Abundance and Diversity of Nematodes and Microarthropods in Established and Newly Converted Agricultural Soils in Western Newfoundland and in Labrador

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Abstract

Nematodes and microarthropods are used as indicators of nutrient flow through complex soil food web interactions and thus are indicators of soil health. In this study, nematodes and microarthropods were extracted from natural and farmed land at two locations in Newfoundland and from biochar treated soil at one location in Labrador. Newfoundland soil had several combinations of crop and manure treatments. All soils were analysed for various abiotic soil fertility parameters. Farmed soil had a more stable and complex nematode community than adjacent natural soils. Manure application did not have an obvious impact on nematode composition but affected the microarthropod community. Biochar treatment resulted in changes to faunal composition and abundance though microarthropod populations were not well established in Labrador soils. Nematode and arthropod compositions were more strongly reflective of variations in soil pH than other measured parameters. All systems had bacterivore dominated nematode communities, an indication of bacterially driven soil metabolism.

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List of Abbreviations

θ_m	Gravimetric Water Content
µL	Microliter
µm	Micrometer
Ag.	Agriculture/farmed land, samples, or soil
BC	Biochar
BD	Bulk Density
CEC	Cation Exchange Capacity
cm	Centimeter
Cmol	Centimole
CT	Conventional Tillage
DI	Deionised (water)
g	Gram
h	Hour
ha	hectare
HF	Hammond Farms
kg	kilogram
L	Liter
LFH	The upper organic soil horizon (Litter, Fermented, Humic layers)
m	Meter
mg	Milligram
min	Minute
mL	Milliliter
Nat.	Natural/forested land, samples, or soil
NT	No Till
NWD	New World Dairy Inc., or New World Dairy Inc. field location in Experiment 1 (2016)
NWDb	New World Dairy Inc. field location in Experiment 2 (2017)
OM	Organic Matter
PD	Particle Density
s	Second
SAR	Sodium Adsorption Ratio
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SWC	Soil Water Content
t	Tonne
TC	Total Carbon
TN	Total Nitrogen
TP	Total Phosphorus

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1 Chapter 1: Introduction

Newfoundland and Labrador (NL), Canada is uniquely positioned in a boreal climate with characteristic podzolic soils. Climatically induced soil limitations, such as high acidity, a short growing season, and extreme harsh winters, make NL distinctively challenging for agricultural development. Soil food webs have been studied poorly in NL cropped and natural soils; biota/soil quality relationships have not been widely investigated resulting in a lack of understanding of soil functions and health. Biochar application is being considered as one of the potential options to improve soil quality as biochar application can result in increased stable organic matter, pH control, and thus nutrient availability, developing a more diverse microbial community.

Natural land is increasingly being converted from forest to agriculture (Government of Newfoundland and Labrador, 2017) to improve food security in NL. Land conversion will inevitably impact soil physical, chemical, and biological properties. Indices of soil health, including free-living nematode and microarthropod communities, will be altered with changes in soil quality. Three hypotheses to address these changes are outlined in section 2.6.

2 Chapter 2: Literature Review

2.1 Nematodes in the food web

Small roundworms of phylum *Nematoda*, free-living nematodes, are diverse and abundant in virtually all terrestrial and aquatic systems. Nematodes are connected closely with all soil food web levels. nematodes are a food source for other soil animals and feed on bacteria, fungi, vegetation, other nematodes, or a combination of resources (Yeates et al., 1993). Functions of nematodes in the soil food web include regulating of faunal populations and redistributing microbial organisms within the soil, sequestering and redistributing carbon, acquiring nutrients through herbivory and bacterial/fungal breakdown, and thereby accelerating soil nutrient turnover rates (Ferris, 2010).

Soil nitrogen mineralisation is enhanced with the presence of bacterial feeding nematodes (Ingham et al., 1985; Ferris et al., 1998). Bacterial feeding nematodes have a higher average C:N ratio than the bacteria that is consumed (5.6 vs. 4.1) because nematodes, at various rates depending on taxonomic classification, excrete excess assimilated nitrogen. The nitrogen is excreted primarily in the form of ammonium (Ferris et al., 1997, 1998) and is available for plant uptake. In addition, nematodes transport microbes on their surfaces resulting in enhanced bacterial colonisation, activity, and increased nitrogen mineralisation (Bouwman et al., 1994). It has been suggested that over 40% of carbon assimilated by bacterivore and fungivore nematodes is excreted by their respiration (Ingham et al., 1985).

2.1.1 Nematodes as indicator of soil health

Given the nematode position in the soil food web, and as nematodes occur in all soils, even those of poor quality, nematodes can be used as biological indicators of soil health. Nematodes respond quickly to management disturbance, are influenced by the physical and chemical parameters of the surrounding environment, and are relatively easily extracted and identified (Bongers and Ferris, 1999; Neher, 2001). The analysis of nematode communities can indicate the flow of resources through bacterial, fungal, and herbivory channels (Ferris and Bongers, 2006). A high bacterivore/fungivore ratio indicates a system that employs bacterially mediated decomposition to rapidly cycle nutrients in comparison to slower, fungal decomposition pathways. A bacteria-dominated system may be both advantageous as nitrogen mineralisation is augmented, and disadvantageous as carbon is cycled quickly through the system and is not available for higher trophic organisms.

Higher biodiversity is generally associated with more sustainable ecosystems (Hooper et al., 2005) indicating that soils with more complex nematode populations (i.e. those including nematodes of all feeding types) are of higher quality and health and thus more resilient. Lower abundance of soil organisms has been associated with lower ecosystem functioning (Wagg et al., 2014) thus more sustainable soil systems have higher nematode abundances. High-input, intensively managed systems tend to have low diversity and favour bacterial driven pathways while low input systems conserve diversity and promote fungal pathways (Bardgett and Cook, 1998).

2.2 Soil microarthropods in the food web

Free-living invertebrates of phylum *Arthropoda,* have an essential role in soil food webs. arthropods feed on detritus, vegetation, fungi, and soil animals including other arthropods and microbes. Many arthropod groups are not limited to one food type and feed on a variety of sources (Culliney, 2013). Functions of arthropods in the soil food web include improving soil porosity and aeration, mixing of soil layers, contributing to nutrient turnover through the transformation and movement of detritus and thus the growth, dispersal, and regulating of microbial populations (Culliney, 2013; Chakravarthy and Sridhara, 2016).

2.2.1 Microarthropod feeding habits

Orbatida, an order of mites, are often dominant in mature forest soil and abundance is higher in coniferous soil than deciduous (Wallwork, 1983). Orbatida eat plant material, fungi, bacteria, and fecal matter. Agriculture soils are usually rich in Collembola (springtails) that feed primarily on fungi as well as hyphae, spores, pollen, feces, and other springtails. Diplopoda (millipedes) are often found in calcareous soils and primarily consume leaf litter and wood. Isopoda (woodlice) are abundant in natural grasslands and feed on leaf litter, wood, and feces (Culliney, 2013).

2.3 Particularities of boreal soil

Boreal soils are predominantly podzolic with low pH and are of poor quality in terms of fertility, making them unfavourable for cropping (FAO, 2017). Podzols are characterised by an illuvial layer (B horizon) rich in metal oxides and/or organic matter and usually a bleached eluvial horizon overlaying it. Coarse to medium textured parent material

contributes to podzol formation (Sanborn et al., 2011). Coniferous vegetation and substrates are associated with boreal regions and contain waxes and lignin that are resistant to decomposition (Swift et al., 1979). Organic matter decreases rapidly with depth in mineral soils (Allison, 1973). In boreal soil, most organic matter is unstable in the litter, fermented, humic layers (LFH) and have few humic compounds in the subsurface. Fulvic acids, which are more harmful to nematodes than humic acids (Elmiligy and Norton, 1973), dominate in podzolic soils (Harada, 2012). There is a lack of complex microbial communities in the subsoil of natural podzol; the majority of the microbial biomass is found in the top 10-15 cm which includes litter and the upper root-inhabited zone (Nikonov et al., 2001). Root penetration is restricted to upper soil horizons as cementation by gravity can restrict root growth (Sanborn et al., 2011). Fungal development is limited in the subsurface of podzols, fungal mycelium is concentrated in the uppermost horizons and decreases in abundance with decreasing horizon (Nicholas et al., 1965).

It has been suggested that low pH may directly and indirectly impact nematode community structure (Korthals et al., 1996a). Zhang et al. (2016) found that fungal and plant feeding nematode genera were correlated with soil pH with fungivores in particular being positively related. Omnivores and predators are particularly sensitive to acidification in spruce forests (Ruess et al., 1996). Earlier studies have found liming to have no effect on nematodes in boreal soils (Huhta et al., 1986; Hyvonen and Persson, 1990) but more recent research suggested that liming changes nematode composition, might impede fungal decomposition channels, and reduce herbivore abundance (Wang et al., 2015). Microarthropod species have variable preferred soil pH, acidity preferences can vary within

the order grouping (e.g. some collembolans have a wide preferred pH range but avoid pH <2, other collembolans have a smaller preferred pH range that is closer to neutral (Van Straalen and Verhoef, 1997). Nematode community structure varies with soil texture (Ferris and Bernard, 1971).

2.3.1 Impact of deforestation on boreal soils and nematodes

In addition to evidence suggesting that the conversion of land from forest or grassland to farmland significantly reduces soil carbon stocks (Deng et al., 2016), land conversion can result in lower pH due to soil nitrification and the use of ammonium-based fertilisers (USDA, 2011). Podzols that have been transformed from forest to agriculture have shifts in soil hydrology and the potential for increased soil erosion and nutrient loss (Altdorff et al., 2017). Deforestation removes the organic horizon (LFH) of podzols, leaving acid soils that are low in organic matter with poor water holding capacity, minimal nutrient status, small fungal community, and possible bacterial driven degradation. Zalba et al. (2016) found differences in the quality of humus through variations in molecular weight of fulvic and humic acids between pine forest and associated agriculture soils of the same age. Nematode abundance and fungivore/bacterivore ratios are lower in clear-cut soil than in natural boreal soil (Sohlenius, 2002). Additionally, foresting operations decrease intact forest floor biomass and impact microarthropod community structure (Kataja-Aho et al., 2016).

2.4 Impact of disturbance on soil biota

Nematodes have several mechanisms for surviving extreme conditions including dormancy, dauer larvae, and changing of sex ratios (McSorley, 2003). Dauer larva, a

quiescent juvenile state, occur in response to environmental stresses such as limited food availability or overcrowding (Cassada and Russell, 1975; Riddle et al., 1981). The length of dauer stage, unlike regular larval stages, is based on environmental conditions, not growth, and has no effect on post-dauer life span or nematode reproductive ability. Nematodes that enter the dauer state have a longer life-span than those that do not simply by the number of days spent as dauer larvae (Klass and Hirsh, 1976). In juvenile stages, under non-favourable conditions, female nematodes can undergo sex reversal to male or may develop intersexual features (Papadopoulou and Triantaphyllou, 1982).

2.4.1 Water stress

Soil nematodes are aquatic animals and require an aerobic, wet environment to survive as nematodes live in water films in soil. Consequently, soil water content and humidity are essential to nematode diversity and function. Nematode distribution is related closely to soil water (Hu et al., 2016). It has been suggested that nematodes are unable to move at low soil water content (Wallace, 1958). Known to withstand long periods of water stress and desiccation, nematodes enter an anhydrobiotic state and coil in dry soil (Freckman and Mankau, 1977; Townshend, 1984). The coiling is in response to matric water potential (i.e. suction forces) acting upon the nematode as soil dries (Demeure et al., 1979). Coiling has therefore been correlated to both soil water content and salinity. Nematodes were found to un-coil rapidly when soil is rewetted and, for example, are most active in times of snow melting events in Antarctic dry valleys (Treonis et al., 2000; Treonis and Wall, 2005). There is evidence to suggest that coiling aids in survival by reducing the surface area of the nematode cuticle and therefore reducing water loss (Womersley, 1978).

Nematode desiccation survival is species dependent (Kung et al., 1991; Patel et al., 1997) and nematodes can form aggregates to avoid desiccation (McSorley, 2003). Patel et al. (1997) suggested a slow rate of drying can allow for necessary biochemical changes in the nematode. In addition, it has been shown that LEA (Late Embryogenesis Abundant) proteins, associated with desiccation tolerance, have been expressed in nematodes that are undergoing desiccation stress (Browne et al., 2002, 2004).

2.4.2 Changes in soil organic matter quantity and quality

Litter quality and concentration of microbial biomass appears to be the driver of forest soil fauna food webs with higher quality litter having more soil organism biomass (Scheu et al., 2003). Land conversion from forest to agriculture results in a decrease in microbial biomass (Raiesi and Beheshti, 2015) and C losses (Mann, 1986; Guo and Gifford, 2002; Murty et al., 2002; Beheshti et al., 2012). Moreover, deforestation results in shifts and redistribution of organic carbon and results in changes of humic acid speciation (Abakumov et al., 2010). Matlack (2001) found that nematode richness and abundance was significantly lower in sites plowed for tree planting and in loose soil that was recently excavated than in natural forest soil. Fungal and bacterial biomass have been shown to have a positive relationship with reforestation age in degraded soil resulting in increased fungivore/bacterivore nematode ratios and the dominance of fungal decomposition channels (Hu et al., 2016). Conversion of grasslands to agriculture land results in reduced nematode diversity (Postma-Blaauw et al., 2012).

Nematode generation time can range from 3 to 15 days under laboratory conditions but is species dependent and varies with abiotic conditions (Vancoppenolle et al., 1999).

Enrichment opportunists, generally bacterivorous nematodes with short-life cycles and fast generation, are more abundant with disturbance that results in addition of organic matter that accelerate organic matter mineralisation, and thus are associated with enrichment of microbial populations (Bongers and Ferris, 1999). Omnivorous and predatory nematodes, as well as their complex linkages in the soil food-web, are most susceptible to disturbance and have long regeneration times (Ferris et al., 2001). In barley and potato rotations in the Netherlands, bacterial growth was increased and bacterivorous nematodes were dominant due to crop residue inputs (Postma-Blaauw et al., 2010). Matlack (2001) found fungal feeders corresponded to organic matter and indicated that stable organic matter is expected to support fungi and therefore fungivore growth.

Microarthropod abundance and diversity is reduced with agriculture intensification, including the conversion of natural land to agriculture, likely due to disturbance and not due to changes in food sources including organic matter or microbial populations (Osler and Murphy, 2005; Bedano et al., 2006; Postma-Blaauw et al., 2010). However, organic matter (OM) had a significant influence on the abundance of mites in orders Oribatida and Mesostigmata in agriculture soil where OM ranged from 1.15-2.70 % (Bedano et al., 2006).

Soil fertilisation strongly affects soil fauna (Jiang et al., 2014; Zhang et al., 2016). Some genera of nematodes are affected by disturbance and nutrient enrichment in opposite ways (e.g. increasing in abundance in response to tillage but decreasing in response to nutrient application) (Fiscus and Neher, 2002). Organic mulch additions can result in increased total nematode abundance (Porazinskaa et al., 1999) but decreased root-lesion nematode abundance (Forge et al., 2008). The incorporation of cattle manure and maize

stalks results in changes in soil structure, increased soil microbial activity, and total nematode abundance (Zhang et al., 2016); manure application, at least in low quantities, results in higher herbivore abundance (Jiang et al., 2014; Zhang et al., 2016). Fungivore abundance decreases with nitrogen inputs (Li et al., 2013; Zhang et al., 2016) and after consistent nitrogen fertilisation in high quantities, the fungal/bacterial feeding nematode ratio in soil is decreased (Azpilicueta et al., 2014). Nitrogen inputs, especially as ammonia or in materials that can be rapidly mineralised and thus allow for rapid ammonification, can be used as a nematicide for plant parasites but efficacy depends on the nitrogen source and the long-term effect on crop health (Akhtar and Malik, 2000). Omnivores and predators are more sensitive to nitrogen inputs than other, more opportunistic nematode groups (Tenuta and Ferris, 2004). Bacterial feeding nematodes have been correlated to soil phosphorus which may be a reflection of increased bacterial populations in phosphorus-rich soil (Matlack, 2001).

2.4.3 Tillage

Nematode abundance and composition can reflect crop and soil management (Freckman and Ettema, 1993; Neher et al., 1995). Soil tillage alters soil physical and chemical properties (Hendrix et al., 1986; Angers et al., 1997; Six et al., 1999). Tillage practices are primarily responsible for a decrease in soil carbon for the first 25 years following deforestation as tilling leave the soil bare, susceptible to erosion, and oxidative processes outweigh constructive ones (Allison, 1973). No-till (NT) systems are used to minimise soil disturbance and generally have more soil organic carbon in comparison to conventionally tilled (CT) soils (Hobbs et al., 2008). In general, tillage practices have a

negative impact on soil fauna (Kladivko, 2001). Nematode abundance and diversity is greater in NT than CT (Shenglei et al., 2000; Nakamoto et al., 2006; Okada and Harada, 2007). Govaerts et al. (2007) found that plant parasitic and non-parasitic nematode populations were higher in NT than CT systems cropped with maize but found no effect of tillage when cropped with wheat. Postma-Blaauw et al. (2010) suggested that plant parasitic nematode populations were higher under maize monocropping than in rotation due to build-up of plant parasites from continual host presence. Higher populations of bacterivores in CT and fungivores in NT systems reflect the micro-faunal populations in each system (Parmelee and Alston, 1986). House and Parmelee (1985) described significantly greater arthropod abundance in NT than CT systems. Arthropod activity has a substantial role in soil nutrient release especially when tillage is not present to accelerate crop residue breakdown (House and Parmelee, 1985).

2.4.4 Contamination

As nematodes are reliable indicators of soil health and have been suggested as indicators of soil and water quality degradation from contaminants. In short and long-term studies, nematode assemblages have been found affected by heavy metals; Lower total nematode abundance has been found in soils with high concentrations of heavy metals (Zullini and Peretti, 1986; Weiss and Larink, 1991; Parmelee et al., 1993; Yeates et al., 1994; Korthals et al., 1996b). Relative abundance of bacterial feeding nematodes increased with the presence of contaminants (Cu, Ni, Zn) while plant and fungi feeder abundance decreased (Korthals et al., 1996a). Although there is some conflicting evidence (Yeates et al., 1994), predatory and omnivorous nematodes appear to be the most sensitive to soil

contamination (Parmelee et al., 1993; Kammenga et al., 1994; Korthals et al., 1996a; b). Parmelee et al. (1993) found that at moderate copper sulfate pollution levels, total nematode abundance increased due to a reduction of predatory nematodes. Nematodes are Cd tolerant (Williams and Dusenbery, 1990; Kammenga et al., 1994; Korthals et al., 1996a). Bacterivores and fungivores are more tolerant to pentachlorophenol, a pesticide and wood preservative, in soil than other functional groups (Kammenga et al., 1994).

2.5 Impact of biochar on soil quality

Biochar, a porous, high-carbon residue resulting from the pyrolysis of organic material, is being used as an amendment for soils of low quality. Biochar improves soil hydraulic properties (Ahmed et al., 2016; Zhang et al., 2016) and nutrient availability (Glaser et al., 2002; Blanco-Canqui, 2017), reduces nitrogen losses (Zhang et al., 2016), and decreases bulk density (Asai et al., 2009). In addition, biochar application improves soil pH and reduces aluminium toxicity, a common problem in podzolic soils (Shaaban et al., 2018). Biochar molecular structure has been reported to change as biochar ripens in the soil (Mia et al., 2017a). Biochar ageing has been reported to impact its ability to retain and adsorb nitrogen and phosphorus in soil (Mia et al., 2017b). Microbial biomass is increased and community composition is changed with biochar application (Lehmann et al., 2011). Most studies have suggested that mycorrhizal fungi have a positive relationship with biochar in soils but some have shown negative nutritional impacts on fungi (Warnock et al., 2007). Nevertheless, effects of biochar application are dependent on feedstock, pyrolysis temperature, incorporation rate, and soil texture (Atkinson et al., 2010; Gul et al., 2015).

Information regarding interactions of biochar and nematode community structure is limited and conflicting. Several studies have found no effect of biochar, natural or man-made, addition on nematode communities (Matlack, 2001; Pressler et al., 2017; Soong et al., 2017) but Xiao-Ke et al. (2013) found a significant increase in fungivore abundance and a decrease in herbivore abundance with biochar amendment. Evidence suggests biochar reduces plant parasitic infection rates but effectiveness is highly feedstock dependant (George et al., 2016). Castracani et al. (2015) reported that agriculture disturbance had a far greater impact on arthropod distribution and abundance than biochar application.

2.6 Hypotheses

Hypothesis 1: Long-term management

Nematode and microarthropod abundance and diversity will be lower in cropped soil than in natural soil due to management disturbance and negative impacts of long-term agriculture management on soil physicochemical parameters.

Hypothesis 2: Manure treatment

Manure treated soil will have changed physical and chemical parameters to those more favourable for soil biota. Nematode abundance will be greater and community composition will be altered with manure application as changes in physicochemical parameters result in changes in soil microfauna.

Hypothesis 3: Land-use conversion and biochar use

In soils newly converted from forest to agricultural use, the nematode and microarthropod communities are affected by the utilisation of biochar amendments. Accordingly, there will be opportunity for increased nematode and microarthropod diversity and abundance; soil that did not receive biochar will have lower nematode and microarthropod abundance and decreased community complexity versus soil amended with biochar. Labrador soil will be analysed.

Western Newfoundland soils under dairy management will be examined to evaluate Hypothesis 1 and 2 while central Labrador biochar treated soil will be studied to evaluate Hypothesis 3.

3 Chapter 3: Experiment 1 and Experiment 2: Survey of soil fauna in western Newfoundland; 2016 and 2017

3.1 Statement regarding the experimental setup

Soil biota was surveyed in soil with various manure and crop treatments at two farms in the years 2016 and 2017. Changes in experimental setup and uncertainties associated with unexpected management of farmers' fields for the Newfoundland based work, led to the experimental results to be described in two experiments:

1. Experiment 1: A survey of arthropods in soils of dairy farms in western Newfoundland; 2016
2. Experiment 2: A survey of arthropods and nematodes in soils of dairy farms in western Newfoundland; 2017

Both these experiments were carried out on same two farms, but plots have changed for one of them; details can be found below.

Soil tillage, manure application, inorganic fertilisation, herbicide/pesticide application, planting, and harvesting was completed by farmers using their normal practices or by guidelines provided by researchers. All soil samples were collected by researchers.

3.2 Experiment 1: Survey of microarthropods in soils of dairy farms in western Newfoundland; 2016

3.2.1 Methodology for Experiment 1

3.2.1.1 Field site description

Field sites were located on producer-owned land at New World Dairy Inc. (NWD) in the Codroy Valley region, NL (48.2878°N, 58.7373°W) and Hammond Farms (HF) in Little Rapids, NL (48.9941°N, 57.7248°W) (Figure 1). The climate is temperate boreal. The closest weather station to the field location is in Codroy Valley is in Port-aux-Basques, NL; average monthly temperature ranges from -6.4 to 15 °C, average annual precipitation is 1569 mm with 343 mm being snow. Average annual temperature in Corner Brook, NL (the nearest weather station to Little Rapids) ranges from -7.2 to 17.3 °C and average annual precipitation is 1270 mm, 421 mm of which are snow. Both fields used in 2016 were forest to agriculture converted lands, between 10-15 acres in size. Sites have been in agricultural use for approximately 40 years, with consistent repeated manuring at variable rates. Thus both fields were irregularly manured approximately twice per year. Past management included long-term forage grasslands and silage corn monocropping. Crops were planted at HF on 6 June 2016, and at NWD on 9 June 2016. Harvest was completed at appropriate crop maturity.

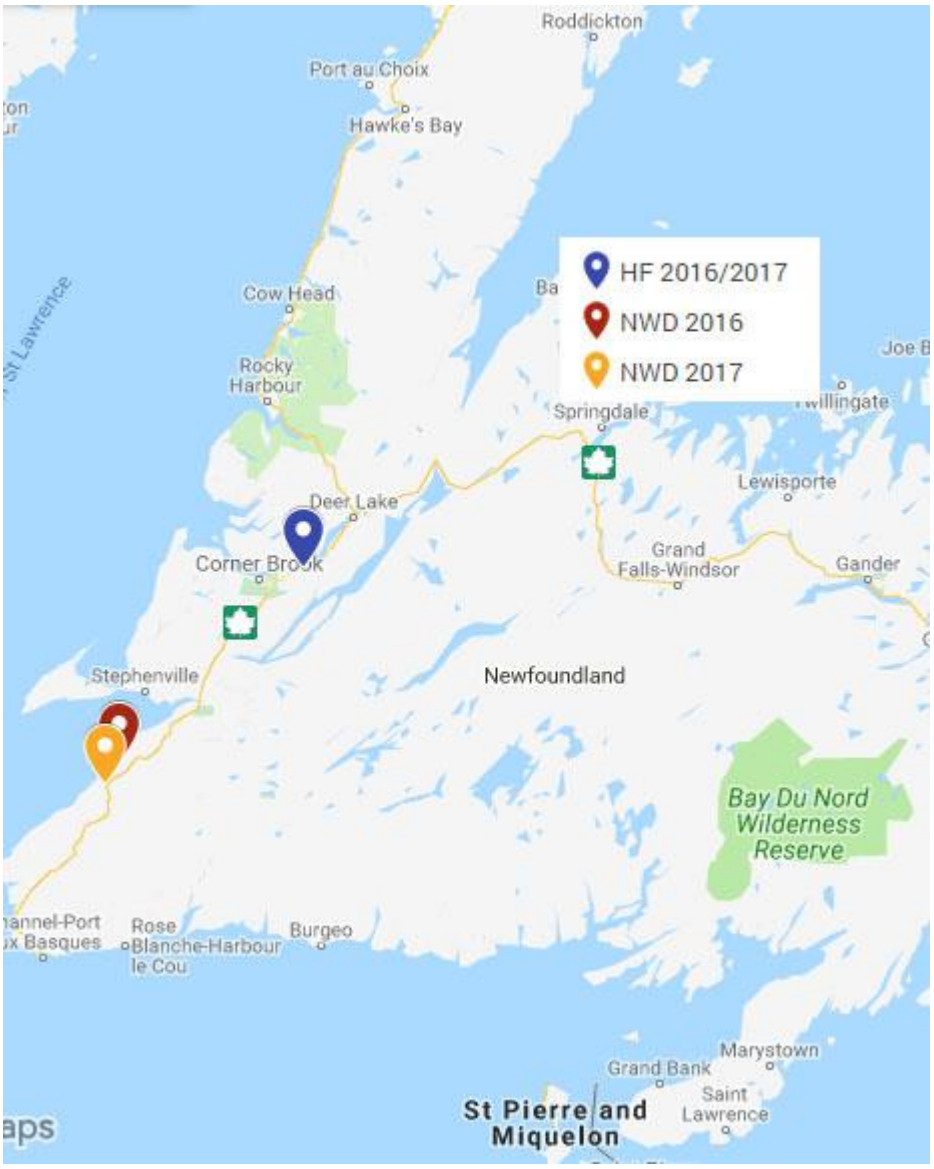

Figure 1 Experiment 1 and 2 field locations for 2016 and 2017 in the Codroy Valley and Little Rapids regions of Newfoundland and Labrador, Canada.

3.2.1.2 Crop treatment

Silage corn (corn AS1047RR EDF), silage oat & pea (oats and peas blend- MAXI SILE), and silage soybean (CRMAX PS0242R2 HCNT 140- pre-inoculated) crops were planted following silage corn in the previous year.

3.2.1.3 Manure treatment

In 2016 two levels of manure treatment, zero and manured, were applied to NWD field; all of HF fields were manured. Liquid dairy manure was applied at HF, partially digested dairy manure was applied at NWD. While the actual rate was not reported, an average estimate is 5000 gal ac^{-1}, dry matter is <2.5%.

3.2.1.4 Experimental design and soil sampling

NWD site was divided into 8 plots based on crop and manure treatment (Figure 2). HF site was divided into 6 plots (Figure 3). For both fields, 5 sample sites (A, B, C, D, E) were located within each plot (Figure 4). Forest reference samples were collected from 5 immediately adjacent sites at NWD and HF. These represent the natural system surrounding the land converted to agricultural use and are therefore assumed to represent the putative state of the agricultural plots had the land not been converted. The natural location at HF was less forested than NWD and was comparable to a natural grassland. From here on the two land use conditions are referred to as agriculture (*Ag.)* and natural (*Nat.)* land or samples, as appropriate.

Two technical replicates were collected for all *Ag.* samples of NWD; the 5 samples sites for HF were used as natural replicates. Duplicates were not collected for *Nat.* samples of either field; the 5 sample sites were employed as natural replicates (Table 1). Soil samples were collected from three depths; 0-10, 10-20, and 20-30 cm. A total of 255 samples were collected from NWD, and 105 from HF. Soil was sampled prior to crop planting.

Dedicated bulk density and extractable arthropod/nematode soil samples were collected (for NWD 25 for bulk density and 35 for arthropods, and for HF 30 for bulk density and 30 for arthropods). Bulk density and extractable arthropods samples were collected from three depths for *Nat.* (upper, organic layer: O horizon, eluviated layer: E horizon, and subsoil layer: B horizon) and from one depth (0-10 cm) for *Ag.* based on crop treatment. Due to the patchiness of microarthropod dispersion arthropod samples were composited. All samples from NWD field and forest were composited according to treatment. Subsequently, each composite sample was split into 3 technical replicates. The same was done for HF. Combining of samples resulted in 9 samples from NWD and 21 from HF.

