear mixed effect models used to test significant effects of treatment, timepoint, and the interaction between treatment and timepoint. An unequal variance model was used for the CI. Linear mixed effects models for the CI utilized an unequal variance model.

Factor	MI		CI		EI	
	F	P	F	P	F	P
Treatment	2.93	0.01	14.29	<0.001	3.5	0.003
Timepoint	17.95	<0.001	27.5	<0.001	15.19	<0.001
Treatment*Timepoint	1.06	0.40	1.89	<0.001	1.52	0.023

Table S2.5. F statistics and P-values for soil C measurements. Linear mixed effect models were used to test significant effects of treatment, timepoint, and the interaction between treatment and timepoint on POXC, mineralizable carbon, and residuals. An unequal variance model was used for POXC and residuals and a log transformed model was used for mineralizable carbon.

Factor	POXC		Mineralizable Carbon		Residuals	
	F	P	F	P	F	P
Treatment	43.40	<0.001	11.2	<0.001	38.11	<0.001
Timepoint	24.18	<0.001	13.83	<0.001	1.04	0.31
Treatment*Timepoint	14.47	<0.001	18.46	<0.001	26.97	<0.001

Table S2.6. P-values for correlations of mineralizable C and POXC with nematode community composition for the conducted vector analysis.

Factor	1991	2021
POXC	0.001	0.040
Mineralizable Carbon	0.023	0.025

Table S2.7. Average nematode abundance (%) and (SE) of nematode feeding groups in early successional and no-till systems in April and June of 2021.

Treatment	Bacterivore	Fungivore	Plant Parasitic	Predator/ Omnivore
March 2021				
Early Successional	13.93 (0.04)	69.91 (0.06)	13.31 (0.03)	1.43 (0)
No-till	33.68 (0.14)	30.02 (0.01)	22.52 (0.07)	6.9 (0.04)
June 2021				
Early Successional	11.2 (1.7)	46.3 (10.6)	33.5 (11)	2.3 (1.34)
No-till	40 (16.6)	49.5 (15.9)	9.7 (4.9)	0.5 (0.3)

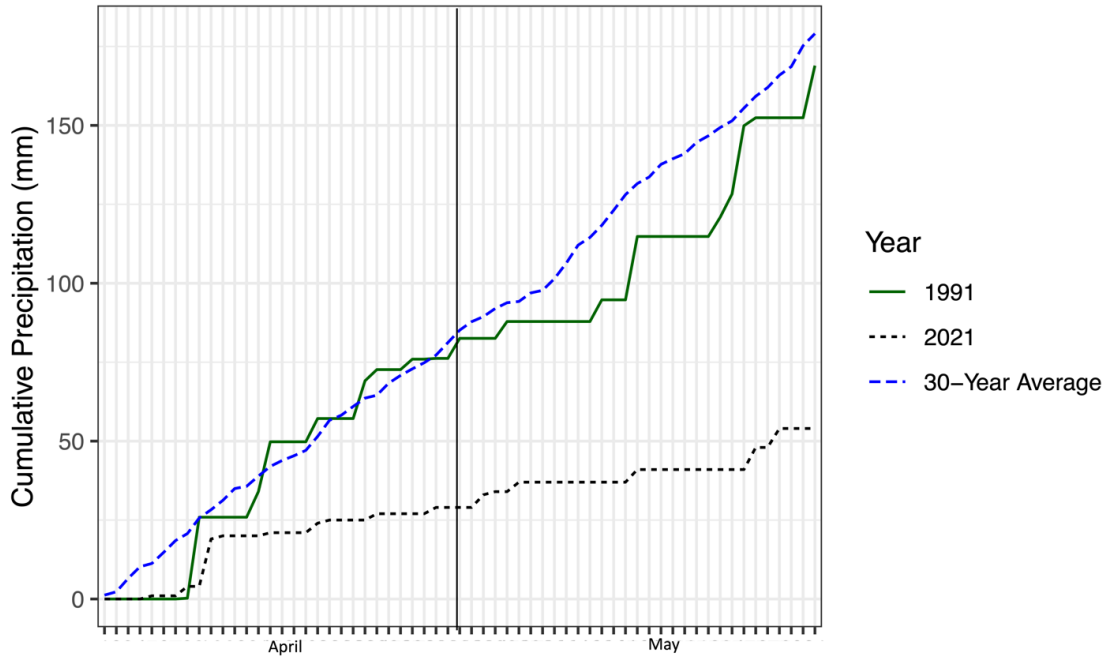

Figure S2.1. Cumulative precipitation in 1991 (green), 2021 (blue-dashed), and the 30-year average (black-dashed) in April and May.

CHAPTER 3: POLYCULTURE PERENNIALS FOSTER SOIL FOOD WEB RESISTANCE AND RESILIENCE TO DROUGHT STRESS IN AGROECOSYSTEMS

ABSTRACT

Variable rainfall is expected to increase with climate change and will lead to more drought stress. The impact that drought has on the soil food web is seldom investigated, and even less is known regarding the role that agricultural management has on soil food web resistance and resilience to drought. Free-living nematodes can serve as bioindicators of climatic stress because they are sensitive to disturbance and span the *r*-*K* strategist continuum. Here I aim to 1) understand how management intensity impacts the resistance of nematode communities to drought and 2) assess how the immediate alleviation of drought impacts soil food web resilience in contrasting agroecosystems. This study was conducted at the W.K. Kellogg Biological Station Long-Term Ecological Research Site in Southwest, Michigan where three rainfall manipulations were induced (drought, variable, and control) in two land uses (an early successional land use and a no-till row-crop). Sampling for nematode communities was conducted prior to drought implementation (pre-drought), six weeks after drought was induced (peak-drought), and two days after rewetting (post-drought). The early successional land use showed little shift in nematode community structure or distribution along an *r*-*K* strategist continuum at peak-drought. In the no-till land use, fungivore *r* and *K* strategist nematode abundances declined with drought stress, indicating overall less resistance to drought. Similar patterns persisted post-drought, whereby nematodes within the early successional land use remained unchanged, while nematodes within the no-till land use were slower to recover. This study demonstrates that enhancing perenniality and plant biodiversity within agroecosystems is a valuable option for fostering soil food webs that are resistant to drought. Moreover, drought stress clearly impacts nematode community dynamics, which have critical implications for ecosystem functioning.

INTRODUCTION

Climate change is causing variable precipitation, which has led to more frequent and intense periods of drought across the globe (Ford et al., 2021). Drought has been shown to disrupt soil biodiversity and ecosystem functioning (de Vries et al., 2012; Schimel, 2018). Yet, there are certain soil fauna that must be further investigated under drought to understand the extent to which drought disrupts soil food webs. It is crucial to identify ecosystems that support soil food webs capable of withstanding drought and that have the capacity to recover quickly, ensuring the preservation of vital ecosystem services. Here, I define resistance as the ability of a system to be

insensitive to pulse disturbances (Shade et al., 2012), and resilience as the ability of a system to return to pre-disturbance levels (Hoover et al., 2014). Under drought disturbance, the resistance and resilience of soil microbial communities are dependent on ratios of slower-growing fungi to faster-growing bacteria (de Vries et al., 2012; Shade, 2023). Although there is substantial evidence on how drought may impact individual organisms within the soil food web, we have yet to understand how multi-trophic organisms within the soil food web are resistant and resilient to drought. Moreover, most studies only account for long-term drought periods (greater than six weeks) and neglect to assess the effect of intermittent drought (three weeks or less) on soil food webs. Equally important, however lacking in investigation, is understanding how dominant life histories within a soil food web are maintained under climatic disturbance. For instance, drought induced shifts in proportions of *r* and *K* strategists that make up the soil food web could directly impact soil functioning and cause a decline of ecosystem services (Grime, 1988; Wan et al., 2024; J. Zhou et al., 2022), however soil food web life-history strategy response to drought has not been investigated.

Determining shifts in life-history strategy of the soil food web is imperative, however issues such as methodology and the sheer diversity of soil fauna make it difficult to quantify life-history strategies for most micro- and macroorganisms (Fierer et al., 2007; Siepel, 1994). That said, free-living nematodes are an exception to this generalization (Franco et al., 2019; Quist et al., 2016). Specifically, nematodes are specialists and will select their prey based on feeding preference; bacterivores will prey on bacteria, fungivores on fungi, herbivores on plants, and predator/omnivores on other nematodes (Bongers & Bongers, 1998). In addition, nematodes within each feeding group range in trophic level complexity and based on their life-history strategies are classified into a group on the colonizer-persister (cp) scale, which aligns directly with the *r*-*K* strategist spectrum (Ferris et al., 2001). Nematode *r*-strategists are characterized as nematode families that have shorter life cycles and are resilient to disturbance, whereas *K*-strategists are characterized as nematode families that have longer life cycles and are more sensitive to disturbance (Ferris et al., 2001). Bacterivore and fungivore nematodes span this *r*-*K* strategist spectrum and vary in their life-history strategies depending on family, whereas predator and omnivore nematodes function solely as *K*-strategist nematodes.

Previous literature has indicated that free-living nematodes usually adhere to life-history strategy theory where stressed environments select for *r*-strategists nematodes, while *K*-strategist

nematodes rapidly decline (Fig.1) (De Deyn et al., 2004; Ferris & Matute, 2003; Powell, 2007). However, we know little regarding how a drought-stressed environment alters nematode life-history strategy distribution. Currently, the extent of our knowledge in relationship to nematode response to drought has indicated declines in nematode predators and total nematode abundance in droughts conditions (Andriuzzi et al., 2020; Franco et al., 2019; Landesman et al., 2011; J. Zhou et al., 2022).

Nematode community structure is also dependent on factors such as plant perenniality and plant diversity, where systems with greater perenniality and plant diversity have nematode communities comprised of sensitive nematodes such as predators and omnivores (Cesarz et al., 2017, p. 201). However, the impact of land use differences such as increased plant diversity on promoting the resistance and resilience of nematode communities to drought is grossly understudied. To my knowledge, only one study currently exists that has tested the effect of land use on soil food web resistance and resilience (de Vries et al., 2012). However, the resistance and resilience of nematode community life-history strategy to drought in systems that differ in land use is seldom tested.

This study aims to test the resistance and recovery of nematode community life-history strategies to a drought disturbance gradient within two land uses that vary in management intensity. My first objective is to understand how land uses that differ in management intensity impact the resistance of the soil food web to drought. I hypothesize that drought stress within a no-till land use will cause nematode communities to be dominated by *r*-strategists, while nematode communities in an early successional land use will remain trophically complex. My second objective is to determine how the alleviation of drought alters soil food webs in land uses that differ in management intensity. I hypothesize that the alleviation of drought will cause an increase in *K*-strategist nematodes and a decline in *r*-strategist nematodes, regardless of land use (Fig. 3.1).

METHODS

Site Description

This experiment was conducted at the W.K. Kellogg Biological Station Long-term Ecological Research (KBS-LTER) Site located at 85° 23’W, 42° 24’ in Hickory Corners, Michigan. The KBS-LTER was established in 1989 and prior to establishment it was a conventionally managed agricultural system. The soil series are Kalamazoo and Oshtemo, and

the soil type is a mixed mesic Typic Hapludalf. The KBS-LTER Main Cropping land use Experiment (MCSE) has seven systems that range in management intensity, perenniality, and plant diversity arranged in a randomized complete block design with six replicates per system. This experiment was only conducted on two of the seven systems: an annual row-crop no-till land used, hereafter referred to as 'no-till' and an early successional land use. The no-till land use consists of a corn (*Zea mays)*-soybean (*Glycine max)*- wheat (*Triticum sativum)* rotation and has not been tilled since prior to establishment of the experiment in 1989. The no-till land use receives external fertilizers (nitrogen, phosphorus, and potassium), amendments (lime), herbicides, and pesticides which are applied at rates recommended by Michigan State University. The early successional land use comprises 11 different species of forbs and grasses and is unmanaged except for a prescribed burn that occurs every spring to prevent the habitation of woody species.

Rain exclusion shelter design

Rain exclusion shelters were designed to exclude all ambient rainfall from the no-till and early successional land uses (Kahmark et al., 2024). Each shelter is 48.5 x 4.3 m and placed in an 87 by 105 m plot. Shelters were deployed on July 1, 2021. Precipitation patterns were simulated on all six replicate plots of the early successional community and four replicate plots of the no-till land uses. Only four replicate plots were used for the no-till land use because of slope effects that impacted the simulated drought (Kahmark et al., 2024). Three shelters were deployed on each plot for all treatments. Thus, the experimental design was a nested randomized-complete-block design. Rain exclusion shelters consisted of three precipitation treatments simulated via irrigation: an irrigated shelter which served as a control (hereafter referred to as irrigated), variable, and drought. The irrigated shelter was watered once a week to reflect the thirty-year average precipitation per week that falls in Southwest, Michigan. The variable shelter had two three-week long drought periods. After every three-week drought, the ambient rainfall that fell over the three-week duration was applied over a two-day period. The drought shelter was irrigated three weeks prior to drought and then a six-week drought was induced. After, the sixth week of drought all shelters were irrigated to end the disturbance period (August 28, 2021). The average amount of irrigation applied under each shelter can be found in Table S3.1.

Soil sampling

I sampled the nematode community three weeks prior to inducing the drought (June 9,

2021, pre-drought), six weeks after shelter deployment (August 23, 2021, peak-drought), and one week after rewetting of all rainout shelters (August 30, 2021, post-drought). Pre-drought sampling was conducted prior to the shelter deployment, whereas peak and post-drought samples were collected under each shelter. For all sampling timepoints, soil samples were collected from a 1.5 x 2 m perimeter plot. Samples were collected down to 10 cm for nematode community analyses using a soil probe with a 2.54 cm diameter. During each sampling period three soil cores were collected from each rainout shelter and composited to form one sample from each shelter, except for pre-drought. Given that there was no rainfall manipulation during pre-drought, one soil core was taken from each plot where the shelters were to be placed and composited to make one soil sample for each land use and replicate. Coordinates were recorded for each of the soil cores to ensure that soil core locations were not re-sampled over the growing season.

Hourly volumetric soil moisture

Campbell Scientific CS655 soil moisture sensors were installed during shelter deployment on July 1, 2021, at a 15 cm depth in each land use and rainfall treatment replicate to measure hourly volumetric soil moisture. Data presented for this study was averaged for each day and was recorded until post-drought. Hereafter, this soil moisture measurement will be referred to as "daily average volumetric water content". There were technical errors and sensor failure earlier in the summer, so there was lower sensor replication (N=0-4), until August 5, when I achieved full sensor replication (early successional: N=6, no-till: N=4). I utilized analysis of variance (ANOVA) to assess the effect of rainfall and land use on daily average volumetric water content measurements on data starting on August 5, 2021, to ensure appropriate replication of daily average volumetric water content measurements. Analysis from this date was sufficient to address my study questions as it captured data for two weeks during and leading up to peak drought (August 23, 2021). Rainfall, land use, and the interaction between rainfall and land use were treated as fixed factors.

Sample processing

Samples were subsampled for gravimetric water content immediately after sampling. Soils were sieved to 2 mm for nematode community analysis and stored at 4 °C for further processing.

Nematode extraction, identification, and colonizer-persister grouping

Nematodes were extracted from 50 g of soil using the Baermann funnel extraction

technique for 72 h (Flegg & Hooper, 1970). Next, nematodes were collected and fixed in a 4% paraformaldehyde solution. Nematodes were counted using a dissecting scope. Afterwards, 100 nematodes were identified to genus at 40-100 x magnification (Bongers, 1990). Each nematode genus was then classified by their life-history strategies into their respective colonizer-persister (cp) grouping on the cp scale for further analysis. The nematode cp scale consists of groupings that range from 1-5 and corresponds to their perspective life-history strategy characteristics (Table 3.1). Each free-living nematode family/genus that was identified was assigned to a corresponding feeding strategy and cp-grouping. Hereafter, I refer to the cp-grouping as life-history strategy.

Statistical Analysis

ANOVA was used to assess the effect of drought and land use on soil moisture, total nematode abundance, nematode community feeding group abundances, and nematode life-history strategies within their respective feeding groups. Rainfall, land use, and the interaction between rainfall and land use were treated as fixed factors. For all ANOVA's sampling timepoint was assessed individually. Given that the rainout shelters were nested within each land use, the unique identifiers of (land use * replicate) and (land use * rainfall * replicate) were treated as random factors. The *lme* package in R was used to assess the effect of fixed factors on nematode communities (Bates et al., 2015). Normality was assessed using studentized residuals with *MASS* in R (Venables & Ripley, 2002). Levene's test was used to assess for unequal variance. Contrasts were applied to understand significant differences of nematode community dependent variables in response to drought within each treatment, p-values were adjusted using Tukey's HSD using the *multcomp* package in R (Hothorn et al., 2008).

I used a permutational analysis of variance (PERMANOVA) to assess the effect of rainfall and land use on nematode community structure using the {adonis} function in the *vegan* package (Oksanen et al., 2013). A post-hoc power analysis was conducted for each factor within each time point to assess the interpretability of significance of PERMANOVA results from understanding the effect of land use and rainfall in peak and post-drought using the *pwr* package (Table S3.2) (Champely, 2020). A harmonic mean was calculated for the power analysis due to unequal sample sizes for early successional and no-till plots. Due to a lack of power, I then conducted PERMANOVAs of the effect of rainfall on nematode community structure within each land use during peak and post drought. Additionally, I conducted a principal coordinate

ordination analysis (PCOA) with Bray-Curtis distance measures using the {vegdist} function with the *vegan* package (Oksanen et al., 2013). The {betadisper} function with the *vegan* package was used to test for homogeneity of variances for factors of land use and rainfall manipulation treatment for each PCOA conducted. Shepard diagrams were plotted in *MASS* between the original distance matrix and the Bray-Curtis PCOA distance matrix to confirm the linear assumption of each PCOA. Five PCOA's were conducted for each sampling timepoint (pre, peak, and post drought) and land use. The effect of rainfall manipulation on nematode community structure within each land use was conducted to maximize power. PCOA's consisted of 43 taxa for pre-drought, and 69 taxa for all PCOA's conducted in peak and post drought. The *indicspecies* package was used to conduct a multi-level-pattern analysis to identify nematode genera that were indicators of the respective rainfall manipulation treatment and land use under pre, peak, and post drought (Cáceres & Legendre, 2009).

