INFLUENCE OF LAND-USE INTENSITY ON SOIL NEMATODE COMMUNITIES AND THEIR RELATIONSHIP TO *RHIZOCTONIA SOLANI* DISEASE SUPPRESSION

by

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ABSTRACT

Soil organic matter (SOM) degradation linked to intensive management across PEI may negatively impact soil nematode communities and disease suppression. Soil nematode communities (Chapter 2), *Rhizoctonia solani* soil concentrations (Chapter 3), and soil properties were assessed across three land-use intensities (pasture/forest systems<four year grain rotations<three year potato rotations) from PEI field soils in 2018 and 2019. Nematode faunal indices were then tested for their ability to predict soils suppressive to *R. solani* disease in soybeans (Chapter 3). Nematode indices reflected degraded nematode communities in high intensity potato rotations, while the community as a whole responded to low intensity vegetation gradients. *R. solani* field soil concentrations were negatively correlated with several indicators of SOM content, while POM C and ACE protein were positively correlated regardless of environmental conditions. Although nematode faunal indices were not good indicators of *R. solani* suppressive soils, the fungivore Aphelenchidae was positively associated with hypocotyl disease ratings.

LIST OF ABBREVIATIONS AND SYMBOLS USED

Abbreviation	Definition
ACE protein	Autoclave citrate extractable protein
C	Total carbon
DNA	Deoxyribonucleic acid
FAME	Fatty acid methyl ester
g	grams
LUI	Land-use intensity
mm	millimeters
ng	nanograms
N	Total nitrogen
Nematode indices	
Ba	Bacterivores
Fu	Fungivores
Om	Omnivores
P	Predators
PP	Plant parasites
MI	Maturity index
PPI	Plant parasitic index
EI	Enrichment index
SI	Structural index
NMDS	Non-metric multidimensional scaling
PEI	Prince Edward Island
OM	Organic matter
PLFA	Phospholipid fatty acid
POXc	Permanganate-oxidizable carbon
qPCR	Quantitative polymerase chain reaction
SOM	Soil organic matter
µg	Microgram

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CHAPTER 1: INTRODUCTION

1. INTRODUCTION

1.1 Land-use intensity

Land-use intensity (LUI) is a non-standardized term used in current scientific literature that generally describes increasing agricultural inputs in order to obtain a greater yield (Shriar 2000). More specifically, increased soil disturbance, increased fertilizer input, and crop diversity reduction are traits of agricultural intensification (Postma-Blaauw et al. 2010). Assessment of LUI can therefore manifest in many ways with assessment at the field or regional level, ranging in a myriad of factors including single factors of tillage, nutrient/pesticide input or rotation or combinations with conventional, organic, no-till, low input treatments which may vary themselves based on regional crops and management practices. The sustainability of intensive management practices in relation to soil physical, chemical and biological degradation is questionable (Foley et al. 2005).

Prince Edward Island (PEI), Canada has long been an agriculturally based island with a range of land-use types and associated intensities where settlers cleared 68% of the island surface between the early 1700s-1900s. As of the year 2000, farming accounted for 39% of the land base in PEI and 48% was forested, indicating reversion of some agricultural land to forest (Commission on Land and Local Governance, 2009). Thus, a large portion of today's forests experienced tillage disturbances if not selective tree cutting, effectively altering forest plant communities (Government of PEI 2018).

Today potatoes (*Solanum tuberosum* L.) are the primary agricultural crop of PEI, with production accounting for 20.8% (83,326 acres) of all PEI cropland in 2016, and an industry which grossed $276.1 million in 2015 (Canadian Ministry of Industry 2017). Potato

cropping systems are perceived as being generally more intensive than others due to the repetitive soil disturbances that are used in production. Tilling, planting, hilling and harvest are all management events which disrupt the soil to varying depths. In addition to tillage, historically short rotations, minimal ground cover while establishing, low potato crop residues at harvest and reduction of soil aggregation have all contributed to erosion issues. Overall, intensive agricultural management has contributed to an island wide trend of decreasing soil organic matter (SOM) (Nyiraneza et al. 2017). In addition to potato production, decreased animal production and increased production of low residue crops, such as soybeans (*Glycine max* (L.) Merr.), are thought to decrease SOM levels (Nyiraneza et al. 2017). Increased risk factors for this region, including high precipitation and sandy soils, exacerbate these trends.

Potato rotations on PEI were lengthened by provincial law to three years as outlined in the Agricultural crop rotation act of 2015 (Government of PEI 2015). The purpose of this act was to not only reduce erosion and prevent water contamination, but also to maintain and improve soil quality and productivity associated with potato production (Government of PEI 2015). Best management practices within this land-use type, such as conservation tillage (Carter et al. 2008) and cover cropping (Edwards and Sadler 1992), may also increase soil residue levels while maintaining or increasing marketable yields (Carter et al. 2008). Increasing rotation lengths has also been shown to decrease disease severity of the fungal pathogen *Rhizoctonia solani* (Carter and Sanderson 2001). This pathogen is significant in PEI as it can cause yield reduction by stem canker and reduce the quality of table potatoes due to black scurf symptoms on tubers. However, Nyiraneza et al. (2017) found declines in SOM even under three-year potato rotations due to the removal of hay and

straw during the non-potato rotation crop phases. Variability in such management practices in potato or other cropping systems may result in differences in many ecosystem services including soil function, biodiversity plant production and disease suppression. Thus, comparison of PEI LUI effects on soil condition and biological function will benefit our current understanding of the ecological impacts and provide a baseline for PEI land-use intensities.

1.2 Soil health indicators

The concept of soil health has evolved over time, where one definition of its meaning is the continued capacity of the soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, maintain the quality of air and water environments, and promote plant animal, and human health (Doran et al. 2000). Unlike soil quality, which is focused on the functions a soil provides, soil health generally includes the sustainability of a soil implying its continued capacity to meet these functions. Soil health may also include value assessments, where crop production may be included as an important outcome. Soil health is generally thought to require certain broad functions including carbon transformation, nutrient cycling; promotion of soil structure; and the regulation of pests and diseases (Kibblewhite et al. 2008).

Regardless of the definition, indicators are necessary as a means of assessing soil health. Soil health assessments include soil chemical, physical and biological related properties in their assessments (Moebius-Clune et al. 2016; Doran et al. 2000). Nematodes are biological indicators that may be linked to soil health, as their communities reflect both ecosystem functions and soil conditions (Ferris et al. 2001). The regulation of pests and diseases is also an often overlooked soil health function, where disease assessments may be

optional and limited to one crop (Moebius-Clune et al. 2016). While this research does not aim for an assessment of soil health, indicators and functions that can constitute a healthy soil will be explored.

1.3 Nematodes and the soil food web

The soil biota is composed of microbes (e.g. bacteria, fungi), micro (e.g. protists, nematodes, rotifers, tardigrades), meso (e.g. mites, springtails) and macro (e.g. earthworms, ants, beetles) fauna, and plant life which interacts to form a soil food web (de Vries et al. 2013). Members of this food web can, singly or collectively to varying degrees, provide soil functions such as nutrient cycling, organic matter degradation and carbon (C) sequestration, and pathogen control (Gattinger et al. 2008; Ferris et al. 2001). Soil food web structure was a good predictor of C and N cycling across Europe, where all nutrient cycling models included one or more soil food web measures (de Vries et al. 2013). Degradation of the soil food web structure in intensive agroecosystems could result in loss of species or functional groups thereby limiting soil function.

Nematodes (Phylum Nematoda) are cylindrical, pseudocoelomate animals, which range in length from 100 µm-2 mm in soils. Nematodes are keystone species, which occupy a central position in the soil food web and range in trophic level (Neher 2010). Yeates et al (1993) categorized nematode genera into the herbivore, bacterivore, fungivore, omnivore and predator trophic levels that are recognized today. Soil nematode feeding can affect bacterial, fungal and plant populations, influencing detrital and herbivorous decomposition pathways. Certain trophic levels and taxa are more susceptible to disturbance or soil enrichment than others (Bongers and Ferris 1999). Due to nematode ubiquity and lifestyle variability, the use of nematode communities as indicators of soil condition and function

across natural and agricultural soils of varying land-use intensities has been of interest (Sánchez-Moreno and Ferris 2007; Ferris et al. 2001).

1.4 Fungal soil-borne plant disease suppression

'Suppressive soil' is a term used to describe soils in which disease incidence or severity remains low despite the presence of a pathogen, susceptible host plant and ideal climactic conditions which promote pathogen growth (Baker and Cook 1974). Soil disease suppression is frequently attributed to the activity of soil communities (bacteria, fungi, beneficial nematodes, mites, and collembolans) that prevent plant pathogen growth (Hoitink and Fahy 1986). The biological mechanisms of disease suppression are categorized into two types: general and specific. General suppression includes soil conditions which are suppressive toward all pathogens, where the activity of the non-pathogenic soil microbes creates a competitive environment limiting the space and nutrients available to plant pathogens. Specific suppression occurs when certain soil microbial populations antagonize a pathogen, through predation or antibiosis, reducing pathogen growth and proliferation (Janvier et al. 2007). Specific and general disease suppression can occur simultaneously, although ecological trade offs may occur between suppression types under varying soil conditions (Schlatter et al. 2017). *R. solani* has exhibited both general and specific suppressive tendencies (Schlatter et al. 2017). Examples indicating general suppression include instances where suppression was nontransferable between soils (Donn et al. 2014) and where many microbial taxa were associated with suppression, although these connections were only based on correlation analyses (Poudel et al. 2016). Support for specific suppression has also been observed, where increases in *Flavobacterium* and Oxalobacteriaceae in the rhizosphere have occurred as a response to infection, and

application of a single isolated bacteria, *Chryseobacterium soldanellicola*, has provided disease control (Yin et al. 2013). In addition to biological disease suppression, soil physical and chemical factors can also influence pathogen growth and plant infection.

1.5 Nematodes and fungal soil-borne plant diseases

Historically, the study of interactions between nematodes and fungal soil-borne pathogens has focused on plant parasitic nematodes. In these studies, synergistic relationships between plant parasitic nematodes and fungal diseases generally have a neutral or positive effect on disease symptoms (Back et al. 2002). This enhancement generally occurs when nematode feeding occurs before the fungus makes contact with the root zone. Punctures in the root, made by the nematode stylet or where the nematode has fully entered the plant root, creates an entry point for the fungus as the integrity of the root's physical barrier has been compromised. Plant cell modifications induced by sedentary nematodes may also make a zone of high nutritive cells for subsequent fungal attack (Back et al. 2002). Several studies have also suggested that some plant parasitic nematode feeding may reduce plant systemic resistance, although these mechanisms are not yet well understood (Back et al. 2002). Most concerning to potato production in PEI is the influence of *Pratylenchus penetrans* along with the fungal *Verticillium* spp. (*V. dahliae* and *V. alboatrum*) in potato early dying complex. While this interaction may be the most concerning, *Pratylenchus* spp. can cause significant plant damage on their own. Contrasting associations have been made in field settings where *Pratylenchus* was associated with a different fungal species, *R. solani*, (Lui et al. 2016) while there was no association found between root lesion nematodes and *R. solani* diseased patches in potato (Björsell et al. 2017). Disease

interactions between these nematodes and *R. solani* in potatoes were found to be nonsignificant (Viketoft et al. 2020).

In comparison, the interaction of free-living nematodes with these soil-borne pathogens is understudied. *Rhizoctonia solani* (Kühn) (Teleomorph. *Thanatephorus cucumeris* (A. B. Frank) Donk) is a fungal soil-borne plant pathogen. This saprophytic pathogen can attack the root and stem tissues of a wide variety of plants, including many agricultural crops (Melzer et al. 2016). Due to the wide host range and saprophytic nature, integration of many management strategies, including those that account for soil biological activity, will benefit disease suppression. Grazing of soil-borne plant pathogens by fungivorous nematodes can mediate soil-borne fungal disease suppression including *R. solani* (Lagerlöf et al. 2011; Bollen et al. 1991). Due to this control mechanism, the study of nematodes as fungal biocontrol agents has mainly focused on fungivorous nematodes. This focus has limited our understanding of how nematode disease suppression functions within the context of the entire nematode community. Moreover, nematode community quadrats are indicative of soil chemical and biological conditions that could influence soil disease suppression. Few studies have assessed soil disease suppression in terms of nematode community indices (Fang 2015; Sánchez-Moreno and Ferris 2007) and there is no literature concerning nematode community quadrat, as determined by Ferris et al. (2001), in relation to soil-borne fungal disease suppression. If these indices significantly relate to disease, they can be manipulated in the field as they are responsive to agricultural management practices (Ferris et al. 2001). Assessment of the effects of nematode community quadrat type on soil-borne plant disease suppression will examine the disease suppressive role of nematodes

within a greater ecological context and determine if nematode community type is indicative of *R. solani* soil suppressive conditions.

2. CONCLUSIONS

Assessment of physical, chemical and biological soil metrics along with nematode communities will benefit our understanding of how the known PEI soil degradation trend affects the biological components and implied functioning of agricultural soils. Currently, there have been limited assessments of cross-PEI nematode communities. For example research conducted by Kimpinski primarily focussed on plant parasitic nematodes in potato production rotations and corn fields (Kimpinski 1987, Kimpinski 1977). Study of the interaction of nematode communities and soil-borne plant pathogen populations across land-use intensities will expand our understanding of multi-trophic level effects. Assessment of field soils with natural soil populations will show the relative contribution of these organisms compared to other suppressive soil agents.

Therefore, this study will have a greater focus on how land-use intensity affects the free-living nematode community, as these nematodes are fundamental to sustainable agricultural soil functioning. Assessment of different agricultural land-use intensities may also help us infer if our classification of land-use intensity ranges is robust and is linked to changes in long-term soil status and functioning, especially in regard to plant pathogen control.

CHAPTER 2: ASSESSMENT OF NEMATODE COMMUNITIES ACROSS LOW, MEDIUM AND HIGH LAND-USE INTENSITIES IN PEI

1. INTRODUCTION

1.1 Soil nematode ecology

While many soil chemical and physical properties may directly influence nematode populations, the interpretation of these factors is often attributed to an indirect effect on nematode food sources as opposed to a direct effect on nematode reproduction and metabolism. Classification of nematodes by trophic level allows for the study of population dynamics in relation to food source availability (Yeates et al. 1993). Nematode trophic classification can be assumed based on morphology, where mouth shape corresponds to feeding type, although taxonomic identification is required in some instances for proper assignment of nematodes to trophic level (Yeates et al. 1993). Trophic level populations are linked to the available nematode food sources, and subsequently nematode grazing affects plant and microbial populations. Bacterivore feeding generally increases bacterial biomass as nematode movement and digestion transports bacteria to new nutrient rich soil microcosms (Ingham et al. 1985). Hyphal grazing generally limits fungal growth (Bae and Knudsen 2001) and mineralizes N in relatively low concentrations compared to bacterivores (Ingham et al. 1985).

For nematodes to obtain appropriate food sources, the soil matrix must be conducive to nematode movement. Pore size restricts nematode movement, where smaller bodied nematodes can move into smaller pore spaces than larger nematodes (Briar et al. 2011). Bouwmana and Arts (2000) reported that compaction did not affect total nematode abundance, but compaction treatments increased herbivorous and decreased bacterivorous nematodes. Pratylechidae and Cephalobidae populations were positively associated with

increasing bulk density in Nova Scotian pastures (Mills and Adl 2011). Nematode movement also requires a 1– 5 μm thick water film on pore surfaces (Neher 2010) and an absence of this film may exclude nematodes altogether (Elliott et al. 1980; Hassink et al. 1993). Precipitation can affect nematode abundance as lack of soil moisture restricts nematode movement, limits microbial food sources, and can induce nematode dormancy (anhydrobiosis) (Landesman et al. 2011).

Higher soil nutrient status is generally associated with increased nematode body size (Liu et al. 2015; Mills and Adl 2011), but can also influence trophic abundances. Pan et al. (2015) found some bacterivorous population abundances were positively correlated with whole suites of nutrient levels (organic C, total N, available N, total P and available P), while in another study bacterivores were simply associated with increasing levels of organic N and pH (Mills and Adl 2011). Fungivores were correlated with organic C and soil moisture (Pan et al. 2015). Soil pH has been found to affect nematode populations across many studies (Mills and Adl 2011; Li et al. 2008; Popovici and Ciobanu 2000). Soil pH ranging from 5-8 can favour different nematode trophic groups or taxa, likely due to indirect pH effects on soil chemical and biological conditions (Korthals et al. 1996; Burns 1971). Bacterivores have been shown to be favoured at a higher pH (6.1) compared to that of fungivores (5.4) (Korthals et al. 1996). Total nematode abundance generally decreases at pH 4 and lower (Korthals et al 1996; Burns 1971).

1.2 Nematode communities and cropping systems

Previous nematode community studies concerning cropping intensity range in approach. One method compared intensive production systems (conventional or organic cropland) to natural or extensive treatments (forest or meadows) (Postma-Blaauw et al.

2010; Kimenju et al. 2009). Villenave et al. (2013) used a gradient approach, where natural and cropping systems (permanent meadow, continuous cropping) with varying levels of rotation intermediates (crop with meadow in the rotation, meadow with crop in the rotation) were assessed. Crop management within rotation type has also been studied, which can range in scope from organic to conventional comparisons, to specific tillage, grazing or fertility treatments.

In most studies, total nematode abundance is higher under natural or 'undisturbed settings' (forests, managed forests, meadows) compared to cropping systems (Kimenju et al. 2009: Villenave et al. 2013). Higher nematode densities (nematodes 100 g^{-1} soil) in meadows, over agricultural soils, were attributed to higher plant parasitic (PP) nematode populations (van Ekeren et al. 2008; Villenave et al. 2013; Ponge et al. 2013). Increased PP abundance in perennial systems can be attributed to a stable food source of continually growing live roots. Conversely, short rotations or mono-cropping can increase PP populations due to a continuous favoured food source for specific PP nematodes. Increased plant parasitic nematode populations were observed in fields with continuous planting of strawberries (Li et al. 2016), and increased soybean cyst nematode populations and egg densities were found with greater frequency of susceptible soybean in rotation (Wright et al. 2011; Howard et al. 1998). Increased densities of plant parasites, in response to monocropping, is a common phenomenon across many plant hosts (Mills and Bever 1998). Globally, the plant parasitic nematodes of concern in potato rotations include *Globodera* spp. (*G. rostochiensis* and *G. pallida*), *Meloidogyne* spp., *Ditylenchus destructor*, and *Pratylenchus* spp. (Lima et al. 2018).

Changes in the bacterivore and fungivore populations across ecosystems can be related to food source abundance or disturbance (Ferris and Matute 2003). Bacterivores and fungivores generally dominate cropped soils, while strict and facultative fungal-feeders are frequent in forests (Villenave et al. 2013; Okada and Kadota 2003). Consistently disturbed agroecosystems tend to be bacterially dominated, where long term increases of bacterivorous nematodes were observed with land use conversion from grassland to arable soils (Postma-Blaauw et al. 2010). While opportunistic bacterivore populations thrive in cropping systems due to the tillage and fertility disturbance effects on bacterial food sources (Ferris et al. 2001), other bacterivores less responsive to food abundance, have remained stable across crop system intensity (Villenave et al. 2013). Although fungivorous nematodes are linked to natural ecosystems (Kimenju et al. 2009), their populations may be more dependent on long term organic matter inputs. Organically managed tomato systems had higher fungivore populations than riparian soils depending on soil aggregate fraction (Briar et al. 2011). Fungivore abundance was more similar than that of bacterivores between organic and conventionally managed asparagus, as both perennial systems received similar residue returns (Tsiafouli et al. 2015). Soils amended with synthetic fertilizer had lower fungivore populations compared with organic amendments (Bulluck et al. 2002). However, organic amendment addition may not increase bacterivore and fungivore populations, as a top-down food web control by predatory nematodes can limit microbivorous nematode populations (Wardle et al. 1995).

Omnivorous and predatory nematodes are sensitive to physical disturbance resulting in reduced populations in high tillage cropping systems (Bongers and Ferris 1999). Omnivorous and predatory nematodes were most abundant in woodland and fallow land

compared to rice and corn fields (Ou et al. 2005). In some cases, low initial populations of these nematodes resulted in non-significant differences across management treatments (Bulluck et al. 2002).

Different nematode taxonomic measurements range in their ability to reflect differences in cropping system intensity. Increased nematode richness may be linked to diverse and abundant plant residue return typical of natural ecosystems, while agroecosystems with little above ground residue return, and disturbed root biomass have shown reduced genera richness (Ou et al. 2005). Genus richness was lowest in annual cropped soils compared to forests and perennial crops (Kimenju et al. 2009). In a separate study, nematode family richness was not significantly different between crop and meadow sites, although richness did significantly respond to different management types (Cluzeau et al. 2012).

Unlike taxonomic assessments, the maturity index (MI) assesses nematode communities using colonizer-persister (cp) values (1-5), where low cp values reflect r strategists (opportunists) and high values reflect K strategists (Bongers 1990). Stress including soil physical disturbance can reduce K strategist nematode populations in intensive systems, leaving a community with lower cp values and final MI value. Long term stress may even cause localized extinction of high cp value predatory nematodes (Korthals et al. 1996). Nematode MI values generally decrease with physical disturbance. This was shown by Kimenju et al. (2009) where undisturbed forests had higher MI values compared to corn and coffee fields. Postma-Blaauw et al. (2010) also found MI decreases when grasslands were converted to arable land. Furthermore, grassland reestablishment was then followed by a MI increase back to that of the long-term grassland (Postma-Blaauw et al.

2010). In other cases, long term stress may prevent reversal of MI values back to pre-disturbed levels. Localized extinction of high cp value predatory nematodes will necessitate a reduction of intensification and potentially species reintroduction before a high MI value can be reclaimed (Korthals et al. 1996)**.** To further complicate the matter, Neher et al. (2005) showed that different ecosystems (wetland, forest and agricultural lands) may respond differently to disturbance due to inconsistent MI value reactions to disturbance. In this case nematode family composition made a better distinction between ecosystems and their disturbance levels than community indices (Neher et al. 2005). Soil fertility amendment also favours low cp value organisms decreasing MI value (Postma-Blaauw et al. 2010; Neher and Olson 1995). However, different soil amendments may have variable effects on nematode MI values. Bulluck et al. (2002) found lower initial MI values in manure and cotton-gin trash amended soils compared to rye-vetch and synthetic fertilizer treatments.

Nematode assessment by combined trophic level (Ba represents bacterivores, Fu fungivores) and reproductive strategy designation (cp values 1-5) can allow us to place nematodes into trophic guilds (Trophic designation cp value, i.e. Ba1). Assessment of the abundance of nematodes in each guild allows for the calculation of enrichment (EI) and structural (SI) indices (Ferris et al. 2001). EI is a measurement of the food web energy flow through opportunistic Ba1 and Fu2 nematodes, which are weighted based on growth and metabolic rates (Ferris et al. 2001). The SI assesses cp3-5 guild nematodes, which are weighted based on disturbance sensitivity. Faunal analysis of nematode SI and EI characterizes communities into quadrats (Fig. Appendix (A)1) that represent different soil conditions (Table A1). As soil management influences these soil conditions, different land-

use intensities generally cluster within these quadrats. Forests and meadows tend to fall in quadrat C, less intensive annual cropland and pastures in quadrat A and B, conventional cropland in A, B or D, and perennial systems in B and C (Ferris et al. 2001). Undisturbed ecosystems or less intensive agricultural soils generally have higher SI values over intensively managed systems, as shown by Li et al. (2008) where abandoned cropland and woodland had higher SI values over cropland soils. Higher SI values of less intensive systems were attributed to increased total N and SOM (Li et al. 2008). Although SI did not significantly differ between conventional or organic tomato, continuous cereal-legume cover and unmanaged riparian corridor, SI tended to be highest under the less intensive riparian corridor (Briar et al. 2011). EI, did not significantly differ between treatments of the same study (Briar et al. 2011). In a grazing intensity study there was no EI difference, and all EI values were high (Mills and Adl 2011). EI similarity, despite intensity differences, was attributed to a well fed system with large labile nutrient pools across the field site (Mills and Adl 2011). EI has been shown to increase in crop systems over meadows (Ponge et al. 2013; Villenave et al. 2013).

Agricultural management generally favours bacterial over fungal decomposition systems (Mills and Adl 2011), thus we generally expect low channel index (CI) values with intensive management. Forests tend to have higher CI values than grasslands and crops (Ruess 2003; Villenave et al. 2013). Fungivore communities are favoured in unfertilized agricultural systems which resulted in higher CI values across land use types (Villenave et al. 2013). Conversely, a number of studies have found no difference in CI across conventional and organic management systems (Briar et al. 2011; Briar et al. 2012a). The basal index (BI) is an index that reflects the abundance of nematodes that are considered

basal to any system which are Ba2 and Fu2 functional guilds. An abundance of these nematodes suggests that there are not adequate microbial carbon resources entering the soil to support enrichment opportunists and there are further disturbances disrupting the food web chain or physical soil habitat that exclude reduce higher trophic populations as well. Generally a higher basal index is considered to represent a biologically degraded soil.

2. OBJECTIVES AND HYPOTHESES

Objective 1- Assess the effect of land-use intensity on nematode abundances and taxon composition at the family level, in relation to soil parameters, across a range of PEI soils.

Hypothesis 1- Fungivorous nematodes will be more prevalent under low land-use intensity soils due to increased SOM content.

Objective 2- Determine the effect of land-use intensity on nematode community structure as measured by maturity indices, faunal assessment and multivariate analysis.

Hypothesis 2a- EI values will increase with increasing land-use intensity as a result of increased opportunistic bacterivore populations.

Hypothesis 2b- The nematode community will be less structured, as determined by SI values, with increasing land-use intensity.

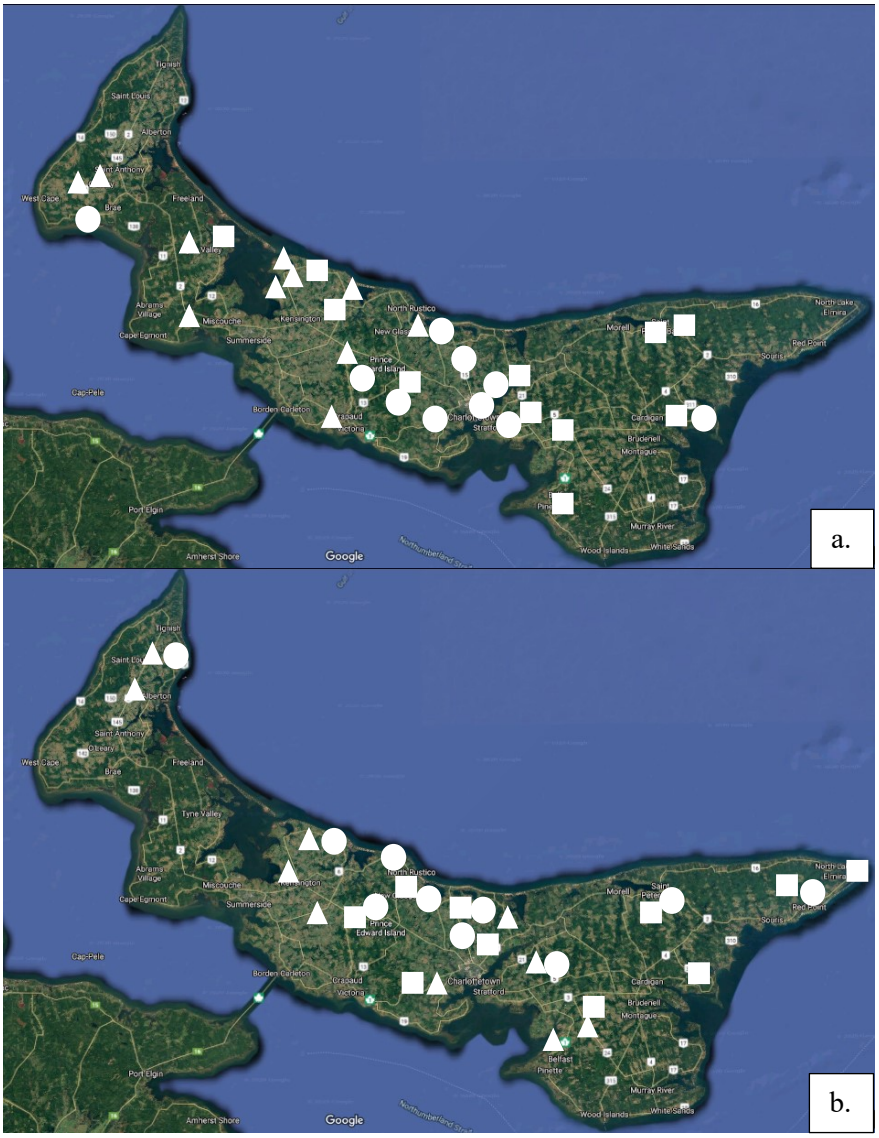
3. MATERIALS AND METHODS

3.1 Sampling

Soil samples were collected from 30 fields across PEI during the 2018 and 2019 field seasons (60 total) (Fig. 1). Agricultural fields were sourced from points within a pre-existing Soil Quality Monitoring (SQM) project established in 1998 by the PEI Department of Agriculture and Land, which was designed on a 4 km x 4 km National Forest Inventory

grid system containing 232 agricultural-land sampling points. Thirty sites per year were chosen based on cropping intensity, where ten sites were selected for high (traditional 2-3 year potato rotation), medium (diversified minimum 4 year rotations) and low intensities (forest and grasslands) (Table 1). One site was lost in 2018, therefore only 29 sites were sampled in this year. Crops in the current sampling year are presented in Table 2.

Figure 1. Map of field sites (n=30) across PEI's SQM project selected for this study in 2018 and 2019.



Note: a. 2018 sites b. 2019 sites. circle: low intensity sites, square: medium intensity sites, triangle: high intensity sites.

High and medium intensities were first selected to represent a wide geographic area and a range of soil organic matter (SOM) within intensity treatment. High intensity management systems growing potato in the year of sampling were selected when possible, while lower intensity management systems were chosen close to high intensity management sites to control for location. Not all low intensity sites (e.g. forest samples) were selected using the SQM database. Soil sampling location at each site was determined using one central geo-referenced point from the SQM project. The remaining four sampling points were collected 20 m north, east, south and west of the central point and georeferenced. Central points for low intensity sites that were not chosen using the SQM project grid were determined randomly.

Sampling was conducted in mid July to early August each year. Eight soil cores were collected per sampling point using a dutch auger from the top 15 cm of soil. Cores were mixed resulting in five composite samples (each ~2 kg) per site.

Table 1. Crop rotation information from 2013-2017 for 2018 and 2019 PEI soil sampling points.