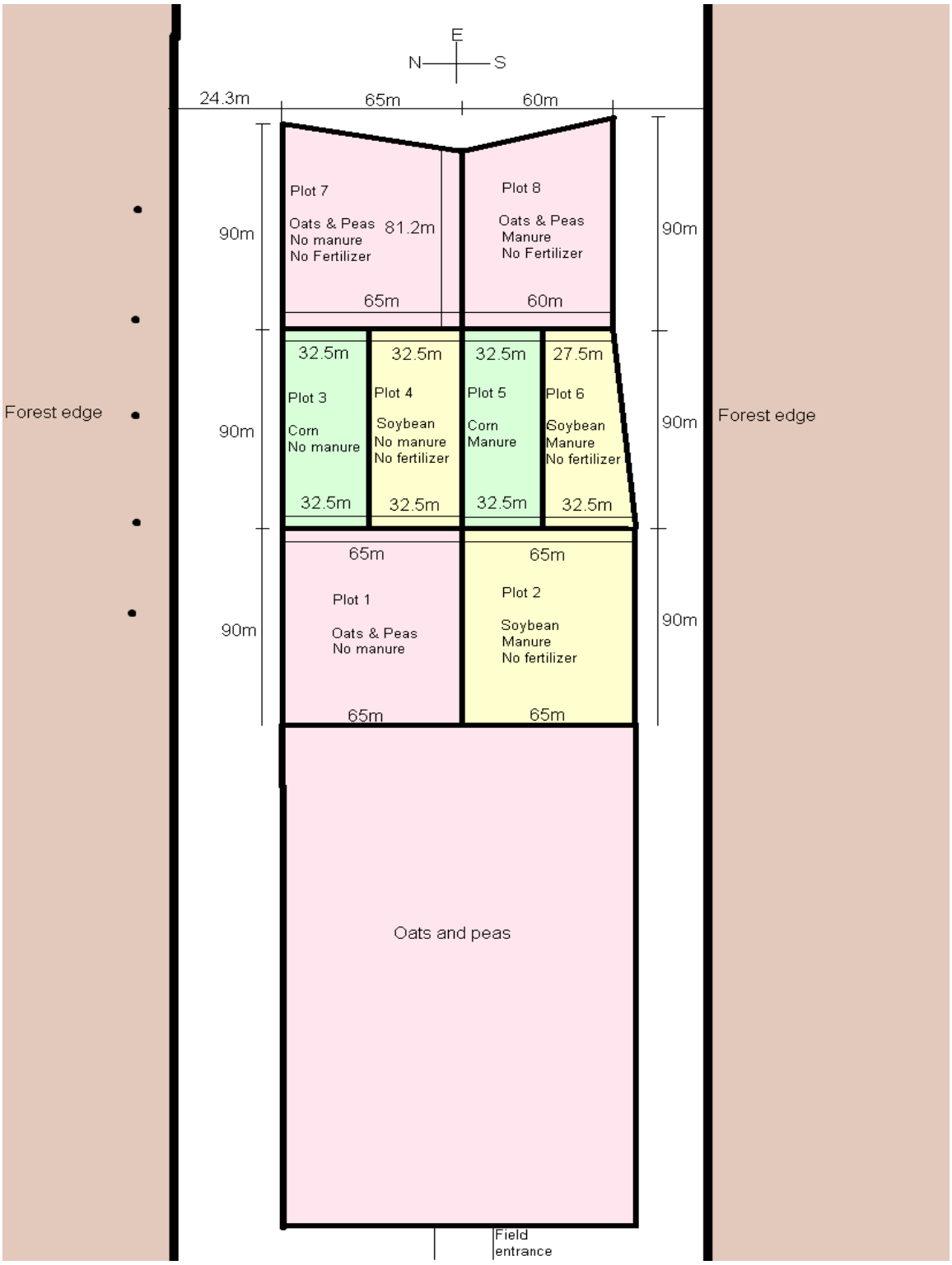

Figure 2 Original crop seeding and treatment plan for Experiment 1 (2016) for New World Dairy.

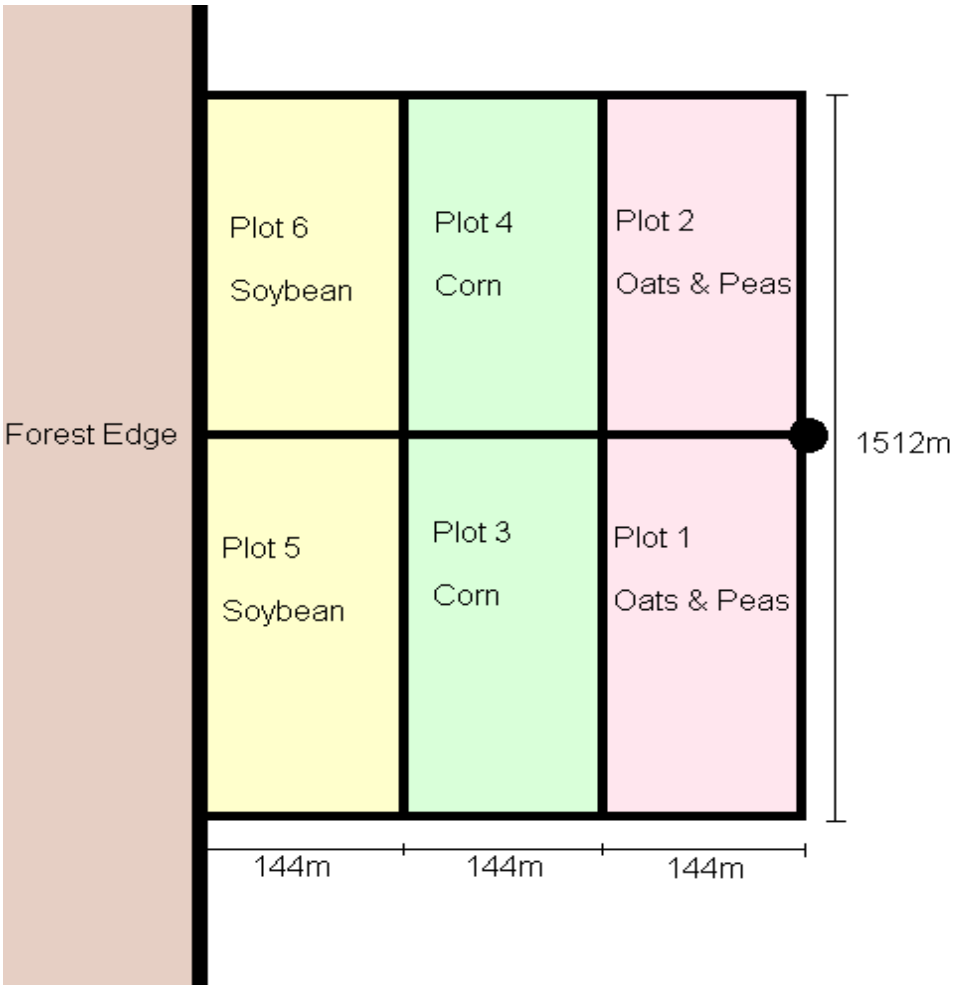

Figure 3 Crop seeding plan for Experiment 1 (2016) at Hammond Farms.

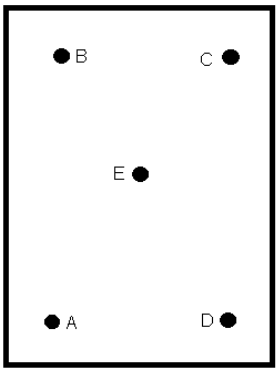

Figure 4 Sample site locations within each plot for Experiment 1 (2016) at New World Dairy and Hammond Farms

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Table 1 Experiment 1 (2016) soil sampling design for New World Dairy (NWD) and Hammond Farms (HF).

Factors			Samples			
Location	Plot	Manure (Yes, No)	Sample Location (natural replicates)	Number of replicates of each sample (technical replicates)	Depth (cm)	Total number of samples
NWD	1	N	A,B,C,D,E	2	0-10, 10-20, 20-30	255
	2	Y		2		
	3	N		2		
	4	N		2		
	5	Y		2		
	6	Y		2		
	7	N		2		
	8	Y		2		
	Natural	N		1		
HF	1	Y	A,B,C,D,E	1	0-10, 10-20, 20-30	105
	2	Y		1		
	3	Y		1		
	4	Y		1		
	5	Y		1		
	6	Y		1		
	Natural	N		1		

3.2.1.5 Soil sampling, handling, and storage

All soil was transported to the lab in coolers with ice within 4 h of collection. Each sample, including all replicates, was hand mixed and split into two equal portions immediately after collection. One portion was frozen at -20 °C, the other was air dried for 48 h, sieved to 2 mm and stored at 4 °C until analysis.

3.2.1.6 Plant monitoring

In 2016, plants were evaluated weekly for emergence date, uniformity, height, leaf numbers, flowering date, and any variability in crop (yellowing of leaves, plant dieback, and deficiency). Seeding was not completed as planned or to satisfaction at NWD (Figure 5); corn plants were sparse and unevenly spaced, soybean was patchy. Due to the lack of replication for the rotational treatment no inferential statistics were completed.

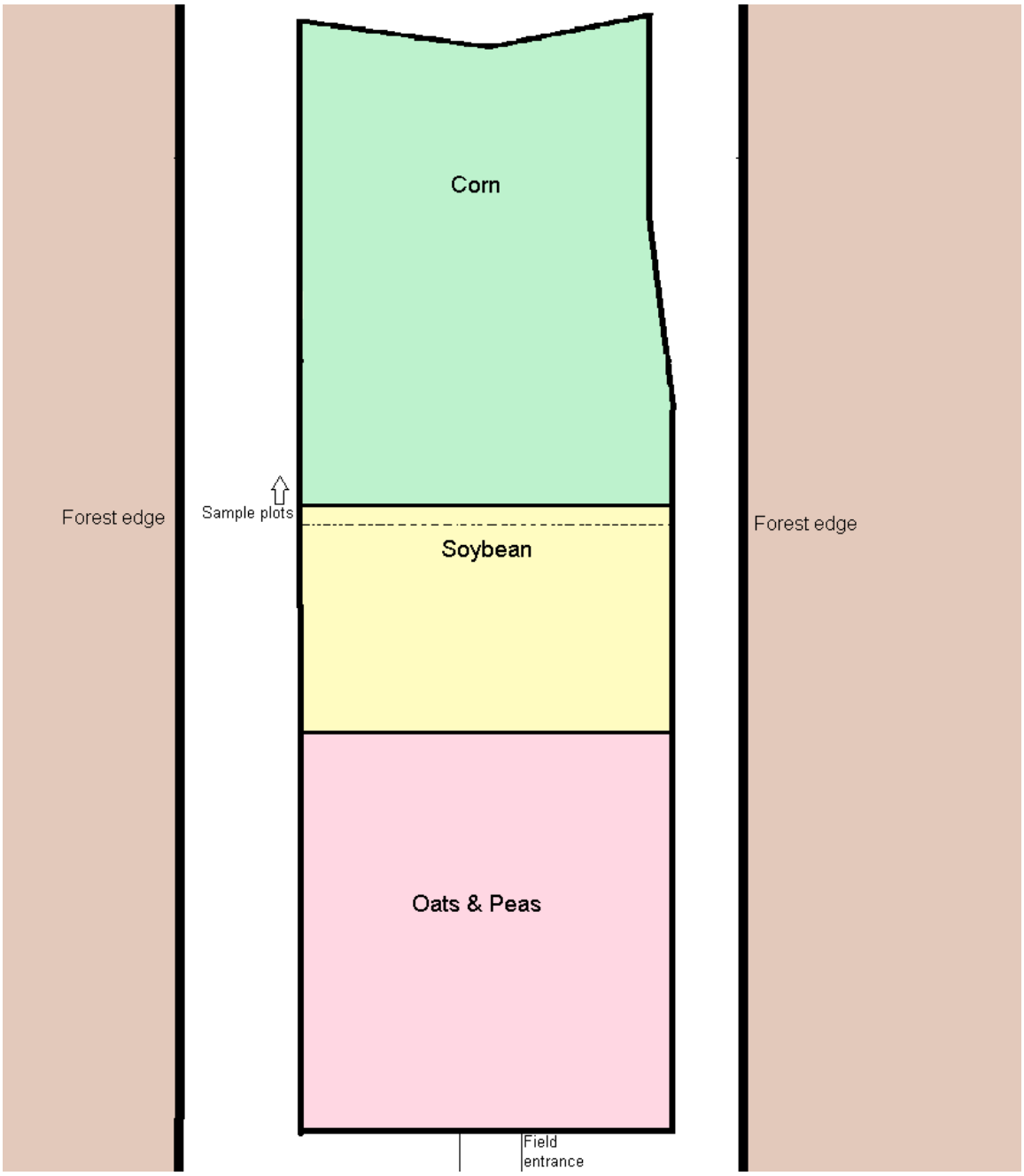

Figure 5 Actual seeding for Experiment 1 (2016) at New World Dairy.

3.2.1.7 Soil physicochemical parameters

3.2.1.7.1 Soil Texture

Soil textural analysis was carried out using a standard methodology as described by (Bouyoucos, 1962) and Carter & Gregorich (2007). Fifty grams of air dried, 2 mm sieved soil was blended with 350 mL of deionized (DI) water and 50 mL of Calgon solution (50 g L^{-1}) using a commercial blender for 5 min on low speed. The soil solution was then placed into a 1 L sedimentation cylinder and DI water was added to the 1 L mark. A second cylinder with 50 mL Calgon (50 g L^{-1}) and 950 mL DI was used as a reference blank solution. The soil solution and the blank were stirred by moving a plunger up and down the length of the cylinder for 2 min (25 strokes). Forty seconds after removing the plunger a Buoyocous hydrometer reading and a temperature reading were recorded. The suspensions were allowed to settle for 2 h before the second hydrometer and temperature reading was taken. For every 1 °C above 20 °C a 0.36 correction was added to the hydrometer reading. For every 1 °C below 20 °C a 0.36 correction coefficient was subtracted. Soil mass was corrected for water content.

$$Oven\ dry\ sample\ mass\ (g) = \frac{air\ dry\ soil\ mass}{1 + gravemetric\ soil\ water\ content\ (\theta_m)}$$

$$Silt + Clay\ (\%) = \frac{corrected\ hydrometer\ reading\ at\ 40\ s}{oven\ dry\ sample\ mass\ (g)} * 100$$

$$Clay\ (\%) = \frac{corrected\ hydrometer\ reading\ at\ 2\ h}{oven\ dry\ sample\ mass\ (g)} * 100$$

$$Sand\ (\%) = 100 - (Silt + clay)$$

3.2.1.7.2 Soil Bulk Density

Bulk density (BD) was determined using the core method as described by Carter and Gregorich (2007). A soil core was extracted using a double cylinder drop-hammer sampler. The soil was removed from the core and oven dried at 105 °C for 48 h to remove moisture.

$$BD = \frac{(total\ mass\ of\ air\ dried\ soil\ (g) - mass\ of\ gravel\ in\ core\ (g))}{volume\ of\ core\ cylinder\ (cm^3)}$$

3.2.1.7.3 Soil Porosity

Soil porosity was calculated using the previously obtained BD measurement and assumed particle density (PD) of mineral soil of 2.65 g cm^{-3}.

$$Soil\ porosity = 1 - \frac{BD\ (g\ cm^{-3})}{PD\ (g\ cm^{-3})}$$

3.2.1.7.4 Soil Water Content

Gravimetric soil water content (SWC) was calculated for fresh soil (SWC at sampling) and for air dried soil to be used to normalize all soil physicochemical parameters (Carter and Gregorich, 2007). Soil weighed prior to and after oven drying at 105 °C for 48 h to remove moisture.

$$SWC(\%) = \frac{(mass\ of\ wet\ soil\ (g) - mass\ of\ dry\ soil\ (g))}{mass\ of\ dry\ soil\ (g)} * 100$$

3.2.1.7.5 Soil Acidity

Soil pH was tested using the calcium chloride method (Carter and Gregorich, 2007). Ten grams of air dried, 2 mm sieved soil were placed in a beaker with 20 mL 0.01

M $CaCl_2$ (pH 5.5 to 6.5, electrical conductivity 2.3 mS cm^{-1} at 25 °C). The solution was stirred intermittently for 30 min. After the solution was allowed to settle for 1 h, pH was recorded using a pH meter (Oakton bench 2700 series, Vernon Hills, IL, USA and Mettler Toledo FiveEasy F20, Mississauga, On, Canada). The $CaCl_2$ solution was tested to ensure a pH of 5.5-6.5 and electrical conductivity (EC) of 2.3 mS cm^{-1}, at 25 °C prior to measuring. The pH meter was calibrated to 3 points (pH 4, 7, 10) prior to analysing each set of samples.

3.2.1.7.6 Soil Organic Carbon

Soil organic carbon (SOC) was measured using the Walkley-Black chromic acid wet oxidation method (Walkley and Armstrong Black, 1934); 400-450 mg of 500 μm sieved *Ag.* soil was added to 250 mL beakers, 150-400 mg of *Nat.* soil was used depending on the estimated quantity of organic carbon to ensure the sample did not reach the endpoint prior to titration. Ten millilitres of $K_2Cr_2O_7$ (1 N) was added to the beakers and swirled until the soil and reagent was mixed. 20 mL concentrated H_2SO_4 was added, the temperature of the solution was checked to ensure that 135 °C was reached. The samples were set aside to allow to cool for 30 min. When cool, the samples were diluted to 150 mL with DI water. An automatic potentiometric titrator (Mettler Toledo G20 compact titrator, with Mettler Toledo DMi140-SC combined platinum ring redox electrode probe, Mississauga, ON, Canada) was used with 0.4 N $FeSO_4$ titrant to approximate 750 mV endpoint. Two blanks, potassium dichromate and sulfuric acid solution without soil, were analysed in the same manner with each set of samples to standardise $FeSO_4$ solution.

$$SOC(\%) = \frac{3(1 - \frac{volume\ of\ FeSO4\ used\ in\ sample\ titration\ (mL)}{volume\ of\ FeSO4\ used\ in\ blank\ titration\ (mL)})}{oven\ dry\ sample\ mass\ (g)}$$

3.2.1.7.7 Total carbon and total nitrogen

Total carbon (TC) and total nitrogen (TN) were analyzed using Perkin Elmer model 2400 CHNS/O Series II elemental Analyzer (Waltham, Massachusetts, USA). The combustion column was set to 925 °C and the reduction column to 640 °C. Into each 8x5 mm tin capsules 8.5-9.0 mg of 500 μm sieved air-dried soil was placed. Calibration was done using 2.0 to 2.5 mg of acetanilide standard, instrumental blanks (nothing), and analytical blanks (tin only).

Acetanilide standard was run to obtain a conversion factor of each element (C, H, N) from the detector. The detector reading was normalized by the weight and the normalized reading divided by the theoretical weight of C, H and N in the standard. The resulting values are K-Factors which were automatically calculated by the analyzer's software (Veysey, 2015).

See Table 2 for CHNS/O blank and K-Factor criteria.

Table 2 Minimum criteria for CHNS/O blanks and K-Factors with reproducibility.

	Acetanilide as sample	Acetanilide as K-Factor	Blank
Percent carbon	71.09 ± 0.40	16.5 ±3.5	<100 ±30
Percent hydrogen	6.71 ±0.40	50.0 ±20.0	200-300 ± 100
Percent nitrogen	6.71 ± 0.40	6.0 ±3.0	<50 ± 16

3.2.1.7.8 Survey of soil cations

Total cation concentrations (Total P, Na, K, Ca, Mn, Zn, Cu, Mg, Al, Fe) were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo Scientific, Burlington, ON, Canada).

3.2.1.7.8.1 Soil digestion

Soil digestion was completed according to EPA method 3050b (U.S. EPA, 1996). For each sample, 0.50 g of air dried, 2 mm sieved air-dried soil was placed into a 50 mL Teflon tube and 10 mL of 1:1 trace element grade HNO_3 was added. The tubes with the samples were then heated at 95 °C ± 5 °C using a digestion block that was capable of digesting 23 samples and one blank. Samples were allowed to cool for 5 min, 5 mL of 70% HNO_3 was added to the slurry and the samples were reheated to 95 °C ± 5 °C and refluxed for 5 min. After samples were cooled, 2 mL of DI water and 3 mL of 30% H_2O_2 was added to each tube. Samples were heated to 60-70 °C and 1 mL of 30% H_2O_2 solution was added to the tubes until no effervescence was observed (not exceeding 10 mL). The samples were cooled to room temperature and DI water was added to 50 mL. The digested samples were then filtered using Whatman No. 41 paper filters. All materials used for the preparation, measuring and digestion of soil were plastic or Teflon and were acid-washed for 12+ h.

3.2.1.7.8.2 Sample preparation and ICP-MS analysis

Na, P, K, Ca, Mn, Zn and Cu were analysed using a 100x dilution with 2% trace element grade nitric acid and 50 ppb of Rhodium as internal standard. A 1000x dilution was used for Mg, Al, and Fe. A Soil Reference Material, 2711a Montana Soil II, obtained

from the National Institute of Standards and Technology (NIST) was used to ensure accuracy. Method blanks were used to ensure minimal contamination.

Calibration curves were obtained on ICP-MS using working standards of 0, 10, 50, 100, 200 and 300 ppb, each containing 50 ppb of Rh as the internal standard (Table 3).

See Table A8.1 for ICP-MS Instrument Detection Limit (IDL) for the measured cations.

Table 3 Calibration curves of different concentrations of each element in the working standard for ICP-MS.

Standard concentration (ppb)	Na (ppb) ± 2.0	P (ppb) ± 2.0	K (ppb) ± 2.0	Ca (ppb) ± 2.0	Mn (ppb) ± 2.0	Cu (ppb) ± 2.0	Zn (ppb) ± 2.0	Al (ppb) ± 2.0	Mg (ppb) ± 2.0	Fe (ppb) ± 2.0
Blank (0)	0	0	0	0	0	0	0	0	0	0
10	95.3	13.0	11.6	15.7	9.6	15.5	6.5	12.5	10.2	12.4
50	117.4	47.7	55.1	61.9	48.4	77.6	45.8	49.6	50.2	51.3
100	147.2	108.1	97.6	104.3	96.9	98.9	96.0	101.4	101.7	103.5
200	208.5	202.4	201.7	208.1	202.0	204.0	202.8	196.5	199.1	198.0
300	264.5	296.0	298.8	290.7	190.0	293.0	300.2	301.9	300.0	299.8

3.2.1.7.9 Sodium Adsorption Ratio

Sodium adsorption ratio (SAR) was calculated using previously determined elemental concentrations.

$$SAR = \frac{Na^{+}(Cmol\ L^{-1})}{\sqrt{\frac{1}{2}(Ca^{2+}\ (Cmol\ L^{-1}) + Mg^{2+}(Cmol\ L^{-1}))}}$$

3.2.1.8 Soil microarthropod extraction, preservation, counts, and identification

3.2.1.8.1.1 Microarthropod extraction and preservation

Microarthropods were extracted using the Tullgren/Berlese funnel method (Tullgren, 1918) with 12” funnels. Mesh with 1 mm openings was cut and placed in the bottom of the funnels and 60W bulbs were used in gooseneck lamps. A 150 g dry weight

equivalent of fresh soil was weighed and placed into the funnel. Beakers with 30 mL of 70% ethanol for preservative were placed under each funnel. Samples were allowed to dry under the light for 5 nights, beakers were checked periodically to ensure the ethanol had not evaporated. The ethanol solution was then transferred to storage tubes until identification.

$$Dry\ weight\ equivalent\ soil = \frac{150\ g\ dry\ soil}{(1 - water\ content\ of\ fresh\ soil\ (\theta m))}$$

3.2.1.8.1.2 Microarthropod counts and identification

Whole extracted samples were placed in a Petri dish and systematically analysed using a dissecting microscope at 40x magnification. Arthropods were identified to order and placed in individual micro-tubes for storage.

3.2.1.9 Statistical analysis

As the studied experimental design was not orthogonal, the influence of factors (land-use, farm, and soil depth) on soil physiochemical parameters and arthropod abundance was determined by using a combination of t-tests, one-way ANOVAs and general linear models (GLM-ANOVA) in Minitab 17.3.1 ("Minitab 17 statistical software," 2010) with α=0.05.

Exploratory statistics were done to assess the differences driven by community structure according to site, farm or crop. Note that the Newfoundland arthropod data was not transformed prior to analysis as the data was already normalized per mass soil.

3.2.2 Results for Experiment 1: Survey of soil microarthropods, 2016

3.2.2.1 Soil physicochemical properties

Texture

Soil texture was similar amongst sites and with depth; soil was classified as sandy loam or loam (Figure 6, Figure 7). *Ag.* soil was significantly sandier at HF ($\overline{x}$=68.90%) than NWD ($\overline{x}$=63.87%) irrespective of depth. NWD had significantly more silt and clay than HF ($\overline{x}$=27.53, 8.60 vs. 24.07, 7.03% respectively) (Table A1.1). *Nat.* soil was significantly sandier at HF ($\overline{x}$=68.49%) than NWD ($\overline{x}$=53.59%) irrespective of depth. NWD had significantly more silt and clay than HF ($\overline{x}$=35.15, 11.26 vs. 22.66, 8.85% respectively) (Table A1.2).

NWD *Ag.* soil was significantly coarser with depth (Table A1.3) while NWD *Nat.* soil had significantly more clay in 20-30 cm than 0-10 cm depth (Figure 8) (Table A1.4). HF *Ag.* soil had significantly more sand in 10-20 and 20-30 cm depths than 0-10 cm, but there was significantly less silt in 20-30 cm than 0-10 or 10-20 cm (Table A1.5). HF *Nat.* soil was not texturally different with depth (Figure 8).

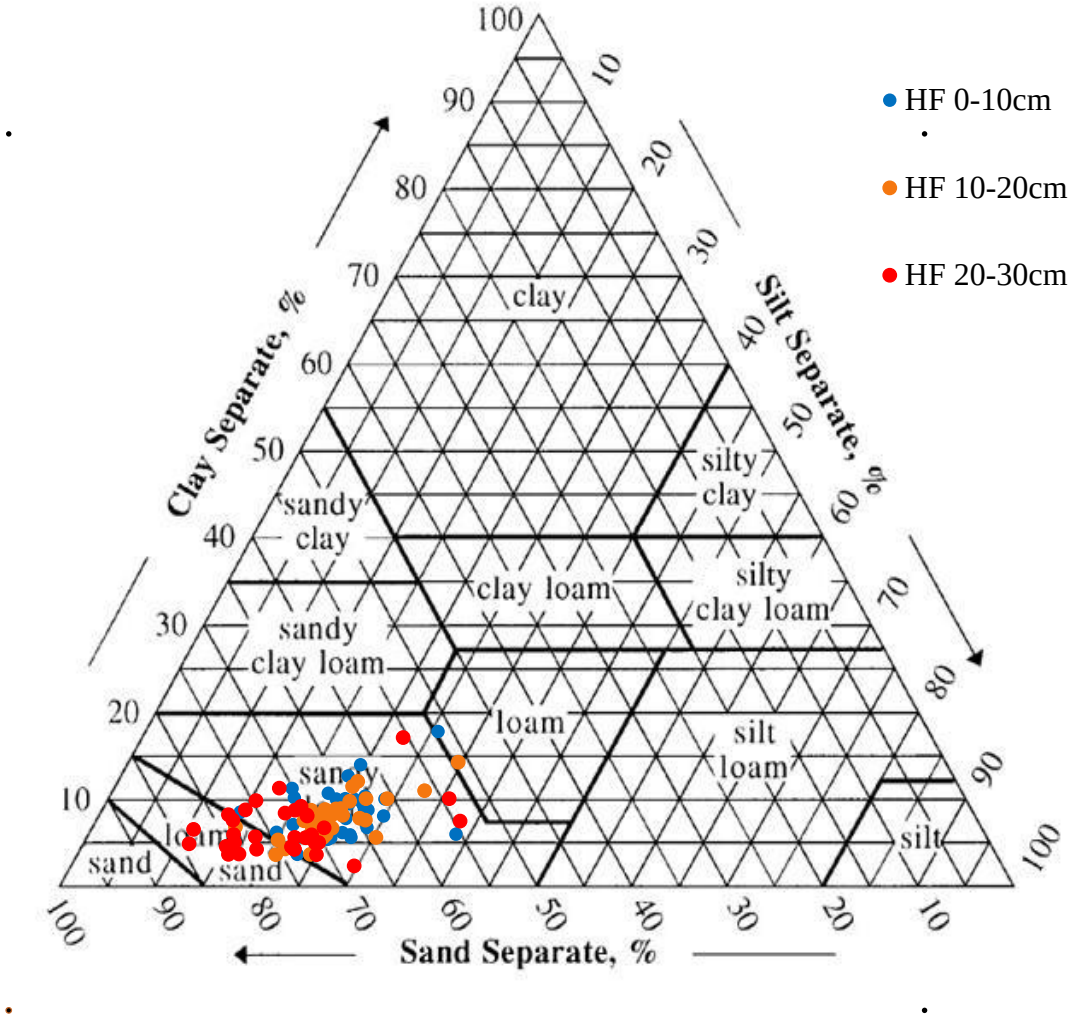

Figure 6 Soil texture for samples of Experiment 1 (2016) from Hammond Farms (HF) soil of depth 0-10, 10-20, 20-30 cm (USDA soil texture ternary plot).