RESULTS

Soil moisture response to drought and rewetting

Gravimetric soil moisture water content remained consistent in the no-till land use where drought appeared to have no significant effect on soil moisture at each sampling timepoint (Table 3.2; Table S3.3). However, daily average volumetric water content measurements indicated contrasting results where rainfall had a significant main effect and a significant interaction effect between land use and rainfall (Table S3.4). Specifically, daily average volumetric water content was significantly reduced by 28 and 23 % in the drought and variable no-till land use, when compared to the irrigated treatment (Fig. 3.2). In the early successional land use the implementation of drought significantly affected both daily average volumetric water content, and gravimetric soil moisture measurements. For gravimetric soil moisture, the drought treatment had significantly lower soil moisture when compared to the irrigated treatment (Table 3.2). For the early successional land use, daily average volumetric water content was significantly reduced by 23% in the drought treatment when compared to the irrigated treatment. However, the variable treatment had significantly greater daily average volumetric water content when compared to the irrigated treatment (Fig. 3.2).

When compared across land use within peak drought, early successional irrigated and variable treatments had on average greater gravimetric soil moisture than the no-till drought and no-till variable rainfall treatments (Table 3.2). Thus, except for the early successional drought

treatment in peak drought, the early successional land use remained wetter than the no-till land use. These trends contrasted the daily average volumetric water content results, where the no-till land use had on average greater volumetric water content (Fig. 3.2). After the rewetting of all rainfall treatments, gravimetric soil moisture became similar across all rainfall treatments and land uses (Table 3.2; Fig. 3.2). These results were similar for daily average volumetric water content in the no-till land use. However, the daily average volumetric water content indicated contrasting results in the early successional land use where the drought treatment volumetric water content remained reduced after rewetting (Fig. 3.2).

Land use alters nematode community structure and life-history strategy

Trends indicate that nematode communities in the no-till and early successional land uses differed prior to drought implementation (Fig. S3.1). Additionally, PERMANOVAs indicate that land use had a marginally significant effect on nematode communities (Table 3.3; p=0.08). Multi-level pattern analysis (i.e. indicator species analysis) revealed that certain nematode genera were strongly associated with each land use. Nematode genera *Paratylenchus* and *Pratylenchidae,* both *r*-strategist plant parasitic nematodes, were significantly related to the early successional and no-till land use, respectively (Table S3.5). Land uses also differed in their distribution of nematode life-history strategies. The no-till land use was dominated by bacterivore and fungivore *r*-strategists, whereas the early successional land use was dominated solely by fungivore *r*-strategists (Fig. 3.3). However, land-use only had a marginal significant effect on bacterivore cp-2 nematode abundances and total bacterivore abundance (Table S3.6; Table S3.7; Table S3.8).

Drought reduces total nematode abundance regardless of land use

Total nematode abundance on average decreased under drought and variable rainfall treatments, in both early successional and no-till land uses, with rainfall having a significant effect on total nematode abundance (Fig. 3.4A; Table S3.9). In the no-till land use, nematode abundance was significantly reduced by 93 % in the drought treatment when compared to the irrigated treatment. Although not significant, early successional land use nematode abundance was 54 and 62 % lower in variable and drought treatments, respectively, when compared to the irrigated treatment.

Drought alters nematode community structure and life-history strategy in the no-till but not early successional land use

Trends from peak-drought indicate that nematode community resistance to drought depends on land use. Rainfall manipulation in the no-till land use had a marginally significant ($p=0.06$) effect on nematode community structure (PERMANOVA; Table 3.3). Additionally, PCOA's depict differing nematode community structure within each rainfall treatment in the no-till land use during peak-drought (Fig. 3.5). In contrast, rainfall manipulation in the early successional land use had no effect on nematode community structure (PERMANOVA; Table 3.3). While PCOA's demonstrate differences in nematode community structure from rainfall manipulation (Fig.3.5), PERMANOVA's suggest a marginal to a non-significant effect from rainfall.

The indicator taxon analysis from the no-till land use showed that certain nematode species were only found in the drought, variable, and irrigated treatments. Specifically, *Tylenchidae,* a plant parasitic *r*-strategist*,* was only present in the drought treatment, *Paratylenchidae,* a plant parasitic *r*-strategist*,* was only present in the variable treatment, and *Aphelenchoide,* a fungivore *r*-strategist was only present in the irrigated treatment (Table S3.10). There was no indicator taxon identified for the different rainfall manipulation treatments within the early successional land use, supporting the previous observation of weaker effects of drought on early successional nematode communities.

The distribution of life-history strategies within each feeding group during peak drought were similar across all rainfall manipulation treatments in the early successional land use, but different in the no-till land use (Fig. 3.6; Table S3.11). In the no-till land use, fungivore *r*-strategists (f2) were significantly reduced in variable and drought treatments when compared to the irrigated treatment (Fig. 3.6). The irrigated treatment in the no-till land use had significantly greater fungivore abundance and a greater fungivore: bacteria ratio relative to the variable and drought treatments (Table S3.12; Table S3.13). Plant parasitic nematode abundance also shifted in response to rainfall manipulation in the no-till land use where the variable treatment had a significantly greater abundance of plant-parasitic *r*-strategist nematodes when compared the irrigated and drought treatments (Fig. S3.3). These changes in the nematode community were not observed in the early successional land use.

Nematode communities during post-drought differ by land use

At post-drought (~48 hours after all plots were rewet) total nematode abundance became similar across all rainfall manipulation treatments (Fig. 3.4B). However, trends suggest that nematode community structure in the no-till land use remained distinct across rainfall manipulations (PCOA; Fig. 3.7A). In the early successional land use, drought and irrigated communities remained similar but appeared to be different from variable nematode communities (PCOA; Fig. 3.7B). However, PERMANOVAs for both the no-till and early successional land uses indicate that rainfall manipulation did not have a significant effect on nematode community structure (Table 3.3). Thus, PCOA trends should be interpreted with caution.

Nematode community life-history strategy distribution also remained similar in the early successional land use but differed in the no-till land use after the alleviation of drought (Fig.3.8). After rewetting all rainfall manipulation treatments in the early successional land use demonstrated similar distributions of nematode life-history traits and were dominated by fungivore and bacterivore *r*-strategists. However, nematode community life-history trait distributions did marginally differ in the no-till land use ($p=0.07$, Table S3.14). Specifically, no-till irrigated treatments had on average, a 39 and 41 % greater abundance of bacterivore nematodes when compared to drought and variable rainfall treatments, respectively (Table S3.15; Table S3.16). In addition, fungivore abundances were elevated in the variable rainfall treatment, but not in the irrigated treatment within the no-till land use (Fig. 3.8).

DISCUSSION

Agricultural land use alters nematode community resistance to drought

I hypothesized that nematode community structure, and their respective life-history strategies would be resistant to drought in the early successional land use. My study confirmed this hypothesis given that nematode communities did not shift in response to drought in the early successional land use despite soil moisture being significantly reduced by drought. Aspects of this system, such as perenniality and plant diversity, may buffer drought impacts on nematode community structure outside of maintenance of soil moisture. I speculate that the resistance of the nematode community to drought in polyculture land uses is most likely caused by stable organic matter decomposition pathways providing a secure food source for nematodes thus creating a nematode community that can resist stress from drought (Sanford et al., 2021; Sun et al., 2019).

In contrast, the annual no-till soybean land use was sensitive to the treatment of rainfall reduction, as indicated by a significant decline in nematode abundance and shifts in nematode community structure within the drought and variable rainfall manipulation treatments. While gravimetric water content measured on the day of sampling did not show significant differences in soil moisture across rainfall treatments, continuous volumetric water content measurements over the six-week drought period revealed that no-till soils in drought and variable rainfall treatments were in fact drier. This suggests that nematode communities in the no-till land use were responding to prolonged drought conditions rather than instantaneous soil moisture levels. Given that nematodes desiccate under shrinking water films (Neher, 2010), the observed community shifts may reflect their susceptibility to moisture loss in a system lacking additional resistance mechanisms to drought disturbance, such as plant diversity (Li et al., 2022). These results indicate that no-till systems may be more vulnerable to extended dry periods, despite gravimetric measurements suggesting otherwise, highlighting the importance of considering long-term soil moisture dynamics when assessing drought impacts on nematode communities.

Fungivore nematode life-history strategies are sensitive to drought stress

I hypothesized that under drought, nematode *r*-strategists would increase because they can withstand stress, and *K*-strategist nematodes would decline (Siebert et al., 2019). I found no evidence supporting this hypothesis; changes in life-history strategies varied by agricultural land use and feeding group but did not include increases in *r*-strategists or reductions in *K*-strategists. Contrary to studies in grassland and desert soils (Franco et al., 2019), drought had no effect on *K*-strategist nematodes in either land use. Given that *K*-strategist abundances are already reduced in agricultural systems (Birkhofer et al., 2008), it is not surprising that there was no significant impact of drought on *K*-strategist nematodes. In the no-till land use, bacterivore nematode *r* and *K* strategist abundances were similar across all rainfall manipulation treatments. This result suggests that bacterial decomposition channels are likely resistant to intermittent drought stress. Moreover, bacterivore nematodes have plastic traits such as anhydrobiosis, which allows bacterivore nematodes to withstand unfavorable conditions such as drought stress (Treonis & Wall, 2005; Vandegehuchte et al., 2015).

Fungivore *r*-strategist nematodes were sensitive to drought and declined. My study indicates that fungivore *r*-strategists may not have the adaptations or plasticity necessary to maintain abundances under drought. One adaptation fungivore nematodes lack is the ability to

form dauer larvae, a developmental stage that allows nematodes to survive harsh conditions without feeding or growing (Gilabert et al., 2016). This may lead to an overall decline in their abundance during drought. Additionally, the decline in the abundance of fungivore nematodes, may be related to a decline in fungal prey availability (Freckman & Caswell, 1985). Specifically, drought has been found to cause a loss of fungal biomass, which in turn may cause a reduction in fungivore abundance (de Vries et al., 2012). Overall, increased drought stress in annual row-crop land uses may shift food webs toward bacterial dominance, reducing fungal-driven functions like slower decomposition and greater carbon (C) accrual (Z. Zhou et al., 2018).

r-strategist nematodes dominated post-drought regardless of agricultural land use

I hypothesized that after drought, *K*-strategists would increase, and *r*-strategists would decrease thus returning to an equilibrium after the end of a disturbance. In early successional land uses, after rewetting, soil moisture became elevated and similar across all rainfall manipulation treatments, indicating that there was a consistent alleviation of drought across all rainfall treatments. After rewetting, bacterivore cp-1 nematodes comprised most of the nematode community in all rainfall treatments, which is not surprising given that these nematodes are known to rapidly increase in abundance in response to pulses of precipitation (Veach & Zeglin, 2020). Regardless of rainfall treatment, nematode life-history abundances shifted from peak-drought after rewetting but remained similar across rainfall treatments post-drought. This underscores the need to consider life-history strategies when assessing nematode community responses to disturbance and their ecological implications.

In the no-till land use, bacterivore *r*-strategist abundances became the dominant nematodes in all rainfall manipulation treatments post-drought. This response to the rewetting event appeared to be stronger in no-till relative to the early successional land use, where the proliferation of *r*-strategist bacterivores contributed to the greater total abundance of nematodes post-drought. The irrigated no-till land use had a significantly greater abundance of bacterivore *r*-strategists when compared to drought and variable treatments. Moreover, there were greater *r*- and *K*- strategist fungivore abundances in the drought and variable rainfall manipulation treatments. Additionally, these results indicate that even though soil moisture, on average, returned to an equilibrium during rewetting, nematode feeding groups in droughted treatments were unable to respond at the same intensity as those in irrigated treatments. Furthermore, the reduced abundance of bacterivore *r*-strategists in drought and variable treatments supports other

studies that have found the release of organic matter and fast decomposition (i.e., bacteria-dominated decomposition) caused by rewetting events is disrupted under drought (Meisner et al., 2015).

Study implications and future research

This study is one of the first to apply life-history strategy theory to nematode communities under drought disturbance. By examining life-history strategies within each nematode feeding group, we can better understand the implications of drought on the soil food web and ecosystem function. My study found that fungivore nematodes, which form the foundation of the fungal decomposition channel (f2), decline under drought conditions. By incorporating life-history strategies, I identified that drought poses a greater threat than previously anticipated—not by reducing K-strategist fungivore nematodes, but by risking the collapse of the entire fungal decomposition channel (f2). This decline suggests that critical ecosystem functions, such as carbon accrual and slow decomposition, may be significantly impaired under increasing drought conditions (Barreto et al., 2024).

My findings reveal the need for continuous soil moisture monitoring in drought experiments, as gravimetric water content point-measurements alone failed to capture prolonged periods of lower soil moisture and obscured our view of the effect of the drought. In the early successional land use, soil moisture declined under drought, yet nematode communities remained stable. In contrast, while gravimetric measurements suggested no-till soils retained moisture, continuous volumetric measurements revealed that they were drier over time, coinciding with significant shifts in nematode communities. These findings suggest that nematode responses to drought are not solely driven by instantaneous soil moisture levels but by prolonged exposure to dry conditions. Drought resistance may therefore be linked to ecosystem functions such as organic matter decomposition, which tend to be more stable in perennial polyculture land uses (Sanford et al., 2021). Furthermore, my results indicate that perennial polycultures foster stress-tolerant soil food webs, as evidenced by the dominance of bacterivore cp-1 (b1) nematodes in early successional land uses. While further research is needed, it is possible that stress-tolerant soil food webs and organic matter dynamics contribute to the observed resistance to drought in perennial polyculture systems, even when prolonged soil moisture loss occurs.

While previous studies have largely focused on peak drought conditions, my findings

highlight that rewetting events following drought can also disrupt the soil food web, potentially altering key ecosystem functions such as nitrogen mineralization and carbon loss. As climate patterns become increasingly volatile, with more frequent drying and rewetting cycles throughout the growing season, these disturbances may intensify, further compromising ecosystem stability. To accurately assess ecosystem resilience to global change, it is essential to evaluate soil food web structure and function not only during drought but also throughout rewetting and recovery periods.

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APPENDIX A: CHAPTER THREE TABLES AND FIGURES

Table 3.1. Colonizer-persister grouping characteristics adapted from Bongers (1990) and Bongers & Bongers (1998).

	Life-history strategy characteristics of colonizer-persister group
1	• Short generation time • Small eggs • Form dauer larvae • Increased growth under enriched conditions
2	• Short generation time • Relatively high reproduction rates but slower than cp-1 • Tolerant to disturbance • Do not form dauer larvae
3	• Longer generation time than cp-2 • Greater sensitivity to disturbance
4	• Low ratio of gonads to body volume • Long generation time • Permeable cuticle
5	• Longest lifespan • Low reproduction rate • Low metabolic rate • Small number of eggs • Extremely sensitive to disturbance

Table 3.2. Average gravimetric water content (H_2O (g)/ soil (g)) for all sampling timepoints (pre, peak, and post drought) under each rainfall treatment and for both land uses. Standard errors represent one deviation from the mean. Treatments with different letters represent significant differences of gravimetric water content within each timepoint across rainfall treatment and land use.

	Early Successional			No-till		
	Irrigated	Variable	Drought	Irrigated	Variable	Drought
Pre-drought	0.04 (0.01)a	0.05 (0.01)a	0.05 (0.00)a	0.11 (0.01)b	0.11 (0.01)b	0.12 (0.01)b
Peak-drought	0.15 (0)c	0.13 (0.02) bc	0.08 (0.02) a	0.11 (0.02)abc	0.08 (0.01)ab	0.09 (0.03)ab
Post-drought	0.17 (0.01)a	0.17 (0.01)a	0.15 (0.02)a	0.14 (0.02)a	0.13 (0.00)a	0.14 (0.01)a

Table 3.3. Permutation analysis of variance (PERMANOVA) of the effect of land use on nematode community structure during pre-drought. PERNAMOVA of the effect of rainfall on nematode community structure in each land use during peak, and post-drought. ·p<0.10*p<0.05, **p<0.01, ***p<0.001

Pre-drought				
Factor	F		p	
Land use	2.01		0.08·	
Peak-drought				
Factor	F		p	
	No-till	Early Successional	No-till	Early Successional
Rainfall	1.5	0.72	0.07·	0.61
Post-drought				
Factor	F		p	
	No-till	Early Successional	No-till	Early Successional
Rainfall	1.7	1.15	0.16	0.31

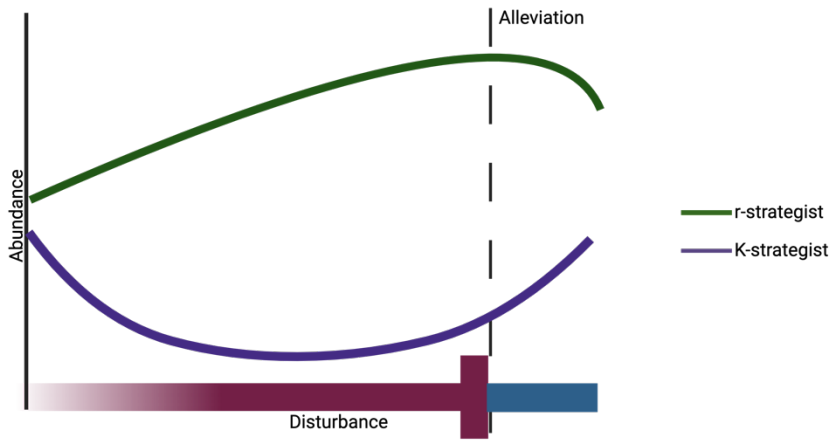

Figure 3.1. Hypothesized effect of increased disturbance and alleviation on *r* and *K* strategist organisms within the nematode community. Color represents abundance of *r* and K strategist nematodes.