2018					2019				
2013	2014	2015	2016	2017	2013	2014	2015	2016	2017
H	PS	PS	FR	PS	FST	FST	FST	FST	FST
PS	PS	PS	PS	PS	FR	G	FR	P	G
PS	P	FR	PS	PS	H	FR	FR	FR	FR
GRS	GRS	GRS	GRS	GRS	H	FR	FR	FR	FR
PS	PS	PS	PS	PS	GRS	GRS	GRS	GRS	GRS
FST	FST	FST	FST	FST	FST	FST	FST	FST	FST
FST	FST	FST	FST	FST	TL	TL	TL	TL	TL
TL	TL	TL	TL	TL	TL	TL	TL	TL	TL
FST	FST	FST	FST	FST	FST	FST	FST	FST	FST
FST	FST	FST	FST	FST	TL	TL	TL	TL	TL
S	C	FR	FR	CAN	NA	S	FR	C	S
G	C	C	P	FR	P	G	FR	P	FR
G	C	C	G	FR	FR	FR	PL	S	C
S	C	FR	P	FR	NA	CR	CR	C	FR
FR	CR	CR	PL	FR	FR	S	G	FR	G
FR	S	CR	S	FR	PS	PS	FR	G	FR
C	FR	FR	CR	CR	GRS	FR	FR	FR	S
GRS	FR	S	GR	FR	PS	CR	CR	G	FR
FR	S	CR	P	G	NA	NA	NA	NA	NA
G	S	C	FR	FR	G	FR	FR	FR	FR
C	FR	FR	M	S	G	G	P	G	FR
FR	P	CR	CR	CR	P	G	P	S	CR
P	FR	O	FR	P	G	G	P	S	G
P	FR	P	G	P	R	G	P	G	S
C	FR	P	G	FR	G	FR	P	G	FR
-	-	-	-	-	FR	P	FR	FR	P
S	P	G	P	G	FR	P	CR	FR	P
C	FR	P	G	FR	G	S	G	FR	P
P	P	FR	G	FR	G	P	G	FR	P
B	FR	P	FR	CAR	H	P	G	FR	P

Note: B= barley, C= cereal, CAN= canola, CAR= carrots, CR= corn, FR=forage, FST= forest, G= grain, GRS= grass, H= hay, M= mustard, NA= not assessed, O= oilseed, P= potato, PL= plowed, PS= pasture, R= radish, S= soybean, TL= treeline. Sampling was conducted in mid July to early August each year. Eight soil cores were collected per sampling point using a dutch auger from the top 15 cm of soil. Cores were mixed resulting in five composite samples (each ~2 kg) per site.

Table 2. Vegetation cover for 2018 and 2019 PEI soil sampling points.

		Crop	
Intensity	**Site**	**2018**	**2019**
Low	1	Pasture	Trees
	2	Pasture (grazed)	Pasture (timothy clover dandelions)
	3	Hay	Grass/weeds
	4	Grass (cut)	Grass/weeds
	5	Wild pasture (not cut)	Grass/weeds (buffer between road and trees)
	6	Trees	Forest
	7	Trees	Tree line
	8	Grass along treeline	Tree line
	9	Trees	Forest
	10	Trees	Treeline
Medium	11	Soybean	Barley
	12	Barley/clover	Potato
	13	Hay	Corn
	14	Barley/clover	Potato
	15	Hay	Soybean
	16	Alfalfa/hay	Barley
	17	Barley	Corn
	18	Triple mix cover; organic	Timothy/Hay (recently cut)
	19	Timothy/hay	Buckwheat
	20	Hay/clover	Potato
High	21	Forage	Potato
	22	Sudan Grass	Buckwheat, lots of weeds, potatoes left in field from 2018
	23	Peas	Winter wheat
	24	Cereal	Oats
	25	Potato	Weeds
	26	-	Red Clover
	27	Buckwheat	Pasture (grass)
	28	Potato	Red clover underseeded to barley
	29	Potato	Sudan grass
	30	Soybean	Hay mix (clover timothy)

Note: Site number corresponds to sites presented in Table A2. Forest sites were generally disturbed Acadian forests, which may contain species typical of Northern Hardwood Forests (Yellow Birch, Sugar Maple, American Beech, White Pine), as well as Boreal Forests (White Spruce, Black Spruce, Eastern Larch, Poplar, White Birch) (Government of PEI 2018).

3.2 Sample processing and storage

After sample collection each composite sample was divided into three fractions where 15 ml of soil was allocated for qPCR analysis and kept at -20 °C, 250 g was stored for nematode analysis at 4°C and approximately 2 kg of soil air dried. Air dried soil was then hand sieved to generate 2 and 8 mm soil samples for later assessments.

3.3 Soil assessments

Soil explanatory variables, including pH, available P and K, texture, C and N content, active carbon, and autoclave-citrate extractible (ACE) soil protein, were determined according to the 2016 Cornell Soil Health Assessment (CSHA) Manual (Moebius-Clune et al. 2016). Methods and any deviations from this protocol, including any additional soil properties assessed, are described as follows.

3.4 pH, available P, and K

pH was determined by adding 20 g of soil to 20 mL of distilled H_2O. Sample was stirred and let stand for 15 min, stirred and let stand for 1 hr. pH was then measured with a Star pH Meter. (Thermo Fisher Scientific, Waltham, United States).

Soil available P and K were extracted using the Mehlich III protocol (Mehlich 1984). Air dried soil samples were passed through a 2.00 mm sieve. 2 g of the sieved soil was weighed into a 50 ml centrifuge tube. 30 mL of Mehlich III solution was added to each tube, and tubes were shaken for 5 min on high. Samples were vacuum filtered through a 2.4 cm Whatman® 934-AH glass filter (Whatman PLC., Maidstone, UK) and collected in 50 mL poly-propylene test tubes. A blank Mehlich III solution sample was included every 50 samples for quality control. Samples were stored at 4°C until analysis.

Inductively coupled plasma-mass spectrometry (ICP-MS) using the 820- mass spectrometer (Varian Medical Systems, Palo Alto, CA, US) was used to quantify available P and K. Control methods included secondary source reference standards as well as prepared check solutions.

3.5 Soil texture

Soil texture procedures generally followed the CSHA rapid soil texture method (Moebius-Clune et al. 2016), although different sized containers and sample sizes were used as described below. Approximately 20 g of 2 mm air dried soil was weighed into snap cap bottles and 65 mL of 3% hexametaphosphate soap solution added to the bottle. Samples were placed on a shaker for 2 hr at 150 rpm min^{-1} at room temperature (approximately 20 °C). The sample was then washed through a 0.053 mm sieve. The sand and particulate organic matter was retained on the sieve, collected on a pre-weighed tin and dried. The silt and clay fraction was collected in a pan, and left to settle for two hours. The clay remained in suspension, which was then decanted after two hours. The remaining silt was placed in a pre-weighed tin, dried and weighed. The clay fraction of the soil was calculated by the difference of the sand and silt fractions from the original soil mass as follows:

Sand % = (oven dry sand mass / original sample mass) x 100%

Silt % = (oven dry silt mass / original sample mass) x 100%

Clay % = 100 - (Sand % + Silt %)

3.6 ACE protein

The autoclave-citrate extractable (ACE) protein assessment involves the extraction, clarification and quantification of soil protein. Protein was extracted by autoclaving soil in a 20 mM sodium citrate buffer with a pH of 7.0. Supernatant containing the protein was

obtained by centrifugation in the clarification process. 10 µL of each protein sample was then quantified by an Epoch™ 2 microplate spectrophotometer (BioTek Instruments, Inc., Winooski, VT, US) at 562 nm following a colorimetric reaction in 200 µL bicinchoninic acid working reagent. Detailed methods used in this study, according to the 2016 CSHA Manual (Moebius-Clune et al. 2016), are provided in the appendix.

3.7 Active carbon

Soil active carbon is a measurement of the readily oxidized soil C using a KM_nO_4 solution of a known concentration, and is therefore termed permanganate oxidizable carbon (POXc). The colormetric reaction (loss of purple colour) that follows is proportional to the amount of soil C oxidized and this change is measured using spectrophotometry at 550 nm .

Before analysis 0.2 M $KMnO_4$ pH 7.2 was prepared by dissolving 11.09 g of $CaCl_2$ and 31.61 g of $KMnO_4$ into 1 L of distilled deionized (DD) water. The sample concentration of active carbon is calculated using a standard curve of known $KMnO_4$ solution concentrations. The standard curve was prepared by diluting 0.5 mL of 0.005, 0.01, 0.02 M $KMnO_4$ with 49.5 mL of water. The standards were made in triplicate, shaken by hand and absorbance determined.

Soil samples were run in duplicate where 2.5 g of 2 mm sieved air dried soil was added to a flacon tube, followed by 18 mL of DD water and 2 mL of 0.2 M $KMnO_4$. Tubes were shaken at 120 rpm for 2 min, then allowed to settle for 8 min. After the settling period 0.5 mL of the solution was transferred to another falcon tube containing 49.5 mL of DD water. The tubes were hand shaken and absorbance observed using Ward's® 2150 UV/VIS Spectrophotometer (Ward's Natural Science, Rochester, United States) at 550 nm.

Calculation of sample POXc:

Active C (mg kg^{-1}) = [0.02 mol L^{-1} - (a + b * absorbance)] * (9000 mg C mol^{-1}) * (0.02 L solution 0.0025 kg^{-1} soil).

Where:

0.2 mol L^{-1} is the $KMnO_4$ concentration

a =standard curve y-intercept

b = standard curve slope

absorbance = sample absorbance

9000 mg C mol^{-1} = For every 1 mol of MnO_4 that is consumed (Mn^{7+} to Mn^{2+}) 0.75 mol (9000 mg) of C is oxidized

0.02 L = volume of $KMnO_4$ solution

0.0025 kg soil = modified according to actual sample mass

3.8 Total C, N and C:N ratio

To measure total C, total N and the C:N ratio, 10 g of 2 mm sieved soil was placed in square French bottles on a roller grinder for 48 hrs. Combustion of a ground, 1 g subsample at 500 °C using VarioMAX CN Elementar system (Elementar American Inc., Mt Laurel, NJ, US) determined the total soil C and N contents. The C:N ratio was determined as the ratio of total C (C) to total N (N).

3.9 Soil Aggregate Size Fraction

Soil aggregate size fractions were determined using a Ro-Tap® Coarse Sieve Shaker (W.S. Tyler, Mentor, OH, US). 100 g of air dried soil was shaken for 15 s over 8, 2 and 0.25 mm nested sieves to generate 2-8, 0.25-2 and < 0.25 mm soil aggregate size fractions. Soil

fractions were individually weighed following shaking while rocks greater than 8 mm were removed.

3.10 Soil moisture

The gravimetric soil moisture content was determined by first weighing approximately 10 g of each soil sample into pre-weighed tins. Soil samples were dried for 48 hrs in a convection oven at 105 °C. Soil moisture was reported as the mass of water per mass of dry soil, in percent.

3.11 Environmental data

Temperature and precipitation data were collected online from Environment Canada's historical weather data (https://climate.weather.gc.ca/historical_data/search_historic_data_e.html). Sites were designated to weather stations based on proximity and typical precipitation patterns that run South-west to North-East across the island. Weather station locations and associated sites are located in Tables A2, A3 and A4. Degree day calculations were based on air temperatures and used a 10°C base temperature. The following calculation was used to determine degree days for each day:

Degree days=((Daily maximum temp + Daily minimum temp) / 2) – Base temp

Total precipitation and degree days were calculated as the sum total from May 1st to July 15th of each year.

3.12 Nematode extraction and identification

Nematodes were extracted using Cobbs sieving and decanting method followed by the sugar centrifugation method (Caveness and Jensen 1955, Freckman et al. 1975). A 1.33 M sugar solution was first prepared by dissolving 454 g of sugar in 1 L of water. Water was added to 100 mL of weighed soil to make up an approximate 650 mL volume in a beaker.

The soil was stirred for 30 s in a figure-eight motion then immediately poured over a stack of 40 mesh (425 µm) over 400 mesh (38 µm) sieves. Cold tap water was then rinsed over the top 40 mesh sieve at an angle. The top screen was removed and the nematodes washed to the base of the angled 400 mesh screen by letting the water cascade over the screen, moving the nematodes indirectly. Nematodes were backwashed into a 50 mL centrifuge tube and spun at 1750 rpm for 5 min. The supernatant was decanted leaving a few mL of water covering the soil pellet. Chilled sugar solution was used to refill the tube. Nematodes within the soil pellet were resuspended by stirring with a spatula, then spun at 1750 rpm for 1 min. The sample was decanted into 500 mesh (25 µm) sieve and nematodes were collected and stored in water at 4 °C until identification. Nematodes were placed on a counting dish and 100 nematodes were identified to family level, using light microscopy. The University of Nebraska- Lincoln, nematode description and identification website found online at https://nematode.unl.edu/index.html was used as an identification guide. The total number of nematodes in each sample were also assessed which allowed for the counts to be adjusted to reflect abundance per 100 g of dry soil.

3.13 Nematode community indices calculations

Nematode indices were calculated using NINJA (Nematode Joint Indicator Analysis) system (Sieriebriennikov et al. 2014) which can be found online at https://sieriebriennikov.shinyapps.io/ninja/. Nematodes were designated into trophic groups as bacterivore, fungivore, omnivore, predator or plant parasite, according to Yeates et al. (1993). Taxonomic families were assigned a cp value of 1–5 according to Bongers (1990) with Monhysteridae re-classified into cp 2 (Bongers et al. 1995).

Table 3. Classification of Family and Genus level nematodes by trophic and cp designations according to Yeates et al. (1993), Bongers (1990) and Bongers et al. (1995).

Trophic Group	Family/Genus	cp value
Bacterivores	Diplogastridae	1
	Panagrolaimidae	1
	Rhabditidae	1
	Cephalobidae	2
	Monhysteridae	2
	Plectidae	2
	Prismatolaimidae	3
	Teratocephalidae	3
	Alaimidae	4
Fungivores	Aphelenchidae	2
	Aphelenchoididae	2
	Diphtherophoradae	3
	Leptonchidae	4
	Tylencholaimidae	4
Omnivores	Dorylaimidae	4
	Qudsianematidae	4
	Aporcelaimidae	5
Predators	Mononchidae	4
Plant parasites	Anguinidae	2
	Paratylenchidae	2
	Tylenchidae	2
	Belonolaimidae	3
	Criconematidae	3
	Heteroderidae	3
	Hoplolaimidae	3
	Meloidogynidae	3
	Pratylenchidae	3
	Longidoridae	5

Four different MI indices were calculated using the formula: MI= Σ[(cp-value) (i) f(i)]/[total numbers of nematodes] where (i) is the individual taxon and f(i) is the frequency of taxa in a sample (Bongers, 1990). Different MI indices assessed different subsets of the nematode community where MI included only free-living nematodes with cp 1-5, PPI included PP nematodes with cp 1-5, MI25 only included free-living cp 2-5 value families and ΣMI included free-living and PP nematodes with cp vales 1-5 (Neher 2005). Faunal indices (EI, SI, BI and CI) were determined accordingly: $e= (Ba_1W_1) + (Fu_2W_2)$ where,

W_1=3.2,W_2=0.8**;** s= ($Ba_n W_n$)+($Ca_n W_n$)+($Fu_n W_n$)+($Om_n W_n$) where, n=3-5, W_3=1.8, W_4=3.2, W_5=5.0**;** b= (Ba_2 +Fu_2)W_2 where, W_2=0.8; EI= 100 (e/e+b); SI= 100 (s/s + b); BI = 100*b/(e+s+b); CI= 100 (Fu_2W_2 / Ba_1W_1+Fu_2W_2) (Ferris et al. 2001).

3.14 Statistical analysis

Soil quality data was analyzed in Minitab 19.0 (Minitab LLC, State College, Pennsylvania, US). Box-Cox transformations were applied to non-normal data and then assessed for equal variance. A mixed effects model using restricted maximum likelyhood (REML) estimation was used to determine significant differences in soil properties across land-use intensity for 2018 and 2019 separately in Minitab 19.0. Random effects included pseudoreplicate nested within site, while fixed effects assessed intensity. Mean comparisons were tested using Tukey's honest significant difference (HSD) post hoc test at a significance level of α=0.05.

Significant differences in nematode family and trophic abundances as well as indices across intensity were analyzed in Minitab 19.0 using non-parametric Kruskal-Wallis one-way analysis of variance due to non-normal data. The nematode counts of five sampling locations were averaged for each site before analysis to account for pseudoreplication. Mean comparisons were carried out using Dunn's multiple comparison test with Bonferroni correction.

Non-metric multidimensional scaling (NMDS) using Bray-Curtis dissimilarities were used to further investigate patterns in the nematode community in Rstudio (RStudio, PBC, Boston, Massachusetts, United States) using the 'vegan' package. Communities were assessed by intensity and crop type. Crop type for NMDS were based on plant family and agricultural use. The forest category consisted of samples taken at minimum 10 m into the

tree line classified under the Acadian forest region. Forest/grass treatments were those sampled 2 m within or outside of the treeline. Grass samples could consist of roadside grass and grass pastures. The cereal category included grain crops, such as corn (*Zea mays* L.), barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.*)*, oats (*Avena sativa* L.) and sudan grass (*Sorghum* x *drummondii* (Nees ex. Steud.) Mills & Chase). Grass/legumes consisted of clover/grass mixes or fields with a cereal underseeded to a clover. Legumes consisted of clovers (*Trifolium* spp.) and soybeans (*Glycines max* (L.) Merr.). All pseudocereal sites were planted to buckwheat (*Fagopyrum esculentum* Moench) and all root vegetable sites were planted with potatoes (*Solanum tuberosum* L.). Nematode counts were converted to proportional abundances and standardized before analysis. Differences in communities within intensity treatments and crop types were tested using ANOSIM with a significance level of $\alpha=0.05$. Dunn's multiple comparison test with Bonferroni correction in Minitab 19.0 was used to assess mean differences for intensities and crops using NMDS coordinates, as there were non-normal distributions and unequal sites per crop in both years.

Soil properties were ordinated over nematode communities using metaNMDS with Bray-Curtis dissimilarities in Rstudio. Soil properties that explained nematode community variation were determined at a significance level of $\alpha=0.05$.

4. RESULTS

Sample site, crop rotation and current crop information can be found in the Table 1 & 2. Three of the traditional grain rotation (medium intensity) sites transitioned to potato production in 2019. Thus, while their current year is in potato production, their rotational histories still reflect the intensity treatments of this study.

4.1 Soil properties

Inherent soil properties such as soil texture showed significant differences in sand, silt and clay % contents between intensities, but showed no trend based upon LUI across both 2018 and 2019 (Table 4 & 5). Sand content ranging from 57-64% across LUI and years, while clay content was consistently below 11.8% for all treatments. Despite textural variation, all of the texture means presented in Table 5 represent sandy loam soils. Precipitation and degree days did not follow a consistent trend across both years (Table 5).

Total C consistently decreased in the LUI order L>M>H and ranged from an average of 1.1 to 2.5 % across both sampling years. Similarly, total soil N showed decreasing trends with increasing LUI in both 2018 and 2019 (Table 5). However, C:N ratios were consistently highest for the low intensity treatment.

While aggregate size fractions were only assessed in 2018, this year's trend showed that medium intensity soils had larger aggregate size fractions. Larger percentages of 2-8 mm and 0.25-2 mm soil aggregate fractions were found in medium intensity soils (Table 5).

POXc trends across intensity in 2018 and 2019 were similar (Table 5). POXc of medium intensity soils were greater than highest intensity soils in both years, and was statistically the same as low intensity systems. ACE protein levels of rotational land-use (medium and high intensities) were lower than low intensity systems in both years (Table 5).

pH, which ranged from 4.47 – 6.72 in 2018 and 4.37 - 7.29 in 2019, showed a consistent trend across both years, where medium intensity soils had the highest pH followed by high and low intensity soils (Table 5).

Table 4. Significance of soil properties due to land-use intensities in 2018 and 2019.

		2018		2019	
Soil trait		**F Value**	**p-value**	**F Value**	**p-value**
C	%	42.06	0.000	7.43	0.001
N	%	25.78	0.000	22.60	0.000
C:N	NA	23.77	0.000	52.01	0.000
Available K	kg ha^{-1}	16.88	0.000	-	-
Available P	kg ha^{-1}	111.99	0.000	-	-
% 2-8 mm	%	3.20	0.044	-	-
% 0.25-2 mm	%	7.37	0.001	-	-
% <0.25 mm	%	11.92	0.000	-	-
% sand	%	3.84	0.024	5.04	0.008
% silt	%	3.12	0.051	6.94	0.001
% clay	%	6.17	0.003	1.51	0.225
Moisture %	%	38.02	0.000	12.90	0.000
POXc	ppm	5.13	0.007	30.33	0.000
pH	NA	62.75	0.000	19.92	0.000
ACE protein	mg g^{-1} soil	43.43	0.000	58.45	0.000
Degree days	Cumulative DD	3.79	0.025	2.61	0.077
Precipitation	Cumulative mm	8.03	0.000	11.61	0.000

Note: POXc= Active carbon, ACE protein= autoclave citrate-extractable protein, % 2-8, % 0.25-2 and % <0.25 = aggregate size distribution percentages indicated based on mm size range. Assessment of available P and K, as well as aggregate size fractions on 2019 soils was not possible due to unforeseen research restrictions.

Table 5. Table of means ± standard error for soil property main effects across PEI with low (L), medium (M) and high (H) land-use intensities in 2018 and 2019.

	2018			2019		
Soil trait	**L**	**M**	**H**	**L**	**M**	**H**
C	2.43 ±0.14a	1.62±0.06b	1.27 ±0.03c	2.181±0.13a	1.42±1.35b	1.10±0.30c
N	0.16±0.01a	0.14±0.01b	0.11±0.01c	0.14±0.01a	0.13±0.43a	0.10±0.04b
C:N	15.32±0.64a	11.66±0.19b	11.30±1.95b	15.72±0.54a	10.74±1.42b	10.51±1.00b
Available K	171.80±11.40c	248.10±25.62b	286.00±12.36a	-	-	-
Avaliable P	55.24±4.53c	131.26±5.88b	199.10±14.03a	-	-	-
% 2-8	23.57±1.30ab	24.45±1.14a	20.43±0.96b	-	-	-
% 0.25-2	40.62±0.89b	43.742±0.69a	40.505±0.80b	-	-	-
% <0.25	36.65±1.30a	32.677±0.95b	40.22±1.32a	-	-	-
% sand	57.56±0.89b	61.15±1.13a	60.02±0.46ab	64.02±0.81a	60.49±7.28b	64.23±2.76a
% silt	30.64±1.30	28.20±0.91	28.40±0.32	25.06±0.71b	27.93±6.54a	24.12±2.23b
% clay	11.80±1.08a	10.46±0.28b	11.78±0.23a	10.92±0.27	11.66±6.19	11.66±0.84
Moisture %	21.54±0.84a	18.74±0.36b	14.53±0.64c	20.68±0.87a	17.54±4.66b	15.19±2.51b
POXc	342.8±0.4ab	383.3±13.4a	296.7±15.7b	435.00±21.3a	397.72±14.5a	283.44±15.2b
pH	5.42±0.69c	6.27±0.06a	5.99±0.03b	5.44±0.07b	6.13±1.77a	5.87±0.47a
ACE protein	26.87±26.08a	6.64±0.18b	5.77±0.10b	9.32±0.45a	6.03±1.82b	4.95±0.67c
Degree days	306.3±0.1ab	296.2±14.1b	397.6±13.1a	370.11±9.7	367.4±24.1	404.0±12.8
Precipitation	233.9±3.2b	225.4±1.6b	226.7±5.6a	268.1±5.4b	290.2±10.7a	254.7±14.9b

Note: POXc= Active carbon, ACE protein= autoclave citrate-extractable protein, % 2-8, % 0.25-2 and % <0.25 = aggregate size distribution percentages indicated based on mm size range. Treatments with the same letters, within the main effects are not significantly different according to Tukey's HSD at α=0.05. Units of measurement are presented in Table 2. Assessment of available P and K, as well as aggregate size fractions of 2019 soils was not possible due to unforeseen research restrictions.

Table 6. Pearson correlation coefficients of soil properties across PEI soils in 2018 and 2019.

C	1	-													
N	2	**0.820**	-												
C:N	3	**0.746**	**0.261**	-											
POXc	4	**0.429**	**0.296**	**0.322**	-										
Ace Protein	5	**0.472**	**0.205**	**0.606**	0.126	-									
Moisture %	6	**0.621**	**0.585**	**0.387**	**0.240**	**0.306**	-								
pH	7	**-0.500**	**-0.176**	**-0.628**	-0.017	**-0.523**	**-0.253**	-							
Available P	8	**-0.492**	**-0.429**	**-0.407**	-0.086	**-0.408**	**-0.491**	**0.338**	-						
Available K	9	-0.046	-0.006	-0.095	**0.206**	-0.086	-0.082	0.003	**0.474**	-					
% sand	10	-0.009	**-0.239**	**0.249**	**0.288**	0.159	**-0.256**	-0.110	0.138	0.072	-				
% silt	11	0.015	**0.281**	**-0.268**	**-0.296**	**-0.191**	**0.242**	0.126	-0.119	-0.020	**-0.959**	-			
% clay	12	-0.007	0.060	-0.119	-0.172	-0.030	**0.201**	0.034	-0.136	**-0.170**	**-0.751**	**0.533**	-		
%2-8 mm	13	-0.029	**0.191**	**-0.274**	-0.070	-0.097	**0.471**	**0.170**	**-0.201**	-0.077	**-0.605**	**0.565**	**0.489**	-	
%0.25-2 mm	14	-0.084	-0.129	0.031	0.079	0.066	-0.017	0.162	0.060	0.045	**0.319**	**-0.309**	**-0.233**	0.035	-
%<0.25 mm	15	0.047	-0.123	**0.204**	0.006	0.003	**-0.533**	**-0.231**	**0.183**	0.039	**0.349**	**-0.320**	**-0.296**	**-0.741**	**-0.316**
		1	2	3	4	5	6	7	8	9	10	11	12	13	14

Note: Significant correlation coefficients, which were determined at a significance level of α=0.05, are indicated in bold.

4.2 Nematode family abundances

Overall, 27 families of nematodes were found in soils across PEI. Nematodes found in over 80% of the field sites include Aphelenchidae, Aphelenchoididae, Belanoliamidae, Cephalobidae, Dorylaimidae, Hoploliamidae, Paratylenchidae, Plectidae, Pratylenchidae, Prismatolaimidae, Rhabditidae and Tylenchidae (Table 7).

Cephalobiade was the most abundant bacterivore followed by Rhabditidae. Plectidae, Prismatolaimidae, Panagrolaimidae, Monhysteridae, Teratocephalidae, Alaimidae and Diplogastridae were found in much lower abundances and at fewer field sites (Table 7, 8 & 9).

Aphelenchiodidae was the family with the highest abundance for fungivores, where they were generally found at least twice as many compared to Aphelenchidae (Table 8 & 9). While Tylencholaimidae was only found in a limited number of fields; when present, these nematodes were found in high abundance in the range of 406-645 nematodes 100 g^{-1} soil (data not presented in table).

Omnivorous nematodes Aporcelaimidae, Dorylaimidae and Qudsianematidae were identified. Only one Qudsianematidae (*Discolaimus* spp.) nematode was found at one site, showing that this taxon is not common in PEI soils.

The only specialist predator family found in PEI was Mononchidae. These nematodes were found in low abundances even under low intensity forest settings (Table 8 & 9).

Pratylenchidae and Tylenchidae were the most dominant plant parasites and were found consistently over all land-use types in 98% and 100% of total fields, respectively. Heteroderidae and *Melodiogyne* spp. were also relatively low compared to Pratylenchidae and Tylenchidae. Of the Hoplolaimidae identified the nematodes were predominantly *Helicotylenchus* spp.

Aphelenchidae, Belanoliamidae, Cephalobidae, Dorylaimidae, Plectidae, Pratylenchidae. Prismatolaimidae and Rhabditidae showed significant differences among land-use intensities during both years (Table 7). Consistent abundance trends, across both years, were found in the majority of nematode families (Table 8 & 9). Aphelenchidae showed a positive trend between abundance and intensity, while Prismatolaimidae abundance decreased with intensity. Belanoliamidae and Cephalobidae showed decreased abundance in low intensity systems. Response of omnivores Dorylaimidae in 2018 and 2019 and Aporcelaimidae in 2018 exhibited the same response to intensity with increased abundance under low intensity systems. Plectidae also exhibited the same trend as the omnivores. Pratylenchidae and Rhabditidae abundances differences were inconsistent between years. Mean abundances of nematodes by intensity were also presented, which showed a general trend of higher nematode abundances in 2019 (Fig. 2b) compared to 2018 (Fig 2a).

Table 7. Significance of nematode taxon abundances due to land-use intensity in 2018 and 2019 as well as field presence percentages.

Nematode Family/Genus	Trophic Group	2018		2019		% presence in 2018-2019 fields (n=59)
		H Value	p-value	H Value	p-value	
Alaimidae	Ba	0.56	0.755	5.12	0.077	61
Anguinidae	PP	5.52	0.063	2.68	0.262	71
Aphelenchidae	Fu	**17.45**	**0.000**	**11.87**	**0.003**	97
Aphelenchoididae	Fu	5.58	0.061	0.06	0.969	100
Aporcelaimdiae	Om	3.84	0.147	**16.65**	**0.000**	78
Belonolaimidae	PP	**31.34**	**0.000**	**19.85**	**0.000**	86
Cephalobidae	Ba	**41.99**	**0.000**	**21.49**	**0.000**	100
Criconematidae	PP	**34.53**	**0.000**	1.86	0.395	68
Diphtherophoradae	Fu	4.78	0.091	1.18	0.555	56
Diplogastridae	Ba	-	-	0.04	0.982	7
Dorylaimidae	Om	**9.08**	**0.011**	**14.11**	**0.001**	90
Heteroderidae	PP	0.81	0.666	0.36	0.837	34
Hoplolaimidae	PP	4.31	0.116	5.1	0.078	88
Leptonchidae	Fu	0.04	0.982	**18.25**	**0.00**	37
Longidoridae	PP	3.61	0.165	1.61	0.447	34
Meloidogynidae	PP	0.33	0.848	**8.55**	**0.016**	75
Monhysteridae	Ba	-	-	1.43	0.489	8
Mononchidae	P	3.72	0.156	0.49	0.782	66
Panagrolaimidae	Ba	**8.65**	**0.013**	0.67	0.714	71
Paratylenchidae	PP	**16.56**	**0.000**	1.49	0.475	92
Plectidae	Ba	**20.69**	**0.000**	**57.58**	**0.000**	81
Pratylenchidae	PP	**43.35**	**0.000**	**10.36**	**0.006**	98
Prismatolaimidae	Ba	**12.13**	**0.002**	**7.52**	**0.023**	83
Qudsianematidae	Om	0.04	0.981	-	-	2
Rhabditidae	Ba	**13.28**	**0.004**	**13.49**	**0.001**	100
Teratocephalidae	Ba	0.35	0.839	0.37	0.832	15
Tylenchidae	PP	0.42	0.811	**45.24**	**0.000**	100
Tylencholaimidae	Fu	-	-	0.36	0.836	5

Note: Ba= bacterivore, PP= plant parasite, Fu=fungivore, Om=omnivore, P= predator. Significant differences were determined by Kruskal-Wallis rank sums test at α=0.05.