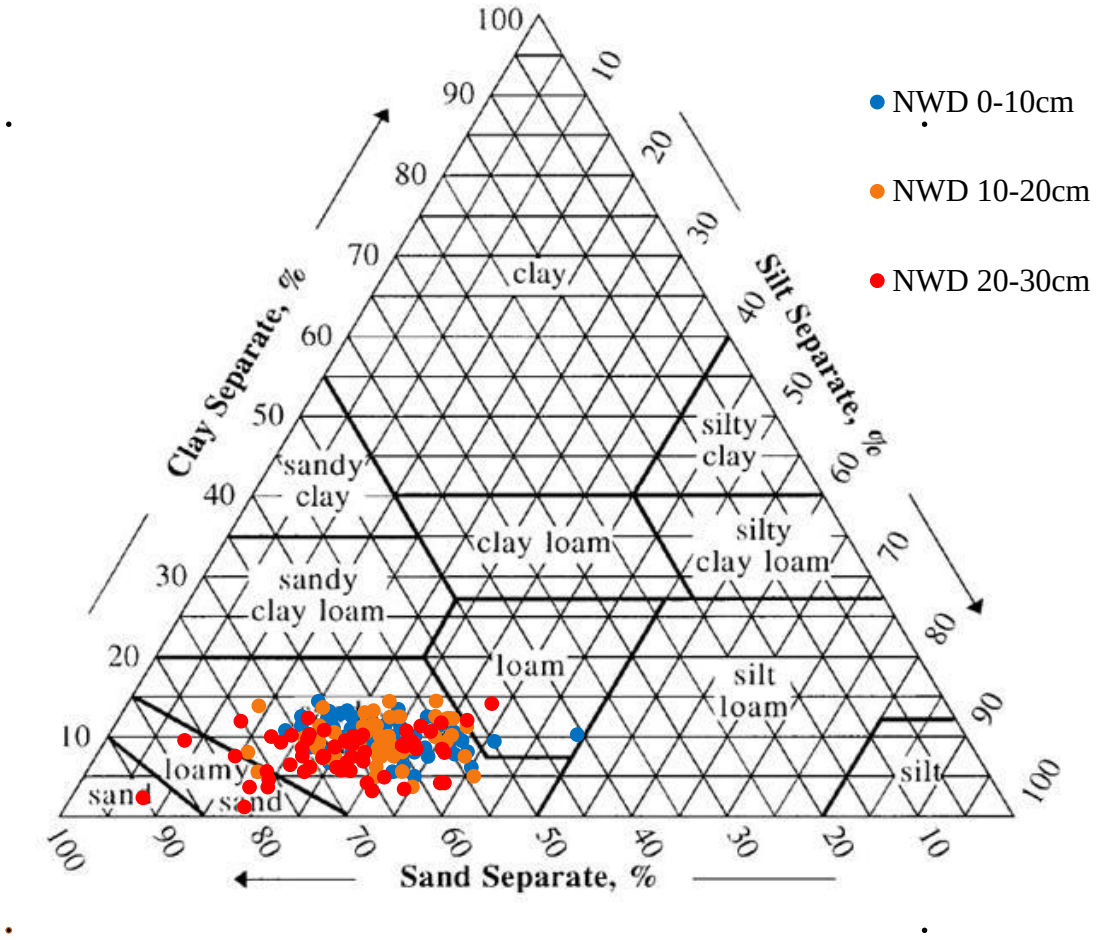

Figure 7 Soil texture for samples of Experiment 1 (2016) from New World Dairy (NWD) soil of depths 0-10, 10-20, 20-30 cm (USDA soil texture ternary plot).

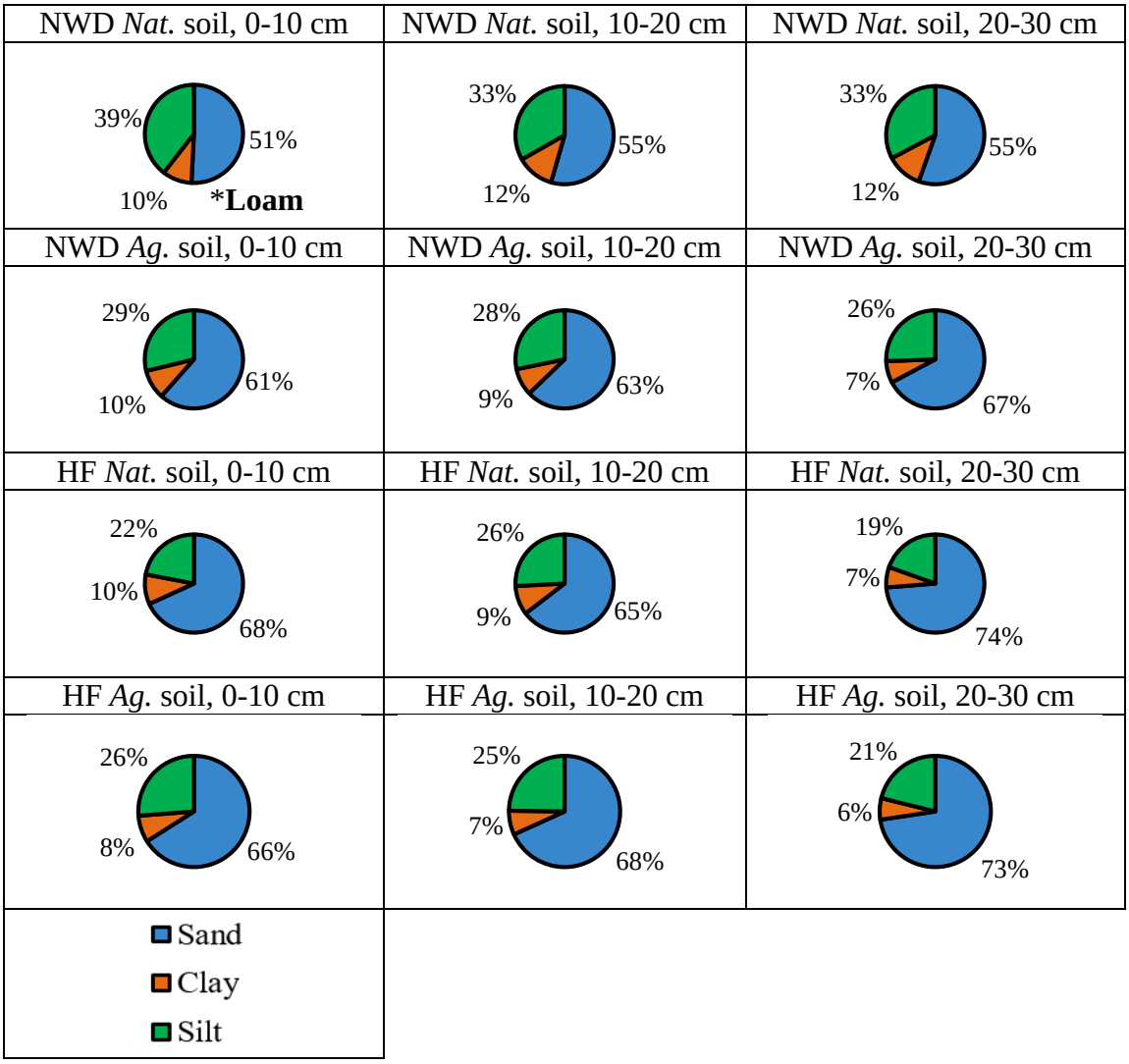

Figure 8 Soil texture for Experiment 1 (2016) New World Dairy (NWD) and Hammond Farms (HF) natural (*Nat.*) and farmed (*Ag.)* soil of depths 0-10, 10-20, and 20-30 cm. Texture is USDA classified as sandy loam unless otherwise noted.

Bulk Density

BD was significantly greater at HF than NWD for *Ag.* and *Nat.* soil ($\bar{x}$=1.25, 1.29 vs. 1.08, 0.97 g cm^{-3}) (Table A1.6, Table A1.7). BD was not statistically different between *Ag.* and *Nat.* soil at HF however, *Ag.* soil had significantly greater compaction than *Nat.* at NWD ($\bar{x}$=1.08 vs. 0.97 g cm^{-3}) (Figure 9) (Table A1.8).

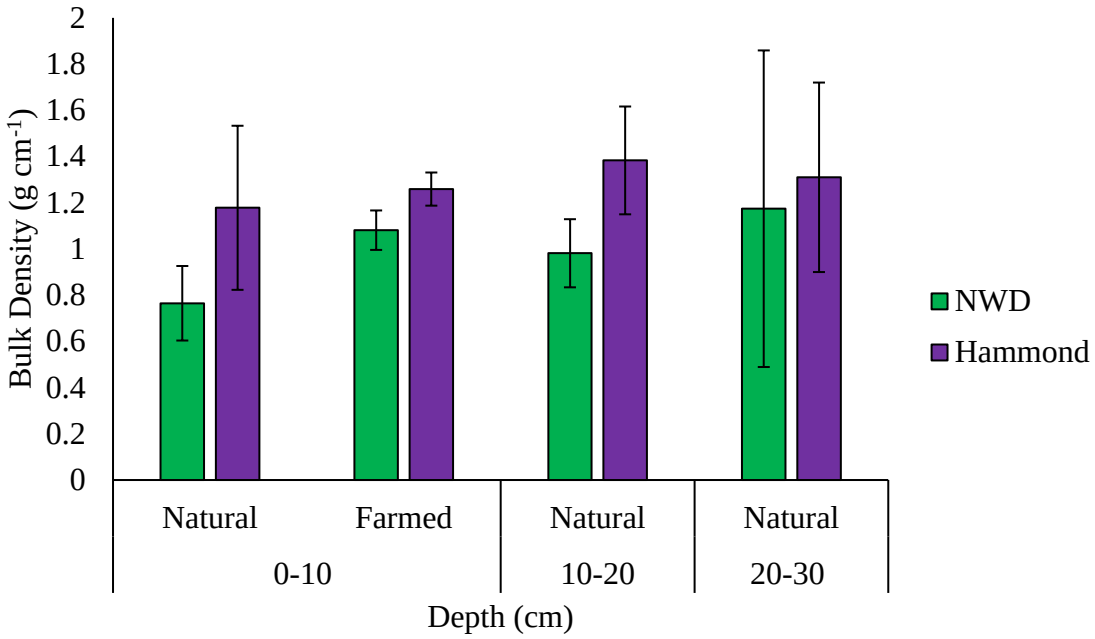

Figure 9 Bulk density (g cm^{-1}) for Experiment 1 (2016) New World Dairy (NWD) and Hammond Farms (HF) natural (*Nat.*) and farmed (*Ag.*) soil of depths 0-10, 10-20, and 20-30 cm. Error term is CI_{95}.

Porosity

Porosity was significantly lower at HF than NWD for *Ag.* and *Nat.* soil ($\bar{x}$=0.53, 0.51 vs. 059, 0.63). Porosity was not statistically different between *Ag.* and *Nat.* soil at HF however, *Ag.* soil had significantly lower porosity than *Nat.* at NWD ($\bar{x}$= 0.59 vs. 0.71) (Figure 10).

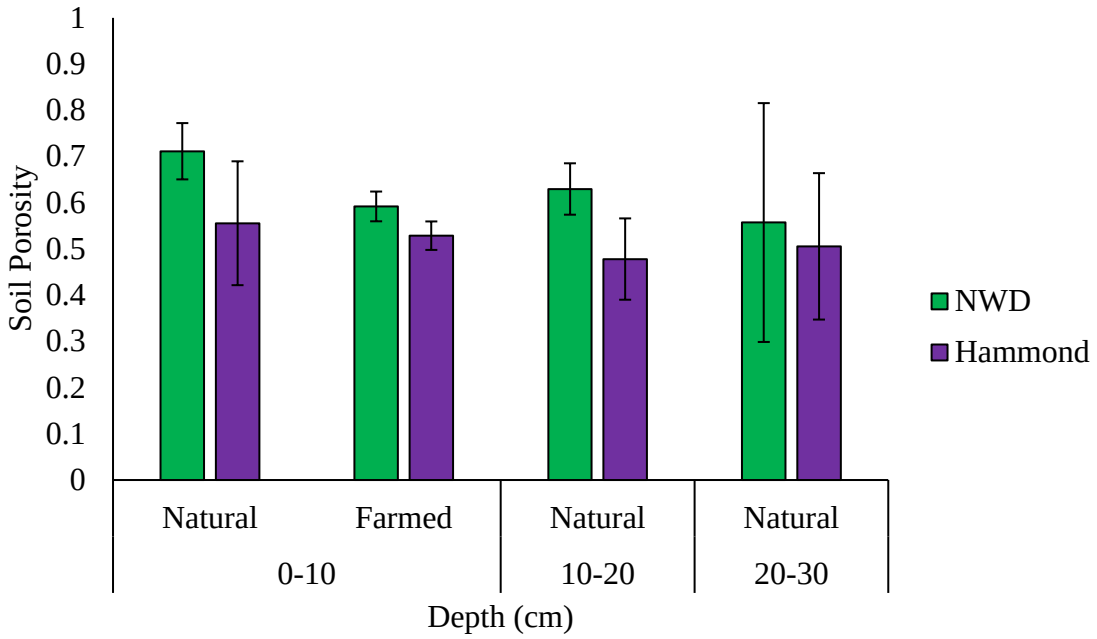

Figure 10 Soil porosity for Experiment 1 (2016) New World Dairy (NWD) and Hammond Farms (HF) natural (*Nat.*)and farmed (*Ag.*) soil of depths 0-10, 10-20, and 20-30 cm. Error term is CI_{95}.

Soil Water Content at Sampling

Ag. soil had significantly greater SWC at NWD ($\bar{x}$=33.84%) than HF ($\bar{x}$=24.98%) irrespective of depth (Table A1.9), a similar trend to the *Nat.* soil where at NWD the SWC was significantly greater ($\bar{x}$=39.53%) than HF ($\bar{x}$=15.06%) irrespective of depth (Table A1.10). NWD *Ag.* and *Nat.* soil had significantly less water at 20-30 cm depth ($\bar{x}$=32.32, 31.57% respectively) than at 0-10 cm ($\bar{x}$=34.94, 50.31% respectively) (Figure 11) (Table A1.11, Table A1.12).

HF *Ag.* soil had significantly less SWC with depth (29.83% at 0-10 cm, 25.67% at 10-20 cm, 19.28% at 20-30 cm) (Table A1.13). On the other hand, HF *Nat.* soil was not different in water content with depth. SWC ranged from 7.95 to 41.21% in HF *Ag.* soil and from 8.34 to 64.35 in NWD *Ag.* soil. SWC ranged from 4.30 to 28.24 in HF *Nat.* soil and from 17.64 to 60.81 in NWD *Nat.* soil.

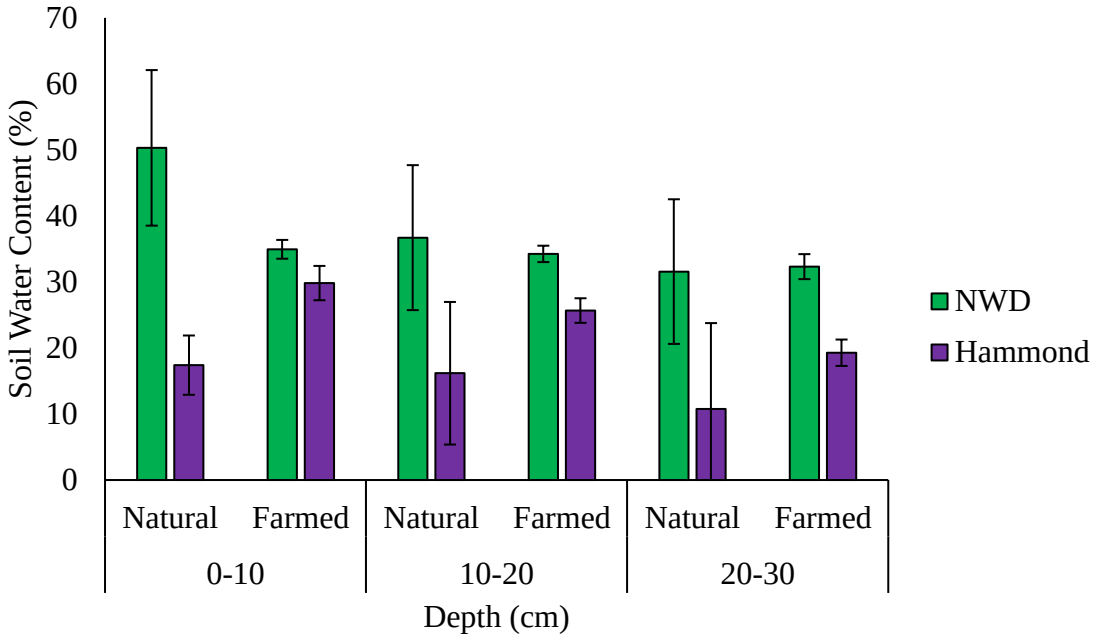

Figure 11 Gravimetric soil water content (%) at sampling for Experiment 1 (2016) New World Dairy (NWD) and Hammond Farms (HF) natural (*Nat.*) and farmed (*Ag.*) soil of depths 0-10, 10-20, and 20-30 cm. Error term is CI_{95}.

Acidity

NWD soils were more acidic than HF soils for both *Ag.* and *Nat.*(Table A1.14, Table A1.15). However, the *Nat* soil was significantly more acidic than *Ag.* soil for both HF and NWD ($\bar{x}$=4.82, 4.41 vs. 6.31, 5.68 respectively) (Table A1.16, Table A1.17). HF *Ag.* soil pH ranged from 6.14 to 6.44, while NWD *Ag.* soil ranged from 5.56 to 6.49. HF *Nat.* soil pH ranged from 4.42 to 5.06, NWD *Nat.* soil ranged from 4.16 to 4.84. (Figure 10).

While NWD *Ag.* soil was more acidic at the deeper 20-30 cm depth ($\bar{x}$=5.47) than for 0-10 cm ($\bar{x}$=5.80) and 10-20 cm ($\bar{x}$ =5.77) (Table A1.18), there was no statistical difference between depths for HF *Ag.* The pH for *Nat.* soil was not significantly different with depth, for both HF and NWD (Figure 12).

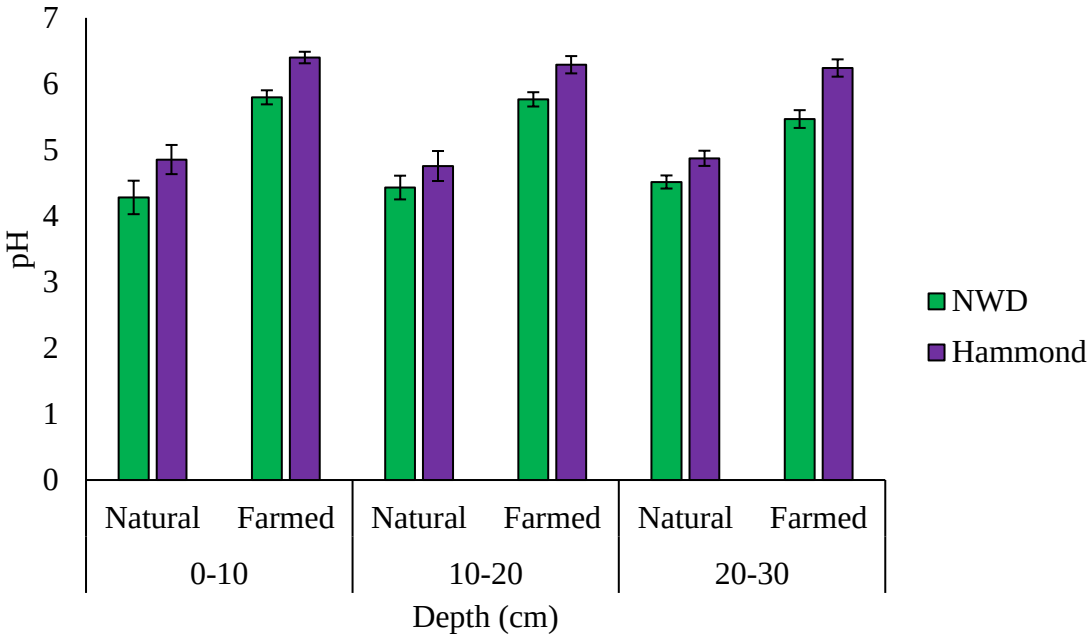

Figure 12 pH for Experiment 1 (2016) New World Dairy (NWD) and Hammond Farms (HF) natural (*Nat.*) and farmed (*Ag.*) soil of depths 0-10, 10-20, and 20-30 cm. Error term is CI_{95}.

Soil Organic Carbon

Ag. soil had significantly more soil organic carbon (SOC) at NWD ($\bar{x}$=3.41%) than HF ($\bar{x}$=2.93%) irrespective of depth. *Nat.* soil had significantly more SOC at NWD ($\bar{x}$=3.97%) than HF ($\bar{x}$=2.28%) irrespective of depth. SOC ranged from 3.47 to 3.82% for HF *Ag.* soil and from 3.31 to 4.05% for NWD *Ag.* SOC ranged from 1.89 to 2.88% for HF *Nat.* soil and from 3.19 to 5.34% for NWD *Nat.*

HF *Ag.* soil had significantly less SOC at the deeper 20-30 cm ($\bar{x}$= 1.9%) than 0-10 ($\bar{x}$=3.66%) or 10-20 cm ($\bar{x}$=3.18%). NWD *Ag.* soil had significantly less SOC with depth (3.83% at 0-10cm, 3.50% at 10-20cm, 2.91% at 20-30cm). HF *Nat.* soil was not significantly different in SOC with depth, while NWD *Nat.* soil had significantly more SOC at 0-10 cm depth ($\bar{x}$=5.34%) than 10-20 cm ($\bar{x}$=3.39%) or 20-30 cm (3.19%) (Figure 13).

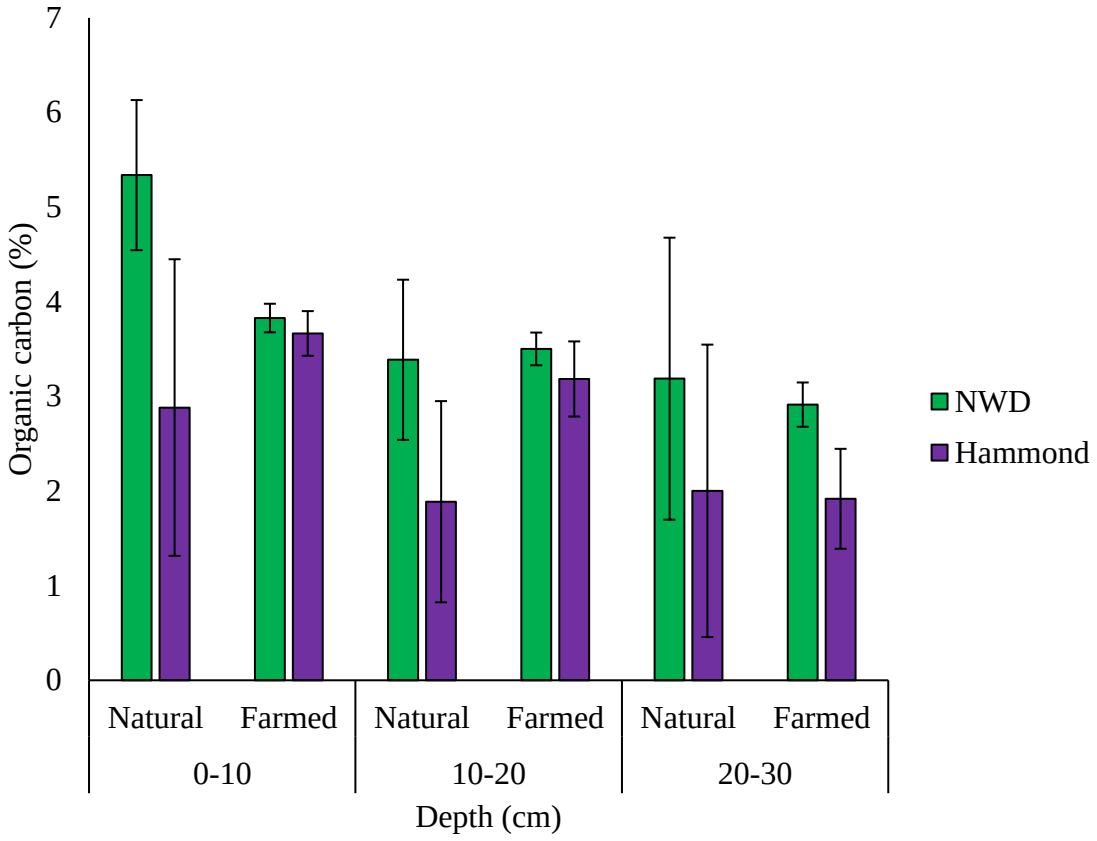

Figure 13 Soil organic carbon (%) for Experiment 1 (2016) New World Dairy (NWD) and Hammond Farms (HF) natural (*Nat.*) and farmed (*Ag.*) soil of depths 0-10, 10-20, and 20-30 cm. Error term is CI_{95}.

3.2.2.2 Soil elemental analysis

There were complex differences between farms, between Nat. and Ag. soils, and among depths (Figure 14).

Between farms

Compared to HF, both NWD *Ag.* and *Nat*. had significantly higher TC ($\bar{x}$=41.20, 46.48 vs. 33.52, 25.28 g kg^{-1} respectively), and AL ($\bar{x}$=12.91, 14.92 vs. 12.13, 10.63 g kg^{-1} respectively). NWD *Ag.* soil was significantly higher than HF in Na ($\bar{x}$=1.14 vs. 0.62 g kg^{-1}), SAR ($\bar{x}$=0.52 vs. 0.24 k kg^{-1}), Mn ($\bar{x}$=7.02 vs. 5.25 g kg^{-1}), and Zn ($\bar{x}$=1033 vs. 541 mg kg^{-1}), there was no significant difference in *Nat.* soils between the farms. TN was

higher in NWD *Nat.* soil than HF *Nat.* soil ($\bar{x}$=2.12 g kg^{-1} vs. 1.42 g kg^{-1}), but higher in HF *Ag.* than NWD *Ag.* ($\bar{x}$=2.59 g kg^{-1} vs.2.19 g kg^{-1}). HF *Ag.* soil also had higher TP ($\bar{x}$=13.65 vs. 11.67 g kg^{-1}) and Mg ($\bar{x}$=3.34 vs. 1.64 g kg^{-1}) but there was no significant difference between the farms for the *Nat.* Fe was higher in HF than NWD *Nat.* ($\bar{x}$=13.00 vs. 12.64 g kg^{-1}) but *Ag.* soils did not differ. K was higher in *Ag.* and *Nat.* soils at HF than at NWD ($\bar{x}$=8.23, 4.54 vs. 5.24, 3.35 g kg^{-1} respectively). There was no difference between farms in Ca or Cu in either *Ag.* or *Nat.*

Table 4 Summary of elemental composition statistical comparisons between farms of Experiment 1.

	Farm with higher concentration		
	NWD	HF	No difference between farms
Both *Ag.* and *Nat.*	TC, Al	K	Ca, Cu
Ag.	Na, SAR, Mn, Zn	TN, TP, Mg	Fe
Nat.	TN	Fe	TP, Na, Mg, SAR, Mn, Zn

Between *Ag.* and *Nat.* (land use within farms)

Compared to *Nat.*, both NWD and HF *Ag.* soil had higher TP ($\bar{x}$=11.67, 13.65 vs.4.93, 4.12 g kg^{-1} respectively), K ($\bar{x}$=5.74, 8.23 vs.3.35, 4.54 g kg^{-1}), Ca ($\bar{x}$=36.39, 36.31 vs.3.94, 5.93 g kg^{-1} respectively), Cu ($\bar{x}$=367, 396 vs.117, 122 mg kg^{-1} respectively), and Zn ($\bar{x}$=1033, 541 vs. 251, 216 mg kg^{-1} respectively). *Ag.* NWD soil was significantly higher than *Nat.* in Mn ($\bar{x}$=7.02 vs. 4.88 g kg^{-1}) though there was no significant difference between land use for HF. *Ag.* HF soil was significantly higher than *Nat.* in TC ($\bar{x}$=33.52 vs. 25.28 g kg^{-1}), TN (=2.59 g kg^{-1} vs. 1.42 g kg^{-1}), Mg ($\bar{x}$=3.34 vs. 2.43 g kg^{-1}), and Fe ($\bar{x}$=13.00 vs. 11.17 g kg^{-1}), there was no significant difference between NWD land-use for TC and TN. *Nat.* NWD soil had higher Mg ($\bar{x}$=2.30 vs. 1.64 g kg^{-1}), Al ($\bar{x}$=14.92 vs. 12.91 g kg^{-1}), and Fe($\bar{x}$= 15.94 vs. 12.64 g kg^{-1}) than *Ag.*, there was no difference in Al at HF with land-use. SAR was higher in *Nat.* soil for both NWD and HF ($\bar{x}$=1.16, 1.39 vs. 0.52, 0.24 respectively).

Table 5 Summary of elemental composition statistical comparisons between *Ag.* and *Nat.* soils of Experiment 1.

	Land use with higher concentration		
	Ag.	*Nat.*	No difference between *Ag.* and *Nat.*
Both NWD and HF	TP, K, Ca, Cu, Zn	SAR	
NWD	Mn	Mg, Al, Fe	TC, TN
HF	TC, TN, Mg, Fe		Mn, Al

Among depth

There was no significant difference with depth in Nat. soil for both farms for TP, K, Na, Ca, SAR, Mn, Cu, Zn, and Fe. Mn increased with depth for NWD *Ag.* soil ($\bar{x}$=6.22 g kg^{-1} at 0-10 cm, $\bar{x}$=7.22 g kg^{-1} at 10-20 cm, $\bar{x}$=7.62 g kg^{-1} at 20-30 cm. TC decreased with depth for NWD Nat. soil ($\bar{x}$=66.62 g kg^{-1} at 0-10 cm, 37.58 g kg^{-1} at 10-20 cm, 35.34 g kg^{-1} at 20-30 cm) and *Ag.* soil of both farms (for HF, $\bar{x}$=42.17 g kg^{-1} at 0-10 cm, 35.20 g kg^{-1} at 10-20 cm, $\bar{x}$=22.88 g kg^{-1} at 20-30 cm, and for NWD $\bar{x}$=45.97 g kg^{-1} at 0-10 cm, 41.90 g kg^{-1} at 10-20 cm, 35.73 g kg^{-1} at 20-30 cm). There was no difference in TC or Mg with depth in HF Nat. soil, Mg was also not different with depth in HF Ag. For NWD Nat. soil Mg was higher in 20-30 cm ($\bar{x}$=3.14 g kg^{-1}) than 0-10cm ($\bar{x}$=1.52 g kg^{-1}), for NWD Ag. Mg was greater in 20-30cm than other depths. ($\bar{x}$=0.60 vs. $\bar{x}$=0.52 at 0-10 cm, $\bar{x}$=0.43 at 10-20 cm). Additionally, there was no difference with depth in HF *Ag.* soil for Na, SAR, Mn, Zn. HF and NWD *Ag*. had significantly more Al in the deepest depth than in 0-10 and 10-20 cm ($\bar{x}$=14.32, 16.40 g kg^{-1} at 20-30 cm, $\bar{x}$=11.41, 11.32 g kg^{-1} at 10-20 cm, and 10.72, 10.99 g kg^{-1} at 0-10 cm respectively), NWD *Nat.* had more Al at 20-30 cm ($\bar{x}$=20.11 g kg^{-1}) than in 0-10 cm ($\bar{x}$=9.03 g kg^{-1}). HF *Nat.* soil had significantly more Al in 10-20 and 20-30 cm soil ($\bar{x}$=12.12, 11.64 g kg^{-1} respectively) than in 0-10 cm ($\bar{x}$=8.33 g kg^{-1}).