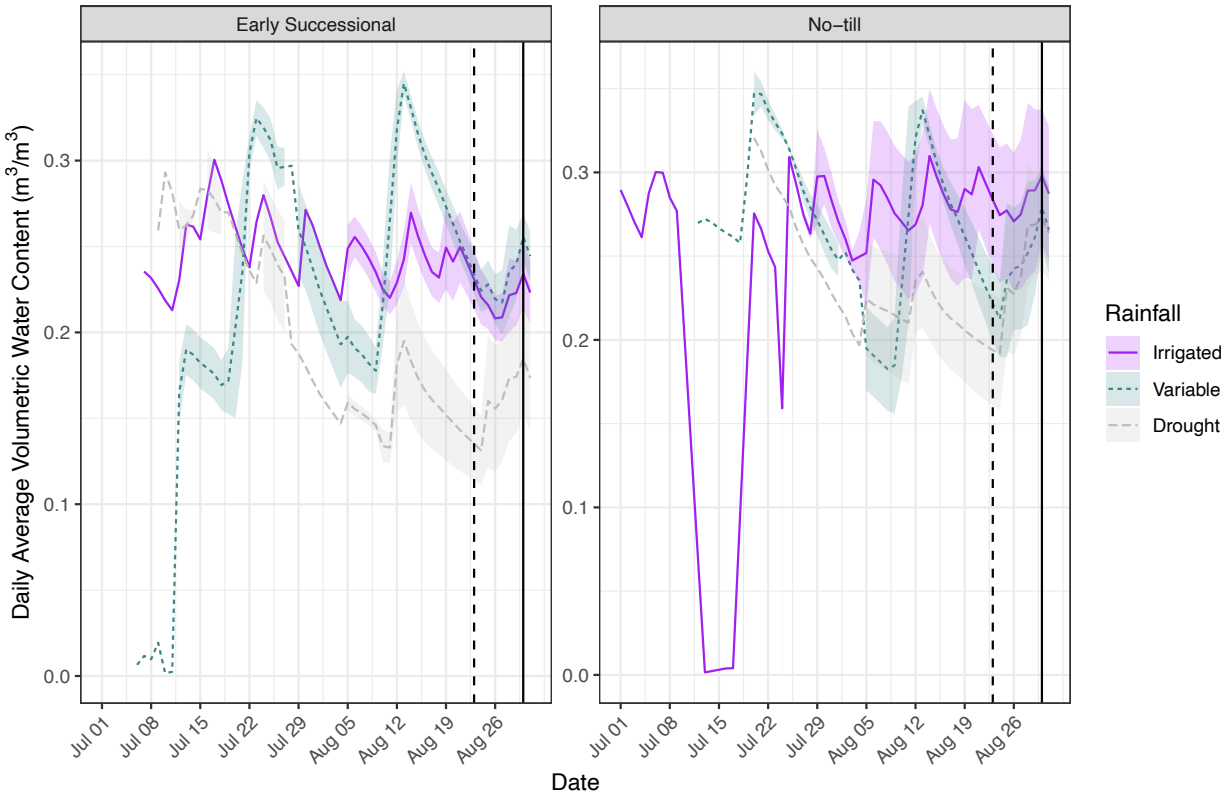

Figure 3.2. Daily average volumetric water content and standard error (shaded lines) for the early successional and no-till land uses within irrigated, variable, and drought rainfall treatments. Vertical dashed lines represent peak drought sampling timepoint (August 23, 2021), and vertical black lines represent post-drought sampling after rewetting (August 28, 2021). Data for all replicates of land use and rainfall combinations were recorded starting August 5, 2021.

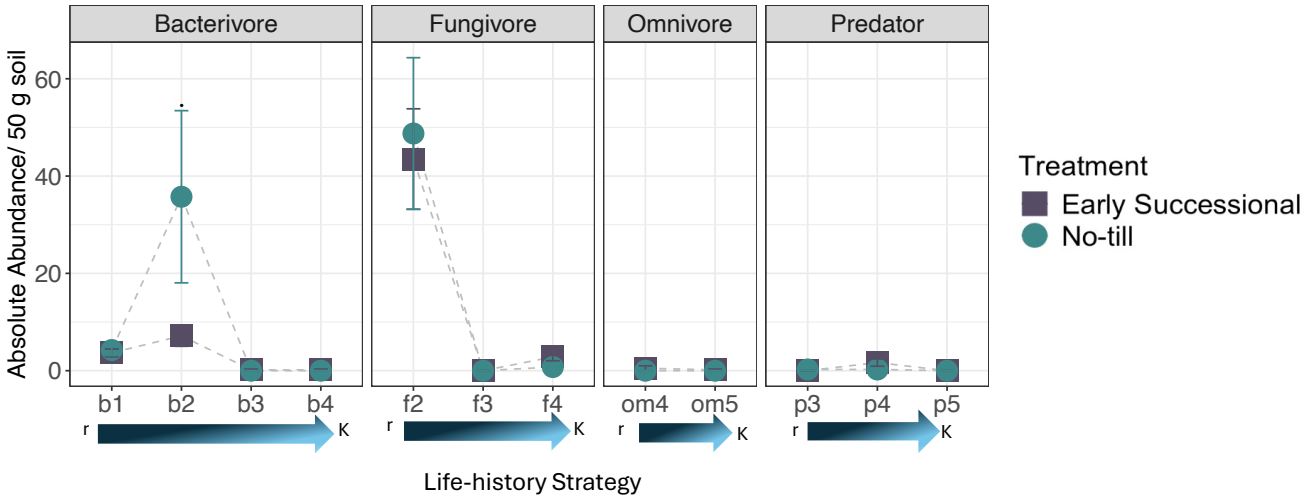

Figure 3.3. Nematode abundance on the *r-K* strategist continuum during pre-drought. Shape and color represent land use. Standard error represents +/- one standard deviation from the mean. *Represent significantly different values ($p<0.05$) of abundance within the corresponding feeding and cp group between each land use. ·$p<0.10$*$p<0.05$, **$p<0.01$, ***$p<0.001$

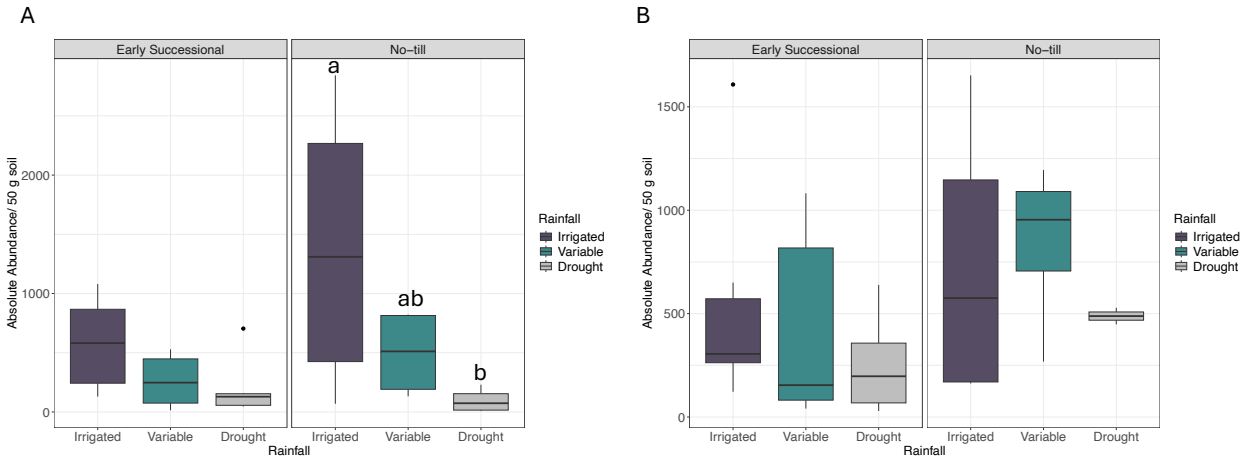

Figure 3.4. Total nematode abundance in the early successional and no-till land use across all rainfall manipulation treatments in A) peak-drought and B) post-drought. Standard error bars represent one standard deviation from the mean. Different letters represent significantly different values of abundance within each land use and across rainfall treatment.

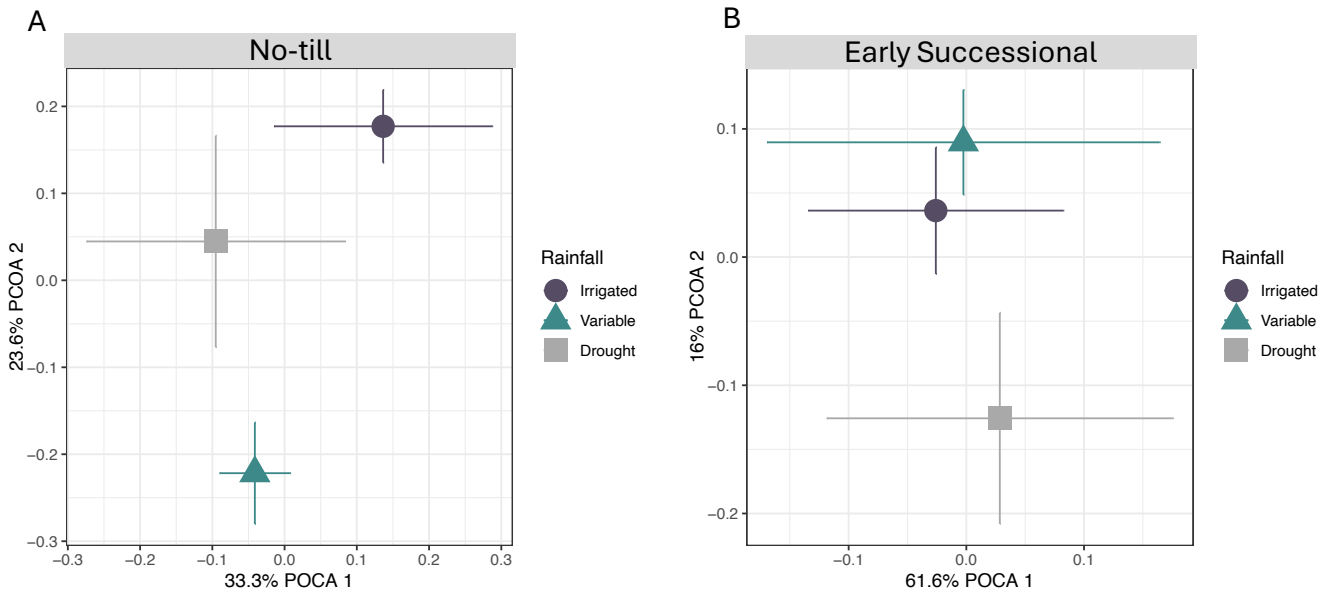

Figure 3.5. Principal coordinate analysis of peak-drought nematode communities within the A) No-till and B) Early Successional land use. X and Y error bars represent standard error from the mean. Color and shape represent rainfall treatment.

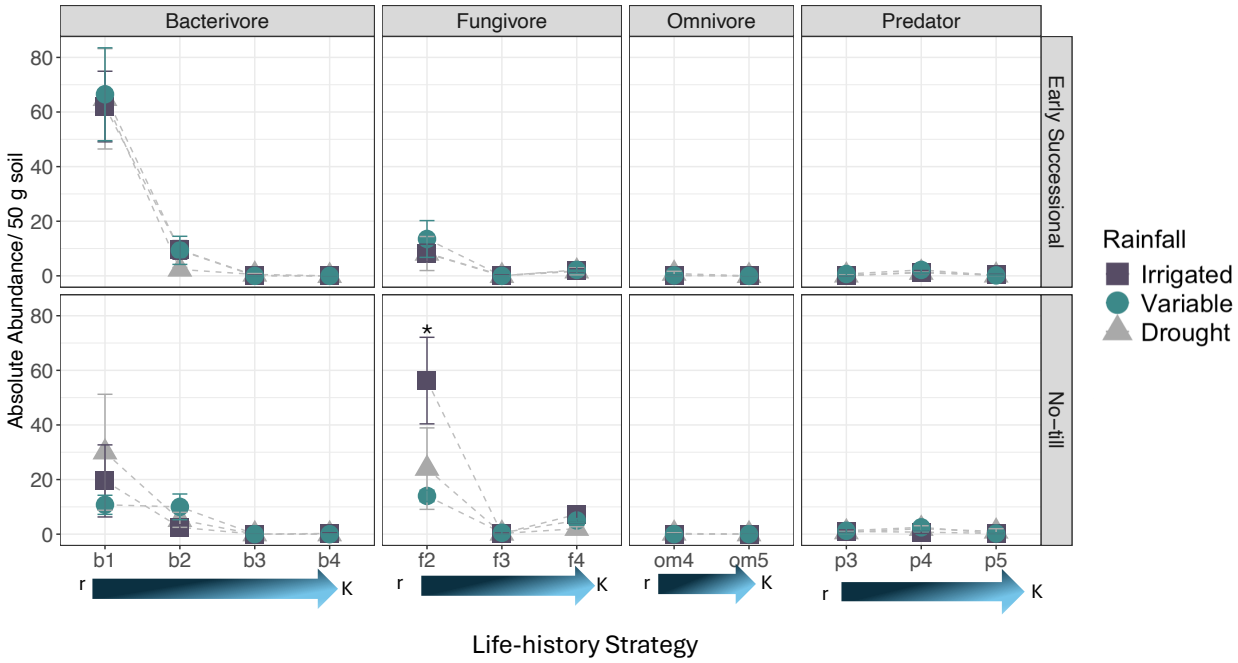

Figure 3.6. Nematode life-history strategy abundance on the *r-K* strategist continuum within each feeding group and land use during peak-drought. Shape and color represent rainfall treatment. Standard error represents +/- one standard deviation from the mean. *Represents significantly different values ($p<0.05$) of abundance within the corresponding feeding and cp group across rainfall treatment within each land use. ·$p<0.10$*$p<0.05$, **$p<0.01$, ***$p<0.001$

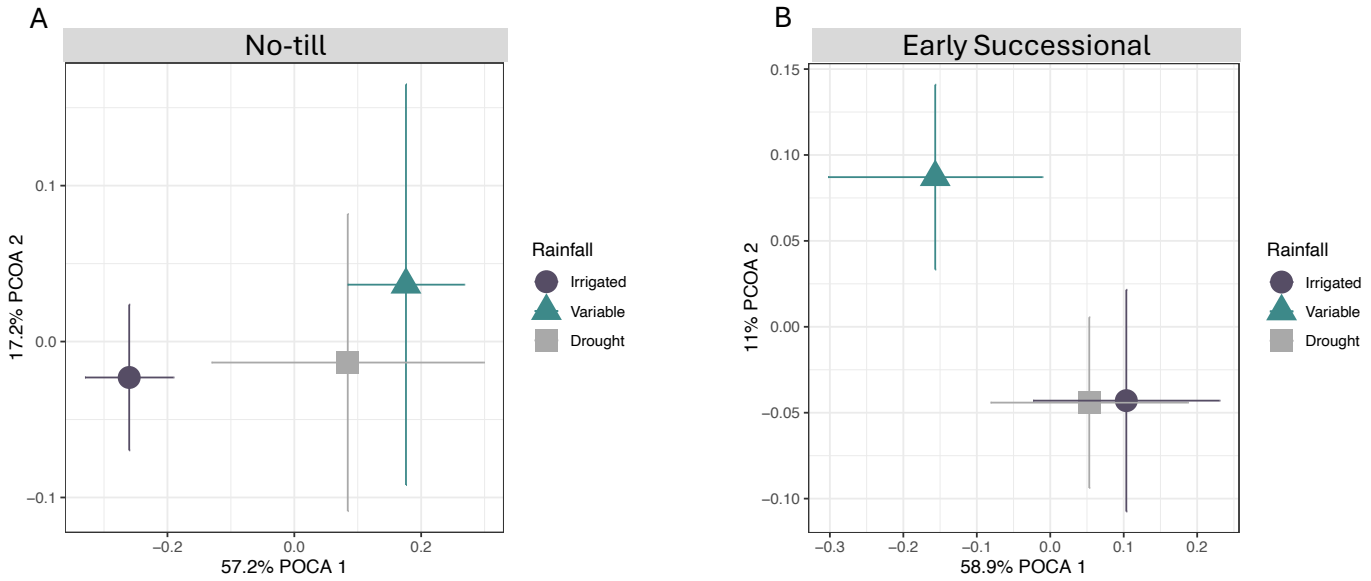

Figure 3.7. Principal coordinate ordination analysis of post-drought nematode communities within the A) No-till and B) Early Successional land use. X and Y error bars represent on standard error from the mean. Color and shape represent rainfall treatment.

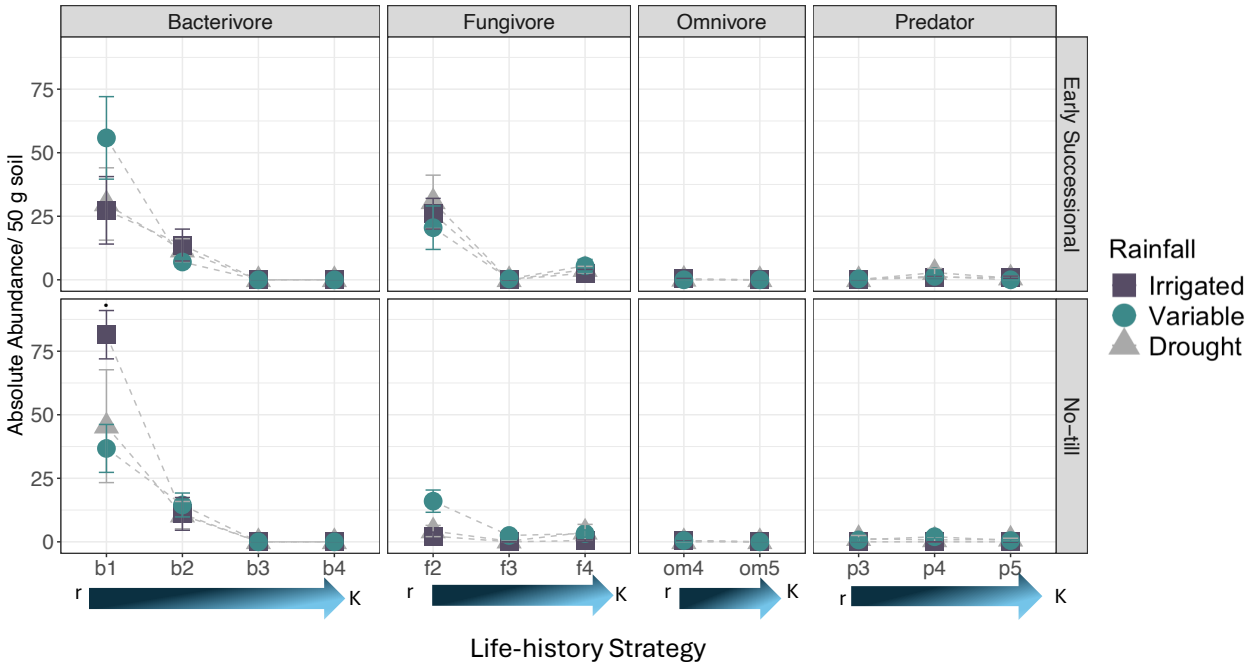

Figure 3.8. Nematode life-history strategy abundance on the *r-K* strategist continuum within each feeding group and land-use land use during post-drought. Shape and color represent rainfall treatment. Standard error represents +/- one standard deviation from the mean. * Represent significantly different values ($p<0.05$) of abundance within the corresponding feeding and cp group across rainfall treatment within each land use. ·$p<0.10$*$p<0.05$, **$p<0.01$, ***$p<0.001$

APPENDIX B: CHAPTER THREE SUPPLMENTAL

Table S3.1: Average and (SE) amount of water applied (liters) for the irrigated, variable, and drought shelters every week during experiment period.