Table 8. Nematode abundance across 2018 land-use intensity sites as population medians.

Nematode Family	Trophic Group	L	Min-Max	M	Min-Max	H	Min-Max
Alaimidae	Ba	0.0	(0-14.7)	0.0	(0-47.2)	0.0	(0-16.5)
Anguinidae	PP	0.0	(0-17.6)	0.0	(0-21.4)	0.0	(0-72.0)
Aphelenchidae	Fu	0.0b	(0-244.9)	6.6a	(0-32.3)	5.5a	(0-44.9)
Aphelenchoididae	Fu	5.9	(0-298.7)	7.7	(0-96.3)	10.1	(0-154.0)
Aporcelaimdiae	Om	0.7	(0-58.9)	0.0	(0-55.3)	0.0	(0-55.9)
Belonolaimidae	PP	0.0b	(0-89.0)	8.3a	(0-110.9)	13.0a	(0-217.0)
Cephalobidae	Ba	30.3b	(0-390.4)	112.7a	(8.1-656.0)	101.1a	(19.8-450.2)
Criconematidae	PP	8.9a	(0-284.4)	0.0b	(0-204.1)	0.0c	(0-7.0)
Diphtherophoradae	Fu	0.0	(0-47.2)	0.0	(0-108.0)	0.0	(0-15.5)
Diplogastridae	Ba	-	-	-	-	-	-
Dorylaimidae	Om	10.5a	(0-186.3)	6.0b	(0-68.0)	3.1b	(0-58.1)
Heteroderidae	PP	0.0	(0-2.4)	0.0	(0-10.5)	0.0	(0-0)
Hoplolaimidae	PP	0.6	(0-578.7)	1.1	(0-507.8)	0.0	(0-30.9)
Leptonchidae	Fu	0.0	(0-10.1)	0.0	(0-5.9)	0.0	(0-0)
Longidoridae	PP	0.0	(0-13.1)	0.0	(0-3.7)	0.0	(0-5.2)
Meloidogynidae	PP	0.0	(0-14.1)	0.0	(0-11.7)	0.0	(10.3)
Monhysteridae	Ba	-	-	-	-	-	-
Mononchidae	P	0.0	(0-22.3)	0.0	(0-11.3)	0.0	(0-24.8)
Panagrolaimidae	Ba	0.0b	(0-37.3)	1.3a	(0-59.0)	0.8a	(0-40.7)
Paratylenchidae	PP	10.3a	(0-463.0)	0.0b	(0-329.5)	0.0b	(0-125.5)
Plectidae	Ba	6.3a	(0-70.7)	0.0b	(0-63.1)	0.0b	(0-15.5)
Pratylenchidae	PP	5.8c	(0-260.8)	86.2a	(0-419.6)	28.6b	(0-208.0)
Prismatolaimidae	Ba	0.0a	(0-51.3)	0.0a	(0-95.8)	0.0b	(0-4.2)
Qudsianematidae	Om	0.0	(0-1.7)	0.0	(0-0)	0.0	(0-0)
Rhabditidae	Ba	27.4b	(0-872.9)	68.2a	(3.4-2311.7)	55.9a	(0-1395.8)
Teratocephalidae	Ba	0.0	(0-28.7)	0.0	(0-0)	0.0	(0-0)
Tylenchidae	PP	23.8	(0-349.3)	25.8	(0-162.1)	22.3	(0-199.4)
Tylencholaimidae	Fu	-	-	-	-	-	-

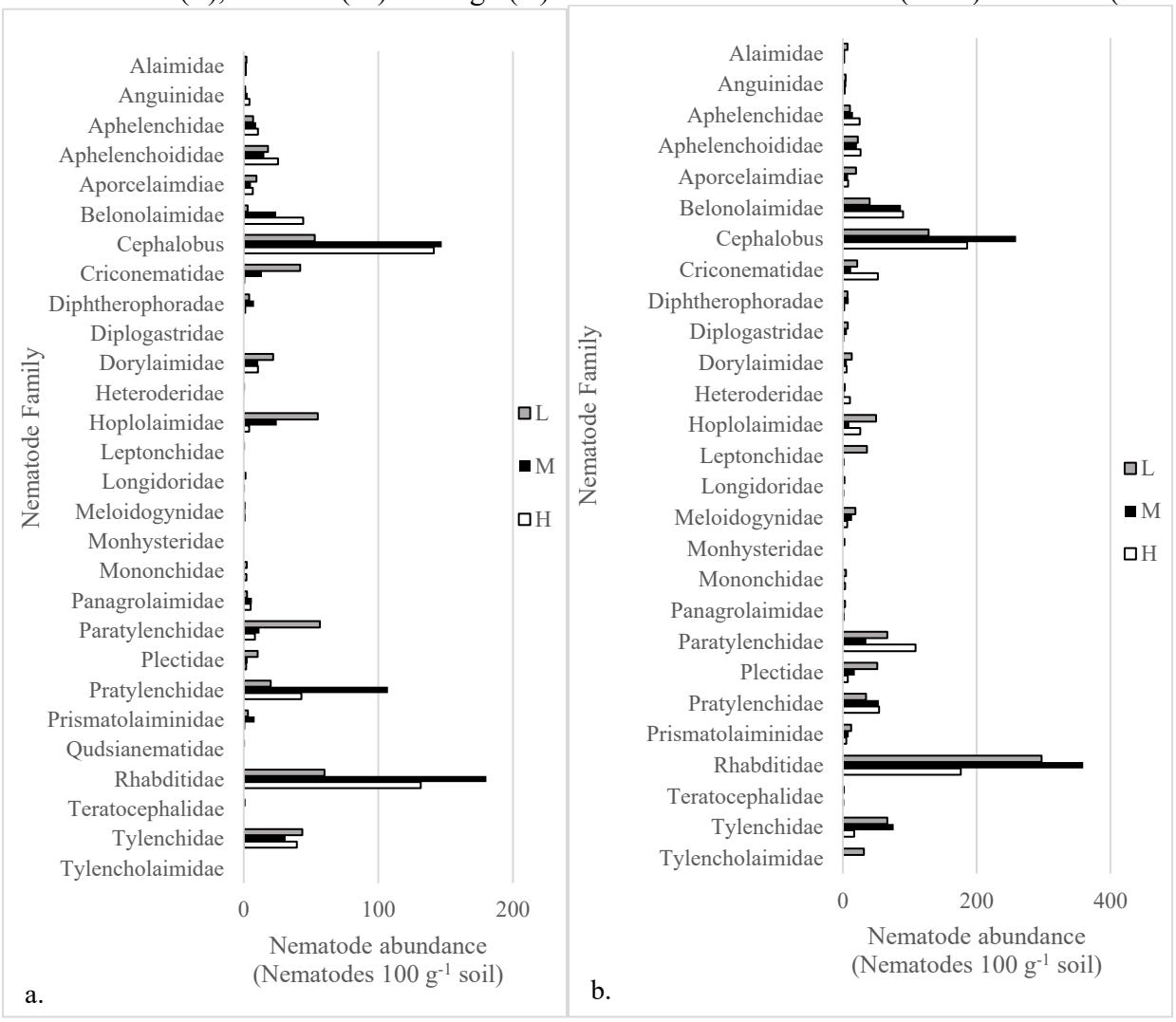
Note: L= low intensity, M=medium intensity, H=high intensity, Ba= bacterivore, PP= plant parasite, Fu=fungivore, Om=omnivore, P= predator. Treatments with the same letters, are not significantly different according to Kruskal-Wallis rank sums test at α=0.05. Absence of letters indicates there is no significant difference.

Table 9. Nematode abundance across 2019 land-use intensity sites as population medians.

Nematode Family	Trophic Group	L	Min-Max	M	Min-Max	H	Min-Max
Alaimidae	Ba	0.0	(0-63.5)	0.0	(0-48.6)	0.0	(0-14.4)
Anguinidae	PP	0.0	(0-59.3)	0.0	(0-26.4)	0.0	(0-14.1)
Aphelenchidae	Fu	4.0b	(0-82.9)	11.5ab	(0-64.7)	11.2a	(0-120.7)
Aphelenchoididae	Fu	13.9	(0-155.4)	16.8	(0-87.6)	76.6	(0-183.8)
Aporcelaimdiae	Om	16.8a	(0-73.1)	4.3b	(0-30.2)	3.3b	(0-50.8)
Belonolaimidae	PP	0.0b	(0-285.5)	39.8a	(0-732.4)	41.4a	(0-369.5)
Cephalobidae	Ba	107.6b	(0-618.2)	237.7a	(30.4-828.4)	167.0a	(27.5-479.0)
Criconematidae	PP	10.6	(0-192.8)	4.9	(0-76.6)	10.6	(0-599.2)
Diphtherophoradae	Fu	0.0	(0-123.5)	0.0	(0-90.5)	0.0	(0-22.8)
Diplogastridae	Ba	0.0	(0-368.0)	0.0	(0-276.1)	0.0	(0-14.1)
Dorylaimidae	Om	10.2a	(0-59.5)	1.3b	(0-32.4)	0.0b	(0-65.0)
Heteroderidae	PP	0.0	(0-51.9)	0.0	(0-20.4)	0.0	(0-249.9)
Hoplolaimidae	PP	10.8	(0-662.1)	0.0	(0-92.4)	5.3	(0-208.4)
Leptonchidae	Fu	4.1a	(0-1015.8)	0.0b	(0-13.2)	0.0b	(0-16.9)
Longidoridae	PP	0.0	(0-26.0)	0.0	(0-5.5)	0.0	(0-11.5)
Meloidogynidae	PP	9.5a	(0-124.4)	8.6a	(0-96.2)	0.0b	(0-40.2)
Monhysteridae	Ba	0.0	(0-61.7)	0.0	(0-0)	0.0	(0-0)
Mononchidae	P	0.0	(0-47.4)	0.0	(0-12.0)	0.0	(0-32.5)
Panagrolaimidae	Ba	0.0	(0-97.4)	0.0	(0-25.1)	0.0	(0-16.3)
Paratylenchidae	PP	10.7	(0-627.3)	13.8	(0-206.3)	16.3	(0-725.2)
Plectidae	Ba	32.9a	(0-182.4)	13.6b	(0-86.4)	0.0c	(0-65.0)
Pratylenchidae	PP	14.7b	(0-234.4)	45.8a	(0-231.5)	38.3a	(0-169.5)
Prismatolaimidae	Ba	7.5a	(0-42.0)	2.7ab	(0-60.3)	0.0b	(0-39.1)
Qudsianematidae	Om	-	-	-	-	-	-
Rhabditidae	Ba	115.5b	(0-3395.1)	189.1a	(33.8-1991.59)	87.8b	(0-916.7)
Teratocephalidae	Ba	0.0	(0-33.8)	0.0	(0-4.4)	0.0	(0-4.9)
Tylenchidae	PP	44.6a	(0-249.6)	57.1a	(0-331.9)	11.9b	(0-90)
Tylencholaimidae	Fu	0.0	(0-645.8)	0.0	(0-0)	0.0	(0-0)

Note: L= low intensity, M=medium intensity, H=high intensity, Ba= bacterivore, PP= plant parasite, Fu=fungivore, Om=omnivore, P= predator. Treatments with the same letters, are not significantly different according to Kruskal-Wallis rank sums test at α=0.05. Absence of letters indicates there is no significant difference.

Figure 2. Nematode mean abundance data represented as nematodes per 100 g of soil across PEI sites with low (L), medium (M) and high (H) land-use intensities in 2018 (n=29) and 2019 (n=30).



Note: a. Nematode family means in 2018, b. Nematode family means in 2019.

4.3 Nematode trophic and indice assessment

There were noticeably increased number of total nematodes (Table A5), predominantly plant parasites (ectoparasites) and bacterivores, in the 2019 samples compared to 2018 samples (Fig. 3). The soils from sites sampled in 2019 (60-64% sand) were typically sandier than 2018 (57-61%) (Table 5), which would have facilitated nematode food acquisition through increased motility. This trend is seen across all land-use intensities and may be also more reflective of

differences in environmental conditions since there were numerically higher degree days and higher precipitation in 2019 (Table 5).

Total nematode abundance was inconsistent where LUI influenced nematode abundances in 2018, but not in 2019 (Table 10). As trophic level increased within the nematode community, more consistent responses were observed to land-use intensity across growing seasons. Bacterivore and fungivore abundances showed increased abundances in soils from the medium and high intensity sites compared to the low intensity in 2018 (Fig. 3). In 2019, the abundance of bacterivores was significantly higher in the medium intensity sites than the high intensity with no difference found in the abundance of fungivores (Fig. 3). Omnivores was the only feeding group that responded similarly to land-use intensity in 2018 and 2019. There were no significant differences in predator abundances as there was only one family identified (Mononchidae) and populations were low across all land-use types (Fig. 3).

Although the EI for medium intensity sites was significantly higher than the high intensity sites in 2019 only, a similar trend was observed across land-use intensity in 2018 (Fig. 4). BI and SI responded consistently to LUI in both years, with the low intensity sites being significantly higher values compared to medium and high intensity for SI and low and high intensity sites having significantly higher BI (Fig. 4).

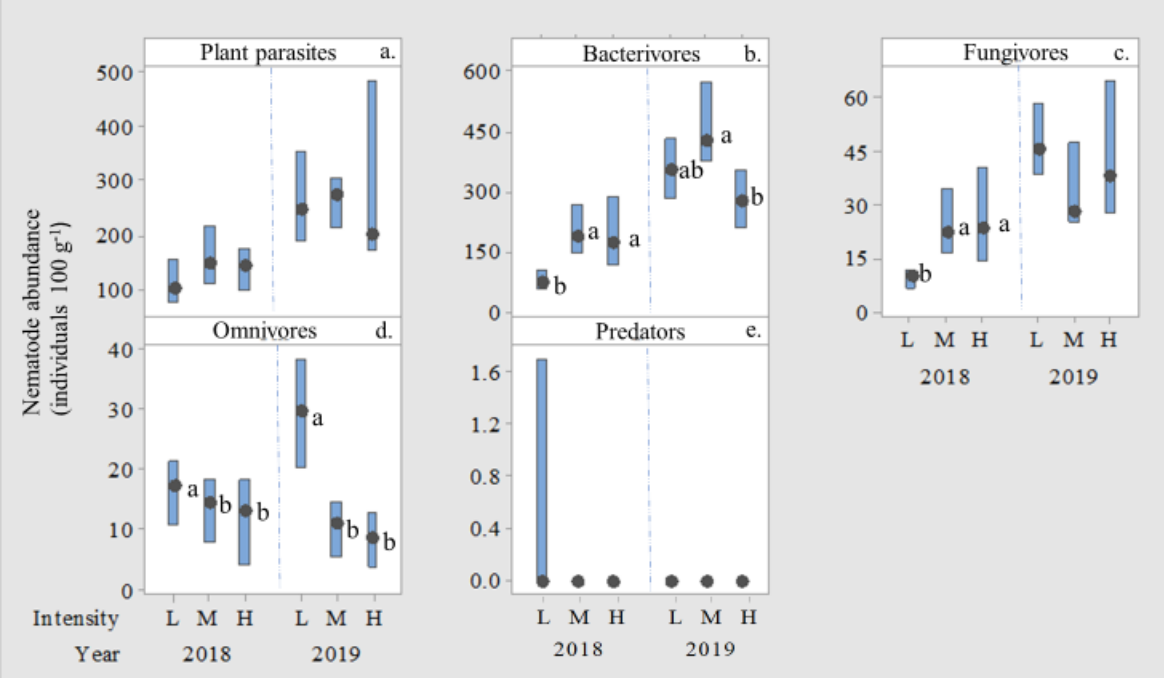
The total abundance of PP and PPI did not show consistent trends across intensity over both growing seasons. Endoparasites were significantly higher in the medium intensity in 2018 (Table A5).

Table 10. Significance of nematode trophic abundances and indices due to land-use intensities during 2018-2019 seasons.

Abundances	2018		2019	
(Individuals 100 g^{-1} soil)	H value	p-value	H value	p-value
Total nematodes	**11.64**	**0.003**	0.93	0.628
Plant parasites	1.89	0.390	0.30	0.863
Endoparasites	**43.16**	**0.000**	2.59	0.274
Ectoparasites	**30.18**	**0.000**	0.06	0.973
Bacterivores	**22.38**	**0.000**	**9.45**	**0.009**
Fungivores	**14.95**	**0.001**	2.91	0.237
Omnivores	**6.38**	**0.041**	**24.50**	**0.000**
Predators	3.72	0.156	0.52	0.764
Trophic proportions (%)				
Plant parasites	5.91	0.052	2.14	0.344
Endoparasites	**40.41**	**0.000**	4.41	0.110
Ectoparasites	**40.58**	**0.000**	0.94	0.625
Bacterivores	**15.97**	**0.000**	**10.33**	**0.006**
Fungivores	**6.94**	**0.031**	5.25	0.072
Omnivores	**23.86**	**0.000**	**23.04**	**0.000**
Predators	**8.03**	**0.018**	0.69	0.707
Indices				
MI	**16.67**	**0.000**	**16.08**	**0.000**
MI2-5	**33.86**	**0.000**	**43.16**	**0.000**
ΣMI	**16.81**	**0.000**	**14.73**	**0.001**
PPI	**7.88**	**0.019**	**29.14**	**0.000**
BI	**14.96**	**0.001**	**20.73**	**0.000**
CI	3.65	0.162	**17.20**	**0.000**
EI	2.18	0.336	**7.33**	**0.026**
SI	**34.52**	**0.000**	**43.33**	**0.000**

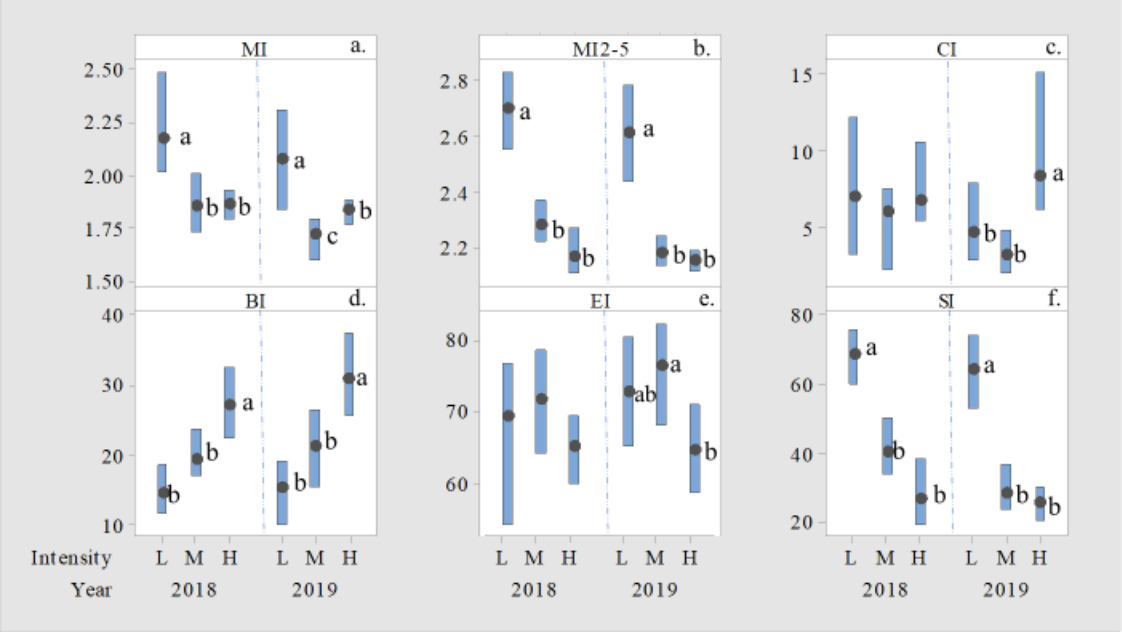
Note: MI= maturity index of all of free-living nematodes, MI2-5= maturity index of free-living cp2-5 nematodes, ΣMI= maturity index of all plant parasitic and free-living nematodes, BI= basal index, CI=channel index, EI= enrichment index, SI= structure index. Significant differences were determined by Kruskal-Wallis rank sums test at α=0.05.

Figure 3. Boxplots of nematode trophic abundances across land-use intensity in 2018 and 2019, where significant differences across intensity were determined separately in each year.



Note: L= low intensity, M= medium intensity, H= high intensity. Significant differences determined by Kruskal-Wallis rank sums test at α=0.05. Absence of letters indicates there is no significant difference.

Figure 4. Boxplots of nematode faunal indices across land-use intensity in 2018 and 2019, where significant differences across intensity were determined separately in each year.

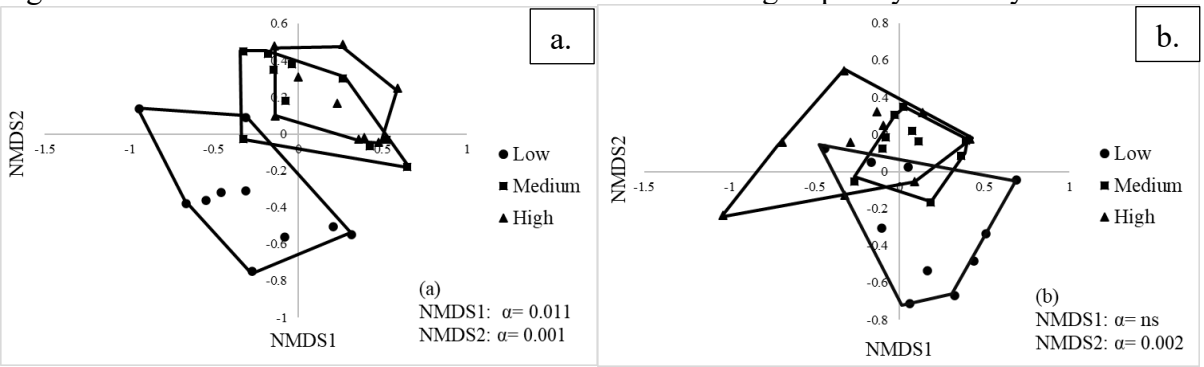


Note: MI= maturity index of all of free-living nematodes, MI2-5= maturity index of free-living cp2-5 nematodes, ΣMI= maturity index of all plant parasitic and free-living nematodes, BI= basal index, CI=channel index, EI= enrichment index, SI= structure index. L= low intensity, M=medium intensity, H=high intensity. Different letters indicate statistically significant differences determined by Kruskal-Wallis rank sums test at α=0.05. Absence of letters indicates there is no significant difference.

4.4 Nematode multivariate analyses

Nematode communities of different intensities were determined to be significantly different by ANOSIM at α=0.001 in 2018 and 2019. Assessment of nematode community composition across LUI via NMDS coordinates in 2018 and 2019 showed significant differences in nematode community composition between low intensity systems and the cropped, medium and high intensity, communities (Fig. 5 & Table 11).

Figure 5. NMDS of 2018 and 2019 nematode communities grouped by intensity treatments.



Note: (Low intensity (forest/pasture systems), Medium intensity (4 year grain rotations), High intensity (3 year potato rotations). (a): 2018 nematode communities, Stress= 0.22. (b): 2019 nematode communities, Stress= 0.23.

Table 11. Means comparison groupings of 2018 and 2019 nematode communities from NMDS coordinate output.

		2018		2019	
Sample	Site sample size (n)	NMDS1	NMDS2	NMDS1	NMDS2
L	9	B	B	A	B
M	10	AB	A	A	A
H	10	A	A	A	A

Note: Different letters represent significant differences in NMDS values (Fig. 5) between LUI treatment.

Soil properties that significantly explained variation in the nematode communities showed some differences in 2018 (Fig. 6) and 2019 (Fig. 7). pH was consistently related to high and medium intensity communities while soil moisture %, ACE protein and total C were consistently related to low intensity systems.

Figure 6. metaMDS of nematode communities across PEI land-use intensities with soil properties from 2018 sites.

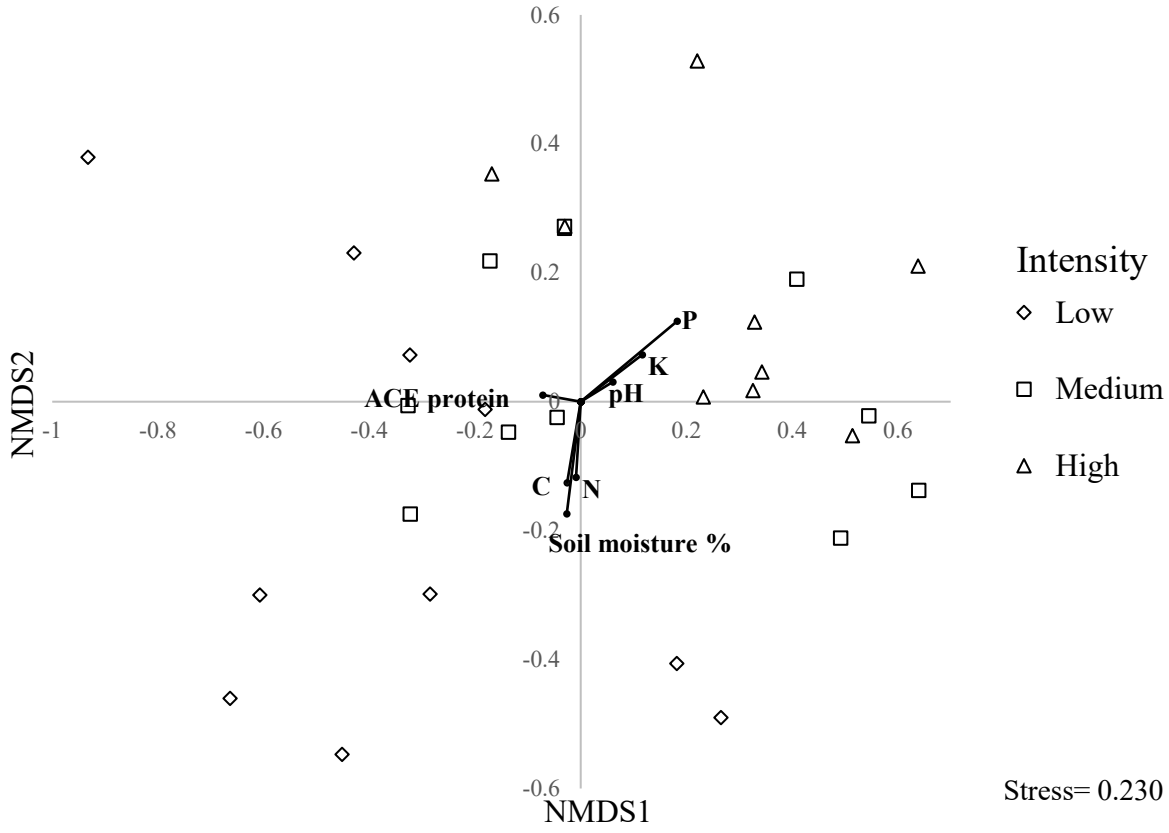
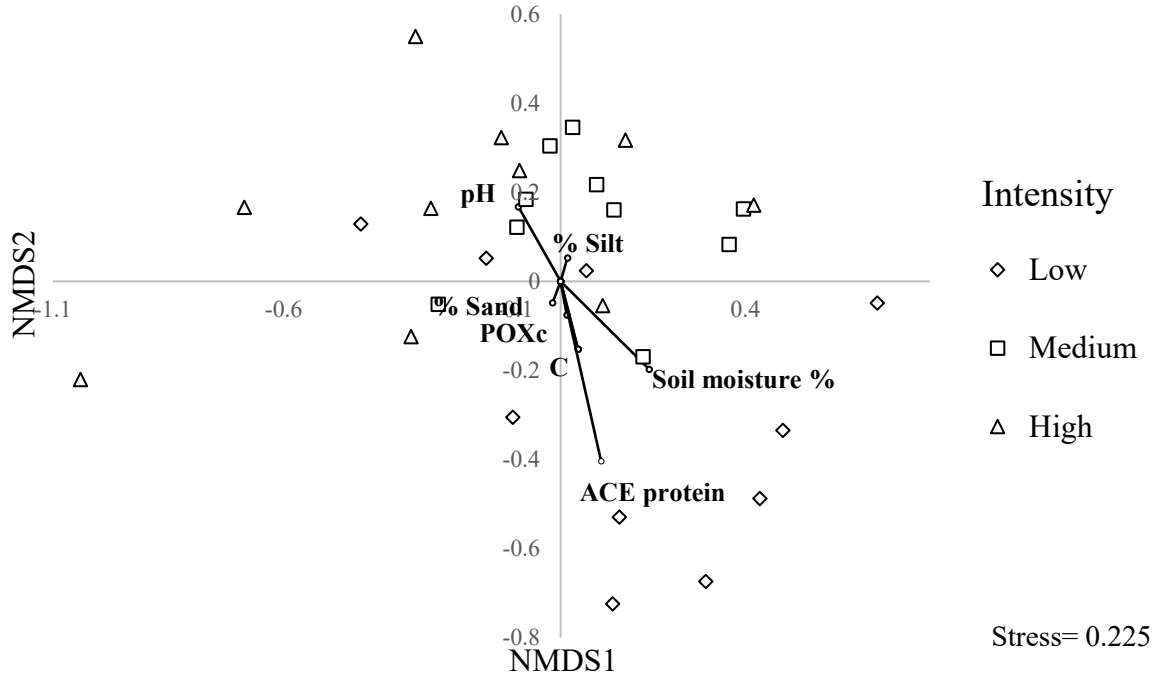
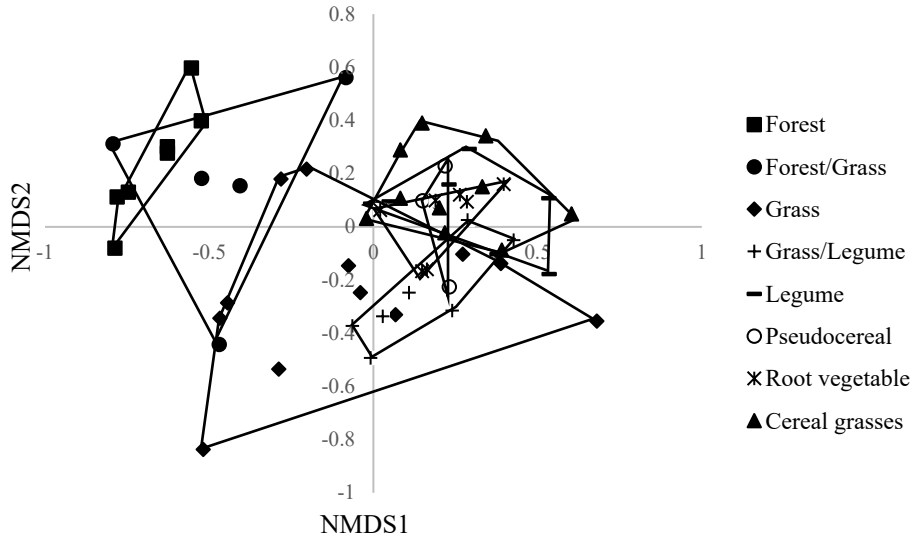


Figure 7. metaMDS of nematode communities across PEI land-use intensities with soil properties from 2019 sites.