In Ag. soil of HF and NWD, TN ($\bar{x}$=3.48, 2.76 g kg^{-1}at 0-10 cm, $\bar{x}$=2.76, 2.23 g kg^{-1} at 10-20 cm, $\bar{x}$=1.48, 1.59 g kg^{-1} at 20-30 cm respectively)., TP ($\bar{x}$=18.86, 14.73 g kg^{-1} at 0-10 cm, $\bar{x}$=15.05, 12.60 g kg^{-1} at 10-20 cm, $\bar{x}$=6.86, 7.69 g kg^{-1} at 20-30 cm respectively), K ($\bar{x}$=9.83, 6.98 g kg^{-1} at 0-10 cm, $\bar{x}$=7.33, 5.05 g kg^{-1} at 10-20 cm, $\bar{x}$=7.47,

5.18 g kg^{-1} at 20-30 cm respectively) decreased with depth. Na ($\bar{x}$=1.17 g kg^{-1} at 0-10 cm, $\bar{x}$=1.10 g kg^{-1} at 10-20 cm, $\bar{x}$=1.16 g kg^{-1} at 20-30 cm) and SAR ($\bar{x}$=0.52 at 0-10 cm, $\bar{x}$=0.43 at 10-20 cm, $\bar{x}$=0.60 at 20-30 cm) were lower in 10-20 cm depth than in 0-10 cm and 20-30 cm in NWD Ag. soil. Cu decreased with depth in HF Ag. soil, ($\bar{x}$=575 mg kg^{-1} at 0-10 cm, 371 mg kg^{-1} at 10-20cm, $\bar{x}$=237 mg kg^{-1} at 20-30 cm), Ca was significantly lower in the 20-30 cm depth than in other depths ($\bar{x}$=22.26 g kg^{-1} vs. $\bar{x}$=39.13 g kg^{-1} at 10-20 cm, $\bar{x}$=47.17 g kg^{-1} at 20-30 cm). For NWD *Ag.*, Ca was significantly higher in 20-30 cm ($\bar{x}$=46.85 g kg^{-1}) than other depths ($\bar{x}$=38.04 g kg^{-1} at 0-10 cm, $\bar{x}$=24.27 g kg^{-1} at 10-20 cm), Cu was significantly 10-20 cm ($\bar{x}$=419 mg kg^{-1}) depth than 0-10 ($\bar{x}$=364 mg kg^{-1}) or 20-30 cm ($\bar{x}$=317 mg kg^{-1}). NWD *Ag.* soil had significantly more Zn in the deeper 20-30 cm soil ($\bar{x}$=2099 mg kg^{-1}) than 0-10 ($\bar{x}$=437 mg kg^{-1}) or 10-20 cm ($\bar{x}$=563 mg kg^{-1}).

Elemental ranges

TC in *Ag.* soil ranged from 6.50 to 37.69 g kg^{-1} for HF and from 9.80 to 40.69 g kg^{-1} for NWD. In *Nat.* soil TC ranged from 8.50 to 44.20 g kg^{-1} at HF and from 24.20 to 76.40 g kg^{-1} at NWD. TN ranged from 2.60 to 5.40 g kg^{-1} for HF *Ag.* soil and from 2.00 to 3.30 g kg^{-1} for NWD *Ag.* HF *Nat.* TN ranged from 0.040 to 2.70 g kg^{-1} while NWD *Nat.* ranged from 1.20 to 6.20 g kg^{-1}. TP ranged from 2.34 to 32.67 g kg^{-1} for HF *Ag.* and from 7.32 to 14.74 g kg^{-1} for NWD *Ag.* HF *Nat.* soil ranged from 2.30 to 7.22 g kg^{-1}, NWD *Nat.* soil ranged from 2.80 to 9.90 g kg^{-1}.

K ranged from 2.98 to 24.69 g kg^{-1} for HF *Ag.* while NWD *Ag.* soil ranged from 4.40 to 7.96 g kg^{-1}. K ranged from 2.77 to 6.10 g kg^{-1} for HF *Nat.* and from 2.01 to 5.20 g kg^{-1} for NWD *Nat.* Na ranged from 0.22 to 1.75 g kg^{-1} in HF *Ag.* soil, NWD *Ag.* soil

ranged from 0.96 to 1.41 g kg^{-1}. In *Nat.* soil K ranged from 0 to 4.32 g kg^{-1} for HF and from 0 to 7.52 g kg^{-1} for NWD. *Ag.* soil ranged in Ca from 8.44 to 161.52 g kg^{-1} for HF and from 23.43 to 49.32 g kg^{-1} for NWD. Ca ranged from 0 to 15.82 g kg^{-1} for HF *Nat.* soil and from 0 to 14.52 g kg^{-1} for NWD *Nat.* Mg ranged from 1.35 to 8.24 g kg^{-1} in HF *Ag.* soil while NWD *Ag.* soil ranged from 0.86 to 2.34 g kg^{-1}. Mg ranged from 1.02 to 4.36 g kg^{-1} in HF *Nat.* soil and from 1.02 to 3.84 g kg^{-1} in NWD *Nat.* SAR ranged from 0.13 to 0.76 for HF *Ag.* soil and from 0.39 to 0.67 for NWD *Ag.* SAR ranged from 0.00 to 5.85 for HF *Nat.* soil and from 0.00 to 5.06 for NWD *Nat.*

Mn in *Ag.* soil ranged from 2.34 to 9.46 g kg^{-1} at HF and from 5.75 to 7.79 g kg^{-1} at NWD. *Nat.* soil Mn ranged from 2.50 to 8.27 g kg^{-1} at HF and from 2.55 to 9.18 g kg^{-1}at NWD. Cu ranged from 0 to 1558 mg kg^{-1} in HF *Ag.* soil and from 260 to 478 mg kg^{-1} for NWD *Nat.* HF *Nat.* soil ranged from 0 to 420 mg kg^{-1}, NWD *Nat.* soil ranged from 0 to 530 mg kg^{-1}. Zn in *Ag.* soils ranged from 0 to 1407 mg kg^{-1} for HF and from 376 to 3676 mg kg^{-1} at NWD. *Nat.* soil ranged from 0 to 550 mg kg^{-1} at HF and from 0 to 1208 mg kg^{-1} at NWD. *Ag.* soil Al ranged from 6.91 to 13.37 g kg^{-1} at HF and from 8.12 to 14.81 g kg^{-1} at NWD. HF *Nat.* soil ranged from 6.91 to 14.31 g kg^{-1}, NWD *Nat.* soil ranged from 4.99 to 23.79 g kg^{-1}. *Ag.* soil Fe ranged from 8.38 to 14.49 g kg^{-1} at HF and from 8.65 to 13.93 g kg^{-1} at NWD. *Nat.* soil ranged from 8.38 to 14.20 g kg^{-1} at HF and from 9.35 to 23.10 g kg^{-1} at NWD.

Table 6 Sodium Adsorption Ratio (SAR) of Experiment 1 (2016) New World Dairy (NWD) and Hammond Farms (HF) farmed (*Ag.*) and natural (*Nat.*) soil. Error term is CI_{95}.

Farm	Land Use	Manure	SAR
NWD	*Ag.*	Yes	0.67±0.00
		No	0.39±0.00
	Nat.		1.16±2.74
HF	*Ag.*	Yes	0.24±0.03
	Nat.		1.39±3.09

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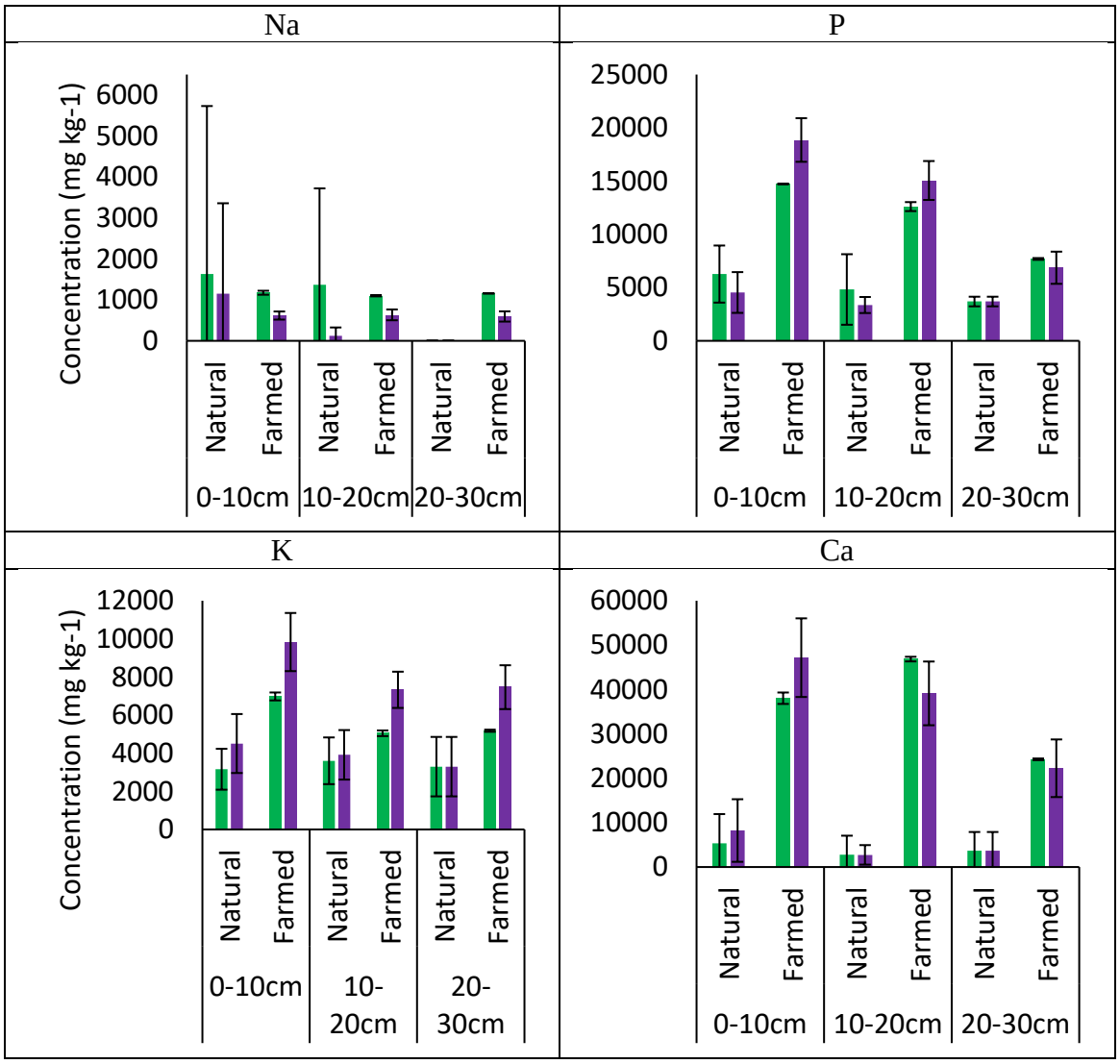

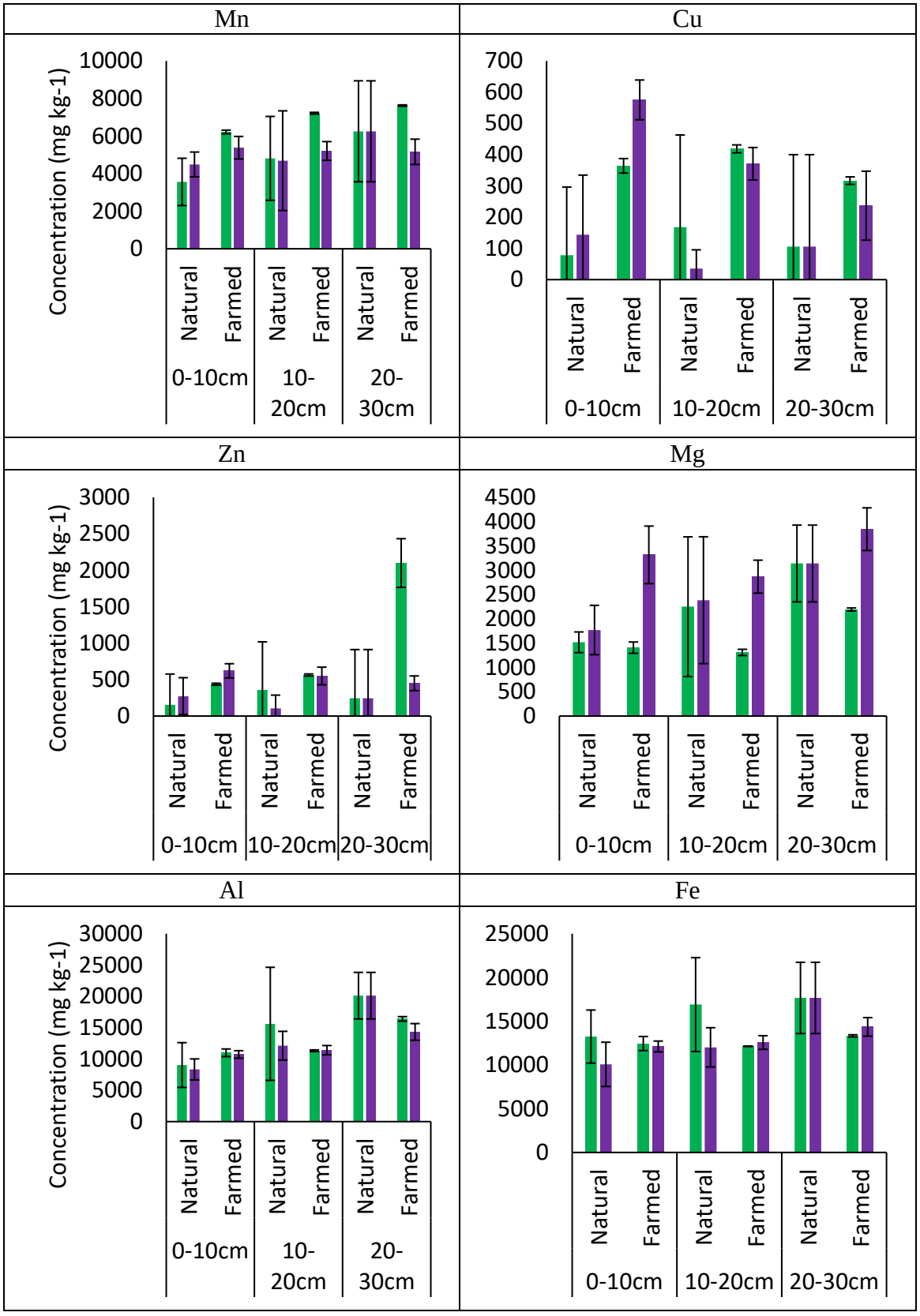
Mn
Concentration (mg kg-1)
10000
8000
6000
4000
2000
0
Natural
Farmed
0-10cm
10-20cm
20-30cm
Cu
700
600
500
400
300
200
100
0
Zn
3000
2500
2000
1500
1000
500
Mg
4500
4000
3500
3000
2500
2000
1500
1000
500
Al
30000
25000
20000
15000
10000
5000
Fe

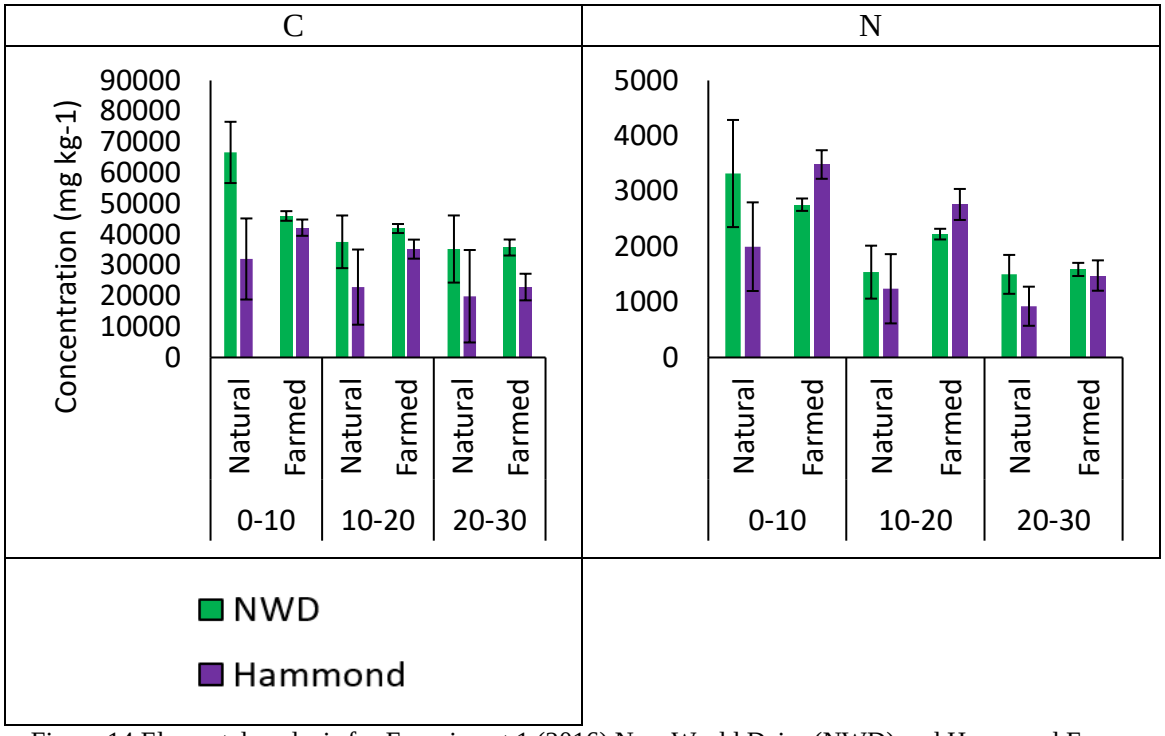

Figure 14 Elemental analysis for Experiment 1 (2016) New World Dairy (NWD) and Hammond Farms (HF) natural (*Nat.*) and farmed (*Ag.*) soil of depths 0-10, 10-20, and 20-30 cm. Error term is CI_{95}.

3.2.2.3 Microarthropod composition

While HF *Ag.* soil had significantly higher microarthropod abundance than NWD *Ag.* soil irrespective of manure treatment ($\bar{x}$=54.7 vs. 6.04 individuals per 1 kg dry soil) (Table A2.1), NWD *Nat.* soil had significantly more arthropods than HF *Nat.* soil irrespective of depth ($\bar{x}$=39.6 vs. 2.67 individuals per 1 kg of dry soil) (Table A2.2).

The top layer (0-10 cm) of *Nat.* soil had significantly more microarthropods than the top layer of *Ag.* soil ($\bar{x}$=94.7 vs. 6.04 individuals per 1 kg of dry soil) at NWD (Table A2.3). The same was not true at HF. NWD *Nat.* arthropod abundance significantly decreased with depth; 0-10 cm soil had significantly more microarthropods than the 20-30 cm depth ($\bar{x}$=94.7 vs. 4 individuals per 1 kg of dry soil) (Table A2.4). HF *Nat.* abundance also decreases with depth, but the trend was not significant (Table 8). NWD *manured*, *Ag.*

soil arthropod abundance was not statistically different than *no manure, Ag.* soil (Table 7).

Discriminant analysis was carried out for depth 0-10 cm for 2016 *Ag.* and *Nat.* soil (Table 9). Ninety-four percent of samples were accurately classified for land management, four samples were misclassified. Abundances of Acari, Collembola, Coleoptera, Diptera, and Pseudoscorpions was significantly different between *Nat.* and *Ag.* lands, while abundances of Hemiptera, Isopoda, and Geophilomorpha were not (Table 10).

Table 7 Experiment 1 (2016) arthropod order abundance and total counts (individuals kg^{-1} dry soil) for manured and no manure farmed soil of 0-10 cm depth from New World Dairy (NWD) and Hammond Farms (HF). Error term is CI_{95}.

	Manure	Acari	Collembola	Coleoptera	Diptera	Hemiptera
NWD	Yes	2.08±1.97	2.50±1.63	0	0	0
	No	1.67±1.89	4.58±3.52	0	1.25±1.32	0
HF	Yes	32.50±21.49	17.50±18.12	0.28±0.54	4.17±4.07	0
		Pseudoscorpions	Isopoda	Araneae	Geophilomorpha	Total Count
NWD	Yes	0	0	0	0	4.58±1.97
	No	0	0	0	0	7.5±5.83
HF	Yes	0	0	0	0.28±0.54	54.72±33.84

Table 8 Experiment 1 (2016) arthropod order abundance and total counts (individuals kg^{-1} dry soil) for three depths (0-10, 10-20, 20-30 cm) of natural soil for New World Dairy (NWD) and Hammond Farms (HF). Error term is CI_{95}.

	Depth (cm)	Acari	Collembola	Coleoptera	Diptera	Hemiptera
NWD	0-10	45.33±53.46	28.00±35.16	9.33±3.20	0	8.00±15.68
	10-20	10.67±15.24	5.33±7.62	4.00±5.23	0	0
	20-30	1.33±2.61	0	1.33±2.61	1.33±2.61	0
HF	0-10	0	0	0	1.33±2.61	1.33±2.61
	10-20	0	0	0	1.33±2.61	0
	20-30	1.33±2.61	0	0	0	0
		Pseudoscorpions	Isopoda	Araneae	Geophilomorpha	Total Count
NWD	0-10	1.33±2.61	1.333±2.61	1.33±2.61	0	94.67±65.78
	10-20	0	0	0	0	20.00±19.38
	20-30	0	0	0	0	4.00±5.33
HF	0-10	0	0	0	2.67±5.23	5.33±10.45
	10-20	0	0	0	0	1.33±2.61
	20-30	0	0	0	0	1.33±2.61

Table 9 Confusion matrix for discriminant analysis of 2016 arthropod order abundance (individuals kg^{-1} dry soil) of 0-10 cm depth of Farmed (*Ag.*) and Natural (*Nat.*) soil from New World Dairy and Hammond Farms.

Group	*Ag.*	*Nat.*
Ag.	56	4
Nat.	0	6
Total samples	56	10
Correct samples	56	6
Proportion	1	0.6

Table 10 Linear discriminant function for discriminant analysis of 2016 arthropod order abundance (individuals kg^{-1} dry soil) from 0-10 cm depth of Farmed (*Ag.)* and Natural (*Nat*). soil from New World Dairy and Hammond Farms.

	Ag.	*Nat.*
Constant	-0.18	-4.4
Acari	0.15	-0.01
Collembola	0.09	-0.1
Coleoptera	-0.65	8.9
Diptera	-0.14	0.39
Hemiptera	7.65	32.33
Pseudoscorpions	-0.1	6.44
Isopoda	-48.84	-187.46
Geophilomorpha	-3.77	-8.76

3.3 Experiment 2: Survey of arthropods and nematodes in soils of dairy farms in western Newfoundland; 2017

3.3.1 Methodology for Experiment 2

3.3.1.1 Field site description

A new field site was chosen on producer-owned land at New World Dairy Inc. (NWD) in the Codroy Valley region, NL (48.1773°N, 58.7880°W) for 2017 (Figure 1) The field was approximately 10 acres in size and has several slopes >4%. It had been repeatedly manured for several years and used for corn mono-cropping. From here on the new 2017 NWD land or samples will be referred to as NWDb, as appropriate. A more forested site adjacent to HF was used to represent *Nat.* soil in 2017 as it more accurately represented the *Ag.* field prior to conversion than the grassland used in 2016. The field site at HF that was used in 2016 was used again in 2017. The new NWD location was manured approximately twice per year. Crops were planted at HF on 31 May 2017, and at NWD on 2 June 2017. Harvest was completed at appropriate crop maturity.

3.3.1.2 Crop treatment

Silage corn (corn AS1047RR EDF), silage oat/pea (oats and peas blend- MAXI SILE), and silage soybean (CRMAX PS0242R2 HCNT 140- pre-inoculated) crops were planted at NWD while only silage corn (corn AS1047RR EDF) was planted at HF.

3.3.1.3 Manure treatment

Two levels of manure treatment, zero and manured (~5000 gal ac^{-1}), were applied according to farmer practice to both field locations. HF was manured for a second time on 10 November 2017, 14 days after the harvest of the corn.

3.3.1.4 Experimental design and soil sampling

NWD and HF were both divided into 6 plots (Figure 15, Figure 16). Sampling took place 14 days after harvest of each crop. Quadrat sampling was completed as described by Van Bezooijen (2006). Three 10x10 m sample quadrats, representing natural replicates, were randomly placed within each treatment plot at both sites. A minimum of 50 core samples, using a 3 cm corer from a depth of 0-10 cm, were taken from each 10x10 m plot (Figure 17). Samples were taken at HF on 10 November 2017 approximately 3 h following the second manure application. The manure formed a distinct layer on the top of the soil and was easily separated from soil and removed prior to collecting the soil. Given the significantly longer life cycle of nematodes, of 24 to 48 h under ideal conditions (Blaxter, 2011), and that microarthropods have life cycles measured in weeks, this event is very unlikely to have affected the nematode and arthropod population structure or abundance. Forest control samples, representing the natural system prior to being converted to an agriculture system, were collected using the same method at NWD and HF (three replicates at each site). Soil was collected from September 2017 to November 2017. Sixty-three samples, including natural and technical replicates, were collected from both NWD and HF (Table 11).

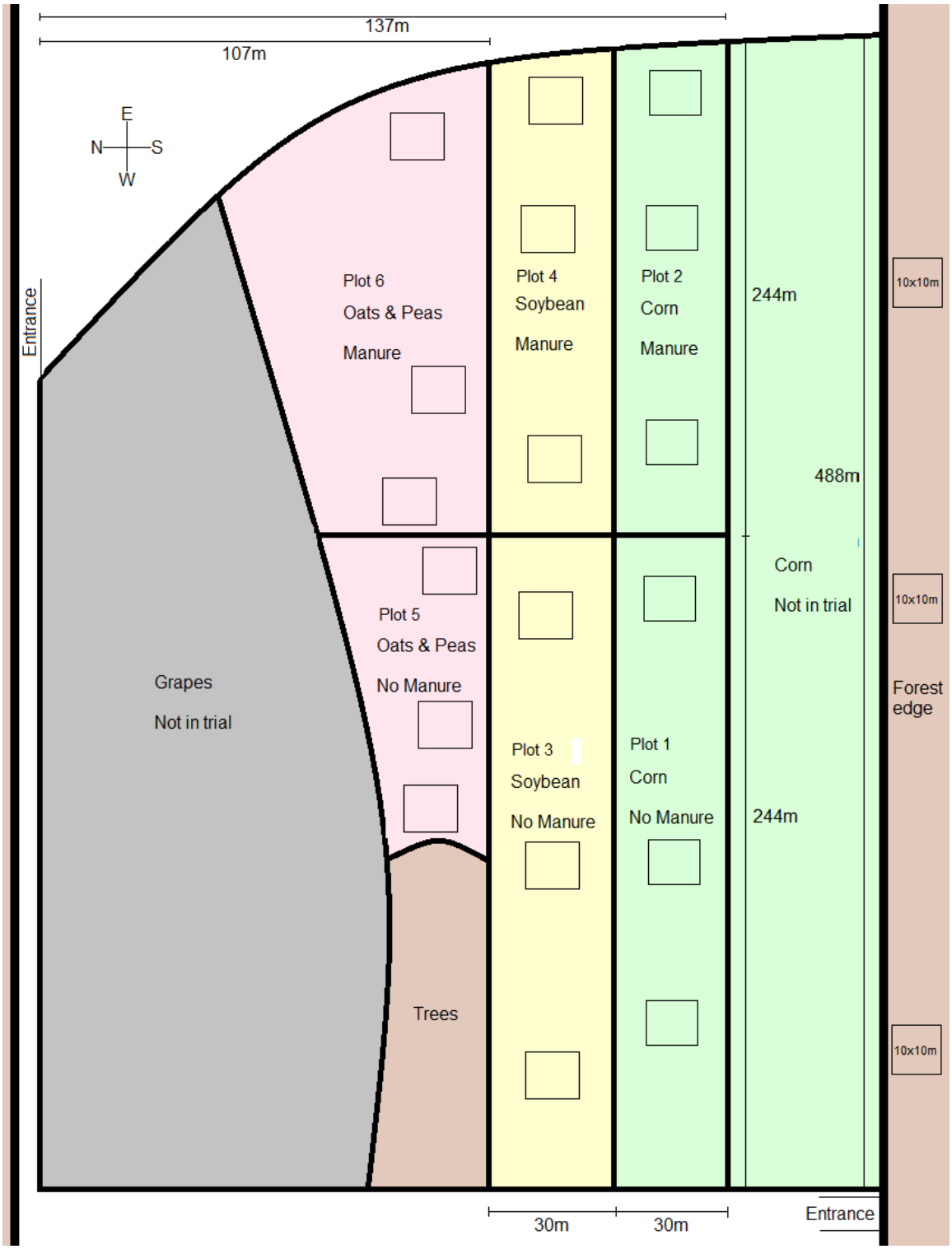

Figure 15 Experiment 2 (2017) crop seeding and treatment plan for New World Dairy. Squares represent 10x10 m soil sampling plots for nematode analysis. Diagram not to scale.