Date	Irrigated		Variable		Drought	
	Early Successional	No-till	Early Successional	No-till	Early Successional	No-till
7/19/21	0	0	908 (8.3)	900 (0)	0	0
7/20/21	0	0	888 (8.3)	901 (9.2)	0	0
7/21/21	0	0	860 (30.5)	855 (45)	0	0
7/22/21	0	0	872 (13.5)	910 (12.9)	0	0
7/23/21	892 (13)	897.5 (6.61)	0	0	0	0
7/28/21	877(12.0)	865 (15)	0	0	0	0
7/29/21	850 (5.8)	890 (0)	0	0	0	0
8/4/21	858 (1.7)	850 (0)	0	0	0	0
8/5/21	860 (0)	870 (0)	0	0	0	0
8/9/21	0	0	880 (0)	870 (0)	0	0
8/10/21	0	0	872 (3.1)	842 (16.5)	0	0
8/11/21	0	0	843 (6.7)	800 (20)	0	0
8/12/21	0	0	849 (3.3)	852 (10.3)	0	0
8/13/21	813 (29.1)	869(3.15)	0	0	0	0
8/18/21	898 (11.7)	897 (2.5)	0	0	0	0
8/20/21	858 (13.6)	912 (27.5)	0	0	0	0
8/27/21	873 (10.1)	840 (30)	860 (11.5)	865 (15)	853 (20.3)	850 (20)

Table S3.1(cont'd)

8/29/21	863 (6.7)	862 (12.5)	847 (8.8)	852 (12.5)	853 (3.3)	880 (0)

Table S3.2. Permutation analysis of variance (PERMANOVA) with a post-hoc power analysis conducted of nematode communities in pre, peak, and post-drought. $^{+}$p<0.01*p<0.05, **p<0.01, ***p<0.001

Factor	F	p	Power
	Pre-drought		
Land use	2.01	0.08^{+}	0.27
	Peak-drought		
Land use	7.24	0.001***	0.41
Rainfall	0.86	0.50	0.09
Land use * Rainfall	1.50	0.17	0.16
	Post-drought		
Land use	2.58	0.05*	0.16
Rainfall	0.62	0.77	0.08
Land use * Rainfall	2.12	0.06 $^{+}$	0.25

Table S3.3. ANOVA for gravimetric water content (H_2O (g)/ soil (g)) for all sampling timepoints (pre, peak, and post drought).

	F	p
Land use	0.15	0.70
Timepoint	50.72	<0.001
Rainfall	2.29	0.11
Land use*Timepoint	31.10	<0.001
Land use*Rainfall	2.08	0.13
Timepoint*Rainfall	2.97	0.02
Land use*Timepoint*Rainfall	0.64	0.63

Table S3.4. ANOVA for daily average volumetric water content (m^3/m^3).

	F	p
Land use	2.18	0.18
Rainfall	155.14	<0.001
Land use*Timepoint	11.94	<0.001

Table S3.5. Indicator taxa and p-values of significant taxa in each land use during pre-drought.

Genus	Statistic	P-value
	Early Successional	
Paratylenchus	0.99	0.012
	No-till	
Pratylenchidae	1	0.007

Table S3.6. Analysis of Variance table of the effect of land use on bacterivore c-p 2 nematode abundance during pre-drought.

Factor	F-value	P-value
Land use	4.21	0.09

Table S3.7. Pre-drought average nematode abundance (%) and (SE) of nematode feeding groups in early successional and no-till land uses. Feeding groups with different letters indicate significant differences at <0.10 between land use.

Land use	Bacterivore	Fungivore	Plant Parasitic	Predator/ Omnivore	Fungivore:Bacterivore	Parasitic:Free-living
Early Successional	11.2 (1.7) b	46.3 (10.6)	33.5 (11)	2.3 (1.34)	4.2 (1)	1.3 (0.9)
No-till	40 (16.6) a	49.5 (15.9)	9.7 (4.9)	0.5 (0.3)	3.5 (2.3)	0.1 (0.1)

Table S3.8. ANOVA table of pre-drought nematode feeding groups where land use was a fixed factor.

Factor	Bacterivore		Fungivore		Plant Parasitic		Predator/Omnivore		Fungivore:Bacterivore		Parasitic:Free-living	
	F	P	F	P	F	P	F	P	F	P	F	P
Land use	5.08	0.07.	0.03	0.87	2.41	0.29	1.1	0.32	0.09	0.76	1.68	0.28

Table S3.9. ANOVA table of land use, rainfall, and the interaction of land use and rainfall on total nematode abundance.

Factor	F-value	P-value
	Pre-drought	
Land use	0.01	0.91
	Peak-drought	
Land use	1.9	0.20
Rainfall	6.1	0.01**
Land use*Rainfall	1.87	0.19
	Post-drought	
Land use	2.14	0.16
Rainfall	0.71	0.51
Land use*Rainfall	0.14	0.87

Table S3.10. Indicator taxa and p-values of significant taxa in each land use and rainfall treatment during peak-drought.

Genus	Statistic	P-value
	No-till Drought	
Tylenchidae	0.7	0.02
	No-till Irrigated	
Aphelenchoides	0.7	0.02
	No-till Variable	
Paratylenchidae	0.8	0.007

Table S3.11. Analysis of Variance table of the effect of land use, rainfall, and the interaction effect of land use and rainfall on bacterivore cp-1, bacterivore cp-2, and fungivore cp-2 nematode abundances during peak-drought.

Factor	F-value	P-value
Bacterivore cp-1		
Land use	8.23	0.02
Rainfall	0.18	0.83
Land use*Rainfall	0.24	0.79
Bacterivore cp-2		
Land use	0.15	0.70
Rainfall	1.33	0.28
Land use*Rainfall	1.02	0.38
Fungivore cp-2		
Land use	9.24	0.006
Rainfall	2.74	0.08
Land use*Rainfall	3.93	0.03

Table S3.12. Peak-drought average and standard error of nematode of nematode feeding groups across rainfall treatments within early successional and no-till systems. Means separation indicates different nematode abundances across rainfall treatments but within each land use.

Rainfall	Bacterivore	Fungivore	Plant Parasitic	Predator/ Omnivore	Fungivore:Bacterivore	Parasitic:Free-living
			Early Successional			
Drought	67.7 (17.7)	9.8 (7.5)	16.8 (8.6)	2.3 (1.1)	1.0 (1)	0.5 (0.3)
Variable	75 (12.5)	15.7 (7.6)	5.7 (3.8)	4 (2.6)	0.4 (0.2)	0.1(0.05)
Irrigated	71.7 (10)	9.7 (3.6)	12.3 (4.4)	2.2 (0.7)	0.2 (0.1)	0.2 (01)
			No-till			
Drought	36.5 (19.8)	26.2 (15.2) a	17 (6.9) ab	4 (2.4)	3.9 (3.4) ab	0.4 (0.3)
Variable	20.7 (5.3)	19.5 (1.7) a	34.5 (9.5) b	4.5 (2.1)	1.2 (0.3) a	0.8 (0.3)
Irrigated	22.7 (14.3)	63.7 (15.3) b	8.25 (2.5) a	2 (1.2)	13.8 (7.4) b	0.1 (0.03)

Table S3.13: Analysis of Variance of the effect of land use, rainfall, and all interaction effects on nematode feeding group abundance during peak-drought.

Factor	Bacterivore		Fungivore		Plant Parasitic		Predator/Omnivore		Fungivore:Bacterivore		Parasitic:Free-living	
	F	P	F	P	F	P	F	P	F	P	F	P
Land use	10.5	0.01	22.3	0.002**	2.5	0.1	0.2	0.7	6.8	0.01*	1.5	0.2
Rainfall	0.1	0.9	2.9	0.07	1.2	0.3	0.8	0.5	2.8	0.1	1.4	0.3
Land use*Rainfall	0.4	0.7	4.2	0.03*	3.7	0.04*	0.1	0.9	3.2	0.1	2.5	0.1

Table S3.14. Analysis of Variance table of the effect of land use, rainfall, and the interaction effects of land use and rainfall on nematode abundance of life-history strategies during post-drought.

Factor	F-value	P-value
Bacterivore c-p 1		
Land use	1.49	0.26
Rainfall	0.66	0.53
Land use*Rainfall	3.16	0.07
Fungivore c-p 2		
Land use	6.35	0.04
Rainfall	0.19	0.83
Land use*Rainfall	1.41	0.27

Table S3.15: Post-drought average and standard error of nematode of nematode feeding groups across rainfall treatments within early successional and no-till land uses. Means separation indicates different nematode abundances across rainfall treatment but within each land use.

Rainfall	Bacterivore	Fungivore	Plant Parasitic	Predator/ Omnivore	Fungivore:Bacterivore	Parasitic:Free-living
			Early Successional			
Drought	417 (12.0)	34.7 (11.2)	11 (6)	3.8 (2.4)	1.2 (0.4)	0.2 (0.1)
Variable	62.8 (15.1)	26.3 (10.6)	9.8 (3.8)	1.8 (0.9)	1.2 (0.7)	0.1 (0.05)
Irrigated	41.2 (11.5)	28.7 (7.03)	18.2 (4.9)	2.2 (0.6)	1.0 (0.3)	0.3 (0.1)
			No-till			
Drought	56.5 (21.9)	8.2 (3.1)	3.7 (2.5)	3.5 (1.5)	0.4 (0.2)	0.1 (0.1)
Variable	51.7 (10.5)	21.7 (5.1)	22.7 (5.8)	3.7 (1.2)	0.6 (0.3)	0.3 (0.1)
Irrigated	92.5 (3.1)	2.7 (1.7)	5 (1.8)	0.5 (0.3)	0.03 (0.02)	0.05 (0.02)

Table S3.16: Analysis of Variance of the effect of land use, rainfall, and all interaction effects on nematode feeding group abundance during post-drought.

Factor	Bacterivore		Fungivore		Plant Parasitic		Predator/Omnivore		Fungivore:Bacterivore		Parasitic:Free-living	
	F	P	F	P	F	P	F	P	F	P	F	P
Land use	2.6	0.1	5.0	0.05	0.4	0.5	0.0005	1	5.6	0.1	0.3	0.6
Rainfall	0.8	0.4	0.6	0.6	1.7	0.2	1.3	0.3	0.3	0.7	0.4	0.6
Land use*Rainfall	2.5	0.1	1.2	0.3	3.9	0.04	0.7	0.5	0.1	0.9	3.4	0.05

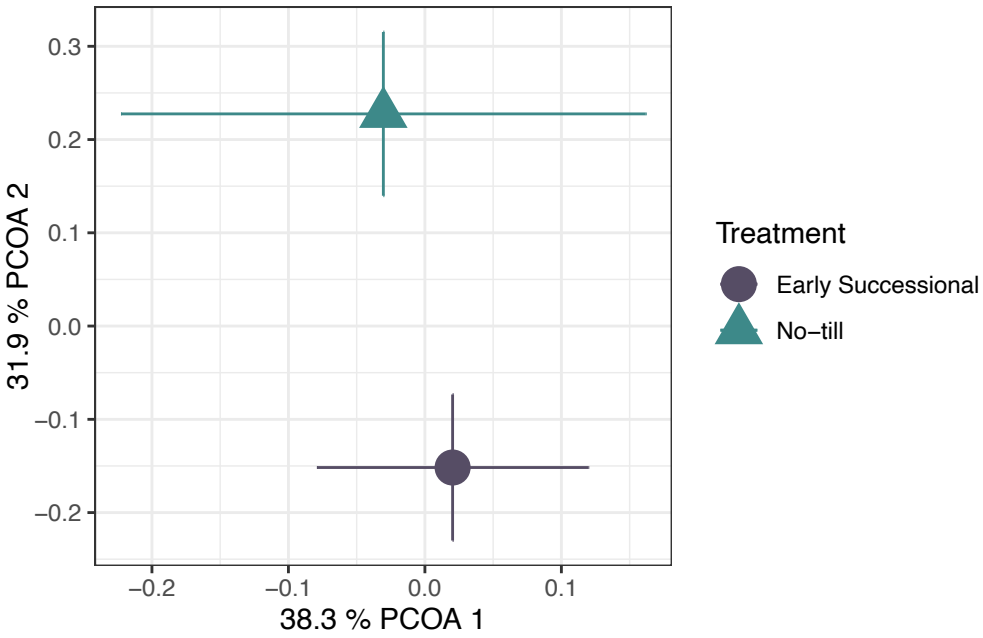

Figure S3.1. Principal coordinate ordination analysis of pre-drought nematode communities. X and Y error bars represent standard error from the mean. Land use is represented by color and shape.

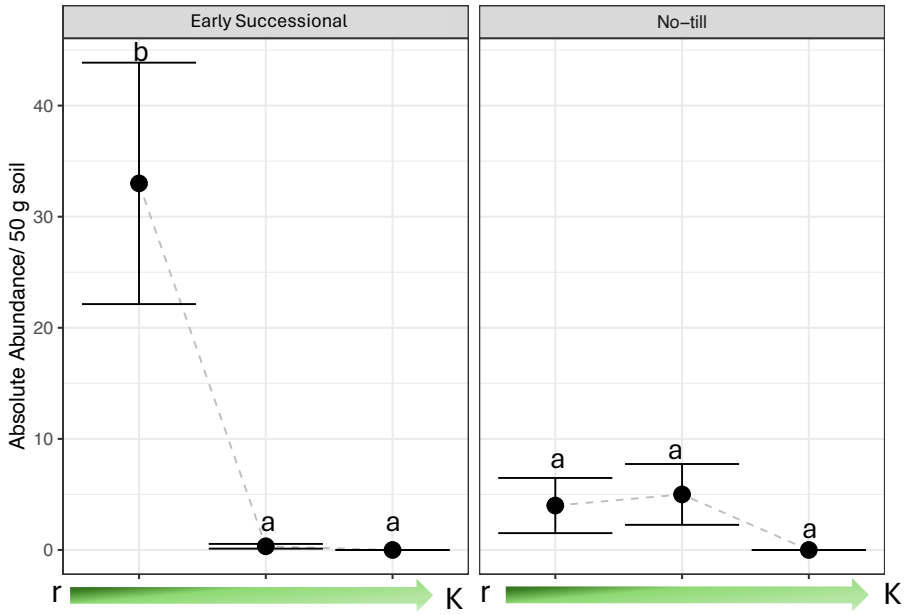

Fig S3.2. Plant parasitic nematode abundance on *the r-K* strategist continuum during pre-drought. Standard error represents +/- one standard deviation from the mean. Different letters represent significantly different values of abundance within each land use.

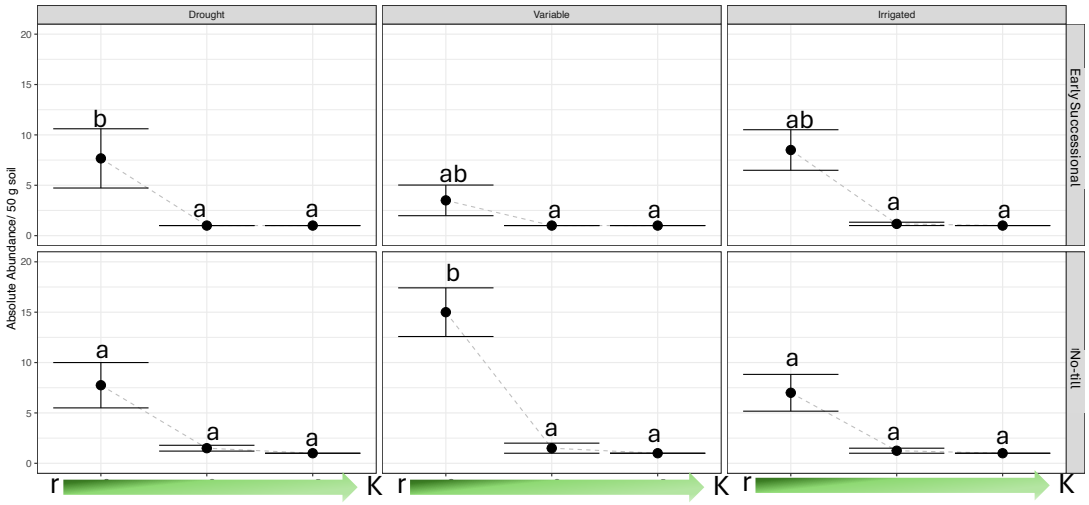

Figure S3.3. Plant parasitic life-history strategy abundance on the r-K strategist continuum within each land use and rainfall treatment during peak-drought. Standard error represents +/- one standard deviation from the mean. Different letters represent significantly different values of abundance within each land use and across rainfall treatment.

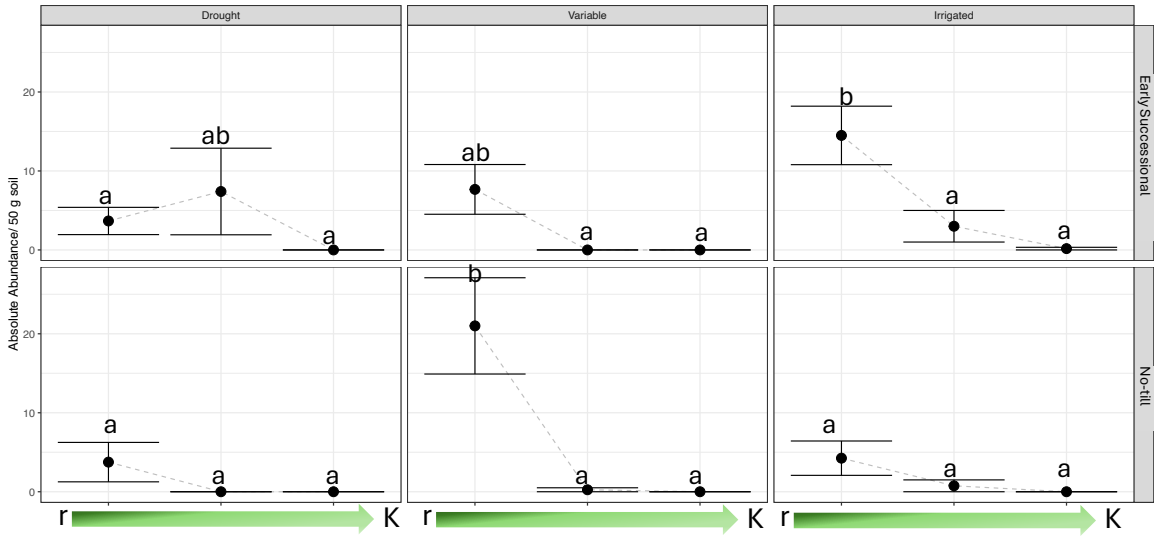

Figure S3.4. Plant parasitic life-history strategy abundance on the r-K strategist continuum within each land use and treatment during post-drought. Standard error represents +/- one standard deviation from the mean. Different letters represent significantly different values of abundance within each land use and across rainfall treatments.