When assessing the effect of vegetation type on the nematode community there were not enough sites for each crop in individual years. For example in 2018 there was only one site representing the forest/grass category, so 2018 and 2019 data were combined before assessment. Nematode communities of different crop types were determined to be significantly different by ANOSIM at α=0.001. When nematode communities were analyzed by crop we see further differences which have implications for the low intensity treatment. There is a distinct gradient from the forest to forest/grass to grass vegetation types (Fig. 8). Within the cropped categories, only cereals were different from grass/legumes in the NMDS2 (Table 12).

Figure 8. NMDS of nematode communities grouped by crop type of combined 2018 and 2019 sites.



Note: Crop type descriptions are provided in the methods section (*3.14. Statistical methods*). Stress=0.249

Table 12. Mean comparison groupings of combined 2018 and 2019 nematode communities by crop from NMDS coordinate output.

Sample	Site sample size (n)	NMDS1	NMDS2
Forest	7	A	C
Forest/Grass	5	AB	BC
Grass	13	BC	AB
Grass/Legume	8	CD	A
Pseudocereal	3	CD	ABC
Root Veg	7	CD	ABC
Cereal	10	D	BC
Legume	6	D	ABC

Note: C=cereal, F= forest, F/G= forest/grass, G=grass, G/L= grass/legume, L=legume, P=pseudocereal, RV= root vegetable.

5. DISCUSSION

5.1 Soil quality traits and environmental variables across PEI land-use intensities

The steady reduction of both total C with increasing land-use intensity (Table 5) can be attributed to tillage intensification, reduction of plant residue returns and reduction of permanent plant roots (Magdoff and Weil 2004). This is consistent with a land-use study where C was significantly higher in forest than fallow and shrub over fallow and arable land (Zajícová and Chuman 2019). Soil moisture was moderately positively correlated with properties relating to SOM

including total C, total N and ACE protein. Thus, decreasing SOM appears to be the driving factor of the degrading function of soil moisture retention in this study.

While POXc is related to particulate organic C (POC), microbial biomass C (MBC), and other soil C fractions (Culman et al. 2012), is more reflective of recent management practices (~1-5 years) relating to organic matter inputs and decomposition (Culman et al. 2012). In this study POXc moderately correlated with total C, which indicates SOM content (Table 6). Inconsistency across year in the low intensity treatment and relatively large standard errors (Table 5) indicate that these land types are more variable when it comes to POXc. Although crops were different, depending on sampling year, medium and high intensity POXc values was consistent across years and always lowest for the high intensity treatment (Table 5). This suggests that the total management practices associated with rotational sites provides a more narrow, consistent range of POXc, perhaps due to reduced total C and increased soil homogeneity. A study by Williams et al. in 2020 found that while POXc responded to management, it was also highly regulated by soil texture across agricultural soils. This is also supported by this study where sand % was also positively correlated with POXc (Table 4). In contrast, in Ontario, Congreves et al. (2015) found that POXc was inversely related to sand content. POXc across all years and intensities were relatively low, but still falling within an acceptable range compared to levels found in residue-tillage trials in Kensington, PEI (200-700 ppm) (Kensington North Watershed Association and PEI Department of Agriculture and Fisheries 2018).

Total N showed strong positive correlations with total C (Table 6). Reduction of total N in medium and high intensity systems is thus likely related to decreasing SOM. The data showed a consistent intensity trend in both years, where the C:N ratio of medium and high intensity

treatments have lower C:N ratios. In a study of different land-use types C:N ratio was significantly different between forests and arable land (Zajícová and Chuman 2019).

ACE protein represents organically bound N which has the potential to be mineralized by soil microbes (Hurisso et al. 2018). This factor was positively moderately correlated with the C:N ratio and negatively moderately correlated with pH (Table 6). The positive correlation may imply that soil protein becomes increasingly less bio-available to microbes. As soil protein was also significantly correlated to total C, variation in SOM content may also explain variation in ACE protein levels. ACE protein is also often positively correlated with aggregate stability (Rillig et al. 2001; Wu et al. 2014). While aggregate stability was not assessed in this study, soil protein did not positively correlate with aggregate size fractions in 2018 (Table 6).

Cumulative degree days and precipitation were not consistently related with site intensity in 2018 and 2019. Despite this, the weather data collected may be biased by distances from fields to weather stations. Plassart et al. (2019) raised the question of how can we separate the effects of land-use intensity on soil conditions if these sites were originally selected based upon site characteristics. Plassart et al. (2019) was able to account for this by first clustering soils by soil properties, then assessing the effects of land-use type (arable, forest, grassland) within clusters in an international survey. In other studies, conducted at smaller regional scales, agricultural soils had higher clay percentages compared to pasture and forest land-use types (Panday et al. 2019) and have shown that land-use was not associated with soil type (Panday et al. 2019; Zajícová and Chuman 2019). Inherent soil properties, such as texture, in this study did not consistently relate to LUI (Tafble 3). Less variability in soil textural components (% sand, % silt, % clay) was observed in this study, compared to other studies which showed larger standard errors (Panday et al. 2019).

5.2 Microbivorous nematodes

Reaction of microbivorous nematodes to LUI disturbances was more evident at the family level, therefore this discussion focuses on specific family niches and sensitivities. The different reactions of individual microbivorous nematode families to LUI were obscured at trophic level assessments. Despite this, the ecological drivers that resulted in family differences were still reflected in higher level nematode indices. Higher level trophic groups, omnivores and predators, partitioned consistently in relation to LUI in both sampling years. Increasing intensity is thought to favour low cp value bacterivores due to small body size and stimulation of bacterial food sources (Postma-Blaauw et al. 2010). Lower cp value bacterivores, Rhabditidae (cp 1) and Cephalobidae (cp 2), were however generally more abundant in soils from medium and high intensity sites (Table 8 & 9). Consistently higher opportunistic bacterivore abundances, primarily determined by Rhabditidae, in medium intensity sites over that of high intensity soils contradicted the hypothesis that their abundance would increase with LUI. Although increasing intensity generally favours smaller organisms, Ba1 also respond to recent management practices that promote bacterial food resource availability such as the addition of fresh residues (Bongers 1999). While organic matter application may support microbial food sources, root abundance and exudation within the depth sampled may also have influenced these communities. Root exudates in low intensity systems may be deposited deeper than depth of soil sampled, while reduced root biomass in fields with row crops may limit opportunistic bacterivores. Medium intensity sites had higher average C, POXc, higher soil moistures and pH which exceeds 6, compared to high intensity sites (Table 5). These conditions would better support soil microbial communities (Delgado-Baquerizo et al. 2016; Curtin et al. 2012), which likely resulted in bottom-up food web effects. However, in a study across 34 diverse farms in Atlantic Canada, Mann et al. (2019), using phospholipid fatty acid (PLFA)

analysis, found higher fungal, mycorrhizal and gram- bacteria associated biomarkers under low intensity management regimes. Overall higher Rhabditidae abundances in medium intensity soils contributed to higher EI values which leads to a rejection of the hypothesis that EI would increase with intensity. Although Ba1 may be more related to short term management, more consistent organic inputs within the medium LUI system may more continually support bacterial and bacterivore communities.

Cephalobidae is thought to be less responsive to food resources than Rhabditidae (Ferris and Matute 2003), thus generally follows Rhabditidae in ecological succession (Ferris and Matute 2003). Despite this difference, these bacterivore families followed similar intensity trends (Table 8 & 9). Therefore, medium intensity sites may span this range of succession, while high intensity sites do not receive or continually deplete organic resources required for this ecological progression. This may suggest that medium intensity sites receive increased organic inputs, and maintain a chemical and physical soil environment that allows for C resources to be shuttled to the tertiary trophic level of bacterivore nematodes.

Higher cp value bacterivores, Plectidae (cp 2) and Prismatolaimidae (cp 3) responded differently, showing an inverse relationship of abundance and land-use intensity (Table 8 & 9). Higher cp designations are more susceptible to stress and disturbance and less responsive to food resources (Bongers and Ferris 1999, Ferris et al. 2001), which account for the different reactions of Ba families in this study. While Villenave et al. (2013) showed land-use of had no effect on Ba234 (bacterivores of cp value 2 through 4) density, this assessment would have included Cephalobidae in the cp234 category across forest, meadow and agricultural land-use intensities. Inclusion of Cephalobidae, which reacted differently to LUI compared to other cp234 bacterivores in this study, may have resulted in this non-significant finding.

While we hypothesized that fungivore abundance would be higher in low intensity systems due to abundance increased with organic matter levels (Tsiafouli et al. 2015; Bulluck et al. 2002) and their association with natural systems (Kimenju et al. 2009), fungivore abundances in this study may be more associated with family specific food resources. Aphelenchidae abundance increased with degradation of more complex SOM (as opposed to recent inputs) and prominence of fungal decomposition over bacterial decomposition pathways (Chen and Ferris 1999; Ferris and Matute 2003). Thus, increasing abundance with intensity (Table 8 & 8) is justified where increasing intensities had lower OM levels (Table 5) and a higher CI in 2019 (Fig. 4) indicating increased fungal decomposition. CI may be related to proliferation of plant fungal pathogens over saprophytic fungal decomposition as organic matter resources in high intensity sites were severely depleted (Table 5). Okada and Kadota (2003) observed increased population growth rates and body size of an Aphelenchidae nematode when presented with plant pathogens, including *Fusarium oxysporum*, *Pythium ultimum* and *Rhizoctonia solani*, as a food source (Okada and Kadota 2003). Increased soil-borne plant pathogens level in these systems may be supporting higher abundances of these nematodes, although *Aphelenchus avenae* may still reproduce on non-pathogenic fungi including *Agaricus bisporus* (Okada and Kadota 2003). Food preferences in natural ecosystems may also vary depending on food source options and abundance. Aphelenchoididae have more generalist feeding, where fungal feeding sources have been found to change over time, and mycorrhizal hosts were most suitable (Ruess et al. 2000). More generalist feeding of Aphelenchidae may have stabilized abundances across land-use intensities, resulting in non-significant differences (Table 8 & 9). This is consistent with the findings of Vazque et al. (2019) where generalists were found across all intensities, while nematodes with narrow niche widths were prolific in agricultural systems.

5.3 Higher trophic level nematodes

Only omnivores consistently reacted to intensity in both years for both trophic abundances and percentages (Fig. 2). Reduced omnivore abundance and trophic percentage in medium and high intensity sites is likely more related to direct management effects, as omnivores are highly sensitive to physical disturbance (Bongers and Ferris 1999), than bottom-up nematode food web dynamics. Dorylaimidae were associated with only 23% of soils in potato production in PEI (Kimpinski 1987). Thus, the 93% presence across all PEI land-use types in this study further reflects the reduced population distribution observed after high intensity tillage. Food web dynamics may still be a driver for omnivorous nematodes as omnivory is thought to be a benefit in more heterogenous environments typical of undisturbed ecosystems (Neher 2010). However, total nematode populations that serve as a food source for these species were consistent across all land-use intensities (Table A5).

Predator trophic level intensity differences were generally nonsignificant (Fig. 3), where only higher trophic percentages of predators in low intensity sites were found in 2018 (Table A5). Increased consistent disturbance, common in agriculture, can reduce predator populations even to the point of localized extinction (Parmelee and Alston 1986). Thus, non-significant treatment differences in agricultural fields may be a result of low or non-existent predator abundances (Bulluck et al. 2002, Parmelee and Alston 1986). This may be a similar case for low intensity sites where previous physical soil disturbance, common in pasture and even forest sites, may have eliminated Mononchidae preventing population re-accumulation. While disturbance may permanently alter nematode communities, initial re-population of omnivores and predators in abandoned cropland has been observed after 2 years (Hánel 2003). While reduced tillage did not affect SI, increases in SI in fallow rotations compared to continuous cropping, were observed in a

one year study (Minoshima et al. 2007). Therefore it is more likely that nematode identification methods may have also biased non-significance across treatments, where identification of only 100 nematodes per sample may mean that these families are underrepresented in samples where there are high proportions of other nematodes. Increasing the number of nematodes identified per sample may improve detection of rare species, although this would also increase sample processing time.

5.4 Nematode faunal indices

Increased omnivore abundances contributed to significantly higher MI in low intensity sites (Fig. 4). Lower MI values in medium and high intensity sites were justified as MI is thought to decrease with increasing disturbance (Postma-Blaauw et al. 2010; Kimenju et al. 2009). However, the MI of medium and high intensity were not consistent across year (Fig. 4). As the MI includes Ba1, this indice is more responsive to recent resource changes in soil conditions, where the decrease reflects an increased proportion of bacterivores in 2019 (Table A5). This metric may be more related to management (e.g. organic matter input, current crop type, tillage) within the sampling year as opposed to long term LUI conditions. Moreover, MI2-5, which excludes Ba1 and is more reflective of long term soil conditions, showed a consistent trend over both growing seasons (Fig. 4).

Nematode indices SI and BI clearly reflected the dynamics of a combination of nematode families. The reduction of higher cp nematodes like omnivores (i.e. Dorylaimidae and Aporcelaimidae) in rotational treatments (Table 8 & 9) as well as reduced higher cp value bacterivores and fungivores contributed to reduced SI values. While it was hypothesized that SI would decrease with intensity, the medium and high intensity sites showed similar food web connectivity as indicated by the SI (Fig. 4). Frequent annual tillage disturbances may result in similar food web connectivity in these LUIs.

Lower BI in low and medium intensity sites reflected the maintenance of higher cp value bacterivores and fungivores that was not observed in high intensity soils. The BI is reflective of an increasing proportion of cp2 fungivores and bacterivores, which are thought to be present in all communities. This indicates that high intensity sites promoted these specific nematode trophic guilds and/or reduced opportunistic Ba1 and cp 3-5 guilds. In both cases, this suggests reduction in ecological niches that support other trophic guilds which is supported by smaller soil aggregate size fractions in high intensity sites (Table 5), indicating increased soil homogeneity.

5.5 Plant parasitic nematodes

The PP abundances and trophic percentages lacked consistent trends across LUI, likely due to plant type variability across treatments. Endoparasites abundances were likely heavily influenced Pratylenchidae in 2018 (Table 8). Dominant plant parasite Pratylenchidae species, notably *Pratylenchus penetrans,* have long been common pests of potato production regions of PEI and New Brunswick (Kimpinski 1987). While this trend was not significant both years, Pratylenchidae abundance appear to be larger in the medium intensity rotation as opposed to the high intensity treatment (Table 8 & 9). While both high intensity and low intensity rotations consistently contain crops, including barley, potato, clover and corn, that are hosts for the most abundant PEI *Pratylenchus* spp. (*P. penetrans* and *P. crenatus*) (Kimpinski 1987), the nematode preference and frequency of these crops in rotation may result in increased population in the medium intensity rotations. Reduced root biomass as well as increased row spacing may also reduce Pratylenchidae proliferation in potato years in high intensity sites. Fallow is generally recommended for nematode control in arid climates (Agrios 2005) and repeated tillage during potato production may mimic this affect, reducing soil Pratylenchidae populations. However, increased tillage is accompanied by degradation of soil health (Mann et al. 2019), with increasing soil erosion and loss of SOM.

Reduced soil moisture in high intensity systems, potentially as a result of repeated tillage, could also have affected nematode distribution in the soil. Pratylenchidae may have migrated deeper in the soil to soil with higher moisture content, beyond the depth of sampling, resulting in reduced abundances in high intensity soils. It is also important to consider that PP root associated populations were not assessed, where nematode abundance in roots may show different trends across intensity compared to soil abundance, especially considering differences in root development and PP reproduction stage at time of sampling. The total number of Pratylenchidae in all possible habitats (increased soil depths, plant roots) should be assessed before it can be determined that reduced populations of Pratylenchidae are associated with high intensity land-use.

Tylenchidae was another dominant PP family (Table 7-9). This family is generally considered to have a non-significant economic effect on most agricultural crops from direct feeding, and have not been associated with any disease complexes. Members of this family may prefer feeding on algae and soil fungi depending on the nematode species (Okada and Kadota 2003). In some cases minimal feeding by diverse plant parasitic nematodes, in conjunction with free-living nematode feeding, have been found to increase plant biomass under high nutrient conditions (Gebremikael et al. 2016). Here it is thought that PP nematode feeding increased root rhizodeposits of carbon, stimulating immobilization of nutrients (N & P) by soil microbes and which nutrients are then released to plants after feeding by microbivorous nematodes (Gebremikael et al. 2016). Thus, it is important for farmers and those working in research and extension to not automatically equate PP abundances and herbivory to agronomic loss, but instead to consider their role in broader ecological function and soil condition.

While specific PP families followed LUI trends, trends in total PP abundances and the PPI were not observed. PPI and MI do not always respond similarly, where fertility and tillage

applications can result in direct or inverse relationships (Bongers et al. 1997; Neher and Campbell 1994). While PP abundances and indicators may be more reflective of recent crop types, Berkelmans et al. (2003) determined that plant parasites continued to indicate management differences one year after disruptive soil management. Despite variability in both current crop and specific management practices in this study, trends in plant parasite family abundances like Pratylenchidae were still observed. As a result, plant parasite family abundances were better indicators of LUI as opposed to trophic abundance assessments.

5.6 Multivariate analysis of nematode community by intensity

While agricultural management practices consistently affect nematode communities in the short term (Ferris and Matute, 2003, Wardle et al. 1995), long-term management in arable soils did not significantly affect the entire nematode community composition. Although nematode indices showed differences across rotational (medium and high) intensities (Fig. 4) in community subsets, multivariate analysis did not yield significant differences at the entire community level (Fig. 5). Medium and high intensity community similarity indicates that the differences in agricultural management across rotational systems are not different enough to cause total community shifts that were associated with low intensity sites. Non-significant differences between long term cropping systems is consistent with the finding of Postma-Blaauw (2010), where diverse cropping only promoted differences in nematode indicators three years after land conversion. These differences were also subject to fertility treatment and not widely found in following years where potato followed barley. Thus, it is expected that sites of the same crop under different long term management (20+ years in this study) would also have similar nematode communities.

Ordination of soil properties over the nematode community data showed the soil properties that significantly explained community variation (Fig. 6). Soil properties relating to nutrient

availability (pH, available K and P) we related to rotational intensities in 2018. Soil properties such as increased soil moisture %, total C and N that are related to undisturbed soil, or lack of tillage, were linked to low intensity communities and ordinated oppositely to rotational intensities (Fig. 6 & 7). These groupings align with our pre-conceived concept of increasing land-use intensity, where increasing fertilizer use and soil nutrient status, as well as increased tillage are thought to affect soil biological communities. Although fertility management and tillage may directly affect nematode community composition, an indirect effect on soil properties may also cause variation in the nematode community as soil properties were also distinctly different between intensity ranges (Table 5). These soil properties may also be proxies for other soil factors that affect the nematode community. Therefore, it is difficult to determine the extent and mechanisms to which soil nutrient and tillage management ultimately affects the nematode community across land-use intensities.

5.7 Relationships between vegetation type and nematode community

When nematode communities were analyzed by vegetation type, vegetation typical of low intensity nematode communities showed a gradient (forest to forest/grass to grassland) (Fig. 7). Transition of disturbed natural ecosystems can take many years if ever return to previous community types (Briar et al. 2012b; Adl et al. 2006). The community gradient observed within the low intensity treatment may reflect time since disturbance. Pastures may be more continuously disturbed by reseeding and cattle movement. Treelines may experience less tillage disturbances, but may still be influenced by compaction if the treeline abuts a road or field. While the majority of PEI forests have been disturbed by previous clear cutting or selective harvesting (Government of PEI 2018), this disturbance would have been decades previous to the recent and consistent disturbances received by treeline and grassland sites.

Other factors such as quality and quantity of residue returns across this gradient may have a significant effect. The transition from forest to grassland may have decreasing residue biomass returns with lower C:N ratio residues due to hay removal and grazing. Residue biomass and nitrogen content altered nematode communities in cover crop trials (DuPont et al. 2009). Changes in plant species and distribution across this vegetation gradient may also more directly affect nematode communities (Coffey and Otfinowski 2019), especially host specific plant parasite populations. The canopy cover would also vary, where forests can receive full shade, treelines may receive intermediate shade (depending on treeline orientation) and pastures no shade. This gradient could affect nematode reproduction rates/development where heating of the soil surface and water retention across vegetation covers would be substantially different.

Different crop types typical of agricultural rotations generally showed non-significant differences in nematode community (Table 12). Common disturbance events between crop types include fertilizer application, tillage and potential use of pesticides, that may be applied with similar frequencies, are factors that may have contributed to nematode community similarity across crop type. Berkelmans et al. (2003) also showed that one year of disruptive soil management was enough to mask management differences (organic, conventional and low-input). Thus, disturbance similarity in both medium and high intensities would have occurred consistently enough to prevent nematode community divergence in the current year of study. The previous year's crops can also have an impact on the nematode community indices (Berkelmans et al. 2003) and, along with management and soil variability, may have contributed to the variability in community within crop observed in this study.

6. Conclusions

Land-use intensity was determined to significantly affect different nematode family abundances. Fungivore nematode abundances did not follow the hypothesized SOM trends that were observed across LUI, as Aphelenchidae nematodes increased with increasing LUI.

Nematode faunal indices across LUI were linked to the variation in nematode family abundances. The EI was related to increased opportunistic bacterivore populations, however these abundances and index did not increase with increasing intensity as hypothesized. Medium intensity sites were the most enriched LUI, potentially due to the association of Ba1 with consistent, increased soil organic inputs as opposed to tillage disturbances. It was hypothesized that the SI, which indicates food web connectivity, would decrease with increasing intensity. Although the structure index generally decreased with intensity, medium and high intensity system SI values were statistically the same. Management in rotational systems, which receive consistent tillage, may be similar enough to maintain similar food web connectivity.

CHAPTER 3: POTENTIAL OF NEMATODE FAUNAL INDICES AS PREDICTORS OF *Rhizoctonia solani* SUPPRESSIVE SOILS

1. INTRODUCTION

1.1 Rhizoctonia solani

Rhizoctonia solani (Kühn) (Teleomorph. *Thanatephorus cucumeris* (A. B. Frank) Donk) is an anamorphic, filamentous fungus which belongs to the division Basidiomycota. This fungus is a soil-borne plant pathogen which can infect a wide range of crops including cereals (e.g. *Triticum aestivum* L., *Hordeum vulgare* L., *Secale cereale* L.), beans (*Phaseolus vulgaris* L.), soybeans (*Glycine max* (L.) Merr.), canola (*Brassica napus* L., *Brassica rapa* L.), chickpea (*Cicer arietinum* L.), corn (*Zea mays* L.), lentil (*Lens culinaris* Medikus), pea (*Pisum sativum* L.) and potato (*Solanum tuberosum* L.) (Melzer et al. 2016; Lootsma 1997). Crop dependent symptoms include causing seed rot, pre- and post-emergence damping off, seedling blight, stem/crown rot, root rot and black scurf of potato (Melzer et al. 2016; Lootsma 1997).

The species, *R. solani*, is composed of 14 multinucleate pathogenic anastomosis groups (AG), that can be further divided into subgroups, which collectively form a species complex (Melzer et al. 2016; Ajayi-Oyetunde and Bradley 2017a). Anastomosis is a process where separate fungal hyphae fuse, exchanging DNA which may or may not result in somatic compatibility. Different *R. solani* anastomosis groupings are somatically incompatible (Ajayi-Oyetunde and Bradley 2017b). AGs vary in host specificity, and pathogenicity where certain AGs can endophytically colonize certain crops (Melzer et al. 2016). *R. solani* is also a saprophytic fungi, which allows for its continued survival in soils during the absence of a host plant.

The wide host range, limited resistant cultivars, and saprophytic ability of *R. solani* can make crop rotation an ineffective management tool. Use of tillage and compost application to control *R. solani* can have mixed results. Generally tillage reduces *R. solani* disease pressure by burying

disease inoculum (Schroeder and Paulitz 2008), however, retention of plant residues at the soil surface increases saprophytic trash microflora at the soil surface which can act antagonistically to *R. solani* (Sturz and Carter, 1995). Organic matter application was least effective at controlling *R. solani* compared to other saprophytic fungi (Bonanomi et al. 2007). In furrow or seed treatment applied fungicides, although not effective in all situations and potentially environmentally hazardous, have been the primary means of *R. solani* control. Susceptibility to different fungicides can be AG group dependent (Kataria et al. 1991) and use of broad spectrum fungicides may help prevent fungicide resistance. Practices which enhance soil conditions, specific antagonists (Salamone et al. 2018), and biotic communities that are suppressive to *R. solani* may provide a more sustainable, long term disease management option.

1.2 Rhizoctonia solani suppression in soil and disease suppression

Soil suppression of *R. solani* disease has been linked to predominantly bacterial populations in the soil microbial community with different potential mechanisms for suppression. Actimomycetes are gram-positive bacteria that are known for the production of antagonistic compounds. Previous studies have suggested *R. solani* suppression from Actinomycete bacteria to be a result of niche competition, nutrient removal or more likely producers of antibiotic compounds that antagonize *R. solani* (Ascencion et al. 2015). Antagonism is often attributed to *Streptomyces* bacteria, although they can exert suppressive or conducive effects in pathogen interactions depending on the strain (Schrey and Tarkka 2008). Yin et al. (2013) have also shown that gram-negative *Chryseobacterium* and *Pseudomonas* exhibited *R. solani* inhibition and may interact with the related species in bulk soil.

1.3 Free-living nematode communities as indicators of disease suppressive soils

Previous studies have assessed the effect of fungivorous nematode populations on *R. solani* disease suppression (Lagerlöf et al. 2011; Bollen et al. 1991). These assessments generally focused on the abundance and effect of fungivorous nematode species. While application of fungivorous nematode populations consistently suppress disease in greenhouse settings, maintaining abundance of field applied fungivores is limited by soil management practices and water availability. Assessment of soil-borne plant pathogen disease suppression in the context of the entire nematode community, which is likely more stable than individual populations, could indicate soil conditions where the nematode community more consistently suppresses plant root disease (Kanfra et al. 2018). The role of multiple nematode genera and trophic levels may also more accurately describe plant-disease interactions; where a recent study found that certain plant parasitic and bacterivore genera populations promoted apple replant disease (Kanfra et al. 2018). Moreover, nematode trophic composition was found to affect C and N nutrient cycling, (Gebremikael et al. 2016) which could influence *R. solani* and plant growth.

Nematode community quadrats are representative of biological and chemical soil factors (N enrichment, fungal or bacterial decomposition channel, C:N ratio, disturbance and food web status) (Ferris et al. 2001). These nematode community quadrats could indicate soil conditions that are suppressive to specific soil-borne plant pathogens. General soil suppression can be considered a function of the evolutionary potential of soil saprophytes and soil nutrient status, where high organic matter contents and limited soil nutrients fuel general suppression (Schlatter et al. 2017). Despite the highly saprophytic nature of *R. solani*, and enzymatic capacity which allows it to utilize substrates ranging in C:N ratio (Papavizas and Davey, 1960) substrate C:N ratio and N availability can significantly affect *R. solani* soil colonization (Davey and Papavizas, 1963). After 3-5 weeks

incubation, increasing N availability across C:N ratios of 0-5, 5-20, 20-100, and 100-400 differently effect % colonization of buckwheat stems by *R. solani* (Davey and Papavizas, 1963). Moreover, differences in nutrient contents and availability in different substrates and surrounding soils can heavily influence *R. solani*, where increased N availability promotes disease symptoms (Bonanomi et al. 2020). These assessments are relevant to the nematode enrichment index (EI) which measures food web enrichment through colonizer microbivorous nematodes, which index is highly sensitive to the amount and nutrient content of organic inputs (Dupont et al. 2009). The soil decomposition pathway, could also be indicative of disease suppressive conditions. Increased competition between saprophytic fungi for organic substrate in fungal dominated decomposition pathway would likely restrict growth of saprophytic fungi. While these nematode indices may be reflective of a large number of soil properties these indices may be less reflective of other soil properties that affect pathogen growth such as, soil texture and pH.

Unlike the enrichment index, the nematode community structure index (SI) can indicate the maturity of an ecosystem. Food web structure is defined by increased number of species and trophic links within a soil food web (Wardle et al. 1995), where limited disturbance fosters structure (Ferris et al. 2001). Increased food web connections, as indicated by increasing SI values, may work to regulate biological communities preventing dominance of any one species, including that of pathogens. Increasing structure index is often related to omnivorous nematode abundances (Ferris et al. 2001) which may feed on fungi at different times in their lifecycle. The SI was positively associated with increasing abundances of larger fungivorous organisms such as mites (Sánchez-Moreno et al. 2009). As a result, the SI may indicate increasing numbers of organisms that provide direct control of the a fungal soil-borne plant pathogen. Although nematode SI is only reflective of nematode community structure, an assessment of *R. solani* disease suppression from compost

application determined that the poorest indicators were those that did not account for ecosystem maturity and stability (Fang 2015). In the same study, nematode MI was not directly correlated to disease suppression, however, this factor was included as one of five disease suppression indicators following multiple stepwise regression.

Soil suppression towards fungal diseases in relation to nematode functional guild levels has not yet been assessed. Moreover, the use of nematodes as indicators of soils conducive or suppressive to *R. solani* could be more readily applied to field management practices compared to microbial assessment, as nematode communities more predictably respond to management.

1.4 Quantification of soil R. solani

Different methods can be implemented to quantify *R. solani* level in the soil. In earlier literature, *R. solani* was typically cultured on a selective media by plating soils or other materials after baiting or elutriation procedures (Spurlock et al. 2015). These techniques determine the propagule number, which determines the number of structures that can give rise to a new individual. It is also possible to distinguish between fungal mycelium and sclerotia using these techniques (Spurlock et al. 2015).

R. solani DNA quantification via quantitative polymerase chain reaction (qPCR) provides a measurement that is reflective of *R. solani* biomass in the soil. Unlike cultural techniques, qPCR may quantify dead *R. solani* where target DNA in dead hyphae or fragments incorporated into SOM are amplified (Emerson et al. 2017). Despite this drawback, free DNA in the soil is generally rapidly degraded although it may be stabilized in soil aggregates (Carini et al. 2016). While this type of assessment may provide a better assessment of the growth of the pathogen in the soil, this assessment does not indicate propagule number.

2. OBJECTIVES AND HYPOTHESES

Section 1

Objective 1- Assess the influence of land-use intensity on the concentration of *R. solani* in the soil.