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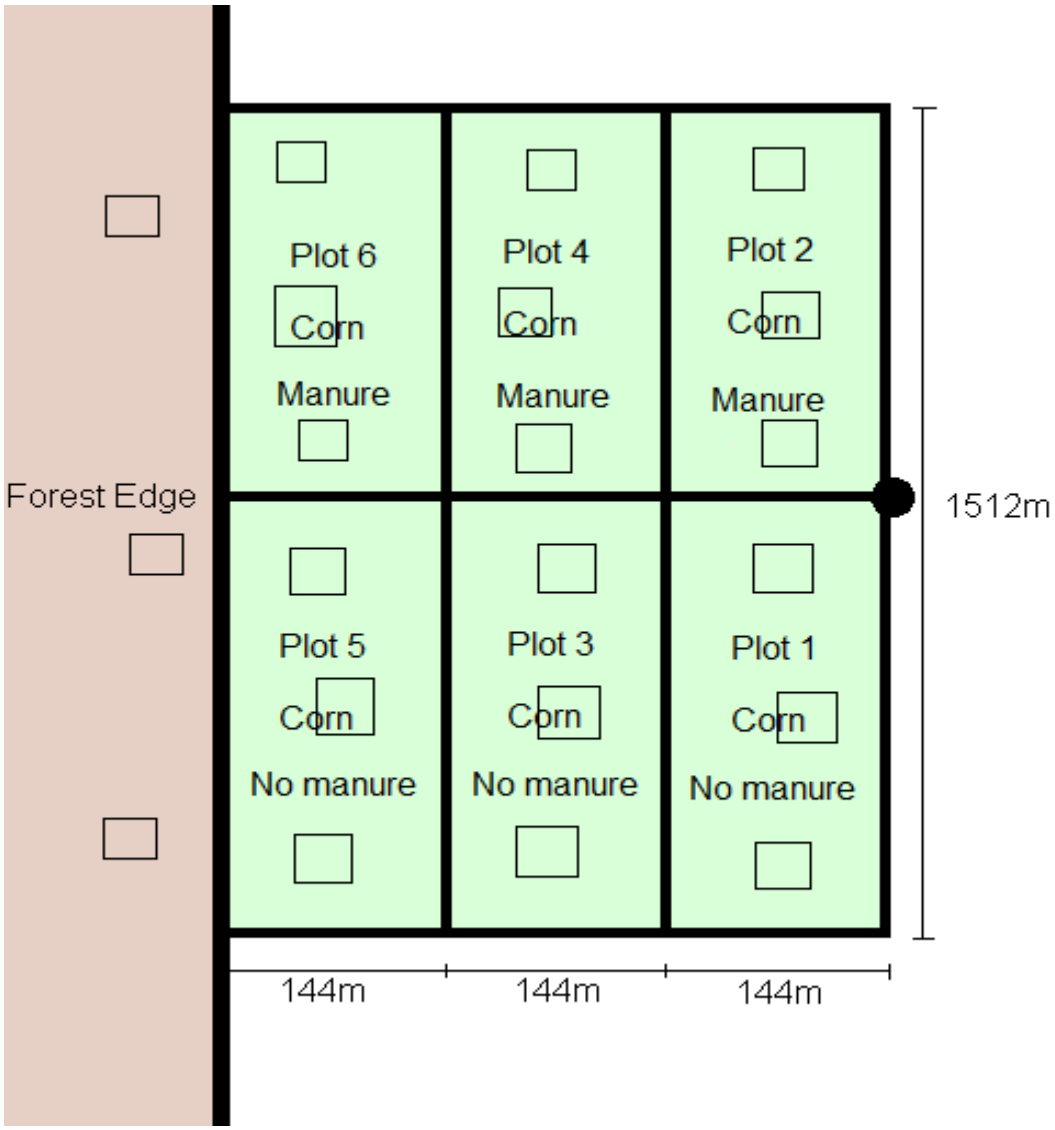

Figure 16 Experiment 2 (2017) crop seeding and treatment plan for Hammond Farms. Squares represent 10x10 m soil sampling plots for nematode analysis. Diagram not to scale.

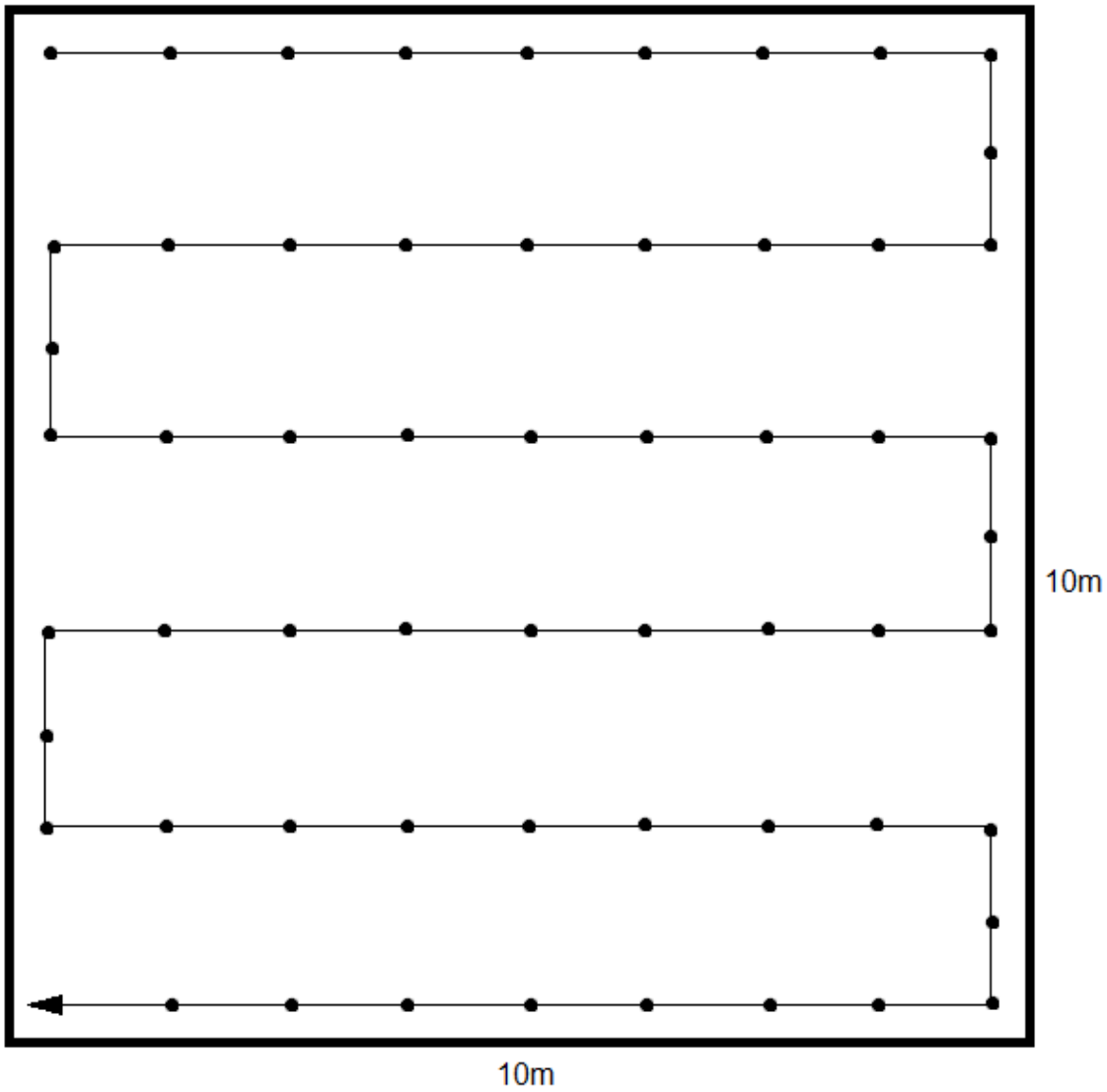

Figure 17 A soil core sampling pattern within each 10x10 m sampling plot at New World Dairy and Hammond Farms for Experiment 2 (2017). Diagram not to scale.

Table 11 Experiment 2 (2017) soil sampling design for New World Dairy (NWD) and Hammond Farms (HF).

Location	Plot	Manure (Yes, No)	Quadrat Location (natural replicates)	Number of technical replicates per sample	Total number of samples
NWDb	1	No	A, B, C	3	63
	2	Yes		3	
	3	No		3	
	4	Yes		3	
	5	No		3	
	6	Yes		3	
	Natural	No		3	
HF	1	No	A, B, C	3	63
	2	Yes		3	
	3	No		3	
	4	Yes		3	
	5	No		3	
	6	Yes		3	
	Natural	No		3	

3.3.1.5 Soil handling

Soil was prepared differently for Experiment 2 (2017) than Experiment 1 (2016) to ensure nematode survival prior to extraction in Experiment 2 soil.

Rocks and herbaceous material were removed from samples, aggregates were gently broken by hand. The soil from each plot was mixed on a 1x1 m tarp; the corners of the tarp were lifted to allow the soil to roll to the opposite corner. Each corner was lifted eight times to ensure homogenisation and the soil was divided into three technical replicates. Each replicate was split into two portions.

3.3.1.6 Soil storage

One portion of each replicate was stored fresh at 4 °C in unsealed bags to be used for microarthropod and nematode extraction, the other was air dried for 48 h, sieved to 2 mm, and stored at 4 °C for physical and chemical analysis. Soil was analysed as soon as possible to ensure little change in the nematode community.

3.3.1.7 Soil physicochemical parameters

Soil texture, pH, organic carbon, and cations were analysed for 2017 samples as described in section 3.2.1.7. Total carbon and total nitrogen was analysed using the method in section 3.2.1.7.7 but with 12-15 mg of soil.

3.3.1.8 Soil microarthropod extraction, preservation, counts, and identification

Arthropod abundance decreased with depth in 2016 (Table 8) and therefore only the top 0-10 cm layer was sampled in 2017. Arthropods were extracted, preserved, counted, and identified as described in section 3.2.1.8.

3.3.1.9 Nematode extraction, cleaning, preservation, counts, and identification

3.3.1.9.1 Nematode extraction and preservation

Free-living nematodes were extracted from soil using the Cobb (Decanting and Sieving) method (Cobb, 1918) as described by Van Bezooijen (2006). The method uses differences in nematodes and soil particle size as well as nematode mobility to separate nematodes from soil. One hundred grams fresh soil was decanted with 1 L of water three times. The suspension was passed consecutively through 710 μm, 250 μm, 150 μm, and 63 um sieves followed by three times through a 45 μm sieve. The debris on the 710 μm

sieve was discarded while the debris on all other sieves was collected. The debris collected from the sieves was placed on two 9" synthetic cow milk filters held by a 7" clamping ring. The filters with clamping ring were placed in an extraction pan filled with 90 mL of deionised water; enough to keep the debris on the filter moist but not cover the filter. The nematodes were allowed to move through the filters into the water for 48h and were then poured into 100 mL jars as a clear suspension. The jars were left to settle at 4°C for 24 h and were reduced to less than 100 mL by suctioning off the top layer of water with a syringe.

3.3.1.9.2 Further cleaning of the nematode extract and sample preservation

Fine organic matter was present in the sample making the sample too dirty to identify individual nematodes from the sample even after using filter pans. The samples were further cleaned using the centrifugal flotation method (Gooris and D'Herde, 1972) as described by Van Bezooijen (2006). Nematodes float in fluids with a specific gravity greater than 1.084 (Andrassy, 1956) while soil particles with a greater specific gravity than the fluid sink. The ~100 mL suspension was transferred to two 50 mL centrifuge tubes and equalized for weight. Kaolin clay (0.1 g) was added to each tube to prevent the pellet from whirling up when the supernatant was poured off. The tubes were mixed thoroughly and centrifuged at 1800 x *g* for 4 min. The supernatant was poured onto a 10 µm sieve to ensure any nematodes that were still floating were not lost. $MgSO_4$ (1.18 specific gravity) was added to each tube and thoroughly mixed to bring the pellet to suspension. The tubes were centrifuged at 1800 x *g* for 3 min to float the nematodes. The supernatant was again poured onto the 10µm sieve, the nematodes were thoroughly rinsed

and collected in a 250 mL jar. After settling for 24h, the sample was reduced to 100 mL using the above method. After an additional 24h the sample was reduced to 10 mL. The sample was homogenised and 2 mL was removed for DNA extraction of a separate experiment. The remaining sample was then allowed to settle for a final 24h, was reduced to 1.5 mL, and preserved by adding 3.5 mL of hot (70 °C) 5% formalin (to kill the nematodes) followed by 3 mL of cold 5% formalin to prevent deformation of nematodes. The final extract was 8 mL of 4% formalin.

3.3.1.9.3 Nematode counts

The preserved nematode solution was homogenised by inverting the tube 5 times and pumping with a 1 mL pipette 10 times. One mL of the homogenised solution was removed and placed in a counting dish. One drop of a soap solution was added to the dish to ensure nematodes sank to the bottom. All nematodes in the 1 mL were counted under 40x magnification. A second count was performed in the same manner. When numbers were greater than 100, a third count was performed if the first two counts differed by more than 5%.

When the extract is homogenised, nematode numbers follow a Poisson distribution, therefore the standard deviation is $\sqrt{x}$ for each count (x) (Southey, 1986). When numbers were less than 100, the above 5% rule was not used, a third count was performed when the first two counts differed by more than the standard deviation ($\sqrt{x}$). The average of the nematode counts was used to extrapolate for the total 10 mL of extract and expressed per 100 g dry soil.

$$N_{dw} = \frac{(average\ \#nematodes\ per\ 1\ mL * 10\ mL)}{Dry\ weight\ of\ sample} * 100g$$

3.3.1.9.4 Nematode identification

The remaining nematode extract was centrifuged at 1800 x *g* for 4 min and allowed to settle for 24 h, the sample was concentrated to 200 µl by suctioning the supernatant with a syringe. The 200 µl of extract was homogenised and 100 µl was removed and placed on a Palmer counting chamber with a cover glass. Each slide was systematically analysed for community composition using a compound microscope under 100x to 400x magnification. The first 150 individuals found on the slide, including juveniles, were identified to feeding habit by observing mouthparts and specific features of the esophagus. Feeding groups include: carnivores/predators (teeth present and/or large mouth cavity), herbivore/plant parasitic (stylet present), omnivore (spear present), fungivore (large/clear mid bulb), and bacterivore (tubular mouth/criteria for other feeding groups not met). Identification of nematodes to feeding groups (vs. detailed identification) is not always accurate; there are exceptions to every classifications (Yeates et al., 1993) (For example, *Trichodoridae* have a spear but is a herbivorous family, individuals can be identified by a unique bent spear). Absolute values for composition were determined using previously found total nematode counts.

3.3.1.10 Statistical analysis

Statistics for determining the influence of factors on physiochemical parameters and arthropod and nematode abundances were carried out as described in section 3.2.1.9.

Permutational multivariate analysis of variance (PERMANOVA) was completed in *Past3* version 3.22 (Hammer et al., 2001). Redundancy analysis (RDA), variation partitioning, and correlation analysis were carried out using the *vegan* package implemented in *R* version 3.5.2 (R Core Team, 2018).

Note that the Newfoundland nematode and arthropod data was not transformed prior to analysis as the data was already normalized per mass soil. For the RDA analyses, soil parameters were normalized in units of standard deviation around the mean to eliminate the impact of the different units commonly employed for various soil parameters.

3.3.2 Results for Experiment 2: 2017 Survey of the Soil Microarthropods and Nematodes

3.3.2.1 Soil physicochemical properties

Texture

Soil texture was similar between sites and land use; sandy loam was the dominant texture (Figure 18, Figure 19).

Ag. soil at NWDb had significantly more clay and silt than at HF ($\bar{x}$=9.75, 45.06 vs. 7.75, 26.19% respectively) (Table A3.1). *Nat.* soil was sandier at HF ($\bar{x}$=66.06%) than at NWDb ($\bar{x}$=45.19%) while *Nat.* soil at HF had significantly more sand and clay ($\bar{x}$=56.46, 13.17 vs. 22.80, 8.03%) and less silt ($\bar{x}$=30.38%) than NWDb ($\bar{x}$=69.17%) (Table A3.2).*Ag.* soil was significantly sandier than *Nat.* soil at NWDb ($\bar{x}$=45.19 vs. 22.80%). However, NWDb *Ag.* soil had less silt ($\bar{x}$=45.06%) than *Nat.* ($\bar{x}$=69.17%) (Table A3.3).*Ag.* soil was significantly sandier ($\bar{x}$=66.06% vs. 56.46%) and had less clay and silt than *Nat.* soil at HF ($\bar{x}$= 7.75, 26.19 vs. 13.17, 30.38 respectively) (Figure 19) (Table A3.4).

Treatments without manure were significantly sandier and had less silt than in manure treatments at HF ($\bar{x}$=67.49, 24.34% vs. $\bar{x}$=64.89, 27.71% respectively). Soil texture was not significantly different between manure or crop treatments for NWDb. Soil texture was significantly different between crop when farm was not considered; percent sand was significantly higher in corn ($\bar{x}$=60.04%) soil than soybean ($\bar{x}$=44.58%) and *Nat.*

soil ($\bar{x}$=39.63%). Oat & pea soil ($\bar{x}$=51.22%) did not differ in texture from soybean, corn or *Nat.* soil (Table A3.5).

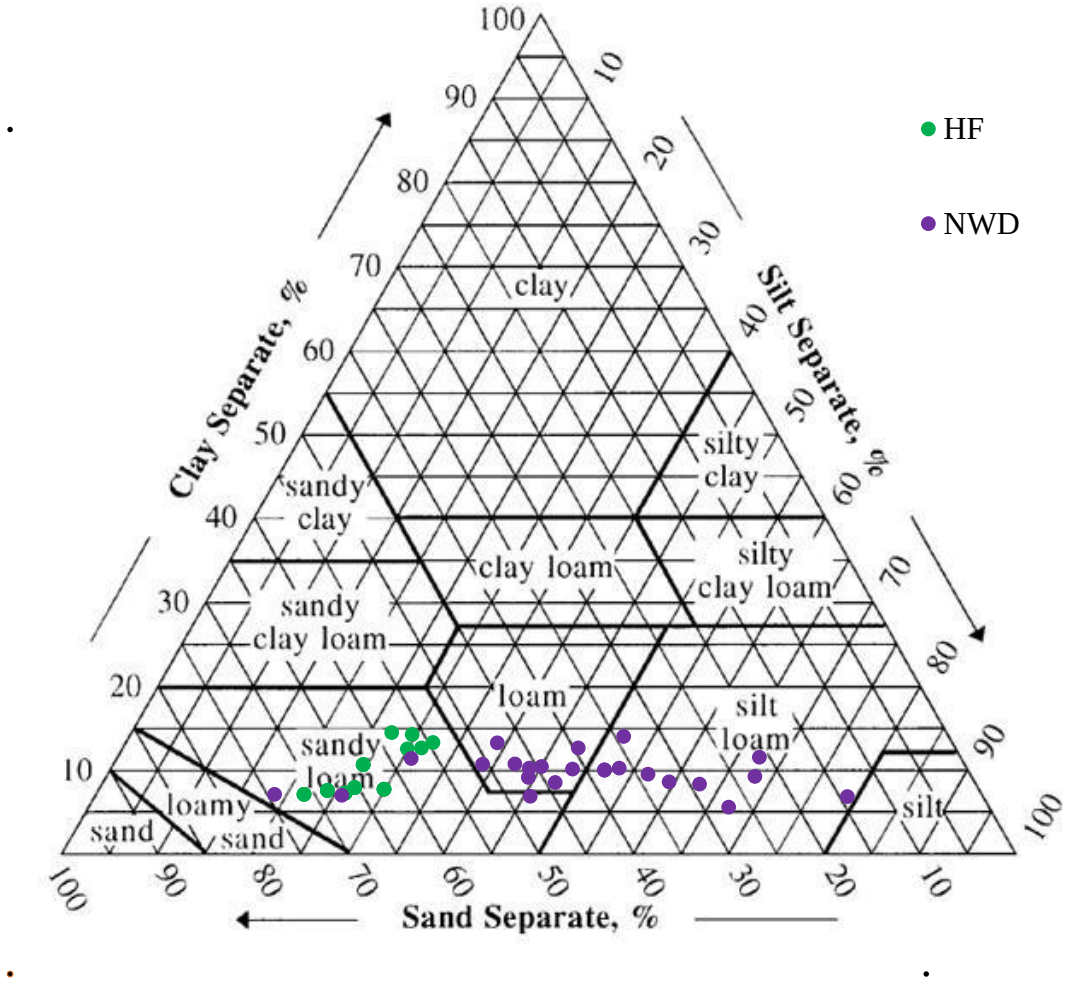

Figure 18 Soil texture for samples of Experiment 2 (2017) from New World Dairy (NWD) and Hammond Farms (HF) (USDA soil texture ternary plot)

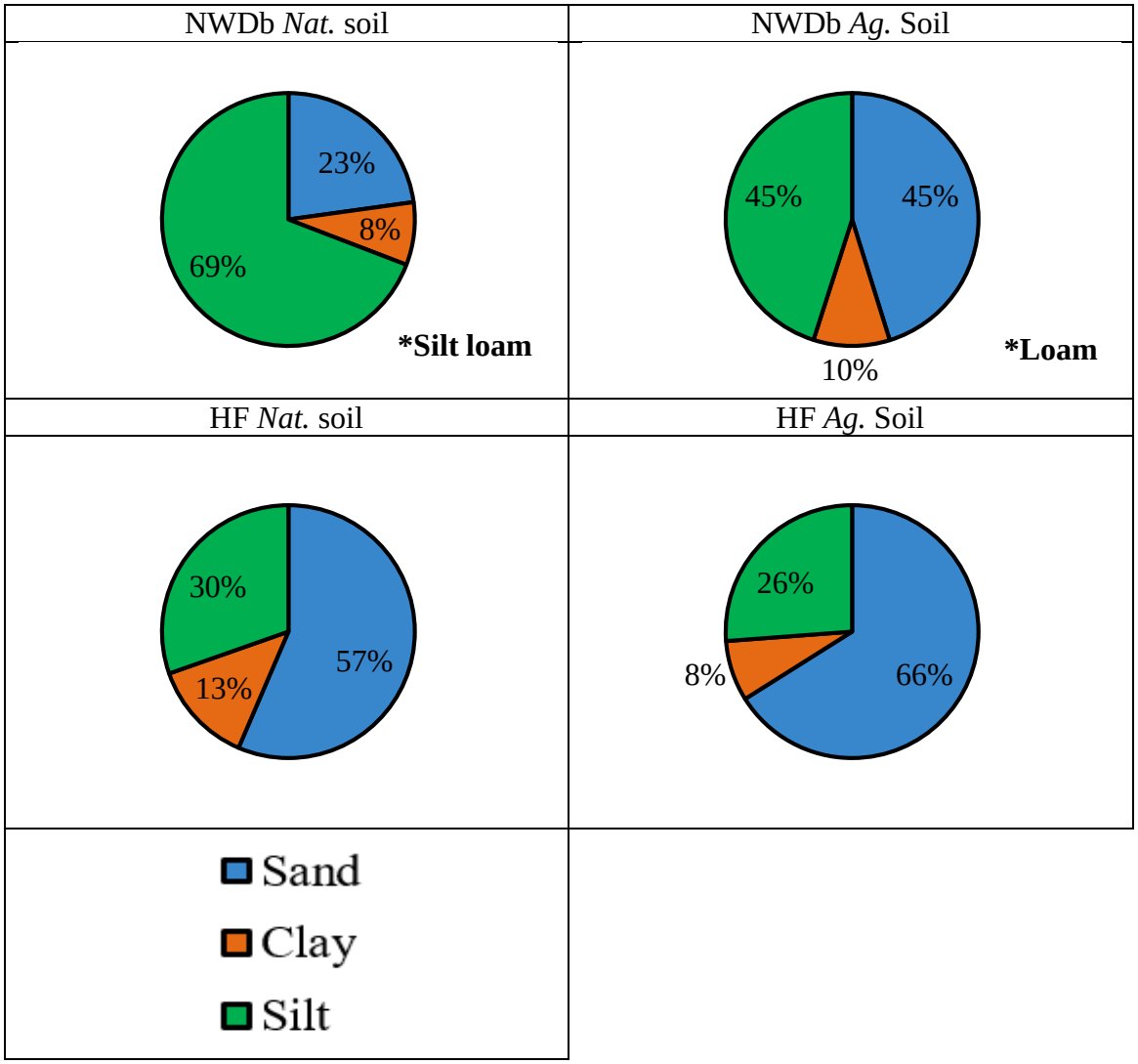

Figure 19 Experiment 2 (2017) Soil texture for New World Dairy (NWDb) and Hammond Farms (HF) natural (*Nat.*) and farmed (*Ag.*) soil of depth 0-10 cm. Texture is USDA classified as sandy loam unless otherwise noted.

Soil Water Content at sampling

SWC at sampling was not significantly different between *Ag.* soil for both farms while NWDb had greater SWC than HF for *Nat.* soil ($\bar{x}$=54.04 vs. 26.61%) (Table A3.6). *Nat.* soil had greater SWC ($\bar{x}$=54.04) than *Ag.* soil at NWDb ($\bar{x}$=34.58) (Table A3.7). Conversely, there was higher SWC in *Ag.* soil ($\bar{x}$= 31.18%) than in *Nat.* soil ($\bar{x}$=26.61%) at HF (Table A3.8). SWC ranged from 26.00 to 36.35% for HF *Ag.* soil and from 14.06 to

42.52% in NWDb *Ag.* soil. SWC ranged from 19.75 to 31.78% for HF *Nat.* soil and from 45.19 to 57.99% for NWDb *Nat.* soil

Manured soil had less water than no manure soil at HF ($\bar{x}$=28.58 vs. 34.36%) although SWC did not differ with manure treatment at NWDb. NWDb oat & pea cropped soil had significantly less water than corn and soybean soil ($\bar{x}$=26.36 vs. 38.45 and 37.83%) (Table A3.9). Alternatively, when farm was not considered, oat & pea cropped soil had significantly less water than *Nat.* soil but corn and soybean soil was not different from oat & pea or *Nat.* soil (Figure 20) (Table A3.10).

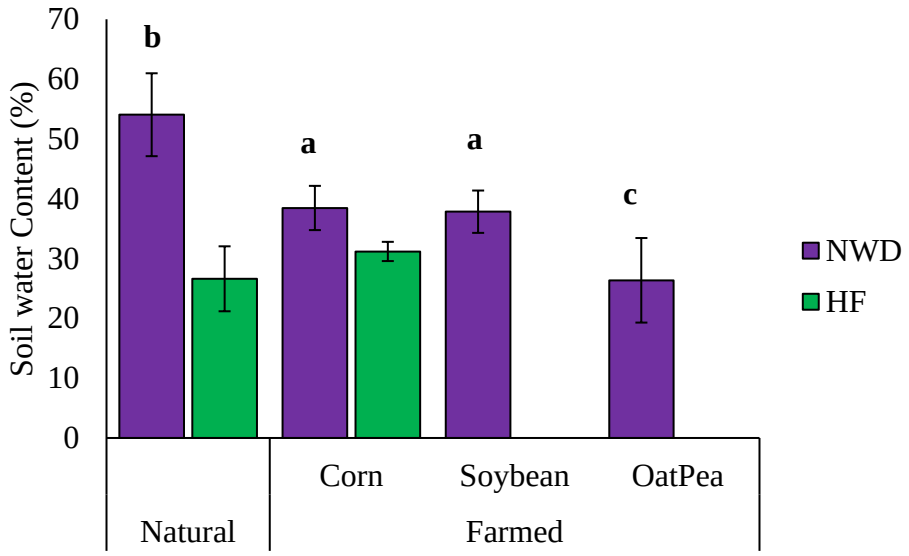

Figure 20 Soil water content at sampling for Experiment 2 (2017) New World Dairy (NWD) and Hammond Farms (HF) natural (*Nat.*) and farmed (*Ag.*) soil of 0-10 cm depth. Error term is CI_{95}. Letters represent post-hoc Tukey test (95% confidence) for NWD crop treatments.

Acidity

For both *Nat.* and *Ag.* soil, pH was significantly higher at HF than NWDb ($\bar{x}$=4.08, 6.14 vs. 3.55, 5.65 respectively) Table A3.11, Table A3.12). *Nat.* soil was more acidic than *Ag.* soil for both NWDb and HF ($\bar{x}$=3.55, 4.08 vs. 5.65, 6.14 respectively)

(Table A3.13, Table A3.14). *Ag.* soil pH ranged from 5.72 to 6.43 for HF and from 5.05 to 6.56 for NWDb. *Nat.* soil pH ranged from 4.21 to 4.19 at HF and from 3.26 to 3.71 at NWDb.

HF manure treated soil was more acidic ($\bar{x}$=6.07) than no manure soil ($\bar{x}$=6.23) however pH was not significantly different between manure and crop treatments at NWDb. Soil acidity was significantly different between cropping systems when farm was not considered; *Nat.* soil had the lowest pH ($\bar{x}$= 3.81) followed by all cropped soil (Figure 21) (Figure A3.15).

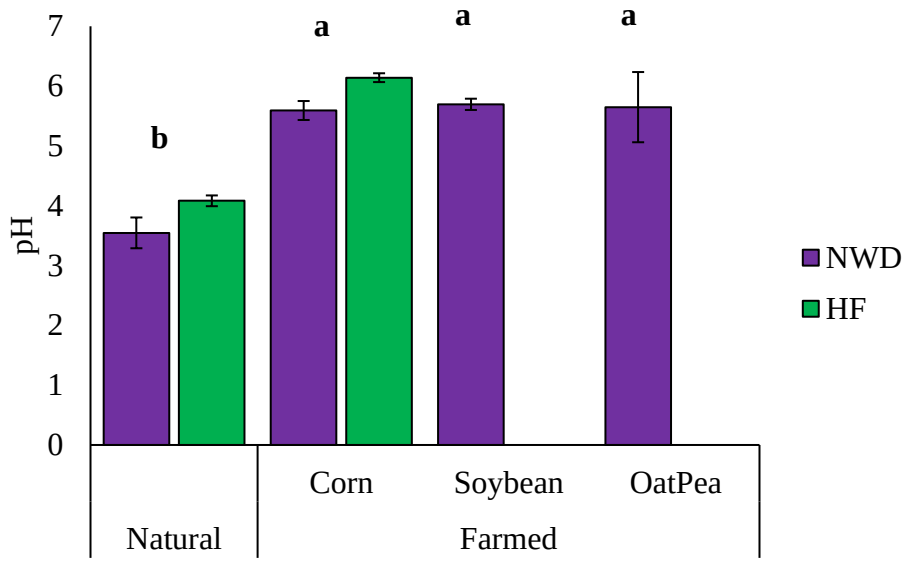

Figure 21 pH for Experiment 2 (2017) New World Dairy (NWD) and Hammond Farms (HF) natural (*Nat.*) and farmed (*Ag.*)soil of 0-10 cm depth. Error term is CI_{95}. Letters represent post-hoc Tukey test for crop irrespective of farm.