CHAPTER 4: BACTERIVORE NEMATODES ARE ESSENTIAL FOR ENHANCED PLANT NITROGEN UPTAKE

ABSTRACT

Bacterivore nematodes (bacterivores) play a vital role in nitrogen (N) cycling through their consumption of bacterial communities, and their direct excretion of plant available ammonium. Maintained N cycling by the food web is imperative for agroecosystems that depend on biologically derived N for plant productivity. Currently, it is unclear if nematode species and their life-history strategies vary in their impact on N cycling. Additionally, it is unclear if soil N cycling is regulated by a single and dominant bacterivore species or if bacterivore diversity is needed for maintained N cycling. Thus, this study aims to 1) explore how the presence and absence of dominant bacterivores with different life-history strategies impact soil N pools and plant N use, and 2) assess how bacterial trophic channels interact with soil nitrogen use efficiency (NUE_{soil}) under the presence of varying bacterivore nematode diversity. This greenhouse microcosm experiment was conducted using soil collected from an organic farm that was removed of all soil fauna (defaunated). Microcosms were treated with four different nematode inoculums: *Acrobeloides nanus* (*A.nanus*), *Rhabditid intermedia* (*R,intermedia*), a co-inoculation of both species, and no nematodes. *A.nanus* and *R.intermedia* were selected because of their dominance in these soils and their variation in life-history strategies. Co-inoculation of bacterivores had the largest significant effect on organic N pools, where they were enhanced regardless of plant presence. Additionally, co-inoculum treatments influenced the relationships between total nematode abundance and belowground N, aboveground biomass, and belowground biomass. These treatments also enhanced the direct link between total nematode abundance and NUE_{soil}, while modifying total nematode abundance associations with bacterial α-diversity metrics. These results indicate that trophic level interactions between microbial communities and soil fauna are essential for overall N cycling. Moreover, this study indicates that the conservation of soil fauna with a diversity of life-history traits is essential for maintained ecosystem functioning.

INTRODUCTION

Free-living nematodes play an integral role in nitrogen (N) cycling (Freckman, 1988; Ingham et al., 1985). Most nematodes have a carbon (C) to nitrogen (N) ratio of 6, while their prey typically has a C:N ratio of 4, much of the excess nutrients that are not required are excreted as ammonia (Ferris et al., 1997). Moreover, bacterivore nematodes, hereafter referred to as

bacterivores, have been found to have the largest impact on N cycling, as bacterivores in the top 15 cm of soil mineralize approximately 1.01 μg N-soil^{-1}day^{-1} (Ferris et al., 1997). Taken together, it is estimated that bacterivores account for up to 30% of N mineralization in conventionally managed agroecosystems (Ingham et al., 1985; Neher, 2001). Given their direct effect on inorganic N pools, bacterivores also play a key role in plant growth and plant N uptake (Gebremikael et al., 2016; Trap et al., 2016). However, the mechanisms that link bacterivore activity to plant N uptake remain poorly understood (Martin & Sprunger, 2021). Addressing this uncertainty requires a better understanding of how bacterivores influence soil nitrogen use efficiency (NUE), broadly defined as the capacity of plants to effectively acquire N from the soil while minimizing N losses (Moll et al., 1982).

Trophic level interactions between nematodes and bacteria largely explain the mechanisms associated with inorganic N pools (Djigal et al., 2004). For instance, greater N mineralization is most likely caused by predator-prey interactions between bacterivores and bacteria. Bacterivores may alter bacterial community richness, biomass, and diversity by selectively feeding, modifying the availability of prey resources within an aggregate structure, and transporting bacteria through the soil matrix (Gebremikael et al., 2016; Irshad et al., 2011; Jiang et al., 2015). Moreover, through altering bacterial community structure there may be direct feed-back loops on N mineralization. For example, bacterivore grazing may alter the bacteria nitrifying community structure and may have more of an effect on N cycling than compared to the direct excretion of ammonium (Buchan et al., 2013; Maboreke et al., 2018). Although bacterivore predation can impact the bacteria prey community, these trophic interactions have seldom been linked to N cycling.

The impact that nematode communities have on ecosystem functioning is generally assessed at the feeding group level. That said, to understand bacterivore impacts on N cycling at a more granular level, species specific assessments are needed (Brondani et al., 2022; Djigal et al., 2004). Free-living nematode species span the *r-K* strategist continuum and have been categorized into their prospective *r-K* strategist groupings based on their life-history strategies (Bongers, 1990). Nematodes are categorized based on a colonizer-persisted (cp) scale, where cp-1 nematodes are sensitive to N enrichment, have short lifespans, and high reproductive rates. In contrast, cp-2 nematodes are more resistant and resilient to environmental disturbances, have longer lifespans, and lower reproductive rates. Lastly, cp-3, cp-4, and cp-5, nematodes

demonstrate increasing sensitivity to disturbance, longer lifespans, and further reduced reproductive rates (Ferris et al., 2001). Currently it is unclear if certain bacterivore species/life-history traits regulate N cycling, or if it is the coexistence/diversity of bacterivore species with varying life-history strategies that may enhance N cycling (Postma-Blaauw et al., 2005). These findings are imperative for understanding if soil biodiversity conservation efforts should also focus on the conservation of a diversity of life-history-strategies in addition to species.

This study aims to investigate how bacterivore species, and their coexistence impact bacteria community structure and N cycling. The first objective of this study is to explore how the presence and absence of dominant bacterivores with different life-history strategies impact soil N pools and plant N use. I hypothesize that bacterivores with faster reproductive capacity and greater community turnover will increase plant N uptake and enhance both soil organic N and inorganic N. My second objective is to assess how bacterial trophic channels influence soil NUE under the presence of varying bacterivore species. I hypothesize that 1) the co-inoculation of two nematode species will reduce bacterial community diversity but increase bacterial richness through selective grazing and 2) the co-inoculation of two nematodes will enhance NUE within a system through bacterial trophic channel predator-prey interactions.

METHODS

Soil collection and defaunation

Soil was collected from the 0-10 cm layer of an organically managed field plot at the W.K. Kellogg Biological Station (42° 24′ N, 85° 24′ W, Hickory Corners, Michigan, USA) prior to planting of corn in June 2023. The collection is an organically certified field that has been utilized for row crop and vegetable research since the early 2000's. Prior to being organically certified, it was used for conventional row crop research. Three-year crop rotations are typical at this site and include corn (*Zea mays*), soybean (*Glycine max*), and winter wheat (*Triticum aestivum*), with a rye (*Secale cereale*) cover crop following corn and a red clover (*Trifolium pratense*) cover crop following wheat. Semi-solid cattle or poultry manure are added prior to corn or wheat crops. The organic field had an existing cover crop of red clover (*Trifolium pratense)* that was roto-tilled prior to soil collection. A light rate of solid manure was added in September of 2021, after which, no external inputs have been added. A subsample from the collected soil (~100 g) was taken for initial nematode community analysis. Soil was stored at 4°C until it was ready for defaunation. Free-living nematodes were removed from the collected

soil through sieving at 6.25 mm, wetting soils, cooling at 4 °C for 24 h, and then heating at 65 °C for 48 h (Franco et al., 2017). Baermann funnels were utilized to extract nematodes from a subsample of the defaunated soil and counts of <10 individuals/ 50 g soil confirmed that the defaunation removed ~99% of the original population.

Nematode inoculum preparation

An initial nematode community analysis from the subsample of the collected soil was conducted to assess the most dominant bacterivore nematodes. Only bacterivore nematode adults were able to be identified to the species level. The most dominant bacterivore nematodes were *Aphelenchoides nanu*s (*A. nanus*) and *Rhabditid intermedia* (*R. intermedia*). *Rhabditid intermedia* is classified as a cp-1 nematode, where this specie rapidly responds to highly enriched conditions, has fast reproduction rates, and a short lifespan. *A. nanus* is classified as a cp-2 nematode, which forms the basis of the soil food web, has slow reproductive rates, is resilient to disturbance, and has a longer lifespan. From this subsample one female individual from both nematode species was isolated and cultures were created through nematode growth on media agar seeded with *E. coli* OPO50. Cultures were maintained until populations met the quota of individuals needed for inoculation (~216,000 *A. nanus* individuals and ~324,000 *R. intermedia* individuals). Abundance of each species for inoculation was calculated based on the natural population that was identified using 50 g of field soil.

Treatment and experimental setup

This microcosm experiment was conducted in a greenhouse at the W.K. Kellogg Biological Station (42° 24′ N, 85° 24′ W). Methods for microcosm assembly were adapted from Franco et al., (2020). Each microcosm consisted of PVC pipes of 10 cm diameter and 30 cm height that were filled with 600 g of sterilized nematode-free sand and 750 g of defaunated soil (Franco et al., 2017). The sand at the bottom was utilized to allow drainage. A cap with a small hole was attached to the bottom of the PVC pipe to allow for drainage of water. A total of 144 microcosms were assembled. Treatments for this microcosm experiment consisted of three factors: plant presence (plant vs. no plant), destructive sampling timepoint, hereafter referred to as "Day", (Day 0, 17, or 24), and nematode inoculation (*A. nanus, R. intermedia, A. nanus + R .intermedia*, or no nematodes added). I selected corn as my study plant to replicate the crop planted in 2021 at the field site where soil was collected. There were six replicates for each treatment combination. The experimental design of this experiment was a randomized complete block design, where there

were three replicates of each treatment within the three blocks (greenhouse benches), to control for temperature and light differences within the greenhouse. All microcosms were watered evenly to maintain 50% water holding capacity. Soil moisture was assessed on each sampling day and showed no significant differences within each day although soil moisture did vary across sampling timepoint (Table S4.1; Table S4.2).

Plant Treatments

To assess if nematode impact on N cycling shifted with or without a plant, half of the microcosms were planted with three corn seeds. Two weeks after germination, all but one corn seedling was removed from the microcosm, to allow for a single plant to grow for the duration of the study.

Nematode treatments and inoculations

Nematode inoculations were conducted three days after the excess corn plants were removed. Nematode treatments consisted of an inoculation of *A. nanus*, an inoculation of *R. intermedia,* a co-inoculation of both *A. nanus* and *R. intermedia,* and a control H_2O inoculation containing no nematodes. The number of nematodes to be inoculated was calculated based on the natural population that was identified using 50 g of field soil. Specifically, ~2 *A. nanus* adults were identified per 1 g of soil and ~3 *R. intermedia* adults were identified per 1 g soil. Thus, for microcosms with 750 g soil the *A. nanus* microcosms were inoculated with ~1,500 nematodes, *R. intermedia* microcosms were inoculated with ~2,250 nematodes, and the co-inoculated microcosms with *A. nanus* and *R. intermedia* were inoculated with ~2,250 and ~1,500 individual nematodes, respectively. Inoculums for each nematode species were prepared through extracting the cultured nematodes using a Baermann funnel apparatus for 24 h. After, extracted nematodes were concentrated into a 1000 mL media jar. Number of nematodes per 1 mL of water were determined and a dilution factor was determined to make sure that 5 mL of water consisted of ~1,500 individuals of *A. nanus* nematodes and ~2,250 individuals of *R. intermedia*. The diluted nematode solutions were the final inoculum used for the microcosm. A control inoculum of deionized water was used for microcosms receiving no nematodes. At day 0 of the experiment 36 microcosms were inoculated with *A. nanus*, *R. intermedia,* a co-inoculation of both *A. nanus*, *R. intermedia,* or water. Inoculations were conducted on "day 0" of the experiment through gently pipetting 5 mL of the corresponding nematode species from the inoculation jar – that was being constantly stirred – into 2-cm deep holes within the soil. For the duration of the experiment the

greenhouse conditions were 20-27 °C and with a consistent photoperiod of 14-hr light/10-hr dark.

Microcosm harvesting

Microcosms were harvested on days 0, 17, and 48 of the experiment. On day 0 microcosm harvesting occurred immediately after the inoculation of nematodes took place. Prior to each harvest, the growth stage for corn plants was recorded. Next, the shoots were clipped at soil level and stored in a paper bag. Then the soil contents were emptied from the microcosm and roots were handpicked, washed over a 2mm mesh sieve, and stored in a separate paper bag. Both the shoots and roots were dried at 65 °C for 48 h, weighed for dry weight, ground using a mortar and pestle, and sieved <2mm. All soil was gently homogenized and immediately subsampled (~3g) and stored at -80 °C for metabarcoding analyses. Soil for nematode community analysis (~100g) was subsampled and stored at 4 °C until further processing. Soils were subsampled for organic and inorganic N pools and dried at 65 °C and ground <2mm. Gravimetric water content was determined immediately, through first weighing 50 g of soil, drying at 65 °C 24 h, and recording the dried weight.

Analyses

Plant and soil analyses

Plant (above and belowground) and soil N were measured using a CHNS elemental analyzer (Costech Elemental Combustion System 4010, Costech Analytical Technologies, Valencia, CA, USA). Methods adapted from Doane & Horwáth, (2003) and Sinsabaugh et al., (2000) were utilized to measure nitrate and ammonium, respectively. Ammonium and nitrate were extracted using 2 M KCl combined with the prepared soil (3g). Supernatant from this extraction was then used for ammonium and nitrate analyses. For ammonium, sample supernatant was mixed with Ammonium salycilate and Ammonium cynurate in a 96 well-plate. After, the well plate was left in a dark drawer for 20 mins and the plate was then analyzed using a spectrophotometer plate reader at 630 nm. Standards from the ammonium analysis were made from an ammonium standard stock solution (100 ppm). Specifically, this stock solution was made using $(NH_4)_2SO_4$ diluted with 2M KCl. The stock solution was then diluted to make standard concentrations of 0, 1, 2, 5, 10, 20, 40, and 60 ppm. Nitrate analysis was conducted through mixing sample supernatant with a Vanadium reagent using a 96 well-plate. After, the well plate was left in a dark drawer for 5 h and the plate was then analyzed using

spectrophotometer plate reader at 540 nm. Standards for the nitrate analysis were made from a nitrate standard stock solution (100 ppm). Specifically, this stock solution was made using KNO_3 diluted with 2M KCl. The stock solution was then diluted to make standard concentrations of 0, 1, 2, 5, 10, 20, 40, and 60 ppm.

Autoclave citrate extractable (ACE) protein was measured to estimate the organic N pool within each microcosm (Hurisso et al., 2018). Briefly sodium citrate was added to 3g of soil, shaken for 5 min, and autoclaved at 121 °C for 30 min, cooled, and centrifuged. Then, the colorimetric bicinchoninic-acid (BCA) assay (Thermo Scientific, Pierce, Rockford, IL) was used to measure the concentration of ACE protein. Colorimetric measurements were quantified using a 96-well spectrophotometric plate reader.

Nematode analysis

At each destructive sampling timepoint, nematodes from each microcosm were extracted from soil (50g) using a Baermann funnel apparatus. Nematodes were then fixed in 4% paraformaldehyde, counted to obtain total abundance, and identified to genus if possible (Bongers, 1990). Nematodes that were in the Cephalobidae and Rhabditidae family were identified to species, if possible, to obtain the number of inoculated individuals that persisted in the microcosms.

Metabarcoding

The bacterial community was characterized through 16S rRNA sequencing. DNA was first extracted from soils stored at -80 °C using the MagAttract® PowerSoil® Pro DNA Kit with the KingFisher™ Flex System. DNA concentration and quality was measured using a Nanodrop Spectrophotometer. DNA was then sent to North Carolina State University Genome Sequencing Laboratory (Raleigh, NC, USA) for library preparation and sequencing. Libraries for amplicon sequencing tarted the bacterial community (16s rRNA, primers 515F and 860R). Pooled libraries were sequences using an Illumina MiSeq instrument on a 250 PE flow cell.

The raw data was processed using the Nexflow nfcore/ampliseq pipeline version 2.11.0 (Ewals et al., 2020). Within this pipeline, the raw data was quality-checked using FastQC and adapters were trimmed with Cutadapt. Reads were then processed with DADA2 to perform quality filtering, ASV identification, chimera removal and taxonomy assignment using the SILVA database v138 (Quast et al. 2013). Sequences of each ASV were aligned using MAFFT (Katoh & Standley, 2013) and a phylogenetic tree was built using FastTree (Price et al., 2009).

The ASV table, the taxonomic information for each ASV, the sample metadata, and the ASV phylogenetic tree were grouped using *phyloseq* v1.46 ((McMurdie & Holmes, 2013). Singletons and sequences identified as "chloroplast" or "mitochondria" were discarded before downstream analyses. Microbiome data were also normalized before calculating relative abundances using the {transform} function from the *microbiome* (Lahti & Shetty, 2012) package in R. Sequencing generated 15,609,997 reads, which, after filtering, resulted in 9,345,490 reads (~ 62,303 / sample).

Statistical analysis and equations

Soil NUE (NUE_{soil}) was calculated according to (Moll et al., 1982) and as shown below.

$$Equation\ 1: NUE_{soil}\left(\frac{g}{g}\right) = \frac{Root\ N\left(\frac{g}{microcosm}\right) + Aboveground\ N\left(\frac{g}{microcosm}\right)}{Total\ Soil\ N\left(\frac{g}{microcosm}\right)}$$

I fit Tweedie generalized linear mixed-effect models with a log-transformed response variable using the *lme4* (Bates et al., 2015) package to assess the effect of nematode species treatment, plant presence, and day on ammonium, nitrate, aboveground N, belowground and aboveground biomass, bacterial richness and diversity, and nematode abundance. For response variables of belowground N and protein normal generalized linear models were conducted the *lme4* (Bates et al., 2015) package. Additionally, a square root transformation was conducted prior to conducting a normal generalized linear model for soil moisture. The factors of day, plant presence, nematode species, and their interactions were treated as fixed factors, while block and the replicate within each block were treated as random factors. Normality was assessed using studentized residuals with *Mass* in R (Venables & Ripley, 2002). Unequal variance was assessed using Levene's test. Post-hoc contrasts were obtained using the *emmeans* package (Lenth, 2023) in R. Tukey's adjustment was utilized to control for significant differences.