Hypothesis 1- Higher soil loads of *R. solani* will be found at high intensity sites over both years of the study and be related to soil properties indicating SOM status.

Objective 2- Determine if *R. solani* soil concentrations are related to the nematode community.

Hypothesis 2- *R. solani* soil DNA concentrations will negatively correlate with high colonizer-persister nematode families.

Section 2

Objective 1- Determine the relationship between *R. solani* disease suppression and nematode EI and SI faunal indices.

Hypothesis 1- Disease suppression will be positively associated with higher SI indices and lower EI values.

Objective 2- To determine soil properties that are related to *R. solani* disease suppression.

Hypothesis 2- Soil properties will significantly affect *R. solani* disease suppression.

3. MATERIALS AND METHODS

3.1 Section 1

3.1.1 Sampling

Soils were sampled across PEI and stored according to the same protocol provided in Chapter 1 (*3.1 Sampling*, *3.2 Sample processing and storage*).

3.1.2 Soil properties and environmental data

The same soil properties were assessed as described in Chapter 1 (*3.3 - 3.10*). Degree day and precipitation measurements were altered to assess cumulative amounts three weeks prior to each sampling date.

3.1.3 qPCR analysis

Genomic DNA was extracted from 0.5 g of each soil sample using the DNeasy PowerSoil Kit (Quiagen, Hilden, North Rhine-Westphalia, Germany). Standards for *R. solani* qPCR quantification were produced by first growing *R. solani* in potato dextrose broth from agar plugs, for 3 days at 25 °C. Mycelial DNA was then extracted using the DNeasy PowerSoil Kit. The DNA concentration was measured using a NanoDrop™ One/OneC Microvolume UV-Vis Spectrophotometer (Thermo Scientific, Waltham, Massachusetts, United States) and DNA purity was assessed based on 260/280 and 260/230 ratios. The DNA was then made into a 10-fold dilution series, producing six qPCR standards.

SYBR green qPCR detection methods were used to quantify soil *R. solani* levels. Forward ITS1 species-specific primer (AGTGTTATGCTTGGTTCCACT) and ITS4 reverse primer (TCCTCCGCTTATTGATATGC), developed by Lievens et al. (2006), were used in order to identify *R. solani* from all AG groups that may be present in field soils. Primers were diluted to 10 µM. Each of the 96 plate wells were loaded with 10 µl of iQ™ SYBR® Green Supermix (Bio-Rad, Hercules, California, United States), 1 µl reverse primer, 1 µl forward primer, 6 µl nuclease-free water and 2 µl of extracted DNA for a total reaction volume of 20 µl. CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, California, United States) was used along with CFX Manager 3.1 software for analysis. Cycle temperatures were set to an initial activation and

denaturation temperature of 95.0 °C for 3 min, and annealing and extension at 60.0 °C for 1 min. Cycle number was set to 40. Samples were run in duplicate.

Multiple *R. solani* AG groups were quantified together based on the chosen primers which were found to amplify AG1, AG3 and isolates were AG group was undetermined (Lievens et al. 2006). *R. solani* soil concentration was not presented in copy number units due to copy number differences between *R. solani* AG groups. Genome sequencing for the AG 2-2 LP genome was not available on GenBank. As a result, *R. solani* concentration was presented in ng DNA g^{-1} soil. As all *R. solani* AG groups were assessed together based on ng DNA g^{-1} soil, some AG groups are likely over represented.

3.1.4 Statistical Analysis

Data were first tested for normality. All non-normal data were subjected to Box-Cox transformations and tested for equal variance before the following assessments. A mixed effects model using restricted maximum likelyhood (REML) estimation, using Kenward-Roger approximations, was used to determine significant differences in *R. solani* soil DNA concentration across land-use intensity and vegetation types in 2018 and 2019. Analyses were performed seperately in Minitab 19.0. Random effects included a pseudoreplicate nested within site, while fixed effects assessed intensity and crop type separately. Mean comparisons were tested using Tukey's honest significant difference (HSD) post hoc test at a significance level of $\alpha=0.05$. Differences in *R. solani* distribution based on the percentage of sample points where *R. solani* was present and the percentage of sample points per field, where *R. solani* was present between intensity treatments, was determined using the same statistical analysis, where only site was included as a random factor.

Pearson correlations were used to explore linear relationships between *R. solani* soil concentration and soil properties as well as environmental variables in Minitab 19.0. Significance of linear relationships was determined at $\alpha=0.05$. Spearman rank correlations in Minitab 19.0 were used to explore linear relationships between *R. solani* soil DNA concentration and nematode family abundances and faunal indices as data were non-normal even after transformations were applied, Significance of monotonic relationships was determined at $\alpha=0.05$.

3.2 Section 2

3.2.1 Growth chamber study

A soybean bioassay was used to determine the effect of free-living nematode community type on *R. solani* disease suppression in soils. A preliminary trial was first conducted to determine a soybean cultivar, AG isolate and inoculum level for the bioassay trial that would provide high, consistent diseases symptoms. Preliminary trial results can be found in Table A8, where soybean cultivar PS0 333XRN, AG 2-2LP and *R. solani* inoculation by mycelial mat grown on filter paper were found to produce acceptable disease levels for further testing.

The main trial included three factors including nematode community EI, nematode community SI and *R. solani* inoculation, which factor allowed for the control of pre-existing soil disease (Table 1). A randomized complete block design was implemented with four replicates.

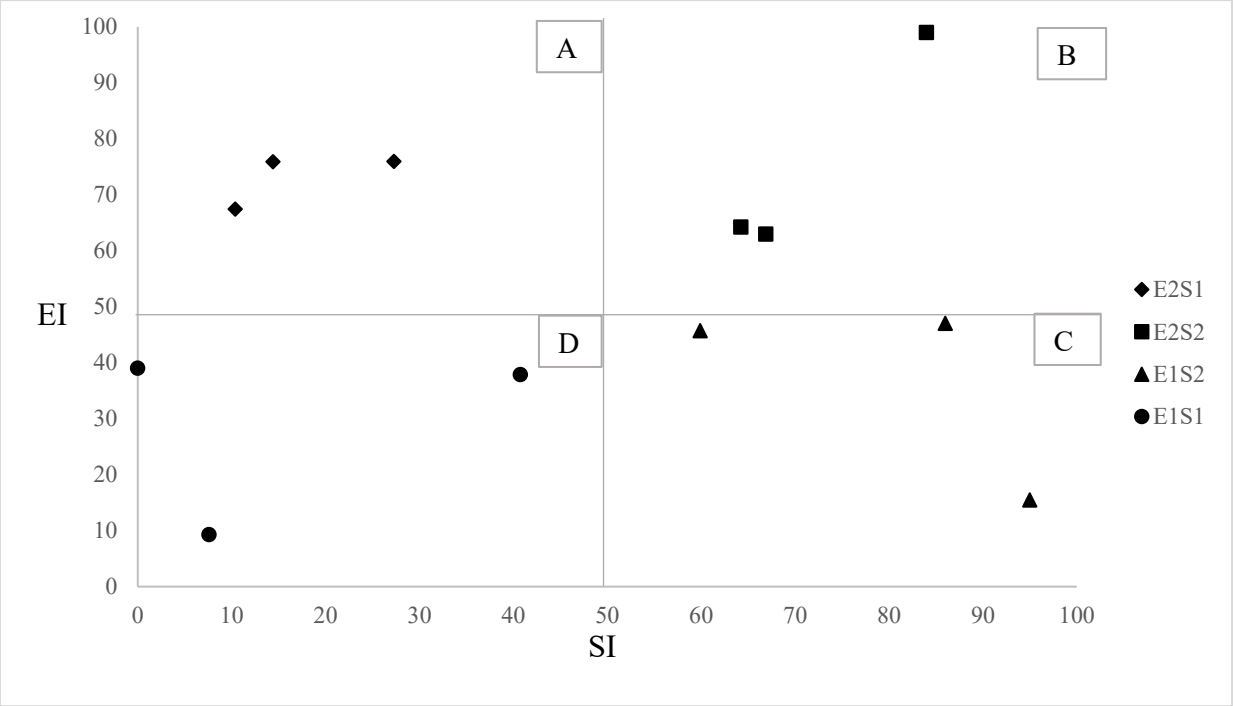
Table 1. Factors and levels included in *R. solani* disease suppression study.

Factor	Level
EI	Low (E1) High (E2)
SI	Low (S1) High (S2)
R. solani inoculation	*R. solani* mycelium No *R. solani* control

Note: An autoclaved soil was included as a single additional treatment.

Soils chosen for this trial (Fig. 1) were selected from amongst the central sampling points of the 2019 across PEI sites as discussed in Chapter 1. An additional 2 kg of soil was collected from all central sampling point in 2019, retained field moisture levels in sealed plastic bags, and stored at 4 °C until the trial was conducted. Each soil chosen reflects a low or high treatment level for both EI and SI, which collectively correspond with the quadrat types (A-D) proposed by Ferris et al. (2001) (Fig. A1, Table A1).

Figure 1. Enrichment and structure index values of soils selected from 2019 cross PEI trial for *R. solani* growth chamber study.



Note: E= enrichment index treatment, S= structure index treatment. Soils in each quadrat were selected from the following sites that were sampled across PEI in 2019 (Chapter 2, Table 2). Quadrat A: sites 14, 17, 29; Quadrat B: sites 8, 9, 21; Quadrat C: sites 1, 3, 5; Quadrat D: sites 10, 26, 28.

Confirmation of similarities and differences in nematode EI and SI of the selected soils between quadrat types were determined using one-way ANOVA tests with Tukey's means comparison at a significance level of 0.05 in Minitab 19.0 prior to bioassay initiation. Significant

differences in EI between E1 and E2 treatments, as well as differences in SI between S1 and S2 treatments were confirmed (Table 2).

Table 2. ANOVA results for enrichment and structure indices of soils selected to represent nematode community quadrat types.

Quadrat- Treatment	EI ± SE	SI ± SE
A- E2S1	80.89 ± 15.12a	22.80 ± 8.65b
B- E2S2	81.920 ± 3.90a	78.74 ± 5.35a
C- E1S2	38.4 ± 17.4b	86.58 ± 13.77a
D- E1S1	37.6 ± 20.5b	21.1 ± 27.2b
F value	7.79	14.4
p-value	0.009	0.001

Note: Means with standard errors. (n=12)

For each soil assessed an inoculated and non-inoculated treatment was applied. Inoculation was applied by culturing three 7 mm plugs of *R. solani* on a 7 cm Whatman® 41 filter paper over potato dextrose agar (PDA) in a Petri plate for two days in a dark incubator set to 25 °C. Non-inoculated soil treatments controlled for pre-existing pathogen presence in each soil. These treatments received a filter paper with sterile agar plugs that were treated the same way as the *R. solani* filter paper cultures in all other aspects. Field soil from high intensity site 22 in the cross PEI trial was autoclaved and used as a control for possible seed-borne disease, which also received a non-inoculated filter paper. Soils were saturated to field capacity before potting. Equal amounts of soil were added to pots at each soil layer based on a dry weigh basis which corresponds to the calculation method of nematode abundances. A bottom layer of soil (approximately 2 cm deep) was lain, followed by the filter paper and then a second layer of soil (approximately 1 cm deep). A 6 day soil incubation period was implemented to allow soil colonization by *R. solani* before planting. Pots were subjected to a 16:8 h light:dark period at 20 °C at relative humidity of 65% in an A1000 Plant Growth Chamber (Controlled Environments Ltd, Winnipeg, Manitoba, Canada). Four PS0 333XRN soybean seeds, which were pre-germinated in moist paper towel at 4 °C for three days, were placed on the soil in each pot. Pots were then topped with soil to bury the seeds, make seeding

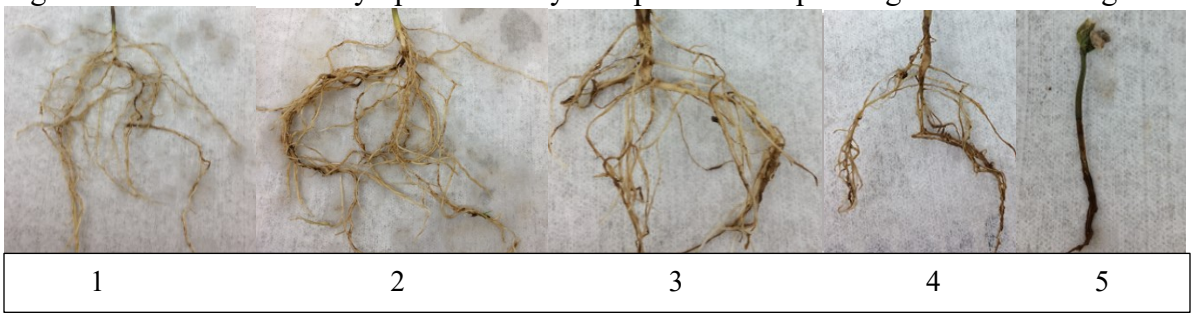
depth approximately 2 cm. The final volume of soil in each pot was approximately 240 mL. Soybeans were grown for 3 weeks under the same conditions as described for the soil incubation period. Pots generally received 5-10 mL of water daily, the amount was regulated to ensure that the soil was always moist enough for pathogen growth but at the same time did not stress the soybeans. Soon after planting it was evident that watering resulted in the disaggregation of select soils, which created variability in the distance from the inoculated soil filter to the soybean seed. To account for this factor soil depth was measured at the side of the pot using a ruler three days after planting.

Soybean emergence was recoded one week after planting. Soybeans were thinned to three pots per plant. In addition to emergence, the following *R. solani* disease traits were determined at the end of the study using the following scales:

Figure 2. Soybean plants exhibiting hypocotyl rot corresponding to disease rating scale.

Note: 0 = no lesions, 1 = lesions <2.5 mm, 2= lesions 2.5- 5mm, 3= lesions >5 mm, 4 = lesions girdling the plant and leaves wilting, and 5 = seedling damped-off or dead. Disease scale as described by Bradley et al. (2001)

Figure 3. Root rot disease symptoms on soybean plants corresponding to disease rating scale.



Note: 1 = no root rot; 2 = 1 to 33% of roots with visible lesions or root rot; 3 = approximately 33 to 50% of the roots rotted or damaged; 4 = 50 to 80% of the roots rotted; and 5 = pre-emergence damping-off and few if any roots. Disease scale as described by (Dorrance et al. 2003).

Root and shoot biomass was collected and recorded on a dry weight basis. Re-isolation of *R. solani* from diseased tissues and microscopic examination confirmed *R. solani* infection.

A subsample (approximately 14 g) of soil was collected in 15 mL falcon tubes and stored at -20°C for PLFA and qPCR analysis. The remaining soil in each pot was collected and stored at 4°C for post-trial nematode analysis, which was performed using the same methods described in Chapter 2 (Materials and Methods, *3.12 Nematode extraction and identification*).

3.2.2 POM C and POM N

In addition to the previously assessed soil parameters of C%, N%, C:N, pH, POXc, ACE protein and texture (Chapter 2, Materials and Methods, *sections 3.4-3.8*), C and N percentages of the soil particulate organic matter (POM) were also assessed on the field soils prior to the study to determine general disease suppressive potential.

Particulate organic matter (POM) was assessed from the sand fraction (0.053 mm – 2 mm) following the soil textural assessments as described in Chapter 2 (Materials and Methods, *3.6 Soil texture*). C and N percentages of POM (POM C, POM N) were determined by combustion of a 1g subsample at 500 °C using VarioMAX CN Elementar system (Elementar American Inc., Mt Laurel, NJ, US).

3.2.3 PLFA Analysis

Phospholipid fatty acid (PLFA) analysis was conducted post-trial to quantify microbial biomass to relate to decomposition pathways and nematode food sources. High-throughput PLFA analysis of soils (Buyer and Sasser 2012) first requires the extraction of phospholipid fatty acids from the soil. Extracted lipids are then separated based on polarity during solid phase extraction (SPE). In the following steps transesterification converts isolated lipids to fatty acid methyl esters (FAMEs), and FAMEs quantification is then achieved via gas chromatography.

Reagent preparation required that the internal standard (40.9 mg of 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine), was dissolved in 20 mL 1:1 chloroform:methanol. This standard was added to the Blight Dyer extractant (200 ml 50 mM K_2HPO_4 in H_2O, 500 ml methanol, and 250 ml chloroform) at a rate of 0.5 μL ml^{-1} extractant just before the extraction process. The transesterification reagent was produced by dissolving 0.561 g KOH in 75mL methanol, then adding 25 mL Toluene.

Extraction

Approximately 1.75 g of soil was added to pre-weighed test tubes, lyophilized over night and re-weighed to determine dry soil weights. An additional test tube was added as a blank. 4 mL of Blight-Dyer extract was added to each test tube. Tubes were sonicated for 10 min at room temperature, then placed in an end-over-end shaker for 2 hrs. After centrifuging the liquid phase was pipetted to clean test tubes with Polytetrafluoroethylene lined screw caps. Each tube then received 1.0 mL of chloroform and water, were vortexed, and then centrifuged for 10 min. The upper phase was removed, and then the lower phase was evaporated at 30 °C.

Lipid separation

96-well silica SPE plates (Phenomenex, Torrance, California, United States) were conditioned with 1 mL methanol three times, followed by chloroform in the same manner. The application of 1 mL chloroform to each test tube and sample transfer to individual SPE plate wells was repeated twice. Wells were washed with 1 mL chloroform, then 1 mL acetone. 0.5 ml of 5:5:1 methanol:chloroform:H_2O was used to elute phospholipids into a 1.5 mL 96-well multi-tier microplate (E&K Scientific Products, Inc., Santa Clara, CA, US). Samples were evaporated at 70 ◦C for 30 min, and then at 37 ◦C until dry.

Transesterification

0.2mL of transesterification reagent was added to each sample, then covered and incubated at 37 °C for 15 min. 0.4 mL of 0.075 M acetic acid and chloroform were added. Samples were allowed to separate after shaking for 10 s. The bottom 0.3 mL of each sample was transferred to a 1 mL Multi-tier plate (E & K Scientific, Inc., Santa Clara, CA, US). Chloroform extraction was repeated where the bottom 0.4 mL was removed and combined to the previous extract. Chloroform was completely evaporated from samples at room temperature. 75 µL hexane was added to each sample, then transferred to GC vials with glass inserts. Samples were stored at -20 °C until further analysis.

Gas chromatography

FAMEs were quantified using an Hewlett Packard 7890 gas chromatograph equipped with a flame ionization detector (FID) (Agilent, Santa Clara, California, United States). FAMEs were assessed using the MIS Sherlock® (MIDI, Inc., Newark, DE, US) and Agilent ChemStation software. The MIDI PLFAD1 calibration standard and naming table were used to identify FAMEs.

Biomarker groupings were composed of the sum of different fatty acids. Arbuscular mycorrhizal fungi (AMF) were represented by 16:1 ω5 fatty acids; eukaryotes by total polyunsaturated fatty acids (Zelles, 1999); gram positive (+) bacteria by iso and anteiso-saturated branched fatty acids (Zelles, 1999); gram negative (-) bacteria, monounsaturated fatty acids and 17:0, 19:0, 20:0 and 22:0 cyclopropyls (Zelles,1999); actinobacteria by 10-methyl fatty acids (Zelles, 1999); fungi by 18:2 ω6 cis (Frostegård and Bååth, 1996); and anaerobes by dimethylacetals (Zelles 1999). Any following mention of microbial groups will refer to the quantified biomarkers.

3.2.4 qPCR analysis

qPCR analysis was conducted to quantify the amount of *R. solani* in the pre and post-trial soil samples. Analysis was performed as described earlier in section *3.1.2 qPCR analysis*.

3.2.5 Statistical Analysis

Ordinal logistic regression was used to determine the effect of *R. solani* inoculation on *R. solani* soil DNA concentration, as well as hypocotyl and root rot disease ratings which were based on ordinal scales. Proportional odds assumptions were tested before analysis. Model terms included *R. solani* inoculation. The logit link function was used. Significance of factors were determined at a significance level of $\alpha=0.05$. Mean comparisons were carried out using Dunn's multiple comparison test with Bonferroni correction.

Outliers in the *R. solani* soil DNA concentration data set were first tested using the Dixon Q test in Minitab 19.0 as a significance level of $\alpha=0.05$. An outlier that was deemed significant was removed from the data set. *R. solani* soil DNA concentrations in control, non-inoculated pots were subtracted from inoculated pots to account for pre-exiting soil *R. solani*. The difference in these values were then used for the analyses which assessed only inoculated treatments. Hypocotyl and root rot disease ratings were assessed on ordinal scales, which prevented the control of disease caused by pre-exisiting *R. solani* in the inoculated treatment disease ratings. Ordinal logistic regression was used to determine significant differences in hypocotyl and root rot disease between the twelve different soils due to pre-existing disease in Minitab 19.0. Proportional odds assumptions were tested before analysis. Model terms included the soil sample, as well as replicate, column, row and their interactions. The logit link function was used. Significance of factors were determined at a significance level of $\alpha=0.05$.

Ordinal logistic regression was used to determine significant differences in *R. solani* soil DNA concentration and hypocotyl and root rot disease symptoms based on nematode faunal indices. Proportional odds assumptions were tested before analysis. When testing intensity affects, model terms included intensity were included in the analysis. EI treatment, SI treatment, EI treatment*SI treatment were included when testing for nematode indice effects. The logit link function was used. Significance of factors were determined at a significance level of $\alpha=0.05$. Comparison of pre-trial and post-trial EI and SI values during the course of the trial was assessed by repeated measures ANOVA in Minitab 19.0. Indice values were raised to the power of two and means were back transformed after analysis. Mean comparisons were assessed using Tukey's HSD post hoc test at a significance level of $\alpha=0.05$.

Monotonic relationships between *R. solani* soil concentration, hypocotyl disease ratings, root rot disease symptoms, and EI, SI and nematode family abundances were assessed in Minitab 19.0 using Spearman's correlations as disease rating data was not continuous. Correlations were determined significant at $\alpha=0.05$.

The relationship of Aphelenchidae abundance to hypocotyl disease symptoms was assessed by high and low hypocotyl ratings based on the median hypocotyl disease rating of 3.6. Aphelenchidae abundances were then related to the reduction of shoot biomass per plant caused by *R. solani* infection (difference in shoot biomass per plant between control and *R. solani* inoculated treatments) according to hypocotyl groupings. Linear relationships between Aphelenchidae abundance and shoot biomass reduction within each hypocotyl disease grouping were assessed by Pearson's correlations in Minitab 19.0. Correlations were determined significant at $\alpha=0.05$.

Monotonic relationships between *R. solani* soil concentration hypocotyl disease ratings, root rot disease symptoms, and soil properties and PLFA group data were assessed in Minitab 19.0

using Spearman's correlations as disease rating data was not continuous. Correlations were determined significant at $\alpha=0.05$.

A mixed effects model using REML estimation, with Kenward-Roger variance estimation, in Minitab 19.0 was used to determine significant differences in microbial group biomarker concentrations between *R. solani* inoculated and non-inoculated controls. Data were transformed to achieve a normal distribution before assessment. Replicate, column and row were included as random terms, while *R. solani* inoculation was included as the fixed term. Significant differences in microbial group biomass were determined at a significance level of $\alpha=0.05$.

The non-linear relationship between *R. solani* soil concentration and actinomycete:fungal biomass ratio was modelled using a double Gaussian function in Genstat 18. Models were significant at $\alpha=0.05$.

Ordinal logistic regression was used to explore soil properties, microbial biomasses and nematode communities as explanatory factors related to *R. solani* hypocotyl disease suppression in Minitab 19.0. Proportional odds assumptions were tested before analysis, and variables that did not meet this criteria were excluded from the model. Interactions of all explanatory variables were included in model testing. Explanatory variables were determined significant at $\alpha=0.05$. The best model was selected based on the highest log-likelihood ratio. In order to graph significant interaction effects, using the binary logistic regression factorial plot function in Minitab 19.0, hypocotyl disease ratings were divided into low (disease ratings 0-2) and high (disease ratings 3-6) groupings for assessment.

A paired t-test in Minitab 19.0 was used to determined significant differences in pre and post-trial Tylencholaimidae abundances when the alternative hypothesis stated that pre-trial Tylencholaimidae- post-trial Tylencholaimidae abundances were greater than 0. A mixed effects

model using REML estimation, with Kenward-Roger variance estimation, in Minitab 19.0 was used to determine significant differences in fungivore abundances between *R. solani* inoculated and non-inoculated pots in Minitab 19.0. Data were transformed to achieve a normal distribution before assessment and assessed for equal variance. Replicate, column and row were included as random terms, while *R. solani* sample time and inoculation was included as fixed terms respectively. Significant differences were determined at α=0.05. Mean comparisons were performed by Tukey's post hoc test.

4. RESULTS

4.1 Influence of land-use intensity on distribution of Rhizoctonia solani in soil (Section. 1)

R. solani soil DNA concentrations showed high variability within intensities in 2018 and 2019 (Table 3 & 4). LUI trends in *R. solani* soil concentration were not consistent across year. In 2018 the number of sites where *R. solani* was detected decreased with increasing intensity, although this trend was not significant. High intensity soils tended to have a higher percent of sampling locations where *R. solani* was present compared to medium intensity soils, although this difference was significant in only 2019. *R. solani* was more commonly detected in each field as intensity increased, although this trend was non-significant in both 2018 and 2019 (Table 3 & 4).

Table 3. *R. solani* soil DNA concentrations and distribution percentages (± standard error) across PEI field sites by land-use intensity in 2018.

Intensity	*R. solani* (ng DNA g^{-1} soil)	% Site *	% Location **	% Field †
L	16.25 ± 7.92b	100 ± 0.00	58 ± 0.07	58 ± 0.08
M	16.21 ± 4.22b	90 ± 0.10	66 ± 0.07	68 ± 0.10
H	170.7 ± 27.18a	77 ± 0.14	71 ± 0.07	73 ± 0.14
F value	15.60	2.56	1.79	0.79
p-value	0.000	0.121	0.171	0.471

Note: *= mean percentage of field sites (n=10) where *R. solani* was present, **= mean percentage of sampling points (n=50) where *R. solani* was present, †= mean percentage of sampling points (n=5) per site where *R. solani* was present in soil.

Table 4. *R. solani* soil DNA concentration and distribution percentages (± standard error) across PEI field sites by land-use intensity in 2019.

Intensity	*R. solani* (ng DNA g^{-1} soil)	% Site *	% Location **	% Field †
L	198.4 ± 37.89a	100 ± 0.0	70 ± 0.06ab	70 ± 0.10
M	45.8 ± 15.16b	100 ± 0.0	68 ± 0.10b	72 ± 0.10
H	118.4 ± 28.14a	100 ± 0.0	94 ± 0.04a	90 ± 0.08
F-value	10.39		4.02	3.56
p-value	0.000		0.020	0.051

Note: *= mean percentage of field sites (n=10) where *R. solani* was present, ** = mean percentage of sampling points (n=50) where *R. solani* was present, †= mean percentage of sampling points (n=5) per site where *R. solani* was present in soil. Different letters indicate statistically significant differences determined by Tukey's HSD mean comparison test at significance level of α=0.05.

Assessment of soil *R. solani* soil DNA concentrations across vegetation and crop types did not show consistent trends in 2018 or 2019 (Table 5). Increased *R. solani* concentration in pseudocereal (buckwheat) planted soils occurred in both years, however, there were few sites sampled in both 2018 (n=1) and 2019 (n=2) (Chapter 2, Table 2).

Table 5. *R. solani* soil DNA concentration (± standard error) across PEI field sites by vegetation type in 2018 and 2019.

Crop	*R. solani* 2018 (ng DNA g^{-1} soil)	*R. solani* 2019 (ng DNA g^{-1} soil)
Pseudocereal	343.4 ± 39.5a	226.5 ± 269.4ab
Root vegetable	162.4 ± 77.2a	82.8 ± 74ab
Legume	125.5 ± 102.9ab	115.4 ± 104.5ab
Cereal	84.3 ± 84.0bc	45.8 ± 28.7b
Grass/legume	21.9 ± 21.9bc	69.7 ± 26.9ab
Forest	2.5 ± 2.6c	355.0 ± 198.4a
Grass	18.81 ± 19.6bc	54.9 ± 49.5b
Forest/grass	7.75 ± 17.0c	164.9 ± 12.9b
F value	7.78	3.84
p-value	0.000	0.001

Note: Different letters indicate statistically significant differences according to Tukey's HSD mean comparison at a significance level of α=0.05.

This study found that *R. solani* soil DNA concentration showed significant correlations with soil and environmental properties when assessed across all LUI categories (Table 6). Environmental factors such as degree days and soil moisture were only significant in 2018, while textural soil components were significant in 2019. During both years of this study *R. solani*

concentration was negatively correlated with total N, total C, POXc and positively correlated with ACE protein in both years of the study. Although significant at α=0.05, most of these coefficients were less than 0.30 showing weak to non-linear relationships.

Table 6. Pearson correlation p-values and coefficients of *R. solani* soil DNA concentration and soil properties across all PEI sampling sites in 2018 and 2019.

		2018		2019	
Soil trait		p-value	r	p-value	r
N	%	**0.000**	**-0.313**	**0.000**	**-0.334**
C	%	**0.000**	**-0.366**	**0.007**	**-0.244**
POM N	%	**0.049**	**-0.165**	**0.006**	**-0.224**
POM C	%	**0.002**	**0.260**	**0.048**	**0.163**
C:N	NA	**0.000**	**0.295**	0.537	-0.056
POXc	ppm	**0.017**	**-0.102**	**0.002**	**-0.251**
ACE protein	mg g^{-1} soil	**0.017**	**0.199**	**0.014**	**0.202**
% Sand	%	0.479	-0.060	**0.04**	**0.169**
% Silt	%	0.591	0.045	**0.005**	**-0.230**
pH	NA	**0.002**	**0.258**	0.307	-0.085
Soil Moisture %	%	**0.000**	**-0.292**	0.971	-0.003
Precipitation	cumulative mm	0.200	-0.107	0.923	-0.008
DD	cumulative DD	**0.005**	**0.231**	0.102	-0.135
Available P	kg ha^{-1}	**0.000**	**0.334**	-	-

Note: POM= particulate organic matter, POXc= active carbon, ACE protein= autoclave citrate-extractable protein. Only soil factors that were significantly correlated with *R. solani* concentrations in either 2018 or 2019 were presented above. Environmental variables were presented in both years regardless of significance. Significant correlations at α=0.05 have been bolded.

Exploration of nematode families which may be linked to *R. solani* suppressive soils showed significant associations (Table 7). Nematodes common in low intensity systems (Dorylaimidae, Paratylenchidae and Criconema; see Chapter 2, Table 6) in 2018 were negatively associated with *R. solani* concentration (Table 7).

Table 7. Significant (α<0.05) Spearman correlation coefficients of *R. solani* soil DNA concentration and nematode family abundances across all PEI sampling sites in 2018 and 2019.