Soil Organic Carbon

While *Ag.* soil had more SOC at HF than NWDb ($\bar{x}$=4.29 vs. 3.75%) (Table A3.16), *Nat.* soil at NWDb had more SOC than at HF ($\bar{x}$=15.30 vs. 4.82%) (Table A3.17). SOC was not different between *Ag.* and *Nat.* treatments for HF. Conversely, SOC was

greater in *Nat.* soil than *Ag.* for NWDb ($\bar{x}$=15.3 vs.3.75%) (Table A3.18). *Ag.* soil SOC ranged from 3.06 to 5.23% for HF and from 5.05 to 5.84% for NWDb. *Nat.* SOC ranged from 4.03 to 5.39% for HF and from 7.35 to 24.05% fir NWDb.

SOC did not differ for manure or crop treatment at NWDb or HF (Figure 22).

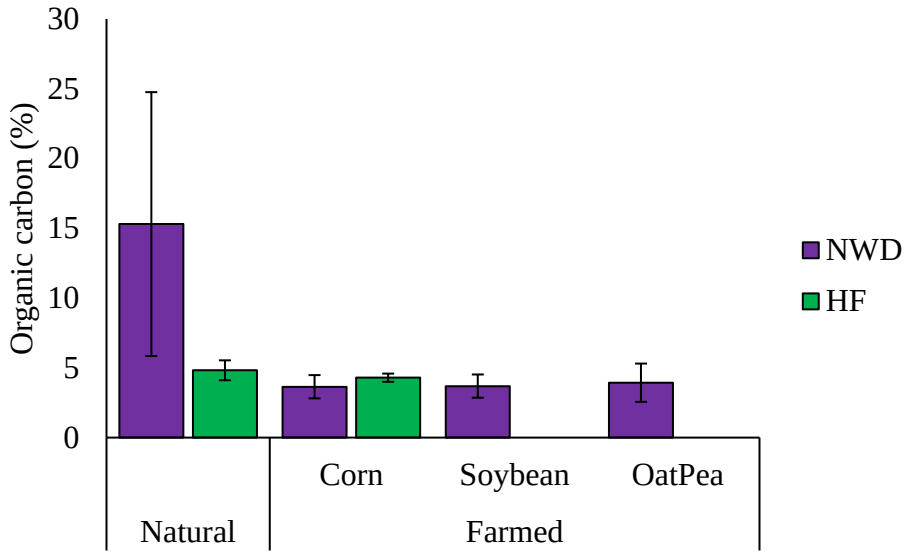

Figure 22 Organic carbon (%) for Experiment 2 (2017) New World Dairy (NWD) and Hammond Farms (HF) natural (*Nat.*) and farmed (*Ag.*) soil of 0-10 cm depth. Error term is CI_{95}

3.3.2.2 Soil elemental analysis

There were complex differences between farms, between *Nat.* and *Ag.* soils, and with manure and crop treatments (Figure 23).

Between farms

TC and TN were significantly higher in *Ag.* soil of HF than of NWDb ($\bar{x}$=42280, 3725 mg kg^{-1} vs. 32050, 2020 mg kg^{-1} respectively) (Table A3.19, Table A3.21). However, there was no significant difference in TC or TN between the *Nat.* soils of NWDb and HF.

Table 12 Summary of elemental composition statistical comparisons between farms for Experiment 2.

	Farm with higher concentration		
	NWD	HF	No difference between farms
Both Ag. and Nat.			
Ag.		TC, TN	
Nat.			TC, TN

Between *Ag.* and *Nat.* (land-use within farm)

Nat. soil had a greater TC ($\bar{x}$=88260 mg kg^{-1}) concentration than *Ag.* soil ($\bar{x}$=32050 mg kg^{-1}) at NWDb (Table A3.20). The same was not found for HF; there was no difference in TC between *Ag.* and *Nat.* treatments. There was significantly more TN in *Nat.* soil ($\bar{x}$=5100 mg kg^{-1}) than *Ag.* soil ($\bar{x}$=2020 mg kg^{-1}) at NWDb (Table A3.22). On the other hand, HF *Ag.* soil had more TN than *Nat.* soil ($\bar{x}$=3725 vs. 2580 mg kg^{-1}) (Table A3.23).

Table 13 Summary of elemental composition statistical comparisons between *Ag.* and *Nat.* soils of Experiment 2.

	Land use with higher concentration		
	Ag.	Nat.	No difference between Ag. and Nat.
Both NWD and HF			
NWD		TC, TN	
HF	TN		TC

Between manure and crop treatments

No manure soil had more TN than manured soil at HF ($\bar{x}$=4200 vs. 3336 mg kg^{-1}) but TN was not different between manure treatments at NWDb. TN was not significantly different between cropping systems at NWDb but was when farm was not considered. Oat & pea and soybean were not significantly different from each other but had less TN than

Nat. samples. TN in corn was not different than in other crop treatments (Figure 23). TC was not different for manure or crop treatment at HF or NWDb (Figure 23) (Table A3.24).

Elemental ranges

TC ranged from 31200 to 6600 mg kg^{-1} for HF *Ag.* soil and from 21900 to 54100 mg kg^{-1} for NWDb *Ag.* soil. TN ranged from 2600 to 6700 mg kg^{-1} at HF and from 1400 to 3300 mg kg^{-1} at NWDb.

TC ranged from 35000 to 56100 mg kg^{-1} for HF *Nat.* soil and from 48800 to 149200 mg kg^{-1} for NWDb *Nat.* soil. *Ag.* soil *Nat.* soil ranged in TN from 1900 to 3300 mg kg^{-1} at HF and from 3200 to 8100 mg kg^{-1} at NWDb.

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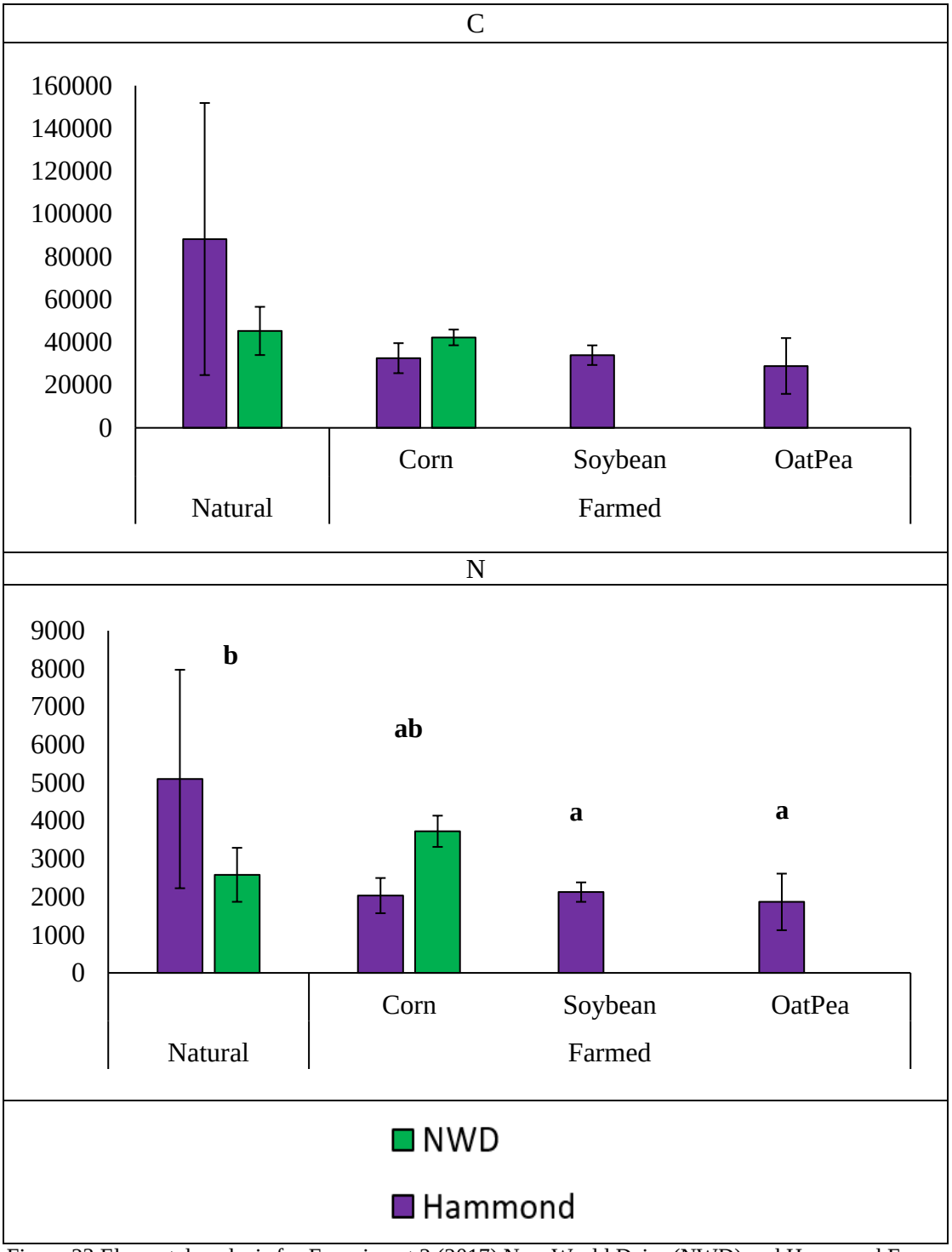
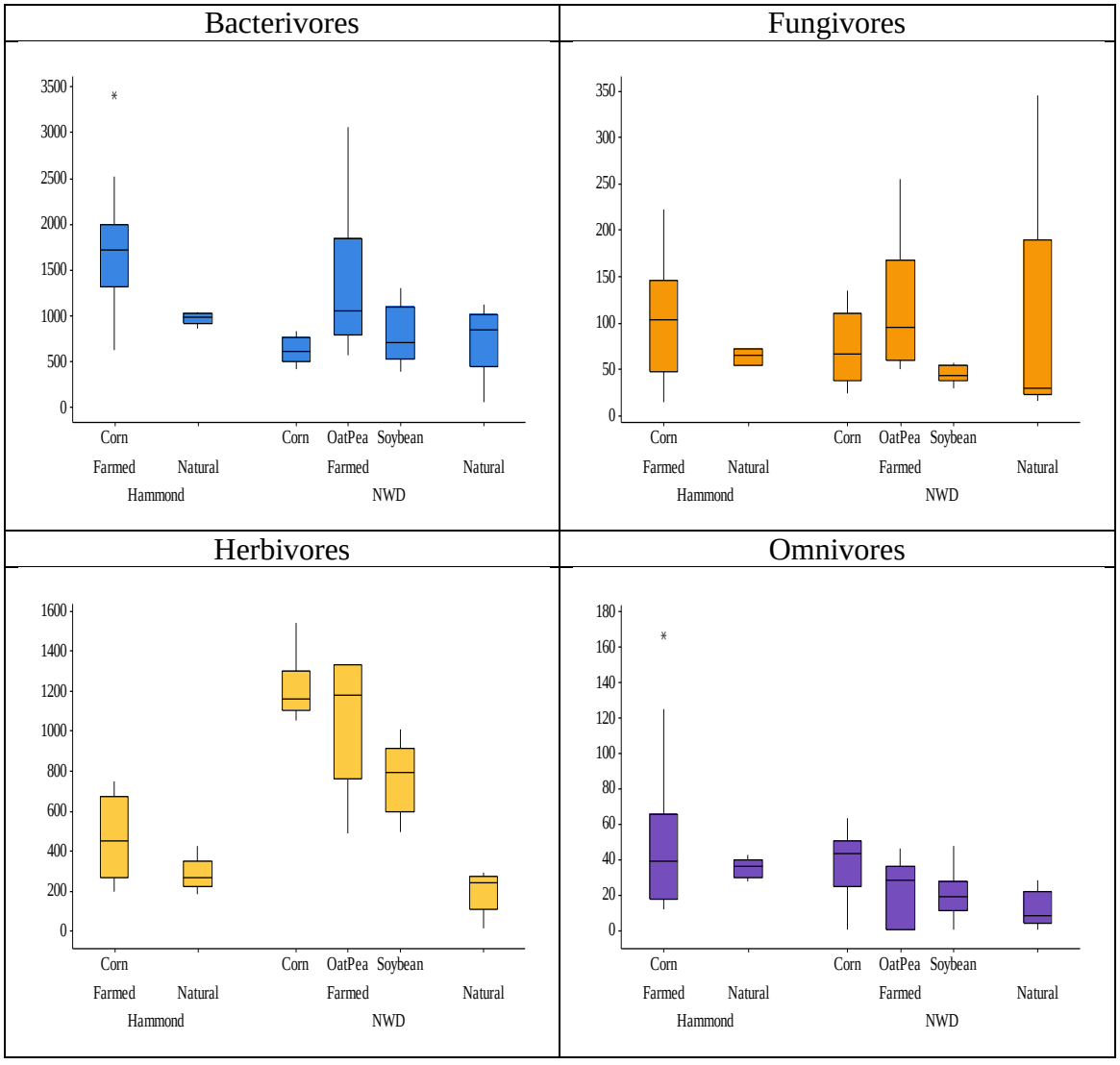


Figure 23 Elemental analysis for Experiment 2 (2017) New World Dairy (NWD) and Hammond Farms (HF) natural (*Nat.*) and farmed (*Ag.*) soil of 0-10 cm depth. Error term is CI_{95}. Letters represent post-hoc Tukey tests (95% confidence) for crop irrespective of farm.

3.3.2.3 Nematode composition

There were significantly more nematodes in *Ag.* soil than *Nat.* soil for both HF and NWDb ($\overline{x}$=2322, 2010 vs. 1375, 1208 individuals per 100 g dry soil respectively) (Table 14) (Table A4.1). There was no significant difference in total nematode abundance between farms for *Ag.* or *Nat.* soil.

Nematode abundance was significantly different between cropping treatments in *Ag.* soil at NWDb (Figure 26) (Table A4.2) and when farm was not considered (Table 15, Figure 24, Figure 25) (Table A4.3).


Bacterivores
3500
3000
2500
2000
1500
1000
500
0
*
Corn
Farmed
Natural
Hammond
Corn
OatPea
Soybean
Farmed
Natural
NWD
Fungivores
350
300
250
200
150
100
50
0
Corn
Farmed
Natural
Hammond
Corn
OatPea
Soybean
Farmed
Natural
NWD
Herbivores
1600
1400
1200
1000
800
600
400
200
0
Corn
Farmed
Natural
Hammond
Corn
OatPea
Soybean
Farmed
Natural
NWD
Omnivores
180
160
140
120
100
80
60
40
20
0
*
Corn
Farmed
Natural
Hammond
Corn
OatPea
Soybean
Farmed
Natural
NWD

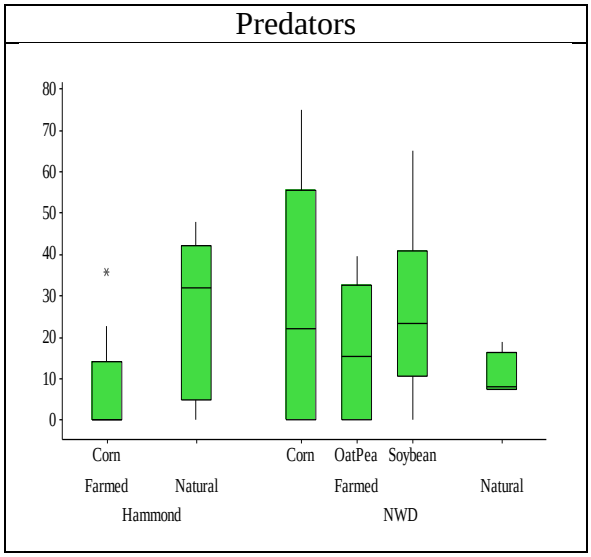


Figure 24 Boxplots of nematode trophic composition (number of individuals per 100 g dry soil) for Experiment 2 (2017) soil of farmed (*Ag.*) and natural (*Nat.*) soil of New World Dairy (NWD) and Hammond Farms (HF). * indicates an outlier.

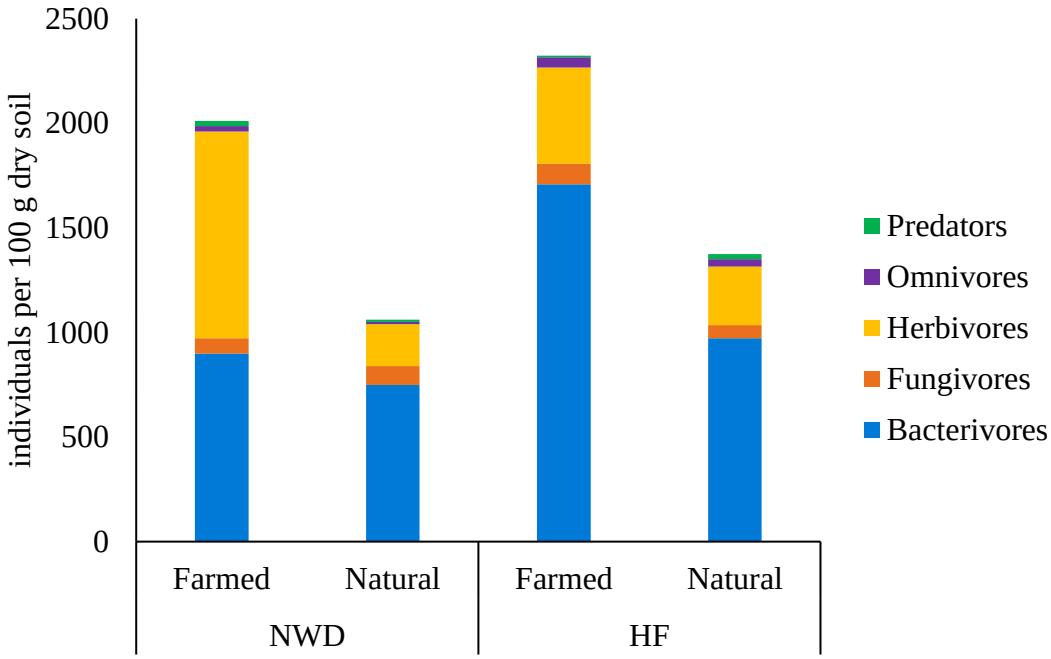


Figure 25 Nematode community composition for Experiment 2 (2017) farmed (*Ag.*) and natural (*Nat.*) soil of New World Dairy (NWD) and Hammond Farms (HF).

Table 14 Nematode abundance for Experiment 2 (2017) *farmed (Ag.) and natural (Nat.)* soil of Hammond Farms (HF) and New World Dairy (NWDb). Error term is CI_{95}.

Farm	Land Use	Number of nematodes/100g dry soil
HF	Farmed	2323±336
	Natural	1375±180
NWDb	Farmed	2010±306
	Natural	1208±148

Table 15 Nematode abundance for Experiment 2 (2017) crop treatment irrespective of farm. Error term is CI_{95}.

Crop	Number of nematodes/100g dry soil
Corn	2240±267
Oat & pea	2552±822
Soybean	1638±405
Natural	1292±109

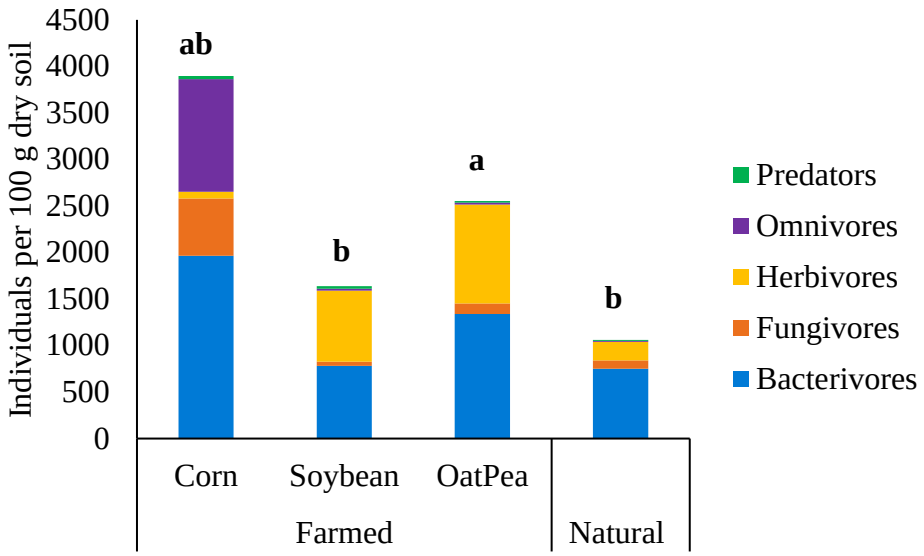


Figure 26 Nematode community composition for Experiment 2 (2017) *farmed (Ag.) and natural (Nat.)* soil of New World Dairy. Letters represent post-hoc Tukey test (95% confidence) for total number of nematodes.

3.3.2.4 Microarthropod composition

Total arthropod abundance was significantly higher in *Ag.* soil at HF ($\bar{x}$=30 individuals per 1 kg soil) than at NWDb ($\bar{x}$=11 individuals per 1 kg soil). Conversely, NWDb had more arthropods in *Nat.* soil ($\bar{x}$=58 individuals per 1 kg soil) than HF ($\bar{x}$=23 individuals per 1 kg soil) (Table A5.1). There were significantly more microarthropods in *Nat.* than *Ag.* soil at NWDb ($\bar{x}$=58 vs. 11 individuals per 1 kg soil) (Table A5.2) but the same was not true for HF.

Manure treatment of *Ag.* soil had a significant impact at NWDb but not HF; NWDb manure soil had fewer individuals than no manure soil ($\bar{x}$=8 vs. 14 individuals per 1 kg soil) (Table 17). Crop had significant impact on arthropod abundance for NWDb (Table A5.4) and when farm was not considered; *Nat.* soil had greater arthropod abundance ($\bar{x}$=40 individuals per 1 kg soil) than corn, oat & pea, and soybean soil for NWDb (Table 16) (Table A5.5).

Table 16 Experiment 2 (2017) microarthropod order abundance and total counts (individuals kg^{-1} dry soil) of farmed (*Ag.*) and natural (*Nat.*) soil for New World Dairy (NWDb) and Hammond Farms (HF). Error term is CI_{95}.

		Acari	Collembola	Coleoptera	Diptera	Hemiptera	Pseudoscorpions
NWDb	*Ag.*	5.68±2.13	2.22±1.2	0.741±0.58	2.72±1.15	0	0
	Nat.	28.9±23.80	14.81±10.18	2.96±3.73	10.37±4.52	0	0
HF	*Ag.*	16.17±6.42	7.78±2.00	1.11±0.98	4.44±1.62	0	0
	Nat.	5.93±5.97	0.741±1.71	6.67±9.92	7.41±5.98	0	0

		Isopoda	Araneae	Geophilomorpha	Orthoptera	Total Count
NWD	*Ag.*	0	0	0	0	11.36±2.88
	Nat.	0	0	0	0.74±1.71	57.80±29.40
HF	*Ag.*	0	0.12±0.25	0	0	29.63±7.50
	Nat.	0	0	2.22±2.56	0	22.96±12.84

Table 17 Experiment 2 (2017) average microarthropod abundance for manure and no manure New World Dairy soil. Error term is CI_{95}.

	Number of arthropods (individuals per kg dry soil)
Manure	8.40±3.70
No manure	14.32±4.37

3.3.2.5 Exploratory and explanatory statistics

3.3.2.5.1 Western Newfoundland nematode analysis

Results show that both farm and crop had an impact on the nematode population structure (Figure 25, 26). While not drastically distinct, the crop type seemed to have a consistently similar effect on population structure (Figure 26). A PERMANOVA analysis confirmed that both farm and crop had a statistically significant role (Table 14, 15). The interaction was not significant suggesting similar community structure behaviour for the same crops independent of farm (Table 15). Manure status was not relevant, either on its own or in the interaction terms with farm or crop (Table 14, 16). Further PERMANOVA

analysis confirmed the impact of farm and crop (Table 21, Table 22) and the lack of impact of manure treatment (Table 23). Most NWD treatments had significantly different nematode populations than HF treatments; NWD, no manure, oat & pea treatment was not statistically different from HF manure and no manure corn treatments. NWD *Nat.* nematode community structure was not distinct from HF *Nat.* community structure (Table 17).

A redundancy analysis (RDA) showed that the best explanatory parameters for nematode community composition were percent sand ($p=0.002$), TN ($p=0.004$), and pH ($p=0.024$) (Figure 29). All three parameters together explained 28.9% of variation in nematode composition but failed to explain 71.1% (Figure 30). Analysis showed that bacterivore abundance was most closely related to percent sand while number of fungivores was most related to pH (Figure 29).

While variation in TN was significantly negatively related to omnivore abundance in *Nat.* soil, bacterivore abundance was weakly positively related to variation in TN for all crops (Figure 31). Bacterivore abundance was significantly positively related to variation in TN for NWD but not HF (Figure 32). Conversely, herbivore abundance was weakly negatively related to variation in TN for all crops (Figure 31) and was significantly negatively related to variation in TN in NWD soil (Figure 32). Bacterivores and herbivores were significantly affected by TN in both manure and no manure soil when farm or crop was not considered, a positive relationship for the former and negative relationship for the later (Figure 33). A linear relationship between total nematode abundance and TN is not clear (Figure 36).

Although not significant at an alpha threshold of 0.05, there was a general negative relationship (i.e. larger alpha values of <0.1 or <0.2) between pH and the abundance of omnivore, herbivores, fungivores, or predators in corn soil (Figure 31). While omnivore, fungivore, predator, and bacterivore nematodes abundances were weakly negatively related to variation in pH for HF, herbivores was weakly positively related (Figure 32). At NWD, omnivore, herbivore, and fungivore abundance was weakly positively related to variation in pH (Figure 32). In manured lands there is a significant relationship between variation in pH and herbivore abundance and a significant positive relationship with fungivore and bacterivore abundance. In no manure soil there was a significant negative relationship between predator abundance and soil pH (Figure 33). There was a trend of increasing total nematode abundance with pH (Figure 35).

Omnivore, fungivore, predator, and bacterivore abundances were weakly negatively related to variation in percent sand (Figure 32) however there was little difference in nematode response to variation in percent sand with manure status (Figure 33). There was a trend of increasing total nematode abundance with percent sand (Figure 34).

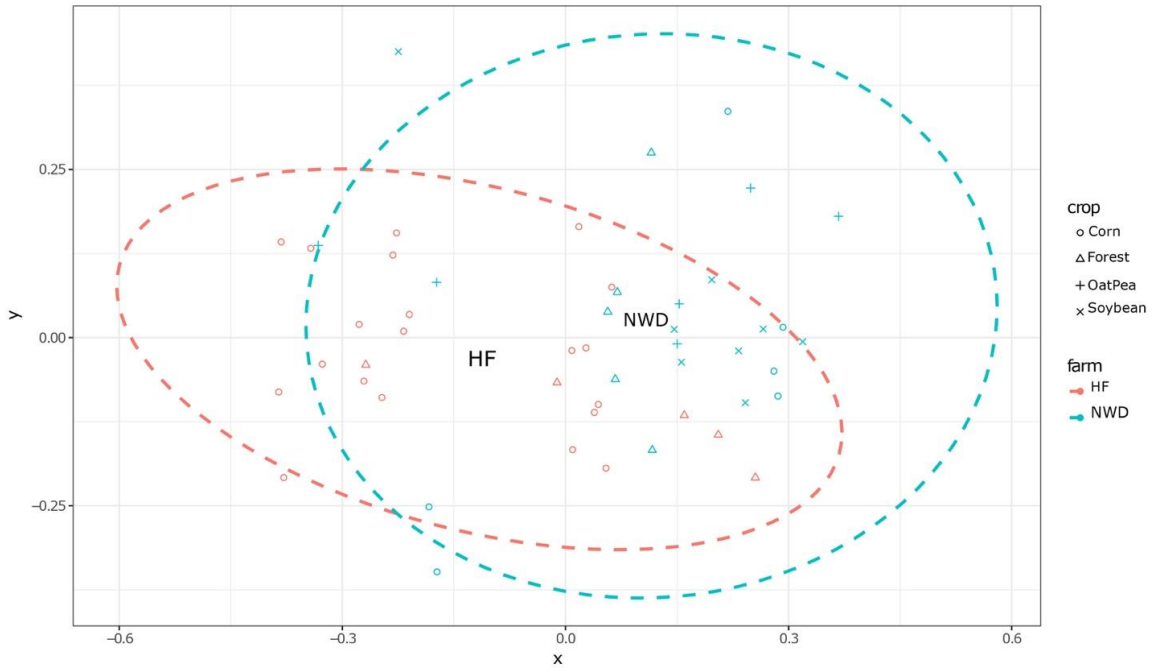


Figure 27. Non-metric multi-dimensional scaling (NMDS) ordination displaying farm and crop for Experiment 2 (2017) nematodes.