To evaluate the effects of total nematode abundance and inoculum treatment on aboveground and belowground biomass, as well as their nitrogen concentrations, I conducted a linear model including both main effects and their interaction. The inoculum treatment with no added nematodes was used as the intercept, meaning all significant effects of inoculum, abundance, or their interaction are interpreted as significantly different from the no-inoculum treatment. A two-way ANOVA was performed using the {lm} function in R, with model fitting conducted via the *lme4* package. Model assumptions, including normality and homoscedasticity, were assessed using diagnostic plots.

Four structural equation models (SEM) were built for each inoculum treatment using data collected from the plant microcosm on day 47. SEM were conducted using *lavaan* (Rosseel, 2012) and *psych* (William Revelle, 2023) packages in R. For each SEM, I tested the direct effect of total nematode abundance, bacteria richness, and bacteria diversity on NUE_{soil}. Additionally, I tested the direct effect of total nematode abundance on bacteria richness and bacteria diversity. I tested the indirect effect of total nematode abundance on NUE_{soil} through bacteria richness and diversity. I chose total nematode abundance as my indicator of the nematode community given that this is a commonly used metric to assess the nematode trophic level and was directly manipulated by my study. Bacteria richness and diversity were used as indicators of the bacterial microbial community given that these α-diversity metrics reflect overall community complexity and ecological stability, providing insight into microbial responses to manipulations of the bacterivore nematode trophic level. A confirmatory factor analysis was conducted on the proposed models, and AIC, RMSEA, and CFI indices were calculated to assess model fit. Significant relationships were determined at $p \leq 0.10$.

RESULTS

Trends suggest that total nematode abundance, as well as *R. intermedia* and *A. nanus* abundances, generally increased over the length of the experiment, with the highest abundances observed on day 47 ($p_{Day}<0.001$; Fig. 4.1). However, in plant treatments, *R. intermedia* abundance was only maintained after day 47 ($p_{Day*plant}<0.001$;Fig. 4.1d). Although not significantly different, trends suggest that by day 47 total nematode abundance was on average 51 % greater in microcosms that had a nematode inoculation regardless of whether a plant was present (Fig.4.1a; Fig.4,1b; Table S4.3). Given that nematode communities recover even after defaunation, by day 47 of the experiment other nematode feeding groups were present. However, bacterivore nematodes made up the largest proportion (Fig. S4.1). Additionally, *A. nanus* composited the largest proportion of bacterivores by day 47 (Fig. S4.2). The abundance of *R. intermedia* was noticeably reduced in all microcosms, suggesting that this species had trouble surviving within the microcosms (Fig. 4.1c; Fig. 4.1d). However, *R. intermedia* abundances were still significantly greater in no plant inoculation treatments that contained *R. intermedia* (Fig.1c; Table S4.4). Within each day, *A. nanus* abundances were not significantly different across inoculum treatments. However, trends suggest that abundances were, on average, two-fold greater in no-plant microcosms with nematode inoculation (Fig. 4.1e; Fig. 4.1f; Table S4.5).

Additionally, in plant microcosms, inoculum treatments containing *A. nanus* had ~25% greater *A. nanus* abundances compared to the other inoculum treatments (Fig. 4.1f; Table S4.5). Both inorganic N pools of ammonium and nitrate differed by day and were also affected by the inoculum treatment and plant presence. In both plant and no-plant treatments, ammonium was initially highest in microcosms with a co-inoculation on day 0 ($p_{\text{Inoculum}*} p_{\text{Day}} <0.001$; Fig. 4.2a; Fig. 4.2b). However, by day 47, microcosms without an inoculum had the greatest ammonium concentrations ($p_{\text{Inoculum}*} p_{\text{Day}} <0.001$; Fig. 4.2a; Fig. 4.2b; Table S4.6). Nitrate was substantially reduced by day 47 in the plant microcosms ($p_{\text{Day}*} p_{\text{Plant}} <0.001$; Fig. 4.2d), however, the no plant microcosm had on average, 22% greater nitrate in microcosms that had a co-inoculum when compared to microcosms that had no inoculation ($p<0.05$; Fig. 4.2c; Table S4.7). In contrast, total nitrogen indicated no significant differences by day, inoculum, or plant presence (Table S4.8; Table S4.9).

Soil protein, an indicator of the organic N pool was significantly affected by both day and inoculum, while trends did not differ across plant presence ($p_{\text{Inoculum}*} p_{\text{Day}} <0.05$; Table S4.10). Protein appeared to substantially increase by day in most inoculum treatments except for the *A. nanus* treatment in the no plant microcosm (Fig.4.2e) and *R. intermedia* treatments in plant microcosm (Fig. 4.2f), where both were reduced after day 17. Overall microcosms that had co-inoculations of *R. intermedia* and *A. nanus* had 21 % greater soil protein by day 47 regardless of plant presence, when compared to all other inoculation treatments (Fig. 4.2e; Fig. 4.2f). Notably, in microcosms without plants, soil protein was significantly greater in co-inoculum treatments compared to those with only an *A. nanus* inoculation (Fig. 4.2e). In plant microcosms, co-inoculum treatments had significantly greater soil protein than all other microcosm treatments (Fig. 4.2f)

Inoculum had no significant effect on aboveground and belowground plant biomass (Table 1) as well as aboveground and belowground total N (Table 2). However, all measures were significantly affected by day ($p_{Day}<0.001$), where aboveground and belowground biomass increased over time (Table 4.1; Table S4.11). Additionally, both aboveground and belowground total N declined over time (Table 4.2; Table S4.11). Similar trends were present for NUE_{soil}, where only day had a significant effect ($p_{Day}<0.001$), with NUE_{soil} increasing overtime (Table 4.3; Table S4.12).

I conducted linear models to understand the effect of total nematode abundance manipulations (defaunation) and inoculation treatment on plant measures at the end of the experimental period (day 47). My goal was to understand how nematode abundance, inoculation treatments, and their interaction influenced plant biomass and N relative to the no inoculation treatment. The results suggest that inoculation treatments, and their interaction with total nematode abundance, have varying effects relative to the no inoculum treatments. Co-inoculum treatments showed a marginally significant negative effect on aboveground N ($p_{R.intermedis+A.nanus}$<0.10), while the *R.intermedia* treatment exhibited a significant negative effect on aboveground N relative to the no inoculation treatment. Furthermore, the negative relationship between aboveground N and nematode abundance appeared to be moderated by the inoculation treatment, particularly for *R.intermedia* ($p_{R,intermedia*\text{Abundance}}$<0.10; Fig. 4.3a). In contrast, the relationship between belowground N and nematode abundance was only marginally moderated by the co-inoculum treatment ($p_{R.intermedis+A.nanus*\text{Abundance}}$<0.10; Fig. 4.3b). Moreover, co-inoculation treatments generally drove increased positive relationships between belowground N and total nematode abundance relative to the no-inoculum treatment (Fig. 4.3b). Positive relationships between nematode abundance and both aboveground and belowground biomass were significantly dependent on the co-inoculation treatment (Fig. 4.3c, 4.3d). Specifically, co-inoculum treatments enhanced the positive relationship between aboveground and belowground biomass and nematode abundance relative to the no-inoculum treatments. However, only the positive relationship between nematode abundance and aboveground biomass was moderated by individual *R. intermedia* and *A. nanus* inoculation treatments ($p_{R.intermedia*\text{Abundance}}$<0.10; $p_{A.nanus*\text{Abundance}}$<0.10; Fig. 4.3c).

Bacteria community richness, and evenness was most affected by where on average, regardless of inoculum treatment and plant presence, richness increased by 53% (p_{Day}<0.001), and evenness by 20% (p_{Day}<0.01), after 47 days (Table 4.4; Table S4.13). The inoculum treatment significantly effected bacteria richness (p_{Inoculum}<0.05), and diversity ($p_{\text{Inoculum*Day}}$<0.01), in plant microcosms on day 0 where *A. nanus* treatments had significantly reduced bacteria richness and diversity (Table 4.4). Evenness was significantly affected by the inoculum treatment in no plant microcosms on day 47 ($p_{\text{Inoculum*Day*Plant}}$<0.01), where the co-inoculation resulted in significantly reduced bacteria evenness (Table 4.4).

I conducted SEMs for each nematode inoculum for day 47 plant microcosms to understand the indirect and direct relationships between nematode abundance and NUE_{soil} within individual inoculation treatments. My results indicate that the effect of total nematode abundance, bacteria diversity, bacteria richness on NUE_{soil} differ based on nematode inoculums (Table S14). Total nematode abundance had a direct positive relationship with NUE_{soil} in co-inoculum and *R. intermedia* treatments, however, the *A. nanus* treatment had no significant relationship. Additionally, the no inoculum treatment had a negative relationship between total nematode abundance and NUE_{soil} (Fig. 4.4). The co-inoculation SEM indicates that total nematode abundance may enhance bacteria diversity, but neither diversity nor richness have significant relationships with NUE_{soil} (Fig. 4.4a). *Acrobeloides nanus* inoculation had no significant relationships between nematode abundance, bacteria community, and NUE_{soil} (Fig. 4.4b). *Rhabditid intermedia* inoculations indicated that total nematode abundance had a significant and negative relationship with bacteria diversity, but a positive and significant relationship with bacteria richness. However, bacteria community α-diversity metrics had no significant relationships with NUE_{soil} (Fig. 4.4c). The inoculations with no nematodes had a negative relationship between total nematode abundance and bacteria richness (Fig. 4.4d). Additionally, bacteria diversity had a negative relationship between NUE_{soil} and bacteria richness and a positive relationship with NUE_{soil} (Fig. 4.4d).

DISCUSSION

I hypothesized that the *R. intermedia* inoculum would enhance soil and plant N pools more effectively due to the species' high reproductive capacity, short lifespan, and responsiveness to N-enriched conditions. However, contrary to my hypothesis, results showed that the co-inoculation of two bacterivore nematodes had the greatest effect on N cycling. Specifically, co-inoculations predominantly enhanced organic N pools, whereas effects on inorganic N pools remained more nuanced. Organic N pools have been seldom linked to bacterivore nematodes, given the lack of measurement of indicators that represent the organic N pool. However, bacterivore nematode grazers can increase N through direct exudation and from biomass contributions of senesced bacterivore nematodes (Wang et al., 2009). Thus, I speculate that some of this nematode biomass may have become protected within the soil contributing to greater soil organic N pools. Additionally, the increased abundance of bacterivores in co-inoculum treatments may have enhanced bacterial turnover through predation, resulting in greater

microbial biomass release and an increase in soil organic N (Trap et al., 2016). This shift toward enhanced soil organic N pools could have important implications for long-term soil fertility and microbial N availability, especially in relation to plant-microbe-nematode interactions.

Co-inoculum treatments had the most significant effect on the inorganic N pool, with enhanced nitrate levels observed in the no-plant treatment. This may be due to resource competition between the two nematode species (Vafeiadou et al., 2022), which could have increased bacterial turnover and boosted the abundance of ammonia-oxidizing bacteria (Xiao et al., 2014), thereby accelerating the conversion of ammonia to nitrate. However, this hypothesis is speculative, as the increased nitrate did not translate into significant changes in plant N.

Nematode inoculation treatments had no significant direct relationships on plant biomass or N, disproving my hypothesis. However, the relationship between total nematode abundance and plant N/biomass was significantly moderated by inoculum treatment. Co-inoculum and *R.intermedia* inoculum treatments, significantly affected the relationship between total nematode abundance and aboveground plant N, where relationships were negative, relative to positive relationships in the no-inoculum treatment. The aboveground N relationships were also synonymous with total plant N (i.e., sum of aboveground N and belowground N) (Fig. S4.3). This relationship may occur, given that greater abundances of nematodes stimulate increased microbial biomass and growth (Neher & Campbell, 1994), thus causing microbes to outcompete plants for N (Griffiths, 1994). However, co-inoculation treatments moderated a positive relationship between nematode abundance and belowground N. Given that organic N pools were enriched in co-inoculation treatments, this may have led to overall greater N cycling rates, thus creating sufficient N for both microbes and plants. The corn plants at day 47 were in the V5 growth phase (Fig. S4), a stage characterized by rapid N uptake for storage ahead of the reproductive phase or allocation for establishment of a robust root network (Garnett et al., 2013). This likely explains the observed negative relationships between total nematode abundance and aboveground N, but positive relationship with belowground N in co-inoculation treatments (Mao et al., 2007; Martin & Sprunger, 2021). In addition to N content, my results indicate that the co-inoculation of bacterivore nematodes can moderate positive relationships between total nematode abundance and aboveground biomass and belowground biomass. My experiment supports the hypothesis that nematodes facilitate greater plant biomass through means other than N dynamics. For example, bacterivore nematodes can increase root proliferation, and thus belowground

biomass, through grazing induced hormone production (Bonkowski et al., 2009; Mao et al., 2007).

Structural equation models were utilized to test my second hypothesis which was that the treatment of a co-inoculation of two nematode species will significantly enhance NUE_{soil} through indirect relationships between nematode abundance and bacteria diversity, rather than through direct relationships. My results disproved my hypothesis where NUE_{soil} was enhanced through direct relationships with nematode abundance in treatments of both co-inoculation and *R. intermedia* (cp-1 nematodes), rather than through indirect relationships. These results corroborate my previous results with co-inoculations bolstering the relationship between N pools and total nematode abundance. Additionally, given that bacterivore cp-1 nematodes are known to be tightly linked to N use and are classified as enrichment opportunists (Ferris & Matute, 2003), it is not surprising that the *R. intermedia* inoculation had a positive relationship between nematode abundance and NUE_{soil}. NUE_{soil} was only enhanced though indirect relationships under the no-inoculum treatment where total nematode abundance had a negative relationship with bacteria richness, but bacteria richness then had a positive relationship with NUE_{soil}. However, both total nematode abundance and bacteria diversity had negative direct relationships with NUE_{soil} in no inoculation treatments. Notably, the relationship between bacteria community composition and NUE_{soil} in the SEMs was only significant when a bacterivore nematode inoculation did not take place. These results suggest that bacterivore nematodes may alter the relationship between bacteria and NUE_{soil}. Given that bacterivore nematodes have a strong effect on N pools, the relationships between bacteria and NUE_{soil} may be lessened when bacterivores are present. Thus, these results imply that without the presence of bacterivores, bacteria communities may then start to directly have significant relationships with NUE_{soil}. Further research is needed to investigate how bacterial communities may influence NUE_{soil} under scenarios of altered soil fauna dynamics, as even partial shifts in nematode populations due to climate factors or environmental disturbances could have cascading effects on N cycling.

My results only indicated partial support for my last hypothesis where I predicted that regardless of plant presence co-inoculation treatments would enhance bacteria diversity but reduce bacteria richness. Specifically, co-inoculation treatments significantly reduced bacteria community richness in no plant microcosms on day 47. This reduction in bacterial richness suggests that co-inoculated bacterivore nematodes may exert top-down control on bacterial

communities, likely through intensified grazing pressure or selective feeding that disproportionately impacts certain bacterial taxa (Sun et al., 2024).

Although inoculum treatments had minimal direct effects on bacterial α-diversity, they altered the relationships between nematode abundance and bacterial richness and diversity in SEMs. Specifically, bacterivore co-inoculation treatments had a positive relationship between total nematode abundance and bacteria diversity. Bacterivore nematodes have been suspected to drive the diversification of bacteria through creating ecological opportunities in predator-excluded spaces within the soil structure, and through feeding preferences (Jiang et al., 2017). For SEMs of individual inoculations, only cp-1 nematode treatments (*R. intermedia)* had significant relationships with the bacterial community, where total nematode abundance had a negative relationship with diversity and a positive relationship with richness. Some bacterivore nematodes have selective feeding preferences, which would decrease diversity, but as certain species are removed other prey species that are adapted to predation may proliferate, thus increasing richness (Neidig et al., 2011). These results further amplify the argument that nematodes within the same trophic group have different biological functions, where an inoculation of cp-1 bacterivore nematodes drives significant relationships with food resources, whereas cp-2 nematode inoculations do not drive significant relationships. No inoculum SEMs also indicate that a lack of bacterivores may have negative implications particularly on bacterial richness, indicating a positive feedback loop may exist between bacterivore nematodes and bacteria community composition (Jiang et al., 2015).

Through inoculating microcosms with bacterivores that vary in life-history strategy, I showed that their coexistence is needed for enhanced N cycling. Given the unexpected mortality of the *R. intermedia* inoculated species, it remains unclear whether the observed trends were driven by the initial presence and activity of the inoculated nematodes or by legacy effects of the inoculation treatment after their decline in abundance. Many nematode microcosm experiments take place on very small scale (petri dish, scintillation vials), and I recommend using smaller microcosms to understand direct nematode species effects on ecosystem functions. I also recommend the use of stable isotopes in future research for investigating bottom-up effects on N cycling, as they can provide precise insights into N transformation pathways, microbial contributions, and the extent to which different nitrogen sources are utilized within the system (Watzinger & Hood-Nowotny, 2019). This approach would help disentangle the roles of soil microbes and fauna in regulating

nitrogen availability and cycling dynamics. Additionally, longer microcosm experiments need to be designed to understand how plant growth stage impacts bacterivore-N interactions. Lastly, given that this experiment only utilized two different nematode species, there is need for more research to be conducted on the fate of N within nematode communities that span varying life-history strategies and trophic groups.

CONCLUSION

Although bacterivores are known to play a critical role in N cycling, the impact of individual species and their interactions on trophic dynamics and ecological function remains poorly understood. My study indicates that bacterivore nematodes are essential for the organic N pool, available plant N, plant growth, and overall NUE_{soil}. Moreover, my study is one of the first to indicate that for bacterivore nematodes a diversity of life-history traits is essential for enhanced belowground N and soil organic N pools. I found that trophic interactions between bacterivores and bacteria communities are life-history dependent, and that the coexistence of nematodes with varying life-history strategies can moderate positive the relationships between total nematode abundance and bacterial community diversity. My findings highlight that enhanced ecosystem functioning depends on the coexistence of nematodes within the same trophic group with complementary life-history strategies, which has been overlooked in past studies focusing on single species or entire communities. This research underscores the importance of conserving fauna with diverse life-history traits to sustain ecosystem functioning and highlights the need for future studies to explore interactions within trophic groups and their role in regulating ecosystem processes.

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APPENDIX A: CHAPTER FOUR TABLES AND FIGURES

Table 4.1. Average and (standard error) for aboveground biomass (g), belowground biomass (g), belowground nitrogen (%), and aboveground nitrogen (%). Standard errors depict one standard deviation from the mean. Different letters represent significant differences between inoculum within day and plant presences after Tukey's adjustment (n=144).