Family	Trophic group	2018	2019
Anguinidae	PP	**-0.277**	-0.086
Belonolaimidae	PP	**0.188**	-0.155
Criconematidae	PP	**-0.183**	-0.097
Heteroderidae	PP	**-0.178**	-0.104
Hoplolaimidae	PP	-0.097	-0.009
Longidoridae	PP	-0.154	-0.032
Meloidogynidae	PP	0.028	**-0.190**
Paratylenchidae	PP	**-0.234**	-0.111
Pratylenchidae	PP	0.138	**-0.212**
Tylenchidae	PP	-0.127	0.000
Alaimidae	Ba	0.147	-0.117
Cephalobus	Ba	0.122	-0.144
Diplogastridae	Ba	-	**0.167**
Monhysteridae	Ba	-	0.154
Panagrolaimidae	Ba	0.084	-0.084
Plectidae	Ba	**-0.192**	-0.014
Prismatolaiminidae	Ba	0.109	-0.032
Rhabditidae	Ba	-0.009	-0.042
Teratocephalidae	Ba	-0.032	0.122
Aphelenchidae	Fu	-0.027	-0.043
Aphelenchoididae	Fu	0.129	-0.086
Diphtherophoradae	Fu	-0.091	0.089
Leptonchidae	Fu	0.111	0.041
Tylencholaimidae	Fu	-	0.056
Aporcelaimdiae	Om	**0.168**	-0.016
Dorylaimidae	Om	**-0.249**	-0.031
Qudsianematidae	Om	0.137	-
Mononchidae	P	-0.128	-0.101

Note: Correlation coefficients with P-values below the significance level of α=0.01 are presented in bold.

R. solani soil DNA concentration did not significantly correlate with any nematode index in 2018 or 2019.

4.2 Relationships of R. solani soil concentration and disease symptoms to nematode communities and soil properties (Section. 2)

4.2.1 Disease symptoms

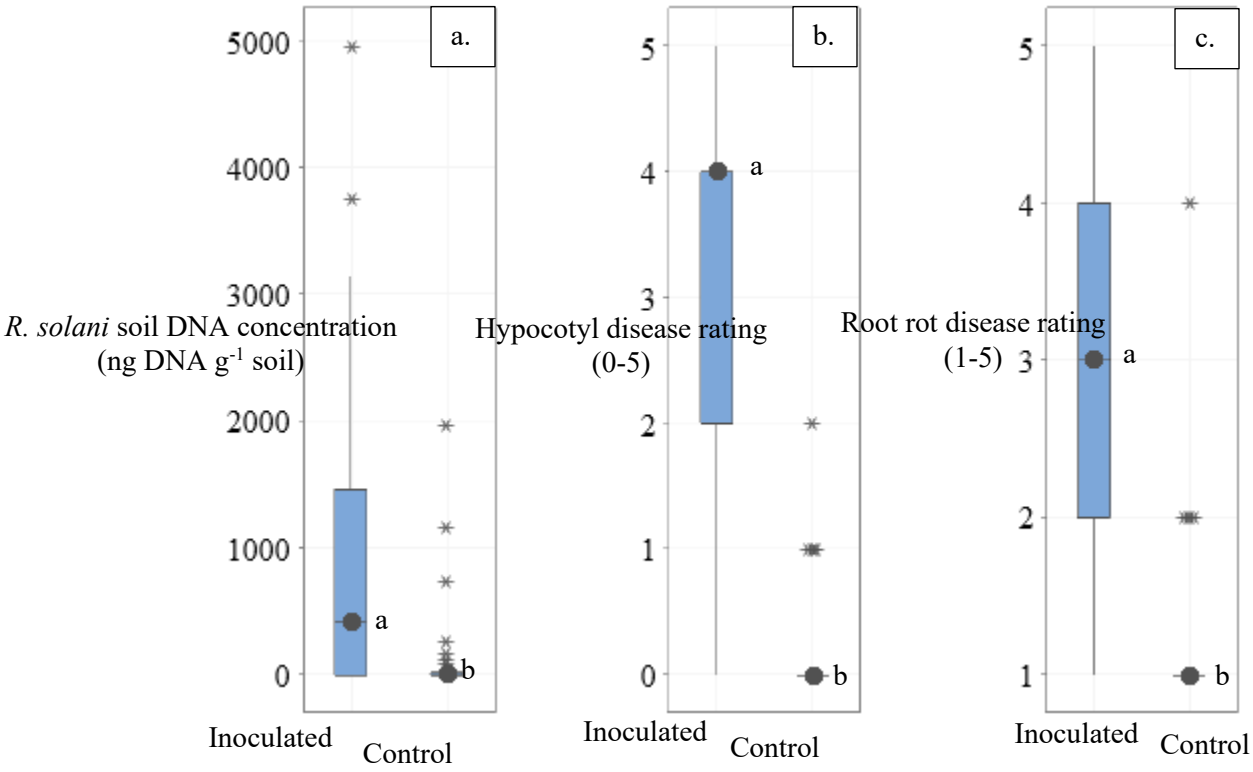
R. solani inoculated treatments had significantly higher *R. solani* soil DNA concentrations and hypocotyl and root rot ratings compared to control treatments (Table 8). Hypocotyl rot disease

ratings ranged from 0-5 in inoculated pots and from 0-2 in control pots (Figure 4). Root rot disease ratings ranged from 1-5 in inoculated pots and from 1-4 in control pots (Figure 4). *R. solani* soil DNA concentrations ranged from 0-4959 ng DNA g^{-1} soil in inoculated pots and from 0-1964 ng DNA g^{-1} soil (Figure 4).

Table 8. Ordinal logistic regression results for *R. solani* inoculation and intensity effects on *R. solani* soil DNA concentration, as well as hypocotyl rot and root rot disease ratings on soybean.

	R. solani DNA concentration (ng DNA g^{-1} soil)		Hypocotyl rot disease rating (0-5)		Root rot disease rating (1-5)	
Factor	Z value	p-value	Z value	p-value	Z value	p-value
R. solani inoculation	2.28	0.022	4.13	0.000	4.30	0.000

Figure 4. Boxplot of *R. solani* soil DNA concentrations and hypocotyl and root rot symptoms by *R. solani* inoculation.

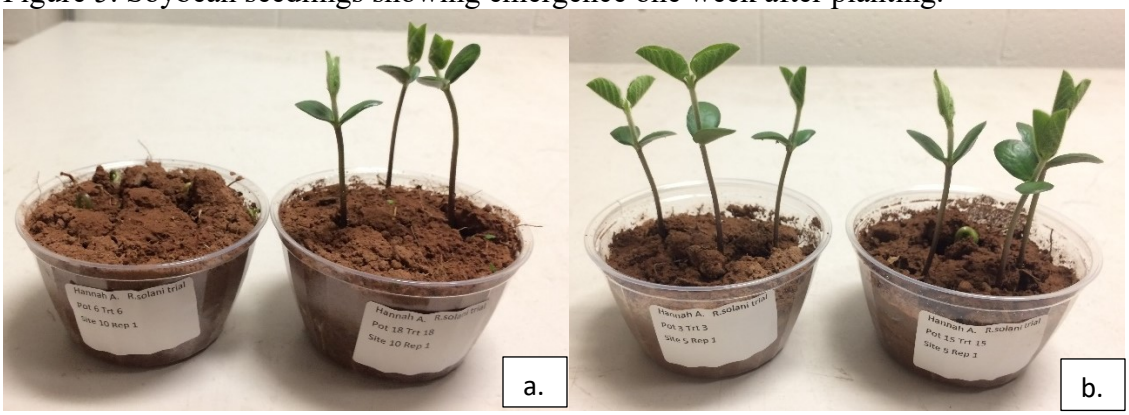


Note: a. *R. solani* soil DNA concentrations by *R. solani* inoculation and control treatments. b. Hypocotyl rot disease ratings by *R. solani* inoculation and control treatments. c. Root rot disease ratings by *R. solani* inoculation and control treatments. Different letter indicate significantly different medians as determined by Dunn's multiple comparison test at a significance level of α=0.05.

Hypocotyl and root rot disease ratings were both negatively correlated with *R. solani* soil DNA concentrations in inoculated soils (Table 12). Significant negative correlations of *R. solani* DNA concentrations with hypocotyl disease ratings were observed in non-inoculated control pots (p-value= 0.014, r= -0.359), while root rot ratings were not significantly correlated with hypocotyl rot disease ratings (p-value= 0.107, r= 0.241).

Variability in the difference of disease symptoms, between soybeans grown in inoculated and control treatments of different soils, indicated differences in soil disease suppression (Figure 5).

Figure 5. Soybean seedlings showing emergence one week after planting.



Note: a. (left) Site 10 *R. solani* inoculated soil, (right) site 10 non-inoculated soil. b. (left) Site 5 *R. solani* inoculated soil, (right) site 5 non-inoculated soil. Inoculated site 10 soybeans are just emerging while the inoculated site 5 treatment soybeans have already fully emerged.

Hypocotyl rot disease symptoms (Z value= 0.00, p-value= 1.000) and root rot disease symptoms (Z value= 0.02, p-value= 0.985) did not significantly differ between the soils that were tested in control pots, due to pre-existing soil *R. solani*.

4.2.2 Relationship of R. solani soil DNA concentration and disease symptoms to nematode community indices

While differences in disease suppression were observed between the individual soils, the pre-trial nematode faunal indices (EI and SI), that were the initial objective of this study, did not significantly predict differences in *R. solani* disease suppressive soils (Table 9).

Table 9. Significance of *R. solani* hypocotyl and root rot disease ratings, as well as soil DNA concentration across pre-trial nematode community indices.

Index	Hypocotyl disease rating (0-5)		Root rot disease rating (1-5)		*R. solani* soil DNA concentration (ng DNA g^{-1} soil)	
	F-value	p-value	F-value	p-value	F-value	p-value
EI	0.00	0.993	0.01	0.908	0.48	0.490
SI	0.08	0.776	0.31	0.591	2.44	0.127
EI*SI	0.37	0.373	0.38	0.549	1.49	0.233

Note: Ordinal logistic regression assessed significance of EI and SI treatments in *R. solani* inoculated pots.

Depending on the initial quadrat type, some nematode faunal indices changed during the course of the trial (Table 10 & 11).

Table 10. Repeated measures analysis of pre-trial and post-trial nematode faunal indices in control pots.

	EI		SI	
Factor	F-value	p-value	F-value	p-value
Time	27.58	0.000	40.15	0.000
Quadrat	14.87	0.000	27.26	0.000
Time*Quadrat	21.49	0.000	14.44	0.000

Enrichment values during the three week trial increased in initially low EI quadrats (C and D) (Table 11). At post-trial assessment all EI values were statistically the same. Large SI values pre-trial (quadrat B & C) saw large significant decreases in post-trial assessment. SI was not statistically the same across post-trial values. Low pre-trial SI value treatments (Quadrat A & D) showed diverging changes in SI, where quadrat A soils increased and quadrat D soils decreased, although quadrat D soils did not change significantly compared to the initial SI value (Table 11).

Table 11. Time *quadrat interaction effect means of pre-trial and post-trial nematode faunal indices in control pots.

Quadrat- Treatment	Enrichment Index		Structure Index	
	Pre-trial	Post-trial	Pre-trial	Post-trial
A- E2S1	80.95a	73.62ab	22.8bc	33.97b
B- E2S2	82.84a	71.74ab	78.74a	33.71b
C- E1S2	40.93c	76.56ab	86.58a	43.01b
D- E1S1	41.15c	68.59b	21.07c	18.99c

Note: Different letters indicate significant differences across quadrat and trial sampling time within each index. Significance determined at α=0.05 by repeated measures ANOVA. Means were compared using Tukey's post hoc test.

Seeing as the nematode community indices changed rapidly during the experiment, assessment of post-trial nematode populations may be more appropriate, as these values likely correspond to the nematodes that were active during the course of the trial.

Post-trial nematode communities indices showed more consistent correlations with of *R. solani* disease symptoms than pre-trial indices. Hypocotyl disease symptoms showed stronger, significant correlations with EI and SI more frequently than root rot and *R. solani* soil DNA concentrations. Post-trial SI showed a weak, negative correlations with hypocotyl disease (Table 12). The EI was related to disease symptoms differently than SI. Post-trial EI showed a marginally significant positive associations with *R. solani* concentrations and significant positive associations with hypocotyl rot (Table 12).

Table 12. Spearman correlation coefficients of pre-trial and post trial nematode indices and their relationship to *R. solani* soil DNA concentration and disease symptoms.

Hypocotyl rot	1	-					
Root rot	2	0.628***	-				
Post-trial soil *R. solani*	3	-0.506***	-0.255**	-			
Pre-trial EI	4	0.294**	0.119	-0.288*	-		
Pre-trial SI	5	-0.027	0.057	0.174	-0.051	-	
Post-trial EI	6	0.270*	0.144*	-0.248	0.217**	0.102	-
Post-trial SI	7	-0.298**	-0.010	0.097	-0.030	0.474**	-0.222
		1	2	3	4	5	6

Note: Significant at * (α=0.1), ** (α =0.05), *** (α=0.01) as determined by Spearman correlations.

4.2.3. Soil nematode and R. solani soil concentration and disease symptoms.

Pratylenchidae abundances showed the highest correlation to *R. solani* soil DNA concentrations as well as hypocotyl and root rot symptoms (Table 13) where correlations also reflected the negative association between *R. solani* soil level and disease symptoms (Table 13). Pratylenchidae abundances were positively correlated with disease symptoms (Table 13). Hypocotyl disease symptoms were associated with the most nematode families. Hypocotyl disease ratings were negatively correlated with higher cp value nematodes including Monhysteridae, Tylencholaimidae and Aporcelaimidae, and positively correlated with the low cp bacterivore Rhabditidae. The fungivore Aphelenchidae was also positively correlated with both hypocotyl and root rot disease symptoms (Table 13).

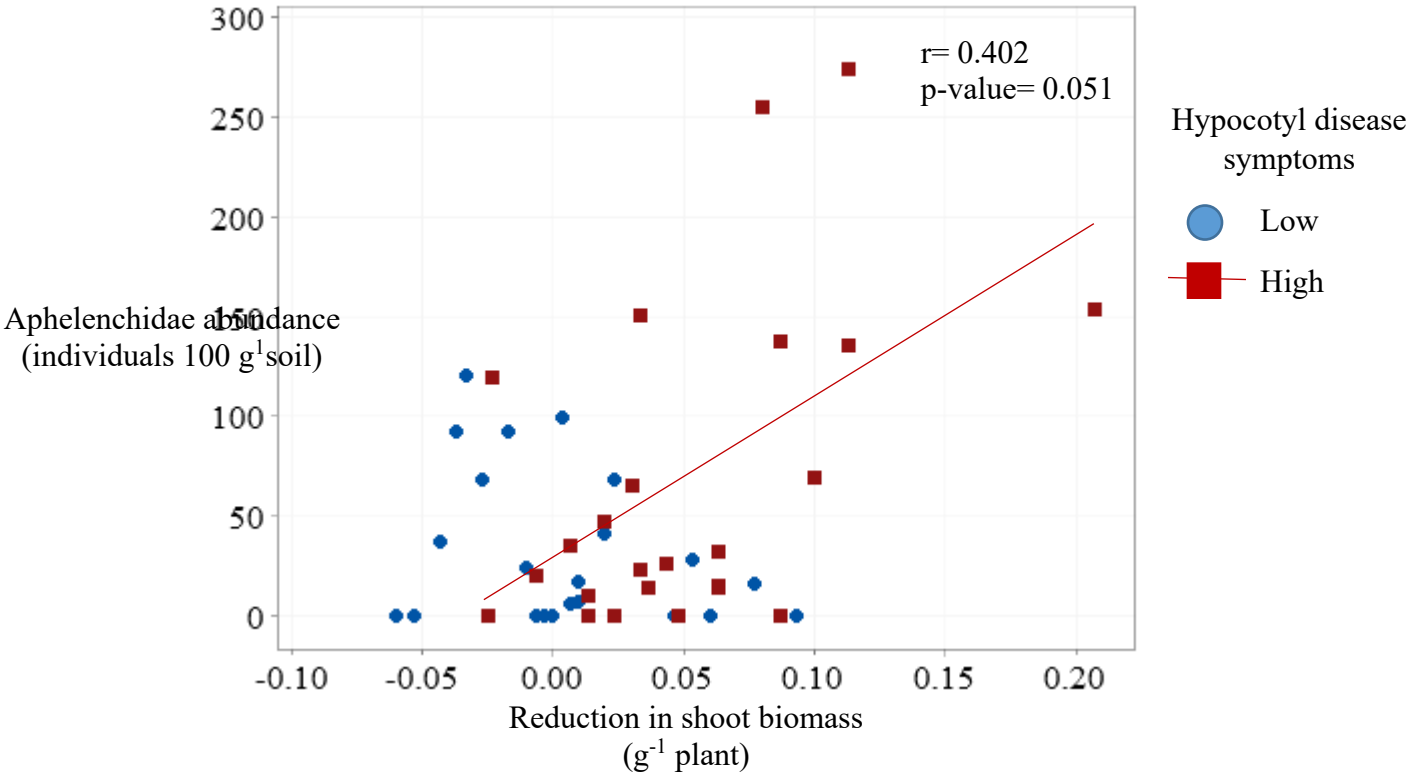
Table 13. Spearman correlation coefficients of *R. solani* soil concentrations and hypocotyl disease symptoms with post-trial soil nematode family abundances.

Family	Trophic Group	*R. solani* soil DNA concentration (ng DNA g^{-1}soil)	Hypocotyl rot disease rating (0-5)	Root rot disease rating (1-5)
Anguinidae	PP	0.083	-0.113	-0.141
Belonolaimus	PP	-0.131	0.034	0.044
Criconematidae	PP	-0.061	0.04	-0.051
Heteroderidae	PP	0.159	0.004	0.043
Hoplolaimidae	PP	-0.108	0.172	0.275
Longidoridae	PP	0.006	-0.017	-0.030
Meloidogynidae	PP	0.154	-0.193	-0.094
Paratylenchidae	PP	0.111	-0.026	0.020
Pratylenchidae	PP	**-0.537**	**0.480**	**0.412**
Tylenchidae	PP	0.120	-0.242	-0.071
Alaimidae	Ba	0.098	-0.013	-0.239
Cephalobus	Ba	-0.064	0.098	-0.210
Diplogastridae	Ba	-0.007	0.078	-0.030
Monhysteridae	Ba	0.287	**-0.304**	-0.053
Panagrolaimidae	Ba	-0.059	**-0.303**	-0.026
Plectidae	Ba	0.154	-0.044	-0.052
Prismatolaimidae	Ba	0.200	-0.231	-0.097
Rhabditidae	Ba	-0.209	**0.298**	0.088
Teratocephalidae	Ba	0.079	-0.210	0.006
Aphelenchidae	Fu	-0.200	**0.363**	**0.356**
Aphelenchoididae	Fu	0.062	0.024	0.007
Diphtherophoridae	Fu	**-0.311**	0.103	-0.003
Leptonchidae	Fu	0.062	-0.125	-0.008
Tylencholaimidae	Fu	0.242	**-0.370**	-0.062
Aporcelaimidae	Om	0.112	**-0.305**	-0.14
Dorylaimidae	Om	-0.122	-0.046	0.161
Mononchidae	P	0.030	-0.040	-0.050

Note: Spearman correlation coefficients with p-values below the significance level of α=0.05 are presented in bold.

Aphelenchidae abundance, was related to the reduction of shoot biomass due to *R. solani* disease (Fig. 6). This was evident when hypocotyl disease was separated by low and high ratings (Fig. 6). When hypocotyl disease symptoms were low, reduction in shoot biomass was not related to Aphelenchidae abundance. When disease symptoms were high, reductions in shoot biomass due to *R. solani* infection resulted in increased Aphelenchidae abundances.

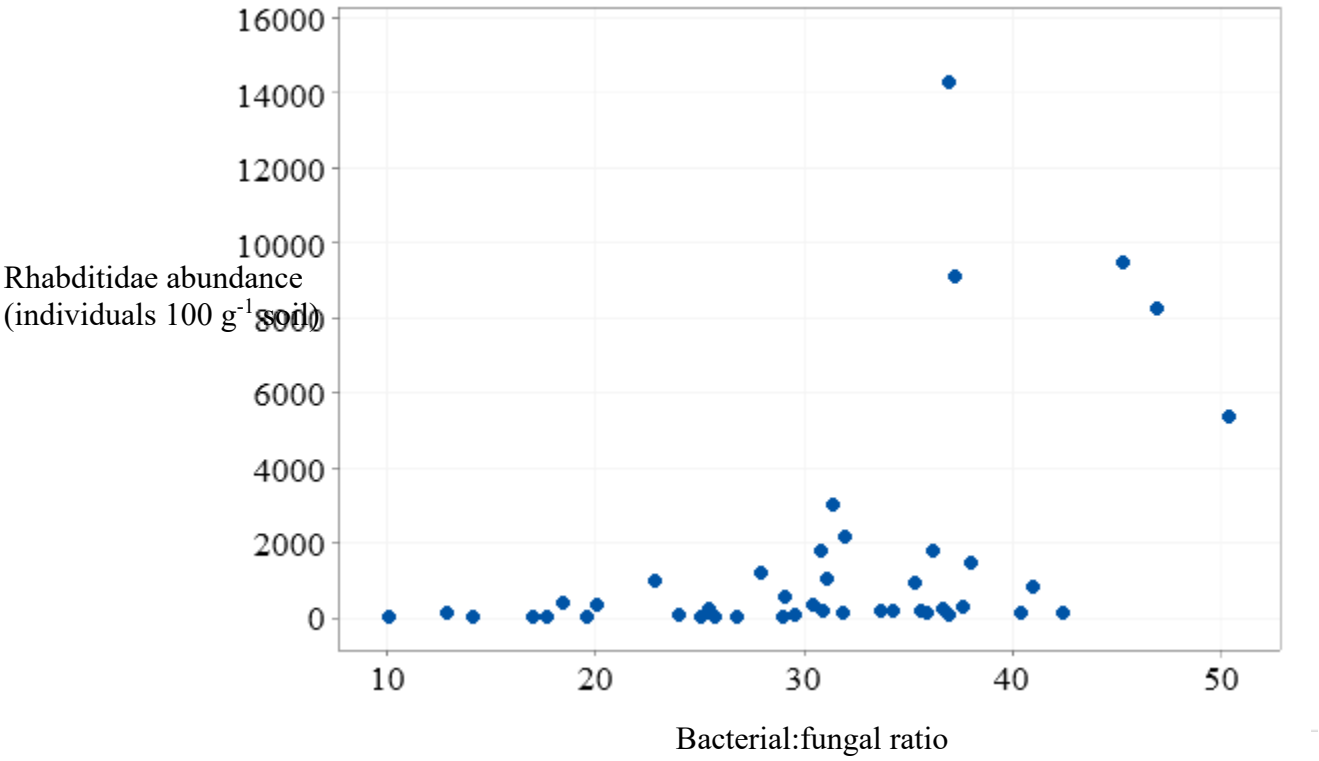
Figure 6. Aphelenchidae abundance in relation to reduction in plant growth as a result of *R. solani* hypocotyl disease symptoms.



Note: A marginally significant Pearson correlation was determined between Aphelenchidae soil abundances and reductions in shoot biomass due to *R. solani* disease under high disease symptoms. Correlations were not significant under low disease symptoms (p-value= 0.457, r=-0.163).

The EI was highly influenced by Rhabditidae nematode abundance as determined by regression analysis (r=0.953, p-value=0.000). Rhabditidae abundances spiked heavily at high bacterial:fungal biomarker ratios (Fig. 7).

Figure 7. Scatterplot of Rhabditidae abundances from post-trial soils.



4.2.4 Soil properties and R. solani soil DNA concentration

R. solani soil DNA concentrations and hypocotyl and root rot disease symptoms showed opposite correlations with % sand and % silt (Table 14). Root rot was not significantly correlated with any soil property, except for the distance in the soil from the *R. solani* inoculum to the planted seed (Table 14).

Table 14. Spearman correlation coefficients of soybean *R. solani* disease symptoms and soil concentrations by soil conditions.

Soil trait	Units	Hypocotyl rot (0-5)	Soil *R. solani* (ng DNA g^{-1} soil)	Root rot (1-5)
% Sand	%	-0.339**	0.423**	-0.026
% Silt	%	0.311**	-0.497***	0.067
Inoculum to seed distance†	mm	0.202	-0.082	0.286*

Note: †= Distance from *R. solani* inoculum to soybean seed. *= Correlations significant at α =0.05. Inoculum to seed distance was measured during the growing period, gravimetric moisture content was measured post-trial and all other soil properties were measured pre-trial.

Soil microbial groups did not significantly responded to *R. solani* inoculation, although fungal and anaerobic biomarkers approached significance (Table 15).

Table 15. Comparison of soil post-trial microbial biomarker group means (± standard error) between *R. solani* inoculated and non-inoculated treatments.

PLFA group	*R. solani* inoculated (nmol g^{-1} of soil)	Non-inoculated control (nmol g^{-1} of soil)	F Value	p-value
G- bacteria	73139 ± 555	61477 ± 5127	2.87	0.094
G+ bacteria	59055 ± 4087	52229 ± 4752	1.76	0.188
Eukaryote	24653 ± 1358	23128 ± 1628	1.13	0.292
Fungi	4727 ± 455	4058 ± 434	3.47	0.066*
AM Fungi	5239 ± 421	4639 ± 433	1.40	0.241
Actinomycetes	21162 ± 1817	17777 ± 1848	2.02	0.159
Anerobes	8894 ± 847	6313 ± 604	3.72	0.058*

Note: * indicates microbial groupings approaching the significance level of α=0.05.

FAME biomarker groups were not significantly correlated with hypocotyl or root rot disease symptoms, although marginally significant and significant correlations were observed for *R. solani* soil DNA concentration (Table 16). The AMF and gram negative associated biomarkers showed significant negative correlations, while gram positive biomarkers showed marginally significant negative correlations with *R. solani* soil DNA concentration (Table 16). 16:1 ω5 was also influenced by the same factors as *R. solani* soil DNA concentration, where it was negatively correlated with % sand (p-value= 0.007, r= -0.405) and positively correlated with % silt (p-value= 0.006, r= 0.413).

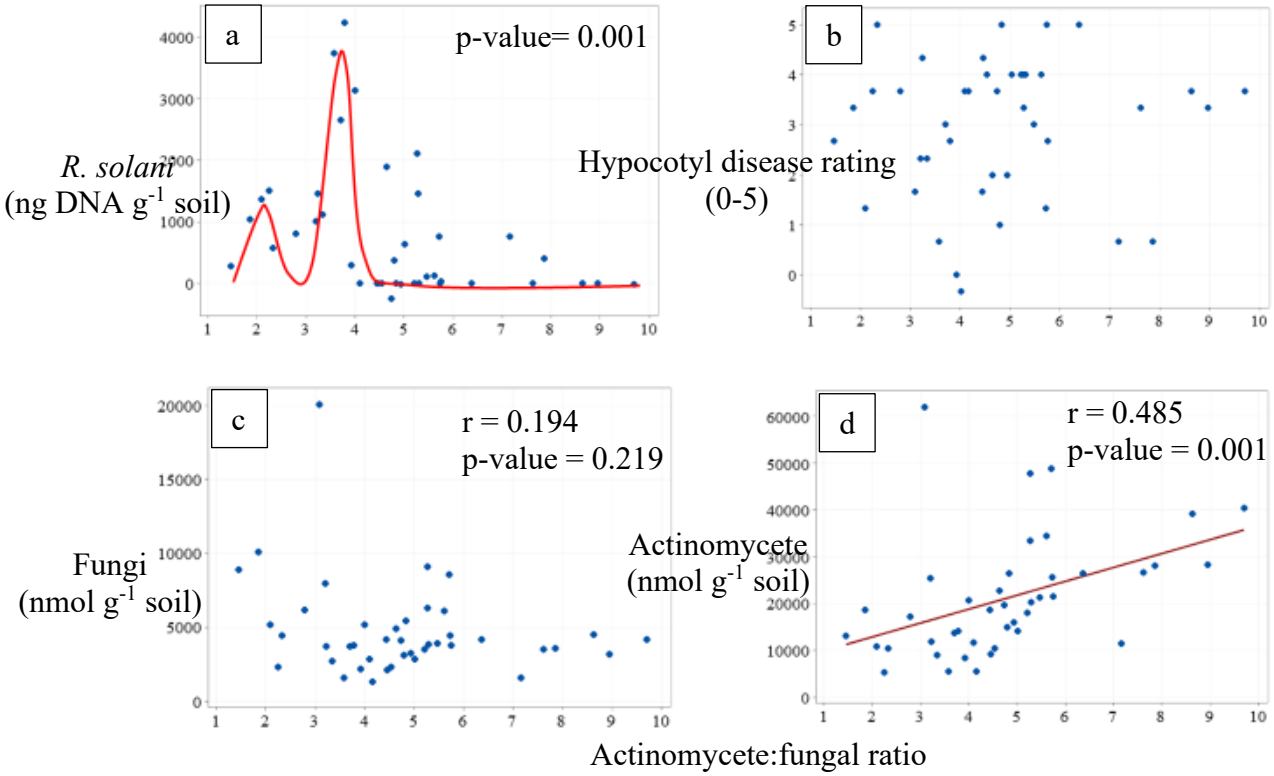
Table 16. Spearman correlation coefficients of soybean *R. solani* disease symptoms and post-trial microbial biomass as determined by phospholipid fatty acid analysis for inoculated treatments.

Hypocotyl rot	-								
Root rot	0.628***	-							
Soil *R. solani*	-0.506***	-0.255**	-						
G- bacteria	0.187	0.052	-0.325**	-					
G+ bacteria	0.223	0.153	-0.269*	0.951***	-				
Eukaryote	0.169	-0.036	-0.133	0.647***	0.774***	-			
Fungi	0.081	0.015	0.116	0.668***	0.681***	0.493***	-		
AM Fungi	0.226	0.145	-0.352**	0.909***	0.900***	0.621***	0.563***	-	
Actinomycetes	0.088	-0.006	-0.262	0.944***	0.730***	0.605***	0.649***	0.908***	-
Anerobes	0.095	-0.058	-0.186	0.767***	0.791***	0.622***	0.487***	0.715***	0.730***
	Hypocotyl rot	Root rot	Soil *R. solani*	G- bacteria	G+ bacteria	Eukaryote	Fungi	AM Fungi	Actinomycetes

Note: Hypocotyl rot= disease symptoms (0-5), Root rot= disease symptoms (1-5), Soil *R. solani*= *R. solani* soil DNA concentration (ng DNA g^{-1} soil, soil microbial groupings= nmol biomarker(s) g^{-1} of soil. Significant at * α =0.1, ** α =0.05, *** α =0.01

Significant relationships between the *R. solani* soil DNA concentration and the actinomycete:fungal ration linked the pathogen to the soil microbial community (Fig. 8). The relationship between *R. solani* soil DNA concentration and PLFA determined actinomycete:fungal concentration ratio was significantly modelled (F-value=9.75, P<fr= <0.001) using a double Gaussian function. This model explained 53% of the variation in soil *R. solani* DNA concentrations. Actinomycete:fungal ratios greater than 5.5 showed consistent suppression of *R. solani* soil DNA concentrations (Fig. 8a). While this ratio was related to *R. solani* soil DNA concentration (Fig. 8a), this ratio did not affect hypocotyl disease ratings (Fig. 8b). Although *R. solani* soil DNA concentration did not follow significant trends across actinomycete and fungal microbial biomarkers (Table 15), the actinomycete:fungal ratio was predominantly influenced by increases in actinomycete associated FAMEs (Fig. 8d) as opposed to decreases in fungal biomarkers (Fig. 8c).