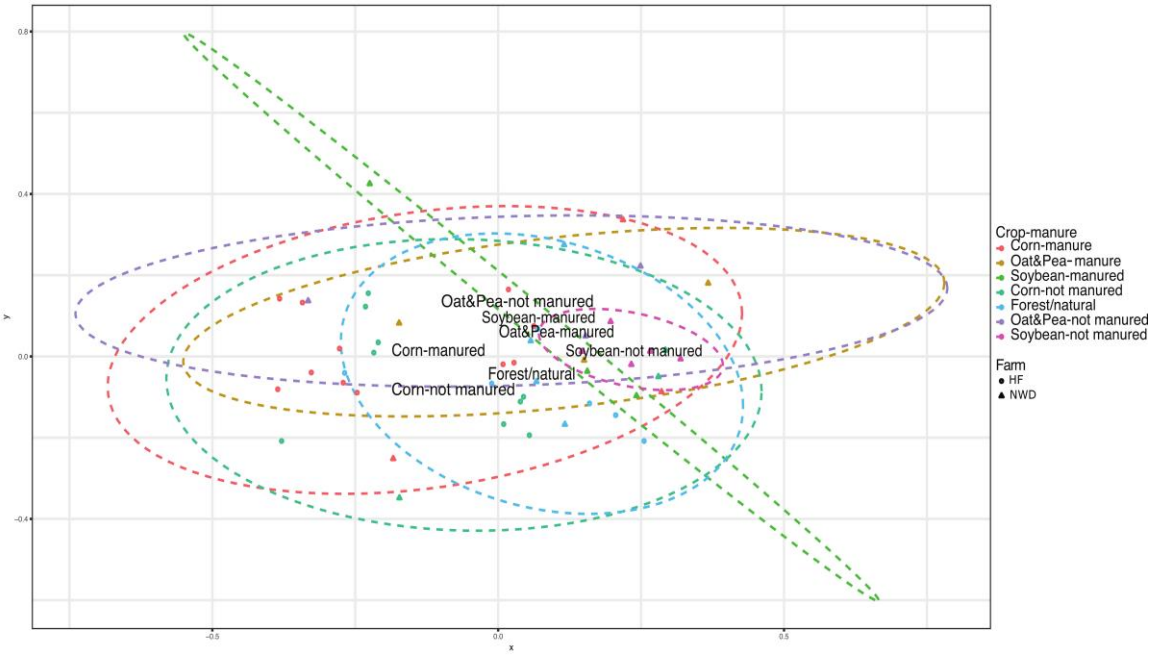


Figure 28 Non-metric multi-dimensional scaling (NMDS) ordination displaying farm and crop-manure treatments for Experiment 2 (2017) nematodes.

Table 18 Two way PERMANOVA of nematode community composition for farm and manure treatment of Experiment 2 (2017) soil. Permutation 9999.

	SS	DF	MS	F	P-value
Farm	0.79	1	0.79	17.51	**<0.001**
Manure	0.036	1	0.036	0.81	0.42
Interaction	-0.10	1	-0.10	-2.33	0.25
Residual	2.06	46	0.045		
Total	2.78	49			

Table 19 Two way PERMANOVA of nematode community composition for farm and crop of Experiment 2 (2017) soil. Permutation 9999.

	SS	DF	MS	F	P-value
Farm	0.79	1	0.79	12.12	**<0.001**
Crop	0.79	3	0.26	4.06	**<0.001**
Interaction	-1.52	3	-0.51	-7.80	0.98
Residual	2.72	42	0.06		
Total	2.78	49			

Table 20 Two way PERMANOVA of nematode community composition for manure and crop of Experiment 2 (2017) soil. Permutation 9999.

	SS	DF	MS	F	P-value
Manure	0.04	1	0.03	0.44	0.48
Crop	0.79	3	0.26	3.18	**<0.001**
Interaction	-1.52	3	-0.51	-6.13	0.97
Residual	3.47	42	0.08		
Total	2.78	49			

Table 21 One way PERMANOVA of nematode community composition for full treatment (combination of farm, manure treatment, crop treatment) of Experiment 2 (2017) soil. H=Hammond Farms, W=New World Dairy, M=manure, NM=no manure, C=corn, F=forest, S=soybean, O=oat & pea.

Permutation N	9999
Total SS	2.78
Within-group SS	0.96
F	8.40
P (same)	**<0.001**

Bray Curtis dissimilarity matrix

	H_NM_C	H_M_C	H_NM_F	W_NM_C	W_M_C	W_NM_S	W_M_S	W_NM_O	W_M_O	W_NM_F
H_NM_C		0.40	**0.009**	**0.004**	**0.005**	**0.004**	**0.014**	0.19	**0.010**	**0.005**
H_M_C	0.40		**0.005**	**0.003**	**0.003**	**<0.001**	**0.001**	0.10	**0.003**	**<0.001**
H_NM_F	**0.009**	**0.005**		**0.019**	**0.017**	**0.007**	**0.019**	**0.019**	**0.017**	0.09
W_NM_C	**0.004**	**0.003**	**0.019**		0.31	**0.018**	0.102	0.20	0.80	**0.017**
W_M_C	**0.005**	**0.003**	**0.017**	0.31		**0.017**	0.10	0.10	0.39	**0.018**
W_NM_S	**0.004**	**<0.001**	**0.007**	**0.018**	**0.017**		**0.035**	0.05	0.55	**0.008**
W_M_S	**0.014**	**0.001**	**0.019**	0.10	0.10	**0.035**		0.10	0.10	**0.017**
W_NM_O	0.19	0.10	**0.019**	0.20	0.10	0.05	0.10		0.30	**0.018**
W_M_O	**0.010**	**0.003**	**0.017**	0.80	0.39	0.55	0.10	0.30		**0.018**
W_NM_F	**0.005**	**<0.001**	0.09	**0.017**	**0.018**	**0.008**	**0.017**	**0.018**	**0.018**	

Table 22 One way PERMANOVA of nematode communities for farm in Experiment 2 (2017) soil.

Permutation N	9999
Total SS	2.78
Within-group SS	1.99
F	18.89
P (same)	**<0.001**

Bray Curtis dissimilarity matrix

	HF	NWD
HF		**<0.001**
NWD	**<0.001**	

Table 23 One way PERMANOVA of nematode communities for manure treatment in Experiment 2 (2017) soil.

Permutation N	9999
Total SS	2.78
Within-group SS	2.74
F	0.64
P (same)	0.56

Bray Curtis dissimilarity matrix

	No manure	Manure
No manure		0.56
Manure	0.56	

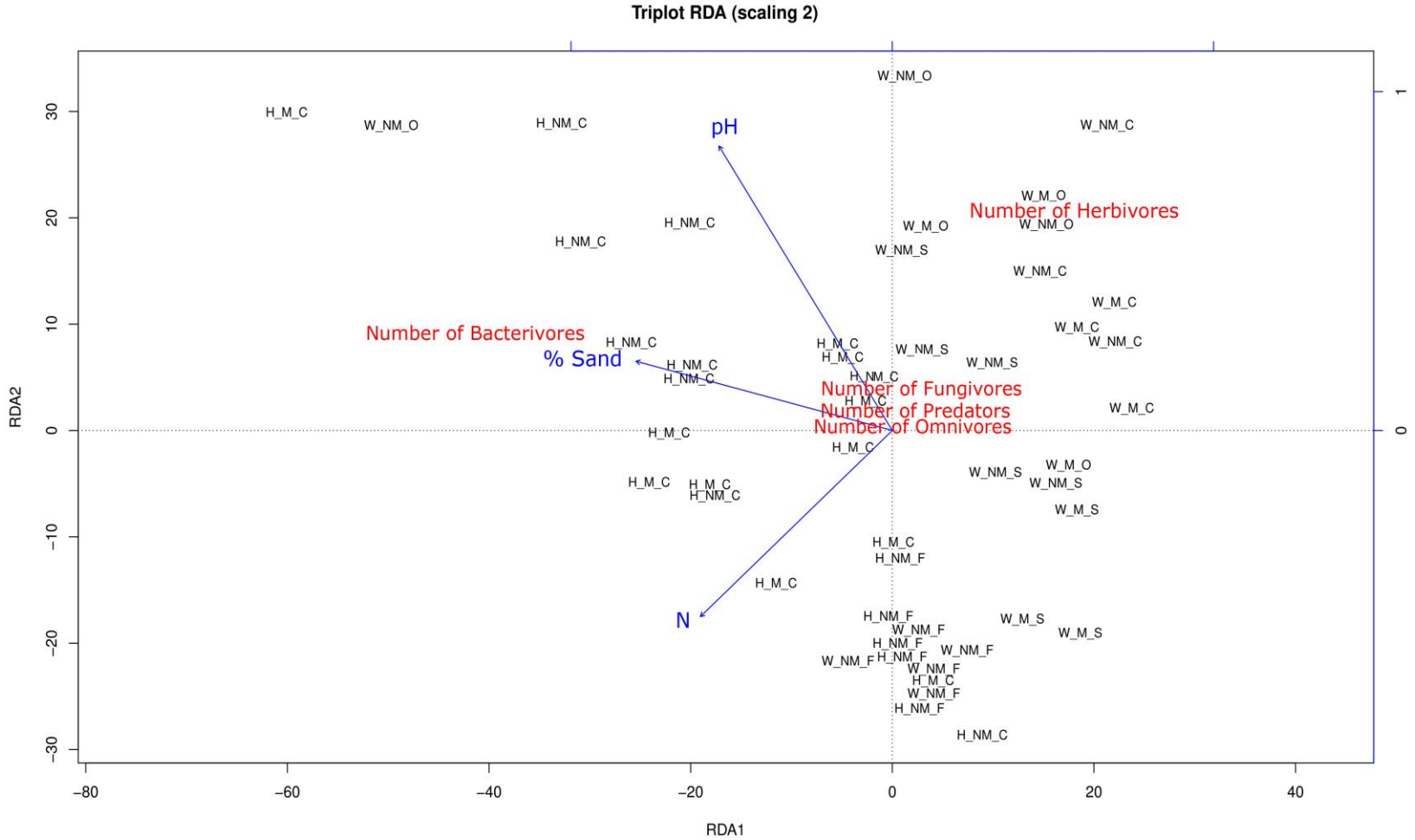


Figure 29 Redundancy Analysis (RDA) triplot of relationship of nematode community composition and environmental variables in Experiment 2 (2017) soil. Labels describe the farm (W=NWD and H=HF), manure status (M=manured, NM=not manured) and the crop (S=soybean, C=corn, O=oat/pea).

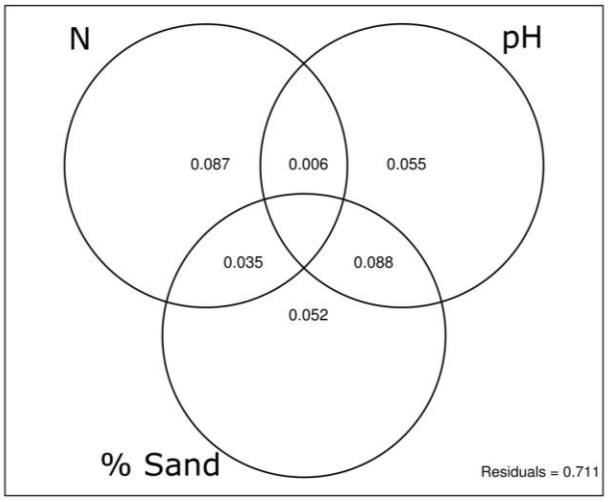


Figure 30 Impact of abiotic environmental parameters on the partition of the variation in nematode composition in Experiment 2 (2017) soil. Only factors identified as significant ($p \leq 0.05$) are presented here.

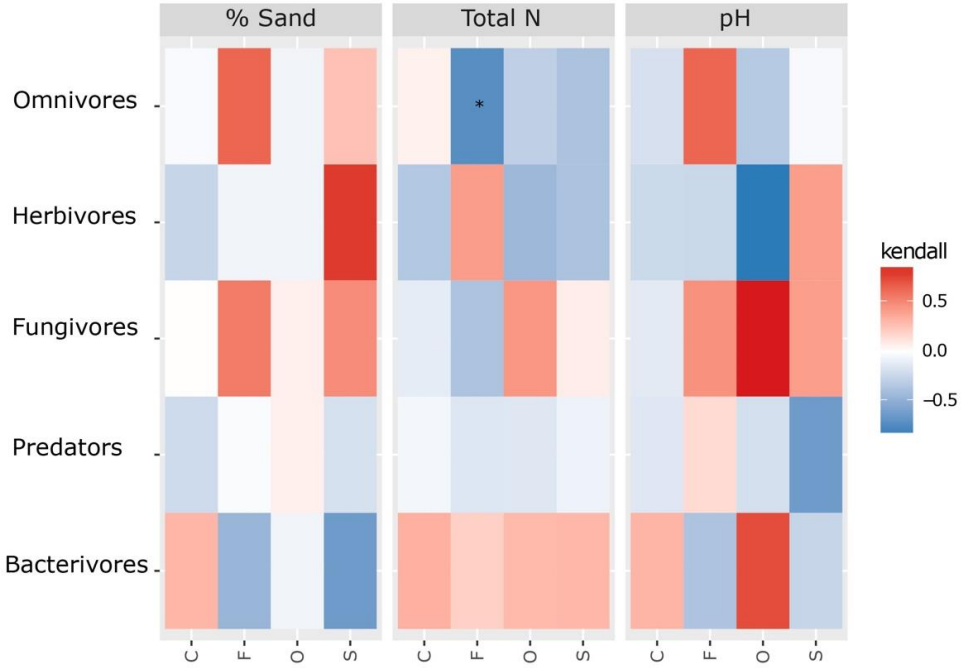


Figure 31 Correlation matrices for nematode composition and environmental variables of crop (corn-C, natural/forest-F, Oat & pea-O, soybean-S) of Experiment 2 (2017) soil. *$P \leq 0.05$.

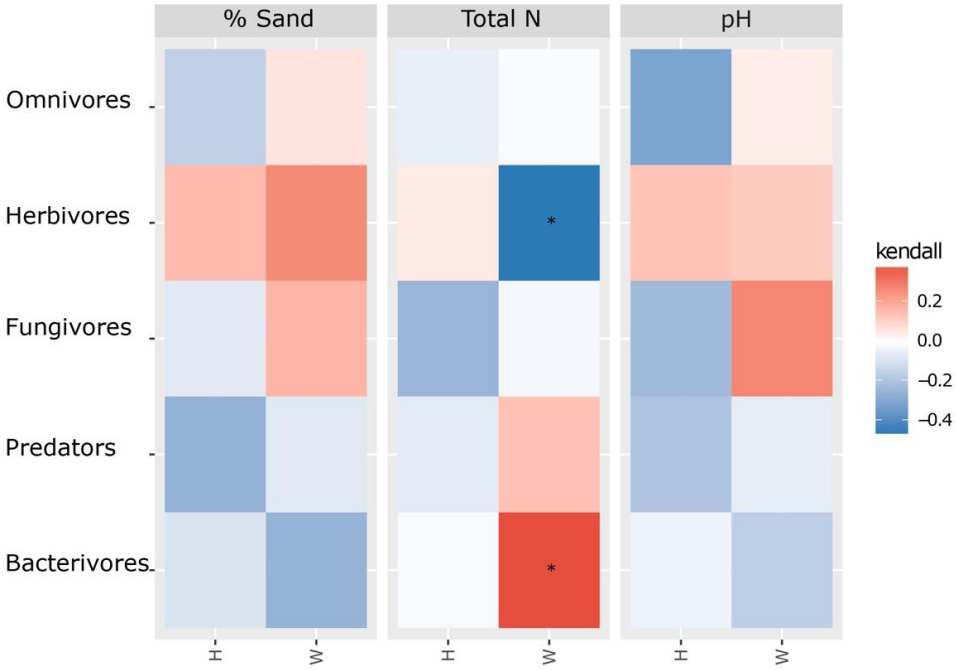


Figure 32 Correlation matrices for nematode composition and environmental variables of farm (Hammond Farms-H, New World Dairy-NWD) of Experiment 2 (2017) soil. *P≤0.05.

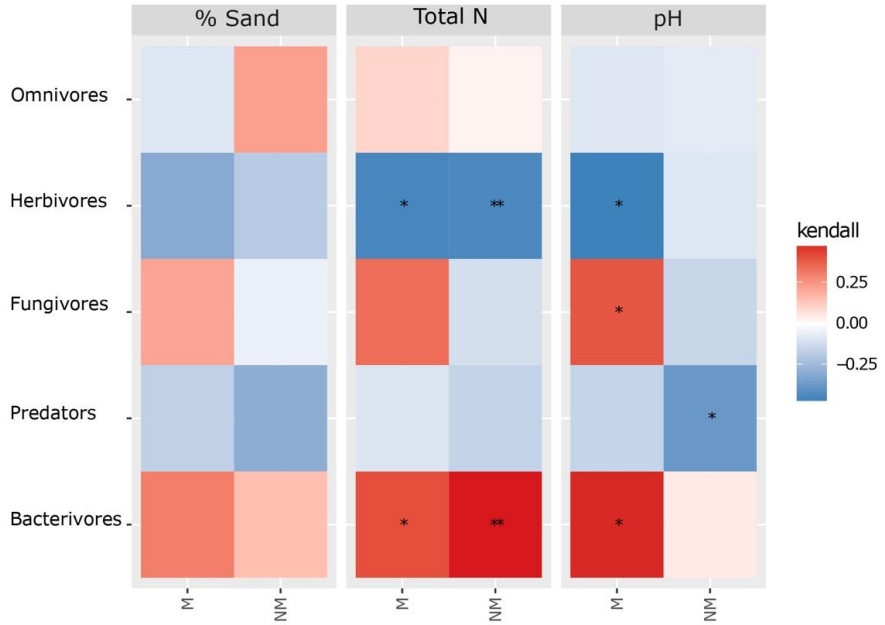


Figure 33 Correlation matrices for nematode composition and environmental variables of manure treatment (Manured-M, No Manured-NM) of Experiment 2 (2017) soil. *P≤ 0.05, **P≤0.01.

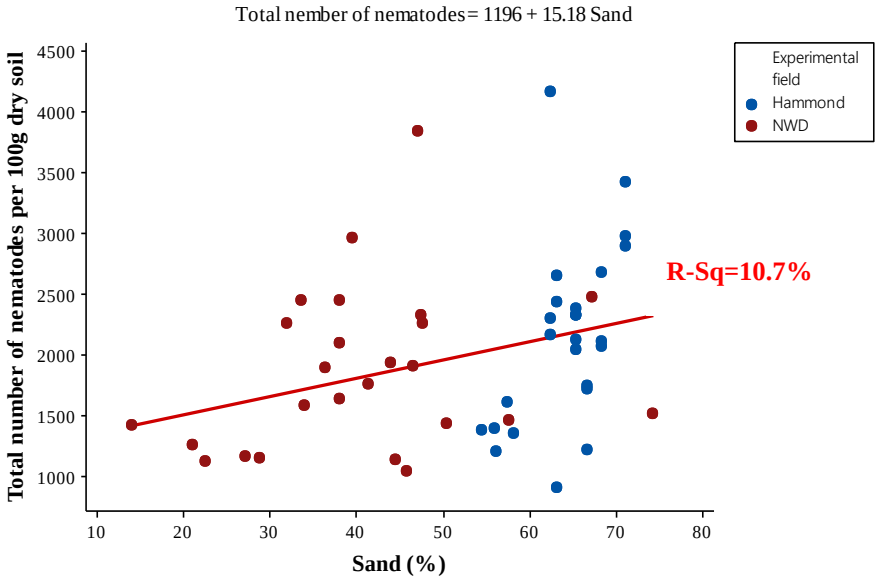


Figure 34 Regression analysis for Experiment 2 (2017) nematode abundance (individuals per 100 g dry soil) for New World Dairy (NWD) and Hammond Farms (HF) with percent sand.

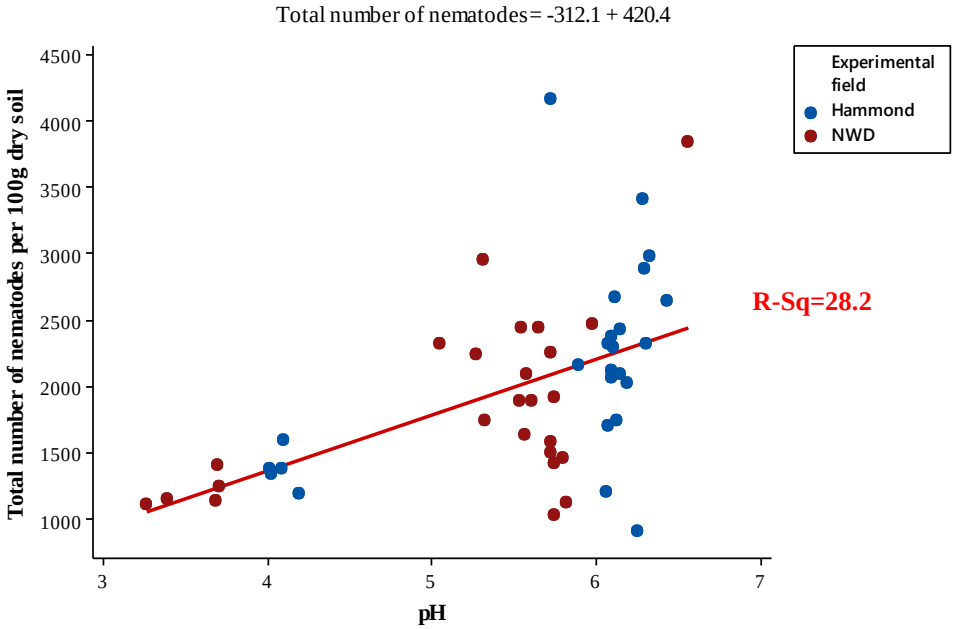


Figure 35 Regression analysis for Experiment 2 (2017) nematode abundance (individuals per 100 g dry soil) for New World Dairy (NWD) and Hammond Farms (HF) with pH.

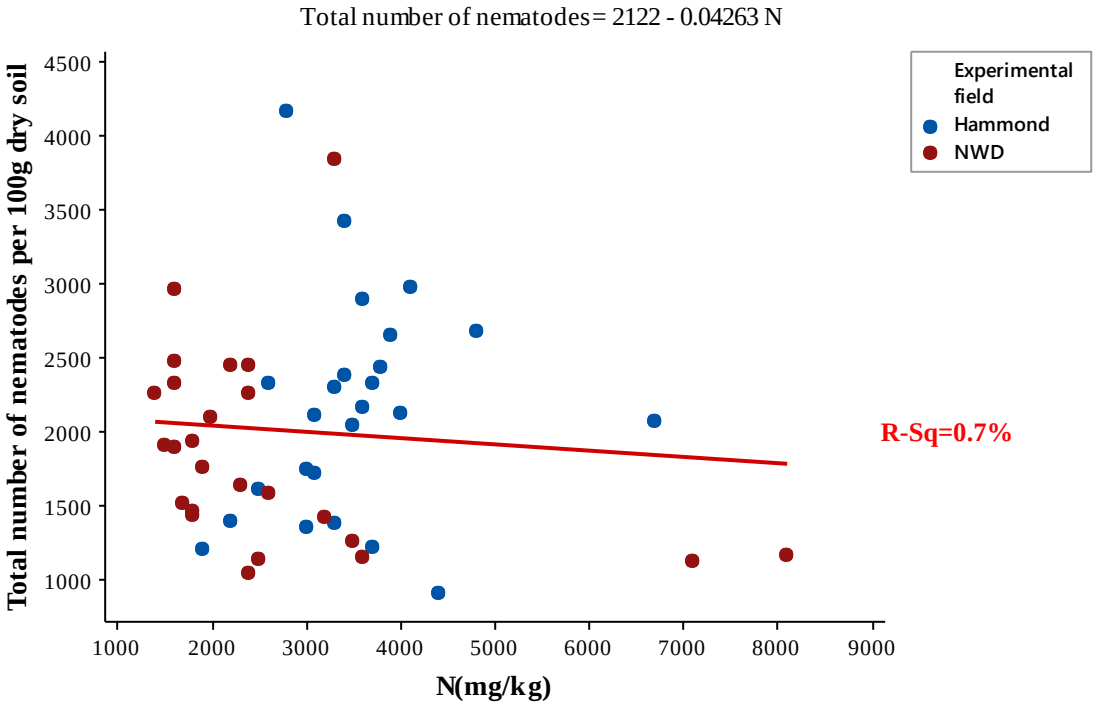


Figure 36 Regression analysis for Experiment 2 (2017) nematode abundance (individuals per 100 g dry soil) for New World Dairy (NWD) and Hammond Farms (HF) with total nitrogen (mg kg^{-1}).

3.3.2.5.2 Western Newfoundland microarthropod analysis

Both exploratory and explanatory statistics were carried out. Exploratory statistics were done to assess the differences driven by community structure according to site, farm or crop. Note that the Newfoundland arthropod data was normalized by Hellinger transformation to eliminate the effect of excessive zeroes prior to analysis. For the RDA analyses soil parameters were normalized in units of standard deviation around the mean to eliminate the impact of the different units commonly employed for various soil parameters.

Initial results did not show a strong impact of farm, crop, or manure on the population structure (Figure 35, 36, 37). Though PERMANOVA analysis indicated that

farm, crop, and manure had a statistically significant role (Table 24, Table 25, Table 26). Further PERMANOVA analysis confirmed the impact of farm, crop, and manure (Table 28, Table 29,Table 30). The interaction between farm and manure, and farm and crop was significant suggesting that microarthropod community structure behaviour is not the same at each farm for manure or crop treatments (Table 24, Table 25) Additionally, the significant interaction between crop and manure suggests that microarthropod community behavior is not the same for manure treatments for each independent crop (Table 26). There was no statistical difference in the arthropod populations between soybean and oat & pea crops (Table 30). Microarthropod communities were not different with manure treatment in HF corn soil. The arthropod community in HF, no manure, corn cropped soil was not different than in NWDb *Nat.* soil. Additionally, *Nat.* soil was sufficiently distinct between NWDb and HF (Table 27).

RDA analysis showed that the best explanatory variables for microarthropod composition were total N ($p=0.002$) and pH ($p<0.001$). Both parameters only explained 6.6% of the variability in arthropod composition but failed to explain 93.4% (Figure 40).

Orthoptera, Geophilomorpha, Coleoptera, and Aranea abundances were significantly negatively related to variation in TN for corn while Collembola was significantly positively related. Geophilomorpha and Aranea were significantly negatively related to TN in *Nat.* soil but not in oat & pea or soybean soil. Most microarthropod groups were weakly positively related to total N in oat & pea soil (Figure 41). Orthoptera, Geophilomorpha, and Aranea were significantly negatively related to variation in TN at NWDb but not at HF (Figure 42). Collembola was significantly

positively related to variation in total N at HF (Figure 42). Orthoptera and Geophilomorpha were significantly negatively related to variation in TN for both manure and no manure treatments (Figure 43), however, Coleoptera was significantly negatively related to total N for manure soil while Aranea was negatively related to total N for no manure soil (Figure 43). Total nematode abundance showed an increasing trend with TN (Figure 45).

Most microarthropod order profiles were weakly negatively related to soil pH in *Ag.* soil but showed a more positive trend in *Nat.* soil (Figure 41). Geophilomorpha abundance was significantly positively related to variation in pH in *Nat.* but not in cropped soil. Acari subclass was significantly related to variation in acidity but only in oat & pea soil (Figure 41). Arthropod groups showed an overall positive trend with variation in pH for both farms with the exception of Diptera. Collembola was significantly positively related to variation in pH at HF but not NWDb (Figure 42). Although not significant, Orthoptera, Geophilomorpha, and Aranea abundances were negatively correlated to variation in pH in manure soil but positively in no manure soil (Figure 43). There was no clear linear relationship between total nematode abundance and pH (Figure 44).

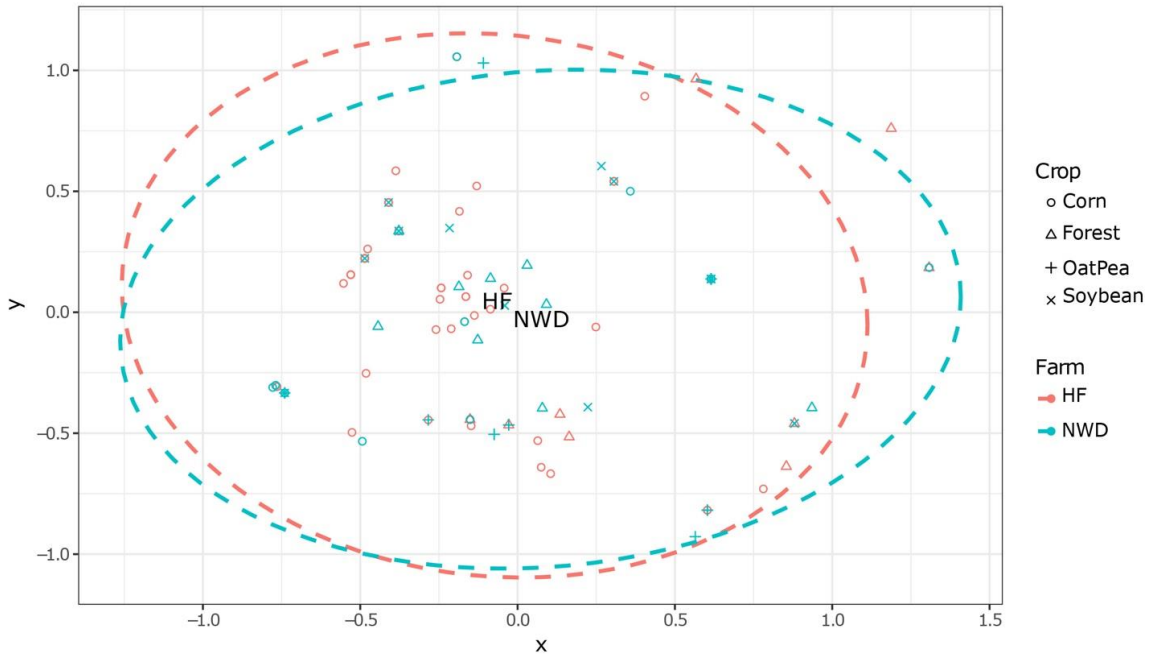


Figure 37 Non-metric multi-dimensional scaling (NMDS) ordination displaying farm and crop for Experiment 2 (2017) microarthropods.