Aboveground biomass (g)

	0	17	47
R.intermedia+A.nanus	0.1 (0.02)	1.18 (0.25)	3.28 (1.05)
A.nanus	0.1 (0.02)	1.32 (0.33)	3.5 (0.4)
R.intermedia	0.1 (0.02)	1.45 (0.24)	3.33 (0.69)
None	0.07 (0.01)	0.98 (0.18)	3.08 (0.6)

Belowground Biomass (g)

	0	17	47
R.intermedia+A.nanus	0.07 (0.01)	0.4 (0.06)	2.17 (0.29)
A.nanus	0.05 (0.01)	0.38 (0.05)	2.07 (0.22)
R.intermedia	0.07 (0.02)	0.4 (0.05)	2.24 (0.3)
None	0.06 (0.01)	0.29 (0.03)	2.11 (0.29)

Table 4.2. Average and (standard error) for belowground nitrogen (%), and aboveground nitrogen (%). Standard errors depict one standard deviation from the mean. Different letters represent significant differences between inoculum within day and plant presences after Tukey's adjustment (n=144).

Aboveground total nitrogen (%)			
	0	17	47
R.intermedia+A.nanus	3.88 (0.12)	4.92 (0.19)	1.98 (0.26)
A.nanus	4.27 (0.12)	4.94 (0.08)	1.7 (0.2)
R.intermedia	4.3 (0.2)	4.1 (0.23)	2.29 (0.53)
None	4.24 (0.26)	4.76 (0.22)	1.9 (0.27)
Belowground total nitrogen (%)			
	0	17	47
R.intermedia+A.nanus	2.86 (0.21)	2.58 (0.2)	1.54 (0.18)
A.nanus	3.05 (0.17)	2.28 (0.23)	1.3 (0.07)
R.intermedia	3.12 (0.13)	2.29 (0.11)	1.31 (0.11)
None	3.1 (0.08)	2.86 (0.13)	1.35 (0.15)

Table 4.3. Average and (standard error) for nitrogen use efficiency (g/g). Standard errors depict one standard deviation from the mean. Different letters represent significant differences between inoculum within day and plant presences after Tukey's adjustment (n=144).

	0	17	47
R.intermedia+A.nanus	3.84 (0.56)	43.57 (8.24)	64.41 (12.41)
A.nanus	4.69 (0.86)	47 (11.03)	58.88 (4.08)
R.intermedia	4.73 (1.03)	46.75 (7.12)	59.64 (8.79)
None	3.16 (0.24)	40.32 (7.45)	49.75 (4.84)

Table 4.4. Average and (standard error) of chao1, Shannon's diversity index, and Simpson's evenness for plant and no plant presence, day, and inoculum treatment. Standard errors depict one standard deviation from the mean. Different letters represent significant differences between inoculum within day and plant presences after Tukey's adjustment (n=143).

Bacteria Richness (Chao 1)

	Plant			No Plant		
	0	17	47	0	17	47
R.intermedia+A.nanus	1396.29 (75.37) b	1648.41 (42.48)	1408.04 (107.82)	1287.97 (56.07)	1496.3 (102.37)	1353.67 (109.3)
A.nanus	1035.66 (152.62) a	1491.05 (101.76)	1565.21 (41.38)	1256.05 (63.23)	1568.97 (63.69)	1312.58 (106.18)
R.intermedia	1392.71 (125.07) b	1655.24 (54.7)	1517.6 (142.09)	1266.75 (70.47)	1357.09 (66.34)	1196.32 (77.23)
None	1363.24 (83.69) b	1731.11 (111.68)	1647.59 (37.33)	1489.54 (48.4)	1508.82 (97.54)	1442.12 (83.68)

Bacteria Diversity (Shannon's Diversity Index)

	Plant			No Plant		
	0	17	47	0	17	47
R.intermedia+A.nanus	6.27 (0.1) b	6.55 (0.04)	6.41 (0.07)	6.22 (0.03)	6.43 (0.07)	6.2 (0.06)
A.nanus	5.95 (0.18) a	6.47 (0.08)	6.52 (0.01)	6.07 (0.09)	6.54 (0.06)	6.27 (0.09)
R.intermedia	6.29 (0.07) b	6.53 (0.08)	6.45 (0.1)	6.19 (0.08)	6.34 (0.07)	6.25 (0.09)
None	6.2 (0.07) b	6.51 (0.08)	6.6 (0.05)	6.34 (0.06)	6.32 (0.07)	6.43 (0.08)

Bacteria Evenness (Simpson's Evenness Index)

Table 4.4 (con't)

	Plant			No Plant		
	0	17	47	0	17	47
R.intermedia+A.nanus	0.1 (0.01)	0.13 (0.01)	0.14 (0.01)	0.09 (0.01)	0.14 (0.01)	0.08 (0.01) a

Table 4.4 (cont'd)

A.nanus	0.12 (0.01)	0.14 (0.01)	0.13 (0.01)	0.09 (0.01)	0.14 (0.01)	0.11 (0.01) ab
R.intermedia	0.12 (0.02)	0.13 (0.01)	0.13 (0.01)	0.1 (0.01)	0.13 (0.01)	0.13 (0.01) b
None	0.09 (0.01)	0.12 (0.01)	0.14 (0.01)	0.1 (0.01)	0.09 (0.01)	0.15 (0.02) b

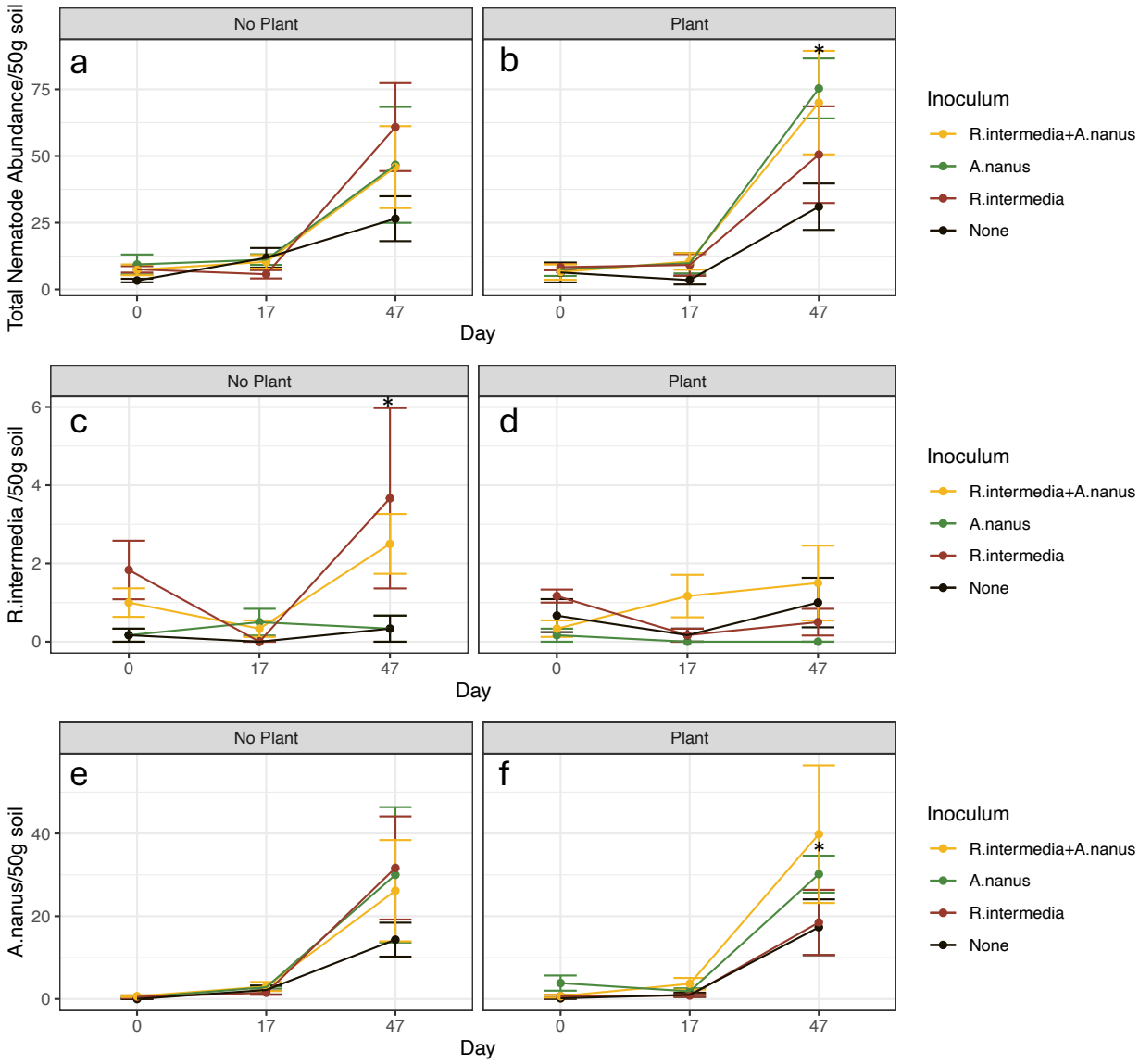

Figure 4.1. Total nematode abundance/50g soil, *R.intermedia* abundance/50g soil, and *A.nanus* abundance/50g soil over the three sampling timepoints and faceted by plant presence. Color represents the inoculum treatment. Standard errors represent one standard deviation from the mean. * Significant differences between inoculum treatments within day and plant presence.

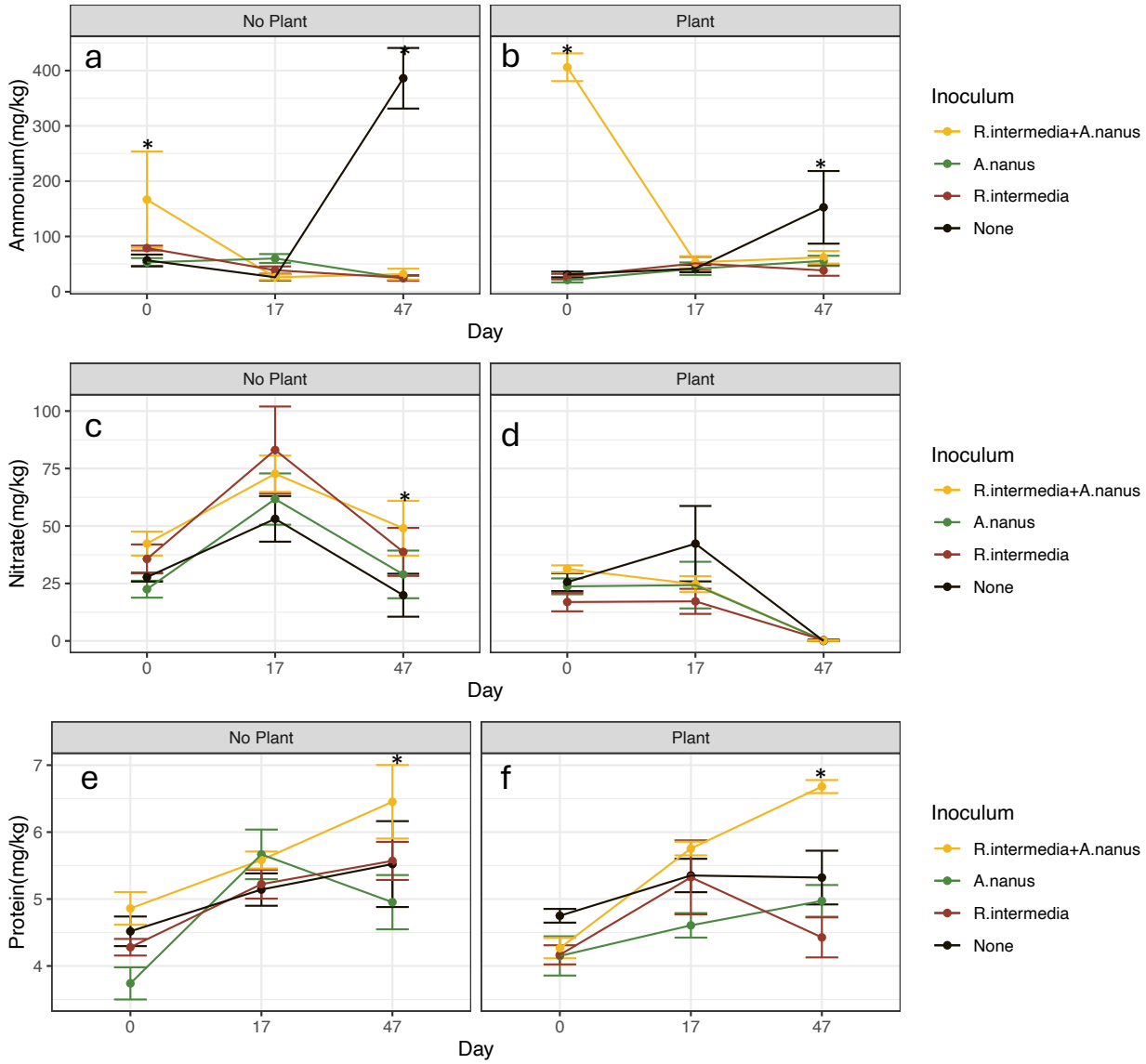

Figure 4.2. Ammonium (mg/kg) soil, Nitrate (mg/kg), and Protein (mg/kg) over the three sampling timepoints and faceted by plant presence. Color represents the inoculum treatment. Standard errors represent one standard deviation from the mean. * Significant differences between inoculum treatments within day and plant presence.

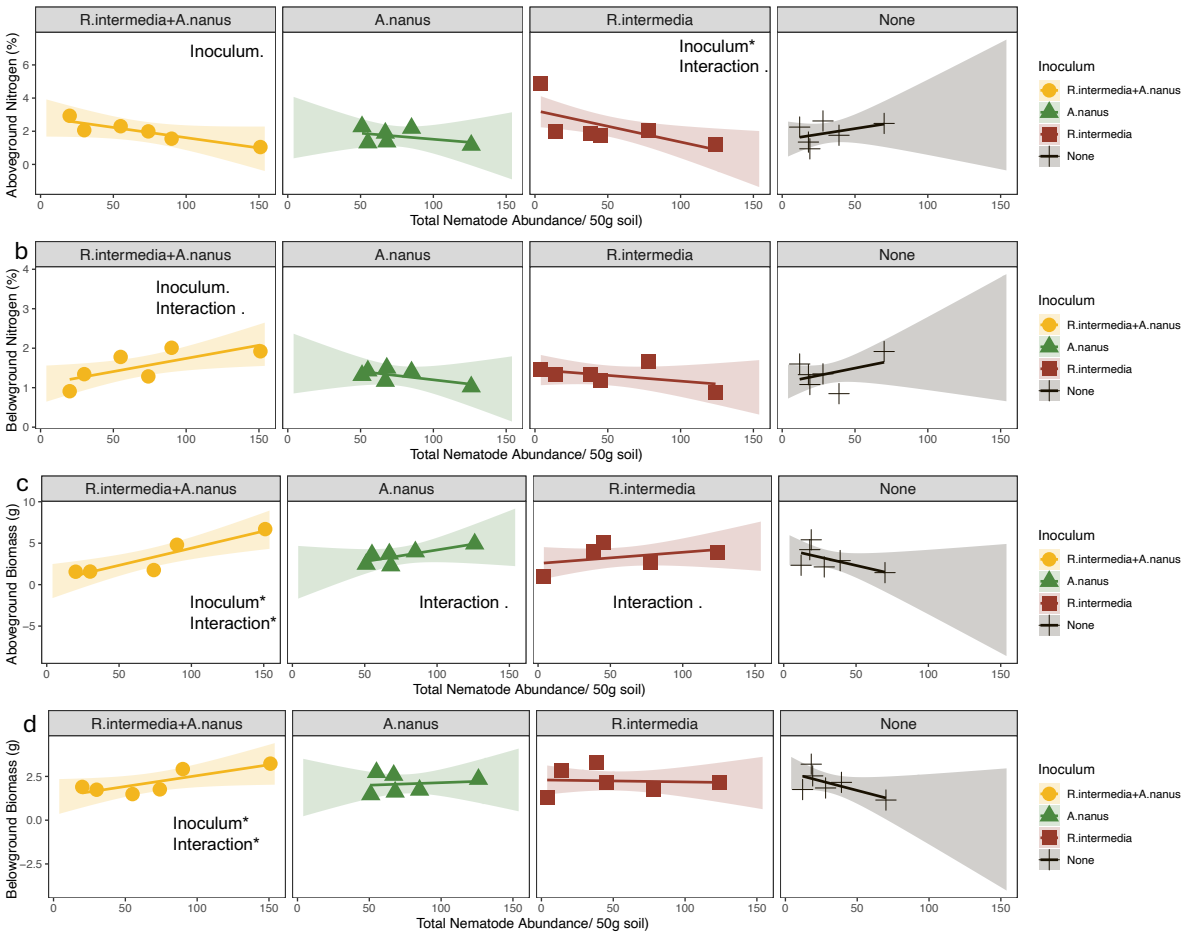

Figure 4.3. Linear models of the main and interaction effect of inoculum treatment and total nematode abundance A) aboveground nitrogen (%), B) belowground nitrogen (%), C) aboveground biomass (g), and D) belowground biomass for day 47 plant presence. Color represents inoculum treatment. Significance relationships are denoted by .p<0.10, *p<0.05.