Figure 8. Relationship of *R. solani* soil concentration and disease symptoms, as well as fungal and actinomycete biomarkers to the actinomycete: fungal biomarker ratio.



Note: Scatterplot of (a) *R. solani* soil concentration by actinomycete:fungal biomarker ratio (b) *R. solani* hypocotyl rot ratings by actinomycete:fungal biomarker ratio (c) fungal biomarker concentration by actinomycete:fungal biomarker ratio (d) actinomycete biomarker concentration by actinomycete:fungal biomarker ratio

The actinomycete:fungal ratio was correlated with many soil properties. Significant positive correlations were observed with N (p-value=0.019, r=0.414), POM N (p-value=0.035, r=0.327), POXc (p-value=0.006, r=0.419) and soil moisture (p-value=0.005, r=0.423). Significant negative correlations were observed with % sand (p-value=0.005, r= -0.425), and % silt (p-value=0.002, r= -0.470).

4.2.5 Soil fungivores and R. solani soil DNA concentration and disease symptoms

Ordinal logistic regression related post-trial fungivore abundance and post-trial *R. solani* soil DNA concentration to *R. solani* hypocotyl disease ratings (Table 17). Both a main effect for

fungivores and an interaction effect with *R. solani* soil DNA concentration were included in this model.

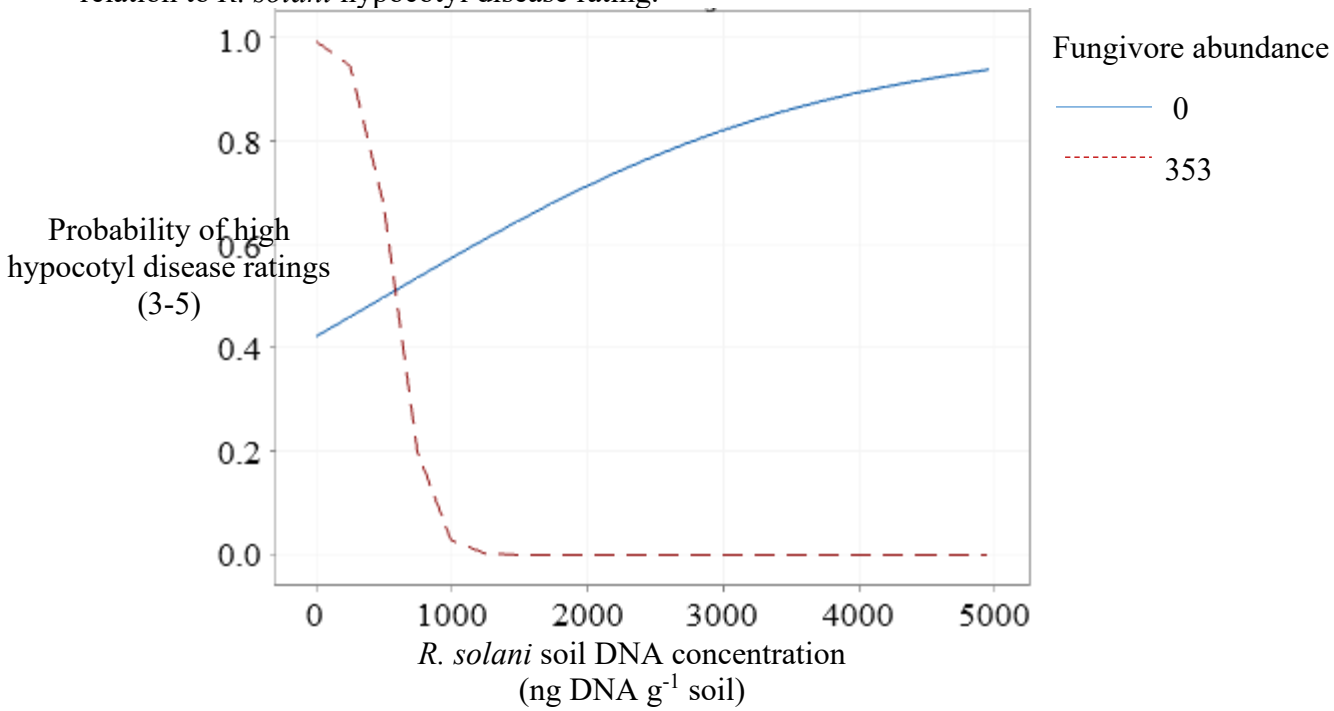
Table 17. Ordinal logistic regression determined significance of variates and model summary explaining *R. solani* hypocotyl disease suppression.

Variate	Coefficient	SE of coefficient	Z Value	p-value
Fungivores	0.1978	0.087	-2.25	0.024
Fungivores* *R. solani*	0.000071	0.000054	2.53	0.011
Model summary				
Log-Likelihood	-63.447			
Somers' D	0.50			
Kendall's Tau-a	0.38			

Note: Fungivores= Fungivore abundance, *R. solani*= *R. solani* soil DNA concentration.

When fungivores were low in abundance there was a positive relationship between the probability of high hypocotyl rot ratings and *R. solani* soil DNA concentration (Fig. 9). At high fungivore abundances an inverse relationship between high hypocotyl rot probabilities and soil *R. solani* DNA concentration was found (Fig. 9).

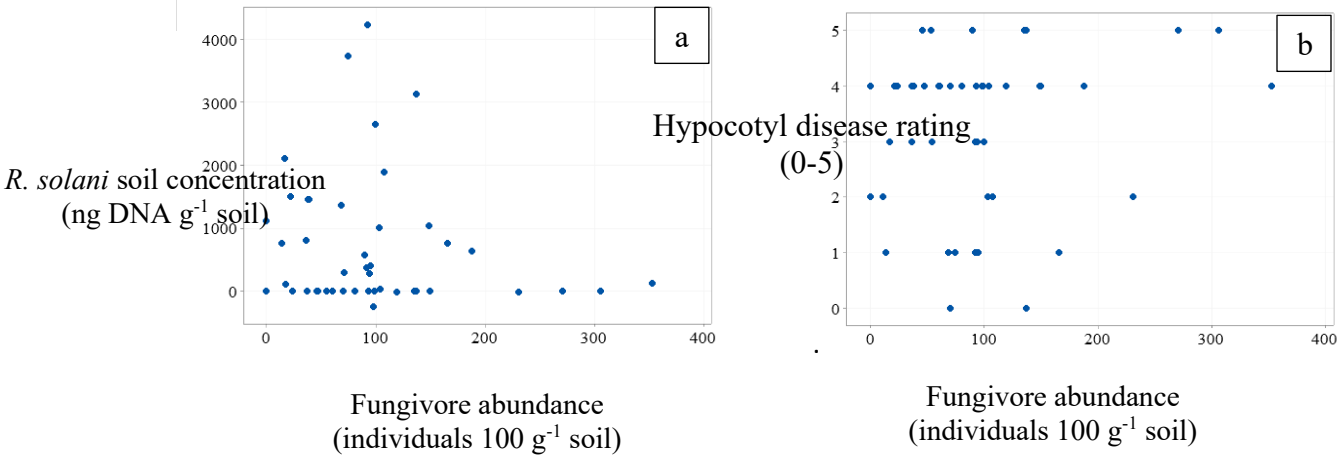
Figure 9. Interaction effect of soil fungivore abundance and *R. solani* soil DNA concentration in relation to *R. solani* hypocotyl disease rating.



Note: Disease ratings (0-5) according Bradley et al. (2001).

At high fungivore abundances (+ 100 individuals g^{-1} soil) there were decreasing *R. solani* soil DNA concentrations (Fig. 10a). Despite this decrease in pathogen inoculum, high hypocotyl disease ratings were also found at high fungivore abundances (Fig. 10b).

Figure 10. Scatterplot of *R. solani* soil DNA concentration and hypocotyl disease symptoms with fungivore abundances in post-trial soils.

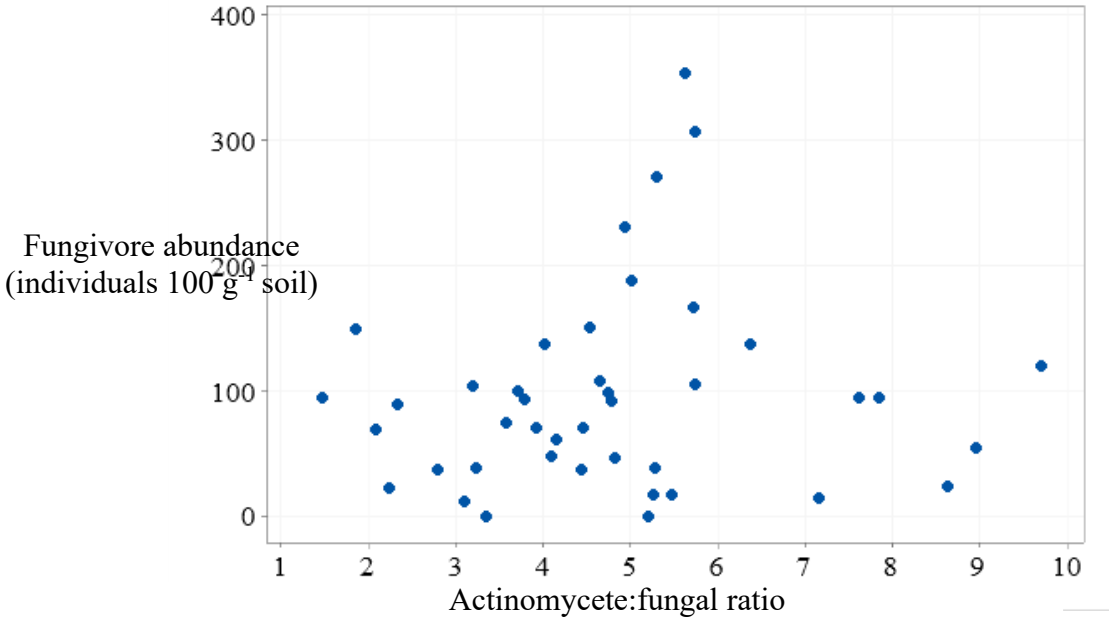


The abundance of different fungivore families reacted differently during the course of the trial. Tylencholaimidae was found in initially high mean abundances (99.4 ± 32.4 individuals) and post-trial their mean abundance was significantly reduced (7.1 ± 3.6 individuals) (T value=4.27, p-value= 0.000). Out of the twelve soils included in this trial only two sites had initial populations of Tylencholaimidae at 522.7 and 645.8 individuals 100^{-1} g soil. Other fungivores showed no change or a small increases in abundance.

Increased fungal food sources in *R. solani* inoculated pots were expected to increase fungivore populations. While post-trial fungivore abundance in inoculated (94.8 ± 11.1) and non-inoculated (79.4 ± 8.6) pots showed no significant differences (F value= 1.27, p-value= 0.262).

Like the *R. solani* soil DNA concentrations, fungivore abundances also may have been influenced by the actinomycete:fungal ratio in select pots (Fig. 11). Several data points from different soils were found to have soil fungivore abundances over 200 individuals and peak around the actinomycete:fungal ratio of 5.5 (Fig. 11).

Figure 11. Relationship of fungivore abundance to the actinomycete:fungal biomarker ratio.



5. DISCUSSION

5.1 Influence of land use intensity on distribution of Rhizoctonia solani in soil (Section. 1)

Environmental conditions appeared to influence pathogen growth. Low precipitation in 2018 resulted in significant correlations of environmental variables such as soil moisture % and cumulative degree days. *R. solani* DNA concentration showed a weak negative correlation with soil moisture in 2018, but not in 2019. Reduced water availability may have limited growth and activity of many biological populations, leading to *R. solani* proliferation. Textural properties, including % sand and % silt, were significantly correlated to the pathogen in 2019 (Table 6), where lighter textured soils promoted *R. solani* growth. This contrast with 2018 indicates that soil moisture retention may be more affected by this intrinsic soil property under average rainfall. In a three year study of AG8 in field soils, *R. solani* AG 8 soil concentrations were also found to positively correlate with maximum temperatures and % sand across all years (Okubara et al. 2014).

R. solani soil DNA concentration did not consistently increase with our characterization of LUI or respond to current crop type. This study assessed *R. solani* as a sum of all AG groups, which have different copy numbers and host ranges. This variability may have obscured *R. solani* LUI trends, where trends in specific AG groups were not assessed. Although *R. solani* soil DNA concentration did not follow intensity trends, soil DNA concentrations were linked to many soil properties that were previously linked to the LUI classifications. In Chapter 2, it was determined that total N and C, decreased with intensity while soil fertility traits such as available P increased with intensity (Chapter 2, Table 5). Negative correlations of *R. solani* with total C, and total N in 2018 and 2019, as well as positive correlations with available P in 2018, indicate that the soil properties associated with increasing intensity promote *R. solani* despite contrasting environmental conditions. Increasing SOM, as indicated by total C, may suppress *R. solani*, however SOM quality could also influence growth (Bonanomi et al. 2010). While SOM was negatively correlated with *R. solani*, POM C and ACE protein were positively associated with *R. solani* concentrations. Organic matter quality traits, including total N and select C functional groups, were most consistently related to the suppression of *R. solani* growth and disease proliferation across organic amendments in lettuce (Bonanomi et al 2020). It is not possible to state whether these soil property associations are direct, or more related to the soil microbial community. Soil microbial communities and their suppressive potential are likely influenced by soil properties, resulting in indirect disease suppressive effects (Liu et al. 2019). Either way, the soil properties that are linked with LUI and soil pathogen suppression, such as total N, total C, available P, POXc, ACE protein and pH, have the potential to be managed in field settings.

The associations with soil traits, over LUI and crop, may also reflect the sampling time in this study. *R. solani* can infect plant tissues during seedling germination and emergence (Melzer et al.

2016), thus intensity and crop trends may be temporal and evident at an earlier sampling time. The samples in this study were collected in late July. Environmental variables, that were significantly related to *R. solani* soil DNA concentrations, could have influenced root growth and post-infection pathogen growth in the soil. It is also possible that our classification of LUI may not reflect the nuances of *R. solani* growth and population dynamics. Ghini and Morandi (2006) found that undisturbed forest, pasture and fallow ground areas, annual crops, perennial crops, and ploughed soil groupings allowed soil properties to explained >90% of in vitro mycelial suppression variation, compared to 51% without taking land-use into account. This may suggest that soil factors variably drive *R. solani* disease suppression under different vegetation covers. Despite this, in 2018 natural vegetation types (forest, forest/grass, grass) had lower *R. solani* levels than most arable land crops which generally had significantly higher pathogen levels (Table 5). These trends were not found in 2019, which may suggest that pathogen suppression during periods of biological stress is based on the resiliency of the former, lesser disturbed, vegetation/crop types. Increased SOM, as inferred by total C, found for the lower LUI classification (see Chapter 2, Table 5) can maintain soil moisture levels and act as reservoirs which more rapidly re-colonize soil after periods of drought (Meisner and de Boer 2018). This would support the hypothesis that *R. solani* soil DNA concentration is influenced by soil factors related to soil biological degradation, although this effect may only be significant in terms of land-use under drought conditions.

Population distribution within intensity may also vary. Different *R. solani* anastomosis groups can infect the same crop (Melzer et al. 2016). Proportions of different AG groups and their distribution within the field may be different across our intensity and vegetation classifications, which has implications for the pathogenicity of these *R. solani* populations. Binucleate *Rhizoctonia* spp. isolates are often less frequently associated with, and less pathogenic toward, agricultural crops

(Melzer et al. 2016; Olaya and Abawi 1994), and may generally play a more saprophytic ecological role. Primers used in this study may not be specific to multinucleate *R. solani* and were tested on isolates specified and non-specified by AG group (Lievens et al. 2006). Due to lack of assessment of specific *R. solani* AG groups, soil DNA concentrations cannot be directly related to disease potential.

Nematode families related to *R. solani* in 2018 reflected the pathogen intensity trend, where soil concentrations decreased with omnivores and plant parasites that were common in low intensity sites. These results may reflect the *R. solani* trend with intensity for that year, as opposed to a direct nematode community association.

5.2 Relationships of R. solani soil DNA concentration and disease symptoms to nematode communities and soil properties (Section. 2)

5.2.1 Relationship of R. solani soil DNA concentration and disease symptoms

R. solani soil DNA concentration were negatively correlated with hypocotyl and root rot disease symptoms in inoculated treatments (Table 12). In this study, large amounts of *R. solani* were added to the soil a week before the infection process could take place. During this time, *R. solani* soil concentrations would have changed across soil treatments, resulting in unequal inoculum between the soils, and soil concentrations would continue to change post infection during the course of the trial. Additional *R. solani* soil quantification throughout the course of the trial, would have benefitted this assessment. This could have been achieved using non-destructive toothpick baiting technique (Spurlock et al. 2015). *R. solani* soil DNA concentrations in non-inoculated pots were also negatively correlated with hypocotyl disease. This is a common phenomenon for *R. solani*, where Bonanomi et al. (2007) determined that disease suppression was only associated with *R. solani* population suppression in less than 30% of the time in a meta-analysis study. Use of AG specific probes may have changed the results in this study.

5.2.2 Relationship of R. solani soil DNA concentration and disease symptoms and nematode community indices

Change of nematode indices over the course of the trial may have occurred due to soil disturbance during potting or original sampling. Increased EI and reduced SI can occur during physical disturbances associated with tillage (Ferris et al. 2001). This convergence of treatment values may have resulted in the non-significant differences in *R. solani* disease suppression and soil concentration that were observed across quadrat. This would imply that if EI and SI do influence *R. solani* disease suppression the soil physical disturbance effects may have already limited these soil control mechanisms before *R. solani* inoculation. Alteration of methods to minimize soil disturbance may have prevented community shifts (Ferris et al. 2001), such as use of undisturbed soil cores. Assessment of post-trial nematode communities is more relevant in our discussion of pathogen suppression, as it likely more accurately reflects the soil organisms that were active or reproducing during the infection. At the same time, associations in post-trial indices in inoculated pots may also reflect changes in nematode populations as a result of *R. solani* presence and disease symptoms.

In post-trial assessments, disease was more conducive with increasing opportunistic nematodes and the resulting higher EI index. While bacterivore feeding may have influenced disease symptoms, these were weak correlations. Increased bacterivore count and subsequent activity here was a response to matricidal hatching in Rhabditidae. Matricidal hatching can be induced by different stressors including: inability to feed due to loss of food source or potential physical barriers to feeding (Chen and Caswell-Chen 2003) as well as increased toxin concentration (Pestov et al. 2011), or bacterial infection (Mosser et al. 2011). Microplastic contamination may be a physical barrier to feeding when ingested. Microplastics were noticed in the samples that we selected, however no associations can be made between matricidal hatching and microplastic

occurrences as neither was directly quantified. Bacterial infection of Rhabditidae can also induce matricidal hatching, and in this study increased Rhabditidae populations were linked to bacterial:fungal biomarker ratios. The relationship to increasing proportions of bacterial biomarkers may however be due to matricidal hatching itself, where intestinal bacteria may colonize the female as a food source for the hatching juveniles (Schulenburg and Félix 2017).

Several higher cp nematodes including Tylencholaimidae and Aporcelaimidae were negatively correlated with hypocotyl disease symptoms. These associations would have resulted in the negative correlation between hypocotyl disease symptoms and the SI that was previously observed. As an omnivore and a fungivore these families may directly suppressed *R. solani* disease proliferation through feeding. They may also indicate suppressive soil conditions. Tylencholaimidae populations were sensitive to physical soil disturbance in this trial, therefore their relationships to hypocotyl disease may signify a response to this disturbance. While there is prior knowledge of each of these family's ecological role, an understanding of how these families singly or collectively related or contributed to this relationship cannot be yet determined.

Fungivorous Aphelenchidae nematodes were positively correlated to both hypocotyl and root rot symptoms. The relationship between this family and hypocotyl disease symptoms will be discussed in a later section (*section 5.2.4*).

Despite correlations with fungivores, opportunistsic bacterivores and many higher cp value nematodes of different trophic groups, the plant parasitic nematode family Pratylenchidae showed the strongest correlaitons. This nematode family also reflected the negative association between *R. solani* soil DNA concentration and the hypocotyl and root rot disease symptoms. Positive correlations between Pratylenchidae and both root and hypocotyl disease symptoms suggests these nematodes may have promoted *R. solani* disease or that increasing *R. solani* disease aided

Pratylenchidae reproduction. Pratylenchidae nematodes are notorious in their role in disease complexes (Back et al. 2002). *Pratylenchus penetrans* have previously been shown to have no interactive effect with *R. solani* in corn (da Silva et al. 2017), and addition of root lesion nematodes reduced stem canker lesions due to *R. solani* in potato (Edin et al. 2019). In contrast, higher field soil abundances have been found in *R. solani* diseased soybean patches compared to healthy stands (Lui et al. 2016) and *Pratylenchus brachyurus* with *R. solani* resulted in a positive synergistic disease interaction, where percent plant mortality was greater than that observed for each singular pathogen (Lindsey and Cairns 1971). While the free-living nematode community may have significantly interacted with the pathogen or disease symptoms in this study, the plant parasitic Pratylenchidae family was the grouping most closely associated with *R. solani* disease.

5.2.3 Soil properties and R. solani soil DNA concentration

Overall *R. solani* disease symptoms showed stronger correlations with physical soil properties as opposed to the biomass of different soil microbial groups, as determined by PLFA analysis. Reduction of porosity in soils which lost their structural integrity may have prevented *R. solani* growth through the soil (Harris et al. 2003). This could explain why root rot increased with increasing distance from the inoculum to soybean seed. Soil properties including % sand and % silt were found to oppositely correlate with *R. solani* soil DNA concentration and soybean disease symptoms, where sandier soils promoted hypocotyl disease symptoms (Table 14). Therefore, pathogen suppression in bulk soil may be mediated by soil factors that increase *R. solani* disease symptoms after infection.

Although *R. solani* soil DNA concentrations were significantly related to physical soil properties including % sand and silt, soil *R. solani* levels were also associated with the biomass of multiple soil microbial groups. The soil microbial community is often thought to be directly

responsible for soil disease suppression by general or specific mechanisms (Schlatter et al. 2017). . *R. solani* concentration was also negatively correlated with gram - and gram + bacteria. Many studies have connected *R. solani* to taxa within these microbial groups (Poudel et al. 2016; Ascencion et al. 2015, Yin et al. 2013), where again antagonism can take many forms competition, predation and chemical antagonism. In this study, soil was conducive to *R. solani* at actinomycete:fungal ratios below 5.5. This ratio correlated with many other soil factors that did not directly relate to the *R. solani* soil levels. *R. solani* soil concentration did not show trends with either fungal or actinomycete biomarkers, thus the ratio appears to be more applicable to *R. solani* control than associations with individual microbial groups.

Suppression mediated by the activity of different community compositions of actinomycetes and fungi may be variable along this gradient. The direct or indirect suppressive effect of each community may have been more relevant at opposing ends of the actinomycete:fungal ratio. Niche competition from other fungi may also provide indirect control (Sarrocco et al. 2009). Direct antagonism may occur due to predation or chemical antagonism.

Many actinomycete bacteria are known for production of antagonistic compounds (Hibbing et al. 2010). Previous studies have shown no suppression of *R. solani* mycelial growth by actionmycete populations (Ghini and Morandi 2006) and increasing suppression of *R. solani* disease symptoms by increasing proportion of actinomycete to fungi (Ascencion et al. 2015). Suppression of soil *R. solani* concentration along this ratio did not result in reduced disease symptoms, as did the study by Ascencion et al. (2015). In the previous studies, actinomycete populations were assessed in terms of CFU as opposed to PLFA analysis (Ascencion et al. 2015; Ghini and Morandi 2006). As *R. solani* suppression was observed after a specific ratio, a biomass threshold may need to be reached before the suppressive capacity of these bacteria can take effect.

This may reflect a quorum sensing response, where a certain cellular number must be reached before increased antimicrobial production as a larger bacterial unit can be coordinated (Hibbing et al. 2010). CFU assessment, as opposed to PLFA analysis, would better reflect cellular density requirements of quorum sensing. In this case, however, the biomass threshold is not representative of the community size, but is relative to fungal biomass. Production of inhibitory compounds or chemical signal degradation by fungi can interfere with quorum sensing signaling of bacteria (Dessaux et al. 2011), although these studies largely focus on suppression of quorum sensing in gram negative bacteria, which uses different mechanisms than the gram positive actinomycetes.

PLFA associated biomarkers are also not always specific to assigned microbial groupings (Frostegård et al. 2011). As a result, trends in PLFA concentrations across different soils may not be reflective of the determined microbial groupings discussed above. Assessment of changes in the microbial community due to soil treatments provide more reliable data interpretations. Microbial groups that were most responsive to *R. solani* inoculation included fungi and anaerobes. This relationship is expected, as application of a fungal inoculant caused disease which promoted anaerobic conditions due to plant death and reduced evapotranspiration. Antagonism between actinomycetes and *R. solani* has been documented in other studies, where Pan et al. (2019) has shown decreases in actinobacteria by up to 10% with inoculation of AG 2-2 LP *R. solani*. An absence of a microbial response to pathogen application does not discredit the control associated with member(s) of that microbial group, but suggests that these communities are likely mediated by soil properties as opposed to their response to *R. solani*.

In this study, AMF (16:1 ω5) was negatively correlated with *R. solani* soil DNA concentration. Pan et al. (2019) showed that *R. solani* 2-2 LP soil inoculation decreased AMF by up to 11%, and suggested this was due to a competitive affect, although the same treatment effect was

not observed in our study. Although other studies have suggested a relationship with the pathogen or disease, indirect relationships of these fungi to soil properties likely occurred in this study. AMF and *R. solani* soil DNA concentrations were oppositely associated with % sand and % silt.

5.2.4 Soil fungivores and R. solani soil DNA concentration and disease symptoms

Fungivore nematode populations are often discussed in terms of direct feeding interactions with soil fungal pathogens (Edin and Viketoft 2017; Bollen et al. 1991), where top down suppression and/or bottom up food resources may be influencing both fungal pathogen and fungivore abundance. In an exploratory analysis using ordinal logistic regression modelling, the interaction term in the model was able to relate high and low fungivore abundances to *R. solani* soil concentration and hypocotyl disease symptoms. At low fungivore abundances increasing trends of soil *R. solani* up to 100 fungivores 100 g^{-1} soil were found, which may suggest that low grazing pressures stimulate fungal growth and/or increasing *R. solani* soil concentration supports increasing fungivore abundances. At low fungivore abundances we found a positive relationship between soil *R. solani* concentration and hypocotyl disease symptoms (Fig. 9). This effectively removed an environmental factor (fungivore abundance) that negatively related *R. solani* concentration and disease symptoms.

The relationship of fungivores to *R. solani* soil concentration and disease suppression at high fungivore abundances appears more complex. The inverse relationship between soil pathogen load and disease symptoms may suggest variable effects on hyphal growth depending on fungivore feeding location. Decreasing *R. solani* soil concentrations at higher fungivore densities suggests direct grazing suppression. This trend was also observed in field soils, where *R. solani* concentrations peaked at low fungivore abundances then decreased (Fig. A2). Suppression of *R. solani* in the soil at high fungivore abundances may have been a combination of fungivore activity

and suppression inferred from soil microbes. The same data points, with high fungivore abundances, were found at actinomycete:fungal ratios just above 5.5. This increase in fungivore abundance may be a reaction to disease symptoms as discussed later in this section.

While suppression of *R. solani* soil concentration at high grazing pressures may have occurred in bulk soil, fungivore feeding may have occurred in diseased hypocotyl tissues as well. Suppression of black scurf symptoms on potato tubers has been attributed to fungivore feeding (Edin and Viketoft 2017), while fungivores also entered *R. solani* diseased stem and root tissues (Edin and Viketoft 2017; Viketoft et al. 2017). While fungivores do not penetrate plant tissues, openings caused by fungal diseases are thought to provide an entryway for these nematodes. Dense, localized fungal food sources present in the diseased tissues likely provided sufficient for fungivore reproduction. While total fungivore abundance did not increase with *R. solani* inoculation in this study, hypocotyl disease severity was found to significantly affect fungivore populations. Only under high disease symptoms did increasing soybean biomass reduction result in increased Aphelenchidae abundances. Under low disease symptoms loss of shoot biomass did not result in higher fungivore abundances. This difference may indicate that there is a quick and direct multitrophic transfer of C resources from plant to fungi to nematode when Aphelenchidae nematodes acquire abundant food resources.

Although increased fungivore abundances are often linked to decreases in *R. solani* disease in most literature sources, these studies generally assessed high densities of inoculated fungivores (Lagerlöf et al. (2011): 17500 nematodes 100 g^{-1} soil fresh weight; Ishibashi and Choi (1991): treatments from 2000-40000 fungivores 100 g^{-1} soil; Bollen et al (1991): 2-7 times greater than standard rhizosphere abundances). Our results probably reflect the effect of fungivores under field conditions, where populations are not large enough to completely suppress *R. solani* in bulk soil.

This effect would have been exacerbated by large *R. solani* inoculum levels used in this study. The findings of Bollen et al. (1991) support this observation, as suppression of *R. solani* disease by fungivores was more effective under lower fungal inoculum levels. The initial nematode abundances used by Bollen et al. (1991) were, however, greater than what would naturally be found in field settings.

Timing of fungivore and pathogen inoculation in literature may also influence disease suppressive effects. Some field level fungivore population studies applied fungivores after primary infection had already occurred (Edin and Viketoft 2017). This sequence would mitigate fungivore feeding affects on the initial *R. solani* soil inoculum levels. As fungivores and pathogen were present before initial infection in this study, fungivore feeding may have affected *R. solani* mycelial growth before pathogen association with soybean roots. This may have contributed to variable pathogen concentrations across treatments during the infection period.

Non-significant associations between soil fungivore abundances and *R. solani* inoculation treatment may have occurred for several reasons. Soil disturbance and rapidly decreasing *R. solani* soil concentrations over the course of the trial, coupled with the fact that fungivore nematodes take longer to respond to disturbance compared to soil microbes, may explain non-significant associations. As trophic associations are temporal, observation of trophic links between fungivores and *R. solani* as well as fungal densities may have required repeated sampling times. Unlike the diseased soybean tissues, niche differences or lower *R. solani* concentrations in the bulk soil may may also prevent fungivore proliferation on *R. solani* hyphae.