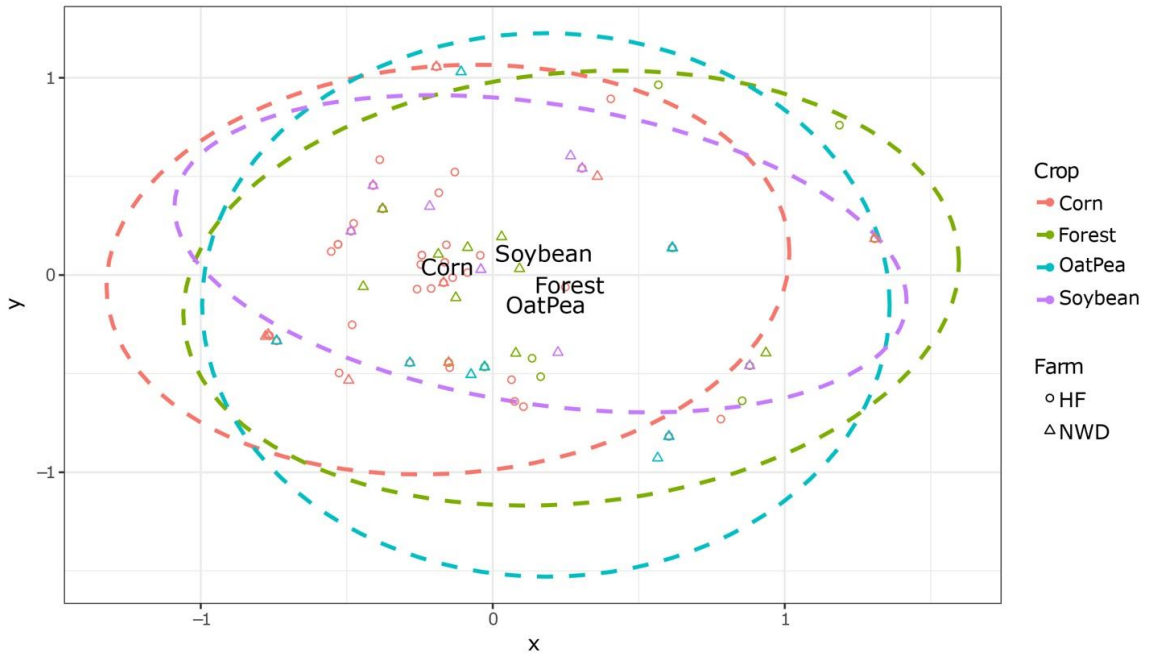


Figure 38 Non-metric multi-dimensional scaling (NMDS) ordination displaying crop and farm for Experiment 2 (2017) microarthropods.

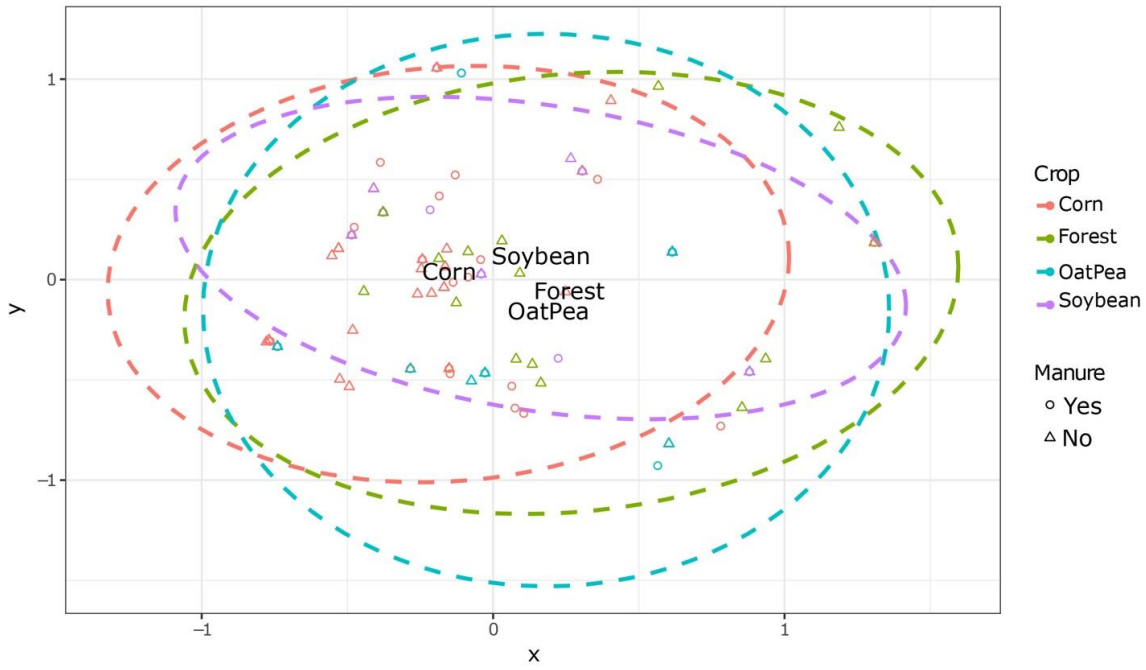


Figure 39 Non-metric multi-dimensional scaling (NMDS) ordination displaying crop and manure for Experiment 2 (2017) microarthropods.

Table 24 Two way PERMANOVA of microarthropod community composition for farm and manure treatment of Experiment 2 (2017) soil. Permutation 9999.

	SS	DF	MS	F	P-value
Farm	1.38	1	1.38	4.91	**0.002**
manure	0.81	1	0.81	2.90	**0.019**
Interaction	-0.29	1	-0.29	-1.04	**0.027**
Residual	34.19	122	0.28		
Total	36.09	125			

Table 25 Two way PERMANOVA of microarthropod community composition for farm and crop of Experiment 2 (2017) soil. Permutation 9999.

	SS	DF	MS	F	P-value
Farm	1.38	1	1.38	2.61	**<0.001**
Crop	2.73	3	0.91	1.73	**<0.001**
Interaction	-30.31	3	-10.10	-19.14	**0.001**
Residual	62.29	118	0.53		
Total	36.09	125			

Table 26 Two way PERMANOVA of microarthropod community composition for crop and manure treatment of Experiment 2 (2017) soil. Permutation 9999.

	SS	DF	MS	F	P-value
Crop	1.38	1	1.38	4.91	**0.001**
manure	0.81	1	0.81	2.90	**0.012**
Interaction	-0.29	1	-0.29	-1.04	**0.023**
Residual	34.19	122	0.28		
Total	36.09	125			

Table 27 One way PERMANOVA of microarthropod community composition for full treatment (combination of farm, manure treatment, crop treatment) of Experiment 2 (2017) soil. H=Hammond Farms, W=New World Dairy, M=manure, NM=no manure, C=corn, F=forest, S=soybean, O=oat & pea.

Permutation N	9999
Total SS	36.09
Within-group SS	30.03
F	2.60
P (same):	**<0.001**

Bray Curtis dissimilarity matrix

	H_NM_C	H_M_C	H_NM_F	W_NM_C	W_M_C	W_NM_S	W_M_S	W_NM_O	W_M_O	W_NM_F
H_NM_C		0.11	**<0.001**	**0.002**	**<0.001**	**0.053**	**0.004**	**<0.001**	**<0.001**	0.14
H_M_C	0.11		**0.003**	0.13	**0.052**	0.423	**0.068**	0.06	**0.010**	0.30
H_NM_F	**<0.001**	**0.003**		**0.009**	**0.036**	**0.045**	0.15	0.15	**0.010**	**0.031**
W_NM_C	**0.002**	0.13	**0.009**		0.15	0.13	0.08	0.14	**0.048**	**0.002**
W_M_C	**<0.001**	**0.052**	**0.036**	0.15		0.79	0.83	0.17	0.88	**0.009**
W_NM_S	**0.053**	0.42	**0.045**	0.13	0.79		0.72	0.23	0.55	**0.047**
W_M_S	**0.004**	**0.068**	0.15	0.08	0.83	0.72		0.35	0.71	**0.035**
W_NM_O	**<0.001**	0.06	0.15	0.14	0.17	0.23	0.35		0.18	**0.011**
W_M_O	**<0.001**	**0.010**	**0.010**	**0.048**	0.88	0.55	0.71	0.18		**0.004**
W_NM_F	0.14	0.30	**0.031**	**0.002**	**0.009**	**0.047**	**0.035**	**0.011**	**0.004**	

Table 28. One way PERMANOVA of microarthropod communities for farm in Experiment 2 (2017) soil.

Permutation N	9999
Total SS	36.09
Within-group SS	34.71
F	4.91
P (same):	**0.001**

Bray Curtis dissimilarity matrix

	HF	NWD
HF		**0.001**
NWD	**0.001**	

Table 29 One way PERMANOVA of microarthropod communities for manure treatment in Experiment 2 (2017) soil.

Permutation N	9999
Total SS	36.09
Within-group SS	35.27
F	2.86
P (same):	**0.025**

Bray Curtis dissimilarity matrix

	No manure	Manure
No manure		**0.025**
Manure	**0.025**	

Table 30 One way PERMANOVA of microarthropod communities for crop in Experiment 2 (2017) soil.

Permutation N	9999
Total SS	36.09
Within-group SS	33.35
F	3.33
P (same):	**<0.001**

Bray Curtis Dissimilarity matrix

	Corn	Forest	Soybean	Oat & pea
Corn		**0.003**	**0.043**	**0.002**
Forest	**0.003**		**0.012**	**0.005**
Soybean	**0.043**	**0.012**		0.55
Oat & pea	**0.002**	**0.005**	0.55	

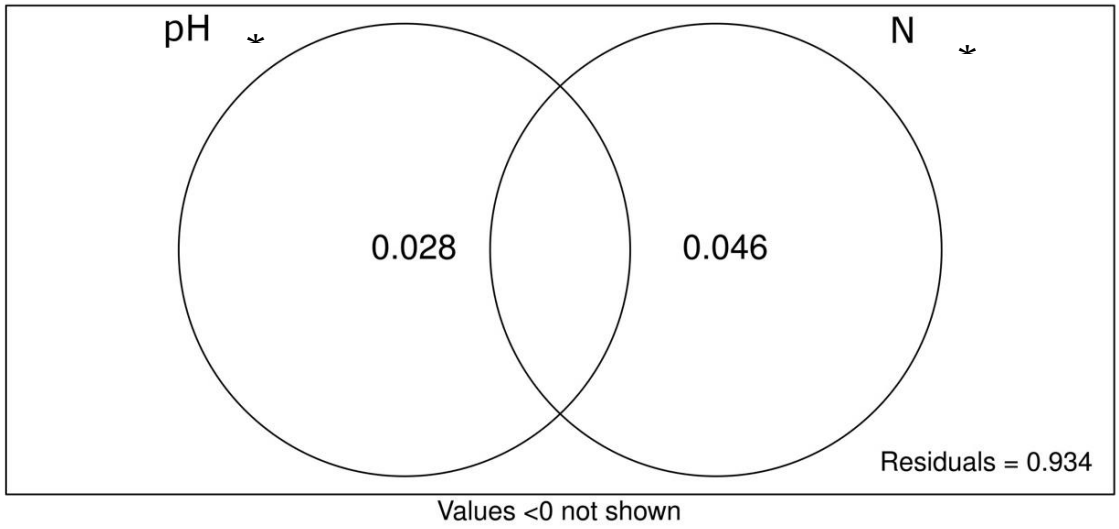


Figure 40 Impact of abiotic environmental parameters on the partition of the variation in microarthropod composition in Experiment 2 (2017) soil. Only factors identified as significant are presented here.

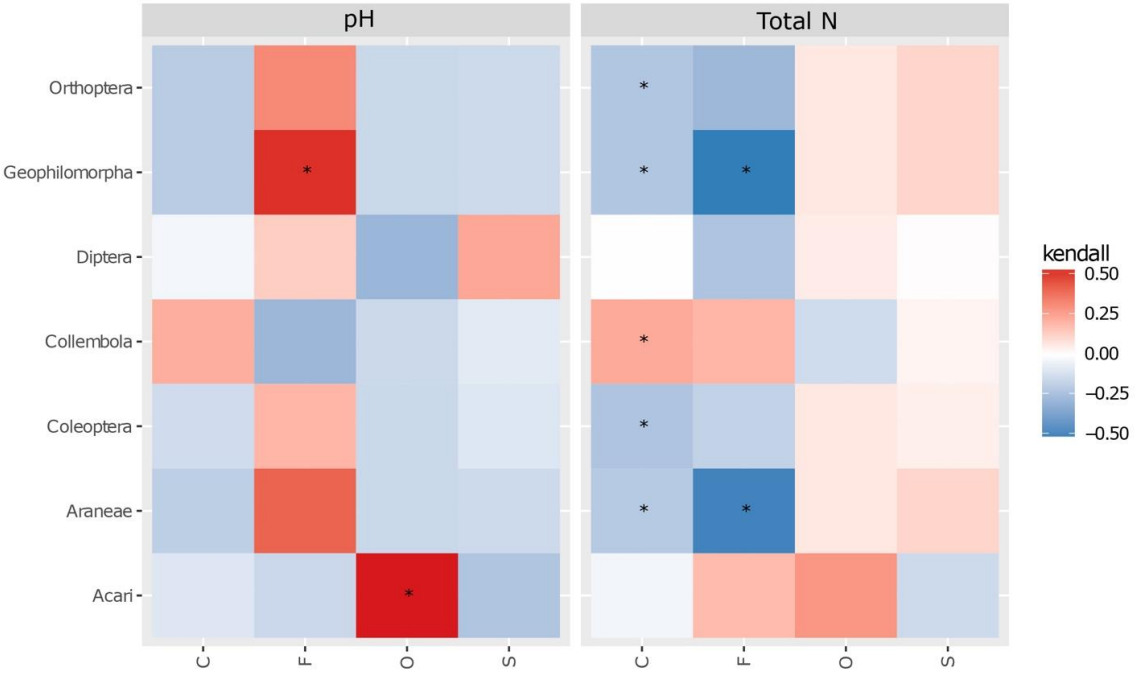


Figure 41 Correlation matrices for arthropod composition and environmental variables of crop (corn-C, natural/forest-F, oat & pea-O, soybean-S) of Experiment 2 (2017) soil. *P<0.05, **P< 0.01, ***P<0.001.

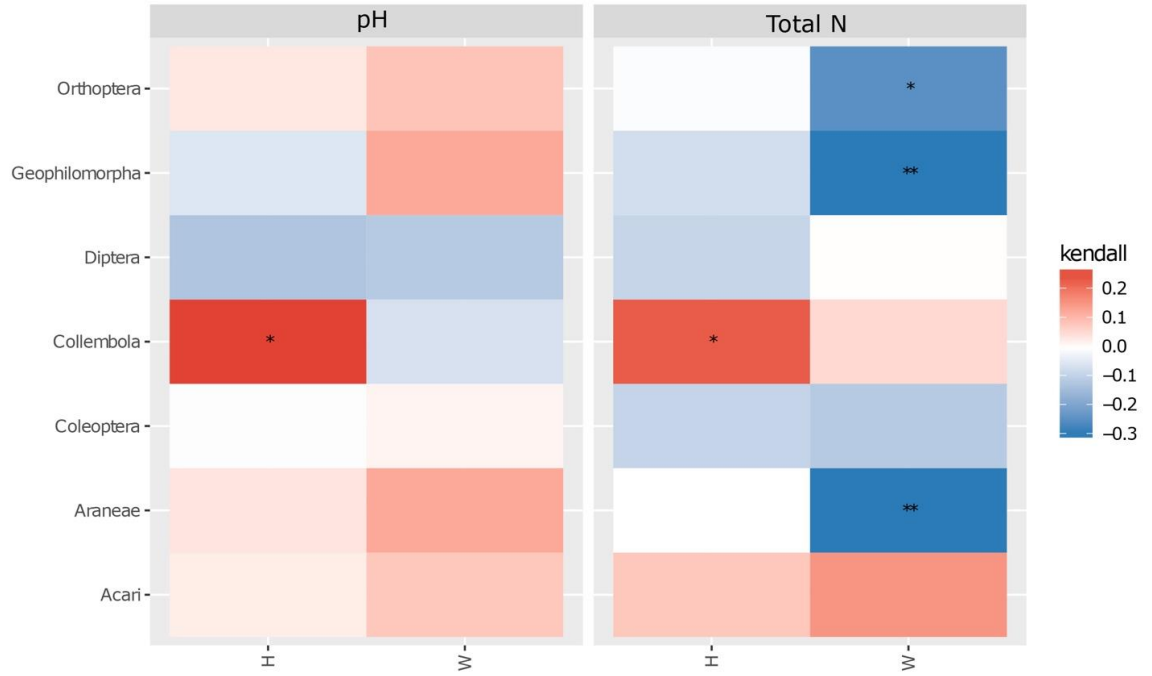


Figure 42 Correlation matrices for arthropod composition and environmental variables of farm (Hammond Farms-H, New World Dairy-NWD) of Experiment 2 (2017) soil. *P<0.05, **P< 0.01.

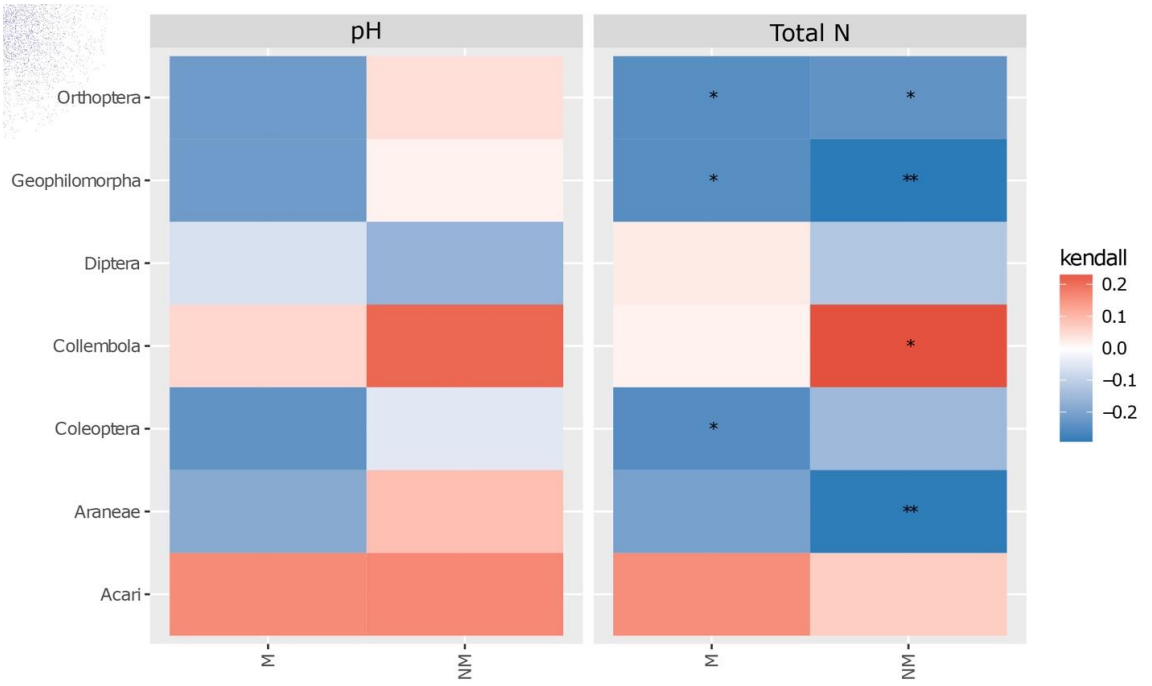


Figure 43 Correlation matrices for arthropod composition and environmental variables of manure treatment (Manured-M, no manured-NM) of Experiment 2 (2017) soil. *P<0.05, **P< 0.01.

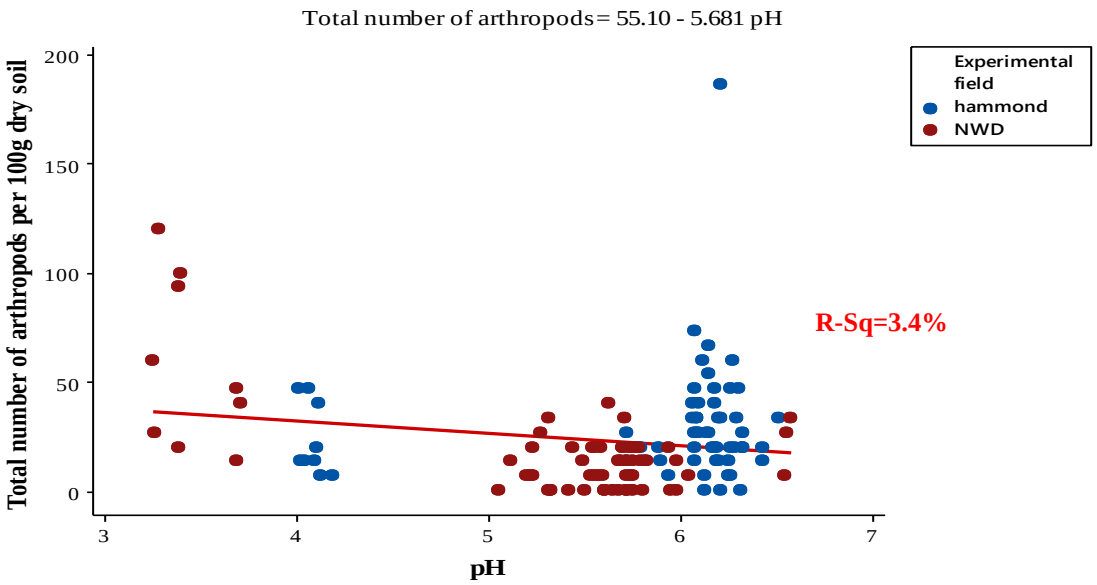


Figure 44 Regression analysis for Experiment 2 (2017) microarthrpod abundance (individuals per 1 kg dry soil) for New World Dairy (NWD) and Hammond Farms (HF) with pH.

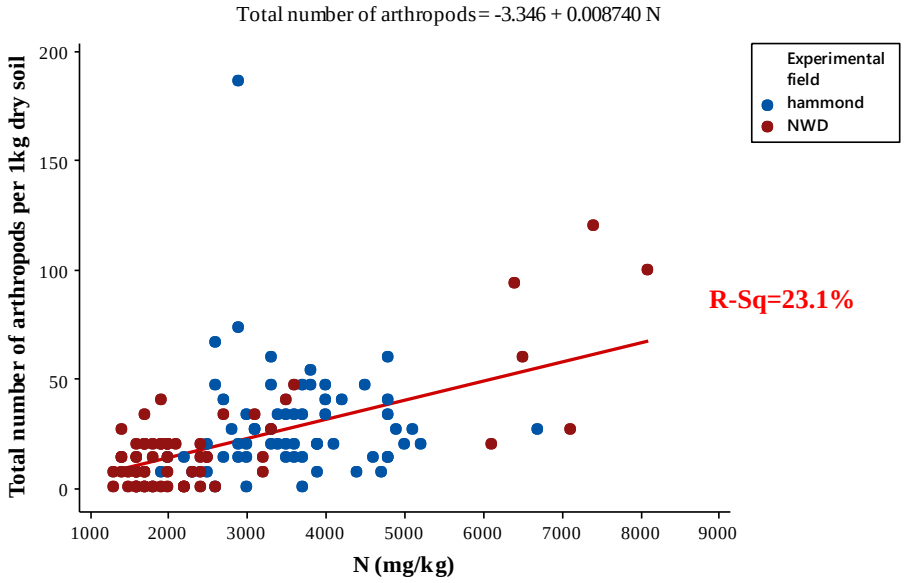


Figure 45 Regression analysis for Experiment 2 (2017) microarthrpod abundance (individuals per 1 kg dry soil) for New World Dairy (NWD) and Hammond Farms (HF) with total nitrogen (mg kg^{-1}).

3.4 Discussion for Experiment 1 and 2: Survey of arthropods and nematodes in soils of dairy farms in western Newfoundland

3.4.1 Soil characterisation

Textural analysis of soils from Experiment 1 and Experiment 2, for both NWD and HF, has shown all samples to be classified as sandy loam. For NWD *Ag.*, NWD *Nat.*, and HF *Ag.* soils the texture became sandier with depth. Podzols typically have medium and coarse textures with high compaction at depth (Sanborn et al., 2011). For both HF *Ag.* and *Nat.* soils the texture was sandier than the respective NWD equivalents. *Ag.* soil was more sand enriched than *Nat.* soil for both farms in Experiment 2. In addition, although slope was not explicitly measured, the experimental fields had undulating areas of variable sloping with some >4% at HF. This allowed opportunity for natural and agriculture-induced soil erosion and thus susceptibility of the higher Al and Fe oxide concentrations of the deeper B horizon to be brought closer to surface. As sloping areas were explicitly avoided during our sampling events this could not be confirmed with our data.

Soil compaction begins to effect plant root growth at greater than 1.40-1.60 g cm^{-3} (USDA, 2014). Soil was not overly compact based on bulk density values at both HF and NWD for neither *Ag.* nor *Nat.* soil.

Low soil pH negatively impacts crop growth and soil biota (Korthals et al., 1996a). Limestone amendments are commonly employed to correct the pH in agricultural fields. While liming history is not fully known for the test farms, however, it is known that both have received lime during their history. Thus, the higher pH of the *Ag.* soils vs. the *Nat.* soils (Figure 12, Figure 21) at all locations, and corroboration with the higher Ca

concentration in the same *Ag.* soils (Figure 14), is very likely indicative of past liming. Liming was reported to impede fungal-mediated decomposition of organic matter and to reduce the abundance of soil herbivore nematodes (Wang et al., 2015). The HF soils had higher pH, by about 0.5 pH units, than the NWD soils for both the *Nat.* and *Ag.* conditions. However, the concentrations of soil Ca were not statistically different between farms for either soil conditions suggesting that the variation in pH between farms might not be entirely due to recent liming activities. Nitrification and utilisation of ammonium by plants and soil microorganisms of ammonium of various origins, including both from manure and ammonium nitrate, which is commonly used in the province, are known to increase soil acidity (Bolan et al., 1991). NWD and HF locations are historically very well manured and are regularly topped up with mineral fertilizers. HF manured soil was significantly more acidic than no manure soil, this trend was not seen in NWD manure treated soil; the latter must be contextualised in the overall already lower pH in these soils. In addition, manure application can lead to P, Zn, and Cu accumulation in soil (Parham et al., 2002; Mantovi et al., 2003); this was confirmed by the higher concentration of these elements in the *Ag.* soils than in the *Nat.* soils for both farms. Significant Na in podzols is not naturally present but can originate from livestock manure (Manitoba, 2015). While the Na concentration was not statistically different between *Ag.* and *Nat.* soils within each farm it was significantly higher in the *Ag.* at NWD than HF emphasising the, likely management driven, distinctiveness of the two locations. SAR was higher in *Nat.* soil than *Ag.* soil for both Experiment 1 locations.

Natural podzols decrease in soil organic carbon (SOC) with depth (Sanborn et al., 2011); over 35% of carbon is often lost from podzols when soil is converted and brought into cultivation (Vandenbygaart et al., 2003). SOC concentration was significantly lower with depth or was lower in the deepest *Ag.* soil samples for Experiment 1 confirming the impacts of recent organic inputs and plant residues in the upper soil layer. Experiment 2 fields did not show variation in SOC with manure application, which may be due to residual impacts of continuous excessive manuring in past years. HF *Ag.* soil had more SOC than NWD *Ag.* soil.

3.4.2 Western Newfoundland nematodes

Initial analysis indicated that HF and NWD had distinct nematode community composition. Moreover, the corn crop induced nematode community structures were distinct from the other crops, this was more obvious for HF. On the other hand, when both farm data were commonly analysed, there was no assessed statistically relevant interaction between crop and manure. This result is possibly obscured by the overall individuality between farms. A PERMANOVA analysis confirmed the distinction between farms and, critically, confirmed that while the manure treatment did not significantly impact nematode composition, crop did. Although not statistically significant, *Ag.* soil had more nematodes at HF than NWD, HF *Ag.* soil had higher pH, more sand, SOC, total C, and N than NWD *Ag.* soil, probably indicating a more favorable environment for nematode survival. This was further confirmed by redundancy analysis that indicated pH was the best explanatory variable for nematode composition. As manure was not relevant on its own or with its interaction with farm or crop it is expected that the history of high manure applications at

the experimental locations influenced the nematode community structure. The one-year manure versus treatments without manure, as implemented during the experimental period, could not overcome the uniformity induced by long term manuring.

A detailed look at the role of the crop has shown oat & pea treatment to have significantly more nematodes than soybean at NWD. The denser surface cover of oat & pea, versus the relatively patchy cover by corn and soybean (see Methodology) presumably led to greater water removal and variations in soil aeration. Thus while nematode distribution and abundance is sensitive to soil water content (Hu et al., 2016), it is not expected that the differences in this region are sufficient to affect nematode population. On the other hand, root density favours nematode abundance.

Dissimilarity indices indicated that the overall nematode population structure was distinct between the two farms. It is likewise important to note that for *Nat.* soils both the nematode community structure and nematode abundance parameters were similar for both farms despite the determination that *Nat.* soil at NWD was less sandy, more acidic, and had more SOC than at HF. Furthermore, a dissimilarity analysis indicated that, within each farm, the nematode composition was distinct between the *Nat.* and *Ag.* soils with significantly greater nematode abundance in *Ag.* soils (Table 14). The nematode populations are thus impacted by agriculture intensification and might have benefited from practices, including liming, as discussed above.

A redundancy analysis has shown that the best explanatory parameters for nematode community composition aside from pH were percent sand and TN. These three parameters explained 28.9% of variation in nematode composition. Bacterivore abundance was most

closely related to percent sand while number of fungivores was most related to pH. These results align with previous reports that show soil nematode composition to vary with texture (Ferris and Bernard, 1971), and also the positive relationship between pH and fungivore abundance (Zhang et al. 2016). While SOC was expected to be a strong explanatory variable (Bongers and Ferris, 1999), this was not confirmed in these experiments. Though SOC speciation was not analysed for this project, it is quite likely that the amounts of SOC in *Ag.* soil are at or above the satisfactory threshold for nematode survival (SOC ranged from 3.06 to 24.05% in *Nat.* and from 3.06 to 5.84% in *Ag.* soil).

Variation in total N in *Nat.* soil was significantly and inversely related to omnivore abundance; Tenuta and Ferris (2004) indicated that, in N solutions, omnivores and predators were most sensitive to ion and osmotic tension effects. Bacterivores were weakly, positively related to total N for all crops and were significantly and directly related to total N in NWD soil but not in HF. The bacterivore/fungivore ratio has been reported to increase with application of nitrogen fertilizer (Azpilicueta et al., 2014). Rapidly mineralising N rich organic materials have been reported to