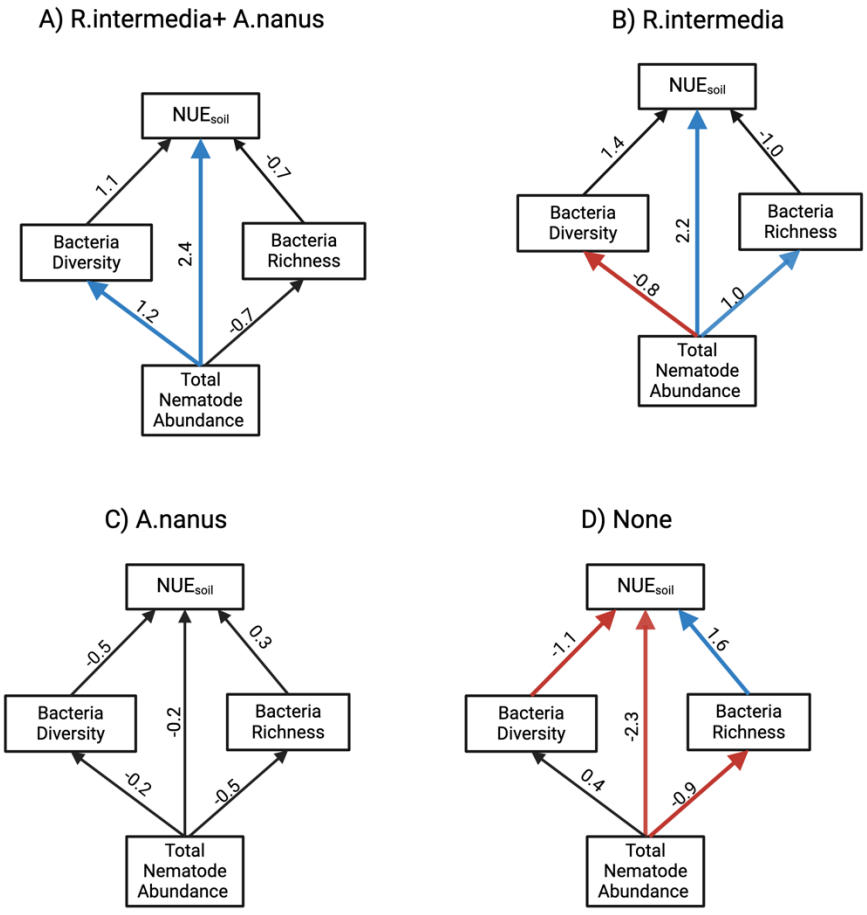

Figure 4.4. Structural equation models conducted for day 47 plant presence data for A) *R.intermedia+A.nanus* B) *A.nanus* C) *R.intermedia*, and D) None. Positive significant relationships are denoted by a blue arrow, where negative significant relationships are denoted by a red arrow. Relationships were deemed significant at $p \leq 0.10$ (n=6).

APPENDIX B: CHAPTER FOUR SUPPLEMENTAL

Table S4.1. Average and (standard error) of soil moisture (g H_2O/g soil). Standard errors depict one standard deviation from the mean. Different letters represent significant differences between inoculum within day and plant presences after Tukey's adjustment (n=144).

	Plant			No Plant		
	0	17	47	0	17	47
R.intermedia+A.nanus	0.19 (0.01)	0.07 (0.01)	0.07 (0.01)	0.19 (0.01)	0.1 (0.02)	0.25 (0.04)
A.nanus	0.19 (0.02)	0.06 (0.01)	0.12 (0.02)	0.17 (0.01)	0.08 (0.01)	0.26 (0.01)
R.intermedia	0.15 (0.02)	0.06 (0)	0.1 (0.02)	0.19 (0.03)	0.12 (0.01)	0.24 (0.02)
None	0.19 (0.02)	0.06 (0.01)	0.12 (0.02)	0.21 (0.02)	0.14 (0.01)	0.27 (0.03)

Table S4.2. Analysis of variance table of the effect of day, inoculum, plant, and all interaction effects on soil moisture. A square root transformation was conducted for this model.

Factor	F	p
Day	1.5	0.2
Inoculum	0.3	0.8
Plant	0.4	0.5
Inoculum*Day	0.1	1.0
Day*Plant	17.7	<0.001
Inoculum*Plant	0.4	0.7
Inoculum*Day*Plant	0.1	0.9

Table S4.3. Analysis of variance table of the effect of day, inoculum, plant, and all interaction effects on total nematode abundance. A Tweedie distribution was conducted for this model.

Factor	F	p
Day	83.3	<0.001
Inoculum	4.3	0.007
Plant	0.1	0.9
Inoculum*Day	0.5	0.8
Day*Plant	2.1	0.1
Inoculum*Plant	0.51	0.7
Inoculum*Day*Plant	1.7	0.1

Table S4.4. Analysis of variance table of the effect of day, inoculum, plant, and all interaction effects on R.intermedia abundance. A Tweedie distribution was conducted for this model.

Factor	F	p
Day	101.8	<0.001
Inoculum	1.1	0.4
Plant	0.9	0.3
Inoculum*Day	0.7	0.5
Day*Plant	43.5	<0.001
Inoculum*Plant	0.8	0.5
Inoculum*Day*Plant	0.8	0.5

Table S4.5. Analysis of variance table of the effect of day, inoculum, plant, and all interaction effects on A.nanus abundance. A Tweedie distribution was conducted for this model.

Factor	F	P
Day	166.8	<0.001
Inoculum	5.7	0.001
Plant	0.3	0.6
Inoculum*Day	0.4	0.9
Day*Plant	1.8	0.2
Inoculum*Plant	1.4	0.2
Inoculum*Day*Plant	1.3	0.3

Table S4.6. Analysis of variance table of the effect of day, inoculum, plant, and all interaction effects on ammonium. A Tweedie distribution was conducted for this model.

Factor	F	p
Day	0.002	1.0
Inoculum	9.7	<0.001
Plant	0.5	0.46
Inoculum*Day	17.2	<0.001
Day*Plant	1.3	0.3
Inoculum*Plant	7.7	<0.001
Inoculum*Day*Plant	5.3	0.001

Table S4.7. Analysis of variance table of the effect of day, inoculum, plant, and all interaction effects on nitrate. A Tweedie distribution was conducted for this model.

Factor	F	p
Day	101.8	<0.001
Inoculum	1.1	0.4
Plant	0.9	0.3
Inoculum*Day	0.7	0.5
Day*Plant	43.5	<0.001
Inoculum*Plant	0.8	0.5
Inoculum*Day*Plant	0.8	0.5

Table S4.8. Average and (standard error) of total soil nitrogen. Standard errors depict one standard deviation from the mean. Different letters represent significant differences between inoculum within day and plant presences after Tukey's adjustment (n=144).

	Plant			No Plant		
	0	17	47	0	17	47
R.intermedia+A.nanus	0.11 (0.01)	0.12 (0.01)	0.11 (0.01)	0.11 (0.01)	0.11 (0.01)	0.12 (0.01)
A.nanus	0.1 (0)	0.12 (0)	0.11 (0)	0.11 (0)	0.11 (0)	0.1 (0.01)
R.intermedia	0.1 (0.01)	0.11 (0)	0.11 (0.01)	0.11 (0.01)	0.1 (0)	0.1 (0.01)
None	0.12 (0.01)	0.1 (0.01)	0.12 (0.01)	0.11 (0)	0.1 (0.01)	0.11 (0.01)

Table S4.9. Analysis of variance table of the effect of day, inoculum, plant, and all interaction effects on total soil nitrogen. A Tweedie distribution was conducted for this model.

Factor	F	p
Day	0.2	0.6
Inoculum	0.5	0.7
Plant	0.3	0.5
Inoculum*Day	0.2	0.9
Day*Plant	0.1	0.7
Inoculum*Plant	0.7	0.6
Inoculum*Day*Plant	1.4	0.2

Table S4.10. Analysis of variance table of the effect of day, inoculum, plant, and all interaction effects on soil protein. A normal linear mixed effects model was conducted for this model.

Factor	F	p
Day	36.4	<0.001
Inoculum	0.8	0.5
Plant	0.1	0.7
Inoculum*Day	3.3	0.02
Day*Plant	0.3	0.6
Inoculum*Plant	0.6	0.6
Inoculum*Day*Plant	1.3	0.3

Table S4.11. Analysis of variance table of the effect of day, inoculum, plant, and all interaction effects on aboveground biomass, root biomass, plant nitrogen, and root nitrogen. A normal linear mixed effects model was conducted for root nitrogen. All other plant measures utilized a Tweedie distribution model.

Aboveground biomass		
Factor	F	p
Day	143.8	<0.001
Inoculum	0.8	0.5
Inoculum*Day	0.3	0.3
Belowground Biomass		
Factor	F	p
Day	466.4	<0.001
Inoculum	0.7	0.6
Inoculum*Day	0.1	1.0
Aboveground Nitrogen		
	F	p
Day	140.4	<0.001
Inoculum	0.1	1.0
Inoculum*Day	1.6	0.2
Belowground Nitrogen		
Factor	F	p
Day	115.7	<0.001
Inoculum	1.2	0.3
Inoculum*Day	1.4	0.2

Table S4.12. Analysis of variance table of the effect of day, inoculum, plant, and all interaction effects on nitrogen use efficiency. A Tweedie distribution model was conducted.

Factor	F	p
Day	244.3	<0.001
Inoculum	0.8	0.51
Inoculum*Day	0.10	1.0

Table S4.13. Analysis of variance table of the effect of day, inoculum, plant, and all interaction effects on chao1, Shannon's Diversity Index, and Simpson's evenness index. A Tweedie distribution model was conducted for each dependent variable.

Chao1

Factor	F	p
Day	13.9	<0.001
Inoculum	3.4	0.02
Plant	5.4	0.02
Inoculum*Day	2.0	0.07
Day*Plant	3.5	0.03
Inoculum*Plant	2.0	0.12
Inoculum*Day*Plant	1.8	0.11

Shannon's Diversity Index

Factor	F	p
Day	26.0	<0.001
Inoculum	1.4	0.2
Plant	10.7	0.001
Inoculum*Day	3.6	0.002
Day*Plant	3.8	0.02
Inoculum*Plant	1.1	0.36
Inoculum*Day*Plant	0.9	0.49

Simpson's Evenness Index

Factor	F	P
Day	11.0	<0.01
Inoculum	0.7	0.53
Plant	3.8	0.05
Inoculum*Day	3.3	0.004
Day*Plant	0.2	0.8
Inoculum*Plant	0.5	0.6
Inoculum*Day*Plant	2.7	0.01

Table S4.14. Goodness of fit indices for each structural equation model.

	R.intermedia + A.nanus	A.nanus	R.intermedia	None
RMSEA	1.4	0.17	1.7	1.2
Test Statistic	10.6	0.7	28.3	7.8
AIC	63.2	73.9	58	44.9
CFI	0.2	0.9	0.3	0.7
n	6	6	6	6

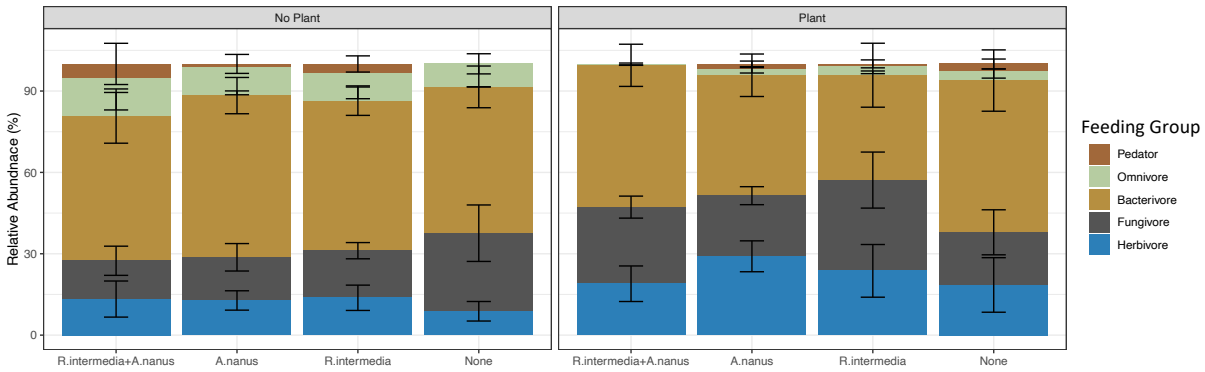

Figure S4.1. Relative abundance of feeding groups within each inoculum and plant treatment on day 47 of the experiment. Standard error bars represent one standard deviation from the mean (n=6).

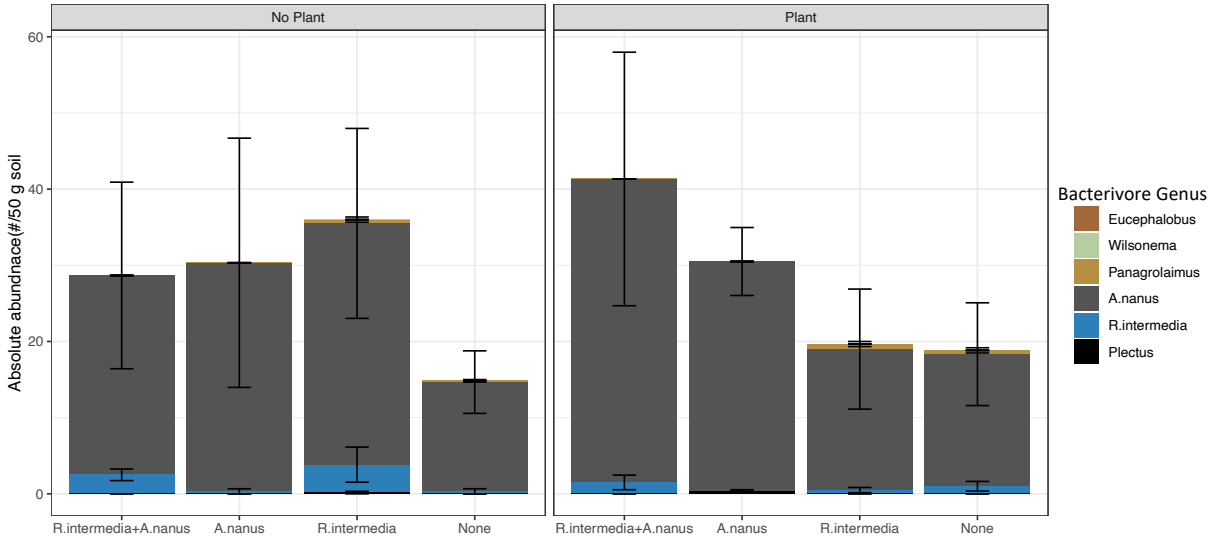

Figure S4.2. Absolute abundance of bacterivore genus nematode distribution within each inoculum and plant treatment on day 47 of the experiment. Standard error bars represent one standard deviation from the mean (n=6).

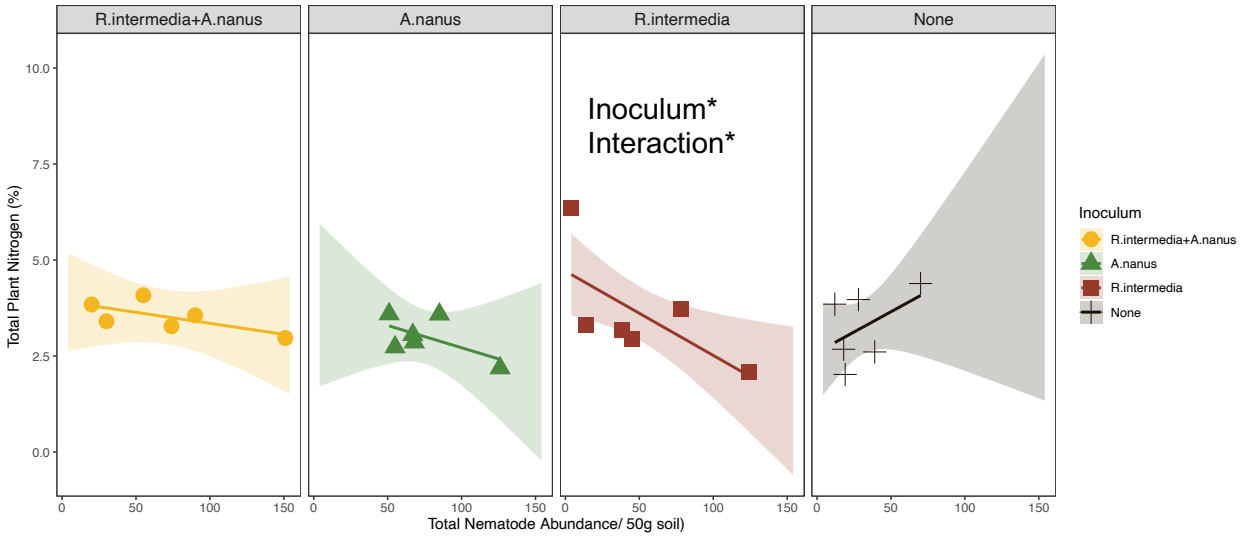

Figure S4.3. Linear models between total nematode abundance and A) total plant nitrogen (%), for day 47 Color represents inoculum treatment. Significance relationships are denoted by *p<0.05 (n=6).

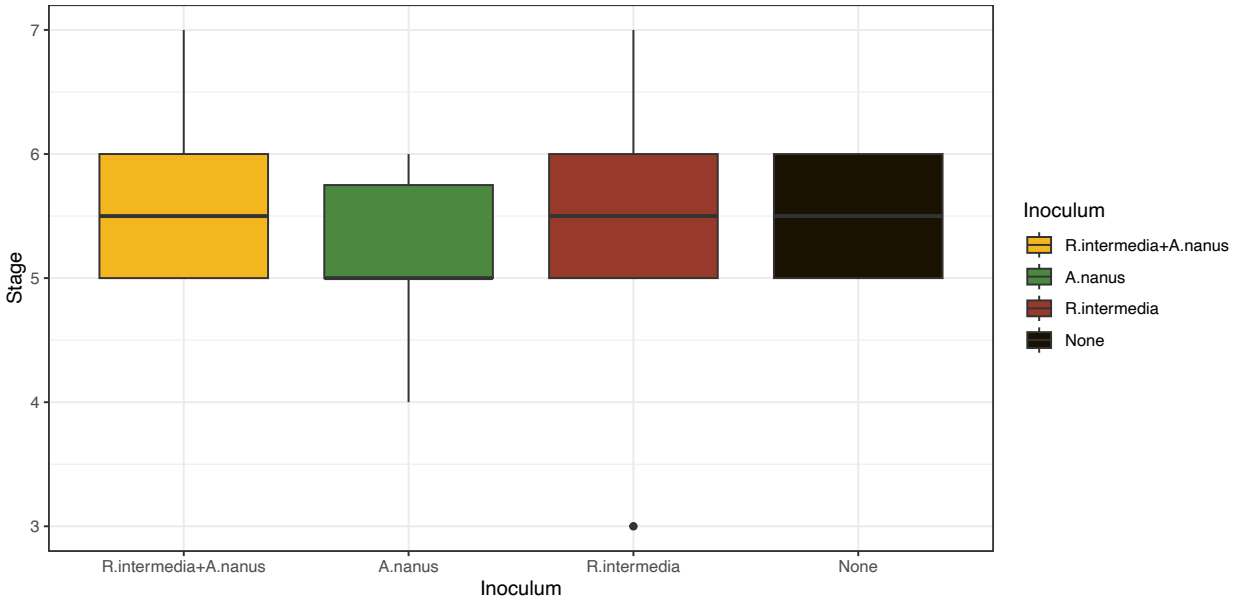

Figure S4.4. Average plant stage on day 47 of the experiment. Standard error bars represent one standard deviation from the mean (n=6).