6. Conclusions

Section 1

Variability in *R. solani* AG group and crops types likely resulted in inconsistent reactions to land-use intensity under field assessments. Despite this, soil organic matter properties that were related to LUI were consistently related to *R. solani* soil DNA concentrations. It was hypothesized that *R. solani* would be related to soil properties indicating SOM status. While increasing SOM, as indicated by total C, resulted in reduced *R. solani* soil levels in both years of study, SOM quality indicators (ACE protein, POM C) were also significantly correlated with *R. solani* soil DNA concentrations. Thus total SOM quantity and quality should both be taken into account in regards to management of this pathogen. Correlations of *R. solani* soil DNA concentrations with nematode families did not show consistent trends across the years assessed. This generally disproved the hypothesis that the nematode ecological indicators would reflect *R. solani* soil suppression under field conditions.

Section 2

Although it was hypothesized that the free-living nematode community indices would be influential in terms of disease suppression nematode community faunal indices, and qPCR determined *R. solani* soil DNA concentrations, were not good indicators of disease suppressive soils. Plant parasitic interactions were more significant in this study than those of the free-living nematode community, as Pratylenchidae abundances showed the strongest correlations to *R. solani* soil levels and disease symptoms. Abundance of the fungivore Aphelenchidae appeared to be responsive to hypocotyl disease severity. These findings were consistent with literature based on the field soil nematode abundances and inoculum levels used in this study. Select nematode families from different trophic levels were related to *R. solani* soil concentrations and disease

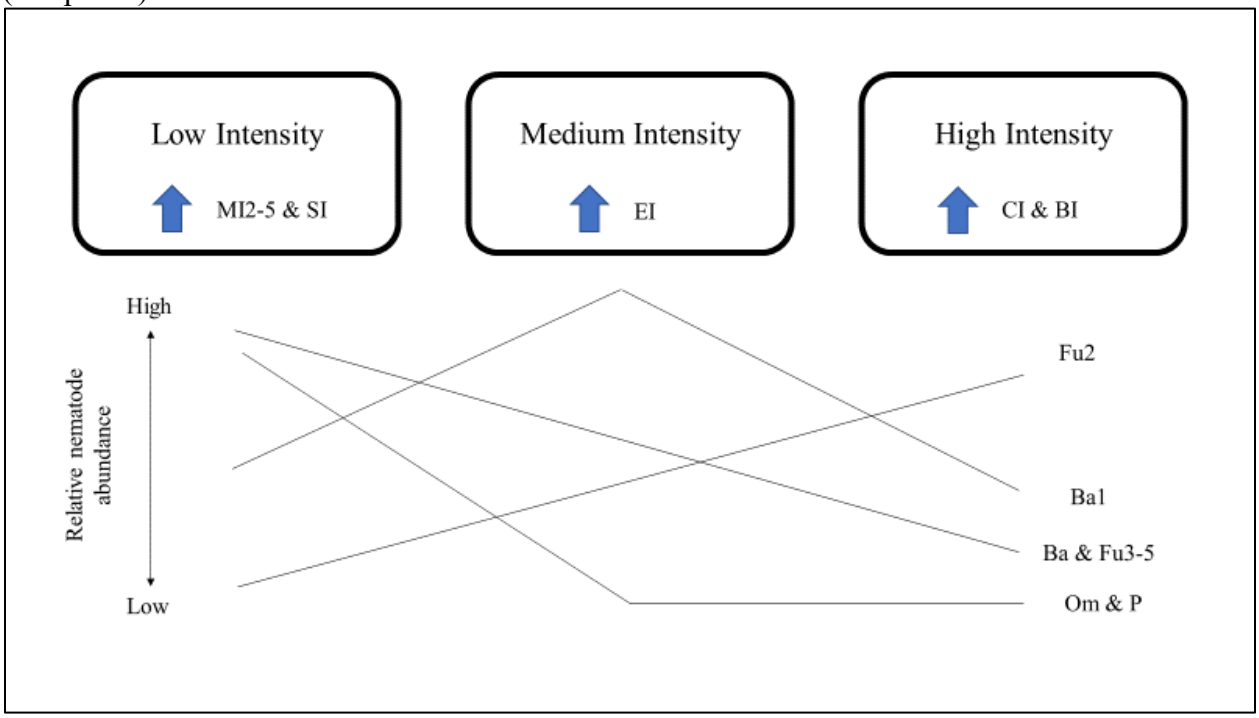
symptoms under controlled conditions. As hypothesized *R. solani* disease symptoms were significantly correlated with several soil properties. Disease symptoms were mainly associated with physical soil properties, while *R. solani* soil DNA concentrations were linked to multiple soil bacterial biomass groupings.

CHAPTER 4: CONCLUSIONS & FUTURE RESEARCH

4.1 Conclusions

Our characterization of land-use intensity was found to affect soil properties and nematode communities. The hypothesis that fungivores would be more abundant under low intensity systems was not supported as fungivore abundances, driven primarily by the Aphelenchidae family, increased with increasing intensity. Although low intensity systems are perceived as having increased fungal decomposition, plant pathogenic fungi in high intensity systems could potentially support higher fungivore abundances. As hypothesized the EI was related to opportunistic bacterivore abundances, however this index did not increase with intensity. Opportunistic bacterivore populations were more abundant in medium intensity soils resulting in a higher EI. This trend may be more related to short term management involving organic matter inputs as opposed to the long-term effects of LUI. Although the SI generally decreased with intensity, rotational intensities (medium and high intensity soils) did not statistically differ in terms of this index. Lower abundances of bacterivores with higher cp values and omnivore predators in rotational intensities resulted in less structured food webs. These differences in nematode trophic composition may result in differences in ecosystem function including nutrient cycling and biological control.

Figure 1. Conceptual diagram of nematode community changes across PEI land-use intensities (Chapter 2).

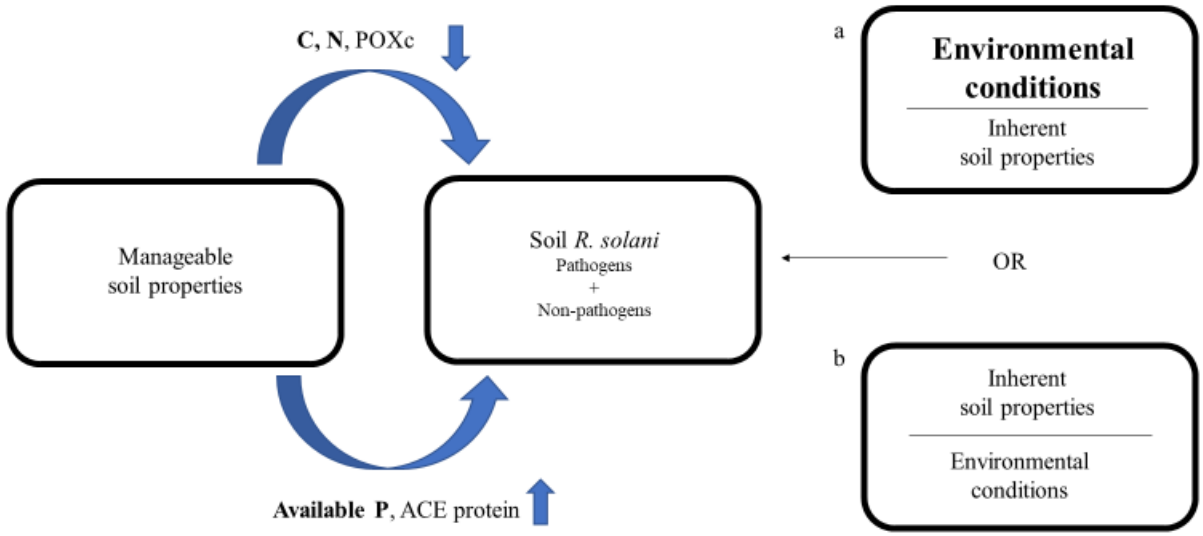


Note: Relative nematode abundance refers to changes across land-use intensity type and does not reflect differences in nematode abundance between trophic guilds.

Assessment of the whole nematode community by multivariate analysis showed significant differences between low and rotational intensities. Soil properties associated with low land-use intensities including increased ACE protein, C and soil moisture %, as well as reduced fertility. Crop type was found to be a driving factor, where vegetation typical of low intensity systems showed a community gradient from grass to forest/grass to forest. Larger differences in residue returns, physical soil disturbances and/or environmental factors across these vegetation types are likely necessary to shift the nematode community. This differed from rotational sites, where intensity did not vary enough to drive community differences. Future soil biological assessments of land-use intensity in PEI may want to consider an additional pasture or grass intensity category (Cui et al. 2014).

In the assessment of *R. solani* over PEI land-use intensities, *R. solani* soil DNA concentration was linked to soil properties and environmental effects over crop rotation (LUI) and crop type. This likely resulted from the assessment of all *R. solani* AG groups, which have different host specificities, combined with crop variability across the sampled fields. Despite the non-significance of LUI, *R. solani* soil concentrations were influenced by manageable soil traits that were linked to intensity, including high C, N, and lower available P. These soil properties consistently suppressed *R. solani* regardless of drought conditions. While this supports the hypothesis that *R. solani* soil pathogen levels are promoted by SOM degradation, the SOM quality indicators ACE protein was positively associated with *R. solani* soil concentrations and was degraded with increasing intensity. Thus, *R. solani* soil concentration in field soils is related to not only to total SOM loss, but also SOM quality. These findings have interesting implications for biological resiliency of soils; however, further investigation of soil biological mechanisms and specific management practices that promote this function are needed. Nematode family abundances did not consistently correlated to *R. solani* soil concentrations, disproving the hypothesis.

Figure 2. Conceptual diagram of mid-summer soil and environmental factors associated with *R. solani* soil suppression (Chapter 3. Section 1).



Note: Arrows related to soil properties reflect relationships to *R. solani* soil concentration across intensity (Arrow up: positive linear relationship, Arrow down: inverse linear relationship). (a) Under extreme environmental conditions soil textural properties are non-significant, (b) under normal rainfall conditions textural properties are significant.

Although EI and SI were related to disease suppression as hypothesized, these indices were not good indicators of *R. solani* disease suppression under controlled conditions. While several post-trial nematode family abundances correlated with hypocotyl disease symptoms, resulting in the weak associations to the EI and SI index, the influence of the plant parasitic nematode Pratylenchidae was more strongly related to disease symptoms and soil *R. solani* concentration.

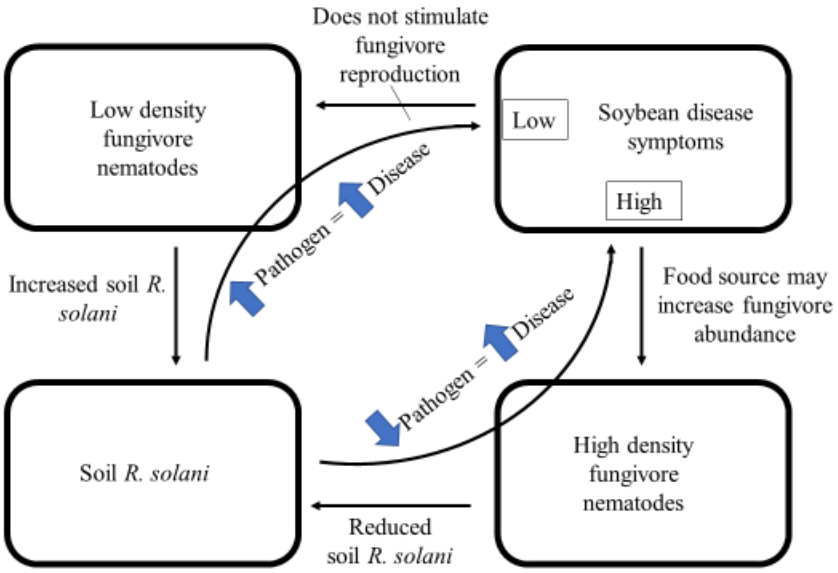
Disease symptoms were related wo soil physical properties, where root rot was related to the distance of fungal inoculum to the soybean seed at planting and hypocotyl rot was promoted by heavier textured soils. While *R. solani* soil concentrations were also correlated with % sand and silt, *R. solani* soil levels were additionally associated with the biomass of multiple soil microbial groups. Soil actinomycete:fungal ratios were implicated in the suppression of *R. solani* soil DNA concentrations, as well as gram + and gram – biomasses. The many weak, significant associations

between soil properties and *R. solani* soil concentrations and disease symptoms found in this study supported our hypothesis. This is consistent with previous literature, where the assessment of many soil properties weakly explains *R. solani* growth and disease.

Fungivores, which abundance was also linked to the actinomycete:fungal ratio, are often related to *R. solani* disease suppression. Increased *R. solani* soil DNA concentrations were found under low fungivore abundances and decreased soil pathogen levels were found at higher abundances. While fungivore feeding activity may be beneficial for long term suppression of fungal mycelium in the soil, rapid increases in fungivore abundance, specifically Aphelenchidae nematodes, only occurred under high disease symptom conditions. This finding may suggest that these nematodes require a dense food source for rapid reproduction. Evidence of direct *R. solani* feeding by fungivores would be necessary to confirm these mechanism. These findings are novel as they reflect fungivore feeding effects, across diverse populations at field soil densities.

Different nematode families, ranging in trophic level, were also associated with *R. solani* soil concentration and disease symptoms. This supports the need for more holistic examinations of the relationship between nematodes and *R. solani* disease interactions.

Figure 3. Conceptual diagram of the affects of nematode grazing pressure on *R. solani* soil concentration and soybean hypocotyl disease symptoms at field level nematode abundances (Chapter 3. Section 2).



Note: Central arrows indicate: Increasing pathogen concentration with increasing disease symptoms under low fungivore densities; Decreasing pathogen concentration with increasing disease symptoms under high fungivore densities.

4.2 Future research

Examination of the intensity and frequency of specific management practices, may have provided greater insights into the nematode communities within each intensity classification. Future research could focus on determining the dominant factors that contributed to the low intensity vegetation gradient. Differences in plant communities, physical soil disturbance, organic matter input and environmental conditions across the forest to grassland gradient may all effect the nematode community to varying degrees.

As previously stated, assessment of specific AG groups across intensity may have allowed for more conclusive results when relating soil concentrations to intensity and crop types. This would have also increased the ability to distinguish between pathogenic and saprophytic

populations. The relationship between land-use intensity/crop type and *R. solani* soil concentrations may have been more apparent at earlier sampling times. Multiple sampling times may have provided a better understanding of the dominant suppressive soil factors at different times throughout the season.

R. solani soil DNA assessments, at multiple time points, during primary disease infection and subsequent proliferation periods, may have provided a better understanding of the relationship between *R. solani* soil concentration and disease symptoms. A more comprehensive assessment of *R. solani* and fungivore quantification in soil, rhizosphere and plant tissues would have also strengthened our explanation of relationships between these communities. Even if nematode and pathogens are found within the same location, the fungivores could feed on other fungal pathogens that co-occure with *R. solani*. Evidence of direct nematode feeding on *R. solani* is necessary to confirm these findings; which could be achieved by labelling *R. solani* enabling the tracing or visualization of potential trophic connections in future research.

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APPENDIX

Figure A1. Nematode community analysis by functional guild used to express enrichment and structural indices (Ferris et al. 2001).

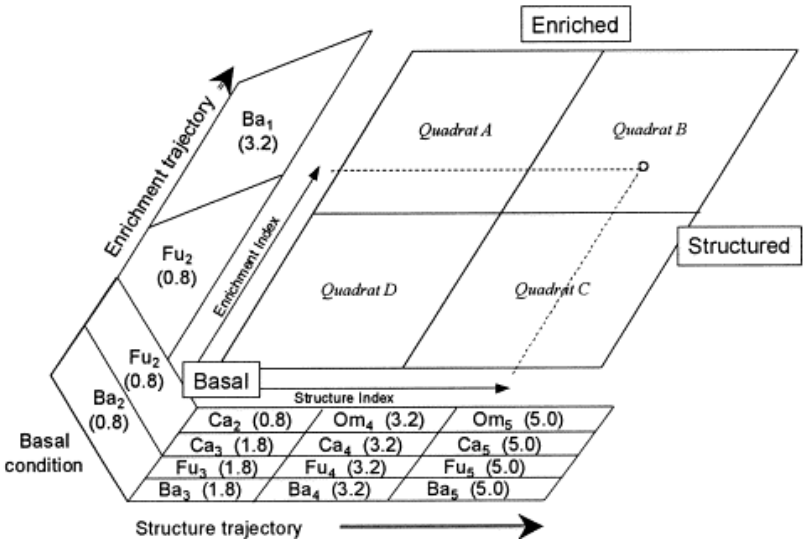


Table A1. Description of inferred soil food web conditions by nematode faunal analysis, corresponding to quadrats A, B, C, and D in Fig. A1 (Ferris et al. 2001).

General diagnosis	Quadrat A	Quadrat B	Quadrat C	Quadrat D
Disturbance	High	Low to moderate	Undisturbed	Stressed
Enrichment	N-enriched	N-enriched	Moderate	Depleted
Decomposition channels	Bacterial	Balanced	Fungal	Fungal
C:N ratio	Low	Low	Moderate to high	High
Food web condition	Disturbed	Maturing	Structured	Degraded

ACE Protein Assessment

Reagent preparation

To make 1 L of 20 mM sodium citrate buffer first place a stir bar in a 1 L glass beaker. Add 5.882 g of Sodium Citrate Dihydrate ($Na_3C_6H_5O_7.2H_2O$) and make to 1L volume with distilled water. Check solution pH and adjust to 7.0 with diluted HCl.

Extraction

1. 3.00 g of air dried, 8 mm sieved soil was weighed into a prelabelled glass test tube, were weigh paper was used to quantitatively transfer soil. Different weigh papers were used between different samples so as to prevent sample contamination. Two replicates were completed for every soil sample and tubes were capped.

2. 24 mL of sodium citrate buffer (20 mM sodium citrate, pH 7.0) was added to each tube using a bottle top dispenser and tubes were capped.
3. Tubes were shook for 5 min at 180 rpm.
4. Samples were swirled to consolidate any soil solids that adhere to the insides of tubes and caps.
5. Caps were fully unscrewed but left on the top of tubes. Samples were autoclaved at 121 °C for 30 min.
6. Samples were removed from autoclave at let cool to room temperature before clarification.

Clarification

7. 2.2 mL microcentrifuge tubes and a set of 1.1 ml open top tubes were labelled to correspond with glass tube labels.
8. Sample caps were closed and samples were shaken for 1 min. Samples were swirled to consolidate solids.
9. 1.75 mL of each sample mixture was transferred with a disposable pipet to the clean 2.2 mL microcentrifuge tube.
10. Tubes were spun at 10,000 xg for 3 min. 1 mL of supernatant was transferred to a 1.1 mL tube using a 1000 µL pipette. Old caps were replaced with new caps.
11. Samples were placed in the refrigerator overnight if quantification needed to be completed the following day.

Quantification

12. Samples and standards were allowed to come to room temperature before step 13. Heating block was preheated to 61.5 °C.
13. Bicinchoninic acid (BCA) working reagent was prepared in a 50 ml 'Falcon' tube by mixing 50:1 parts clear reagent mixture (bicinchoninic acid, sodium carbonate, sodium tartrate and sodium bicarbonate in 0.1N NaOH):blue-green copper sulfate solution (4% (w/v) CuSO4.5H2O). Enough was made for 200 µL per well.
14. 10 µL of 0, 125, 250, 500, 750, 1000, 1500, and 2000 micrograms per milliliter bovine serum albumin (BSA) were pipetted into the first column of the 96-well plate using a multi-channel pipettor. These standards were repeated in the seventh row of the plate. Standards were recapped and set aside.
15. Sample tubes were opened after changing gloves. For each sample replicate two technical replicates were made, therefore for each soil sampled there were 4 wells used. Technical replicates of the first set of samples were placed in column two and column eight, the next set in three and nine, and so forth until all wells were filled with 10 µL of sample or BSA standard.
16. BCA working reagent was dispensed into multichannel pipettor reservoir and 200 µL of working reagent was pipetted into each well.
17. The plate was rolled close with tape seal then placed in a heating block for 60 min. The plate was then removed from the block and left to cool for 10 min undisturbed.
18. The Gen5 plate reader program was set to a 562 nm wavelength. The seal integrity was checked again before the plate was inverted slowly so that all the liquid collected at the base of the plate. The tape seal was removed and the plate placed in the plate reader tray before clicking the 'read plate' button.

19. The generated file was saved. Data was exported to excel and labelled according to sample. ACE protein concentrations were calculated based upon CSHA protocol in excel.

Table A2. Weather station coordinates and associated sampling sites across PEI in 2018 and 2019.

Weather stations	Weather station coordinates	2018 sites	2019 sites
Alberton	46°48'53.090" N. 64°03'40.090" W	No data available	No data available
Charlottetown	46°17'21.000" N 63°07'09.000" W	2, 7, 18	18, 27
East point	46°27'36.000" N 61°59'18.000" W	6, 13, 17	No data available
Harrington	46°20'37.020" N 63°10'11.050" W	4, 9, 10, 15, 19	2, 5, 19, 20
Maple Plains	46°18'10.080" N 63°34'32.040" W	3, 26, 30	No data available
New Glasgow	46°24'32.080" N 63°21'01.040" W	1, 8, 21	3, 4, 10, 16, 17, 30
North Cape	47°03'29.000" N 63°59'55.000" W	5, 28, 29	9, 26, 28
St. Peters	46°27'01.000" N 62°34'33.000" W	11, 12	1, 7, 8, 11, 12, 13, 14, 15, 21, 23, 24
Stanhope	46°24'59.000" N 63°04'59.000" W	-	22
Summerside	46°26'28.000" N 63°50'17.000" W	14, 16, 20, 22, 23, 24, 25, 27	6, 25, 29

Table A3. Cumulative degree days and precipitation by month at PEI weather station sites in 2018.

Weather	Degree days (10° threshold)				Total precipitation (mm)			
station	May	June	July	August	May	June	July	August
Charlottetown	27.5	109.2	351.3	323.8	61.7	154	24.3	115.7
East point	10.4	75.2	314.3	342.3	75.5	127	14.3	100.2
Harrington	25.6	94.1	328.6	316.0	77.9	152.3	9.5	111.0
Maple Plains	16.8	91.8	322.6	310.0	33.0	147	27.2	159.2
New Glasgow	27.7	97.2	333.0	-	83.0	192.2	34.9	74.0
North Cape	21.0	101.6	343.9	354.6	79.9	77.3	19.8	43.9
St. Peters	20.9	96.0	334.6	328.8	65.6	142.7	17.3	128.6
Stanhope	21.1	109.9	344.5	331.6	19.2	137.5	9.6	99.8
Summerside	24.6	110.0	333.5	321.4	77.8	123.8	39.6	129.5
Average	21.7	98.3	334.0	328.6	63.7	139.3	21.8	106.9

Table A4. Cumulative degree days and precipitation by month at PEI weather station sites in 2019.

Weather	Degree days (10° threshold)				Total precipitation (mm)			
station	May	June	July	August	May	June	July	August
Charlottetown	2.6	133.5	282.2	298.3	82.5	152.1	36.2	72.0
East point	-	-	-	-	-	-	-	-
Harrington	1.1	127.7	278.7	284.3	85.5	143.1	49.3	76.8
Maple Plains	-	-	-	-	-	-	-	-
New Glasgow	9.0	141.0	291.3	298.3	95.0	138.3	23.6	122.0
North Cape	4.1	114.7	591.3	301.1	53.6	73.4	124.6	-
St. Peters	3.3	114.4	272.5	283.5	91.2	179.6	49.4	81.5
Stanhope	3.7	128.3	290.2	303.8	86.8	110.3	31.7	77.9
Summerside	2.4	147.3	293.0	284.4	92.3	113.0	26.6	115.1
Average	3.7	129.5	328.4	293.4	83.8	130.0	48.8	90.9

Table A5. Median nematode abundances and indices across land-use intensities during 2018-2019 seasons

Nematode abundance	2018			2019		
(100 g^{-1} dry soil)	L	M	H	L	M	H
Total nematodes	267.9b	466.7a	382.8ab	803.3	738.0	759.6
Plant parasites	99.4	145.8	140.8	245.5	273.1	201.3
Endoparasites	8.4c	89.2a	32.0b	49.5	59.6	61.2
Ectoparasites	81.1a	58.6b	80.3ab	191.2	166.4	160.2
Bacterivores	74.9b	187.4a	173.3a	354.6ab	425.8a	276.4b
Fungivores	9.6b	22.0a	23.2a	45.2	28.2	38.1
Omnivores	17.4 a	14.4 b	13.1 b	29.6a	11.1b	8.7b
Predators	0	0	0	0	0	0
Trophic proportions (%)						
Plant parasites	49.95 a	42.20 ab	32.50 b	29.9	30.5	38.6
Endoparasites	3.20c	19.18a	8.5b	5.6	8.0	8.5
Ectoparasites	42.4a	15.5b	17.6b	24.1	20.4	25.5
Bacterivores	30.90 b	46.25 a	55.60 a	50.00 b	61.90 a	52.25 b
Fungivores	3.8 b	5.0 ab	6.9 a	5.9 a	4.0 b	6.0 a
Omnivores	6.15 a	2.65 b	2.00 b	3 a	1 b	1 b
Predators	0 a	0 b	0 b	0	0	0
Indices						
MI	2.2a	1.9b	1.9b	2.1a	1.7c	1.8b
MI2-5	2.7a	2.3b	2.2b	2.6a	2.2b	2.2b
∑MI	2.6a	2.3b	2.1b	2.4a	2.1b	2.3a
PPI	2.8ab	2.9a	2.8b	2.8b	2.7b	2.9a
BI	14.7b	19.4b	27.1a	15.3b	21.3b	31.0a
CI	7.1	6.0	6.8	4.7b	3.3b	8.3a
EI	69.5	71.9	65.3	72.9ab	76.6a	64.7b
SI	67.0a	40.6b	26.7b	64.2a	28.4b	25.9b

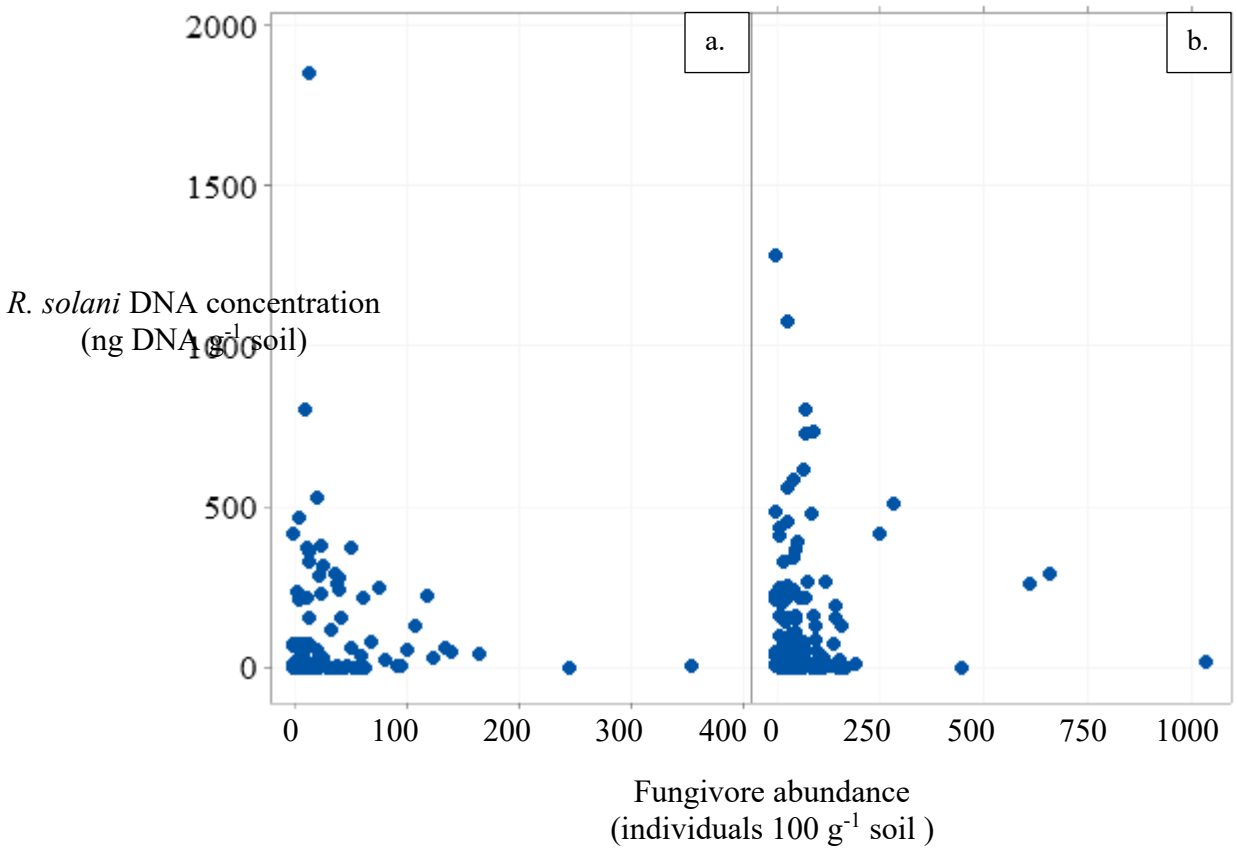
Note: L= low intensity, M=medium intensity, H=high intensity, determined by Kruskal-Wallis rank sums test at α=0.05. Absence of letters indicates there is no significant difference.

Table A6. Factors and levels assessed in preliminary soybean bioassay trial

Factors	Levels assessed	Selected treatment
Plant parasitic resistant soybean cultivar	PS0 333XRN* PS0044XRN*	PS0 333XRN*
Soybean pathogenic *R. solani* isolate	AG4,Alberta RH1-001,Ottawa 1124-001,Ottawa	1124-001,Ottawa
Inoculation method	Soil, *R. solani* filter paper Soil+ *R. solani* mass 1 Soil+ *R. solani* mass 2 Soil+ *R. solani* mass 3	Soil, *R. solani* filter paper
Controls	Soil, No *R. solani* filter paper Soil, No *R. solani* Autoclaved soil, no *R. solani*	Soil, No *R. solani* filter paper Autoclaved soil, No *R. solani* filter paper

*Soybeans with PP nematode resistance

Figure A2. *R. solani* soil DNA concentration as related to total fungivore number in PEI soils in 2018 and 2019.



Note: a. 2018 data b. 2019 data. Non-linear modelling was attempted for the 2018 and 2019 data, but neither were significant. The closest model fit was the 2019 fungivore abundance fitted to a critical exponential curve (α=0.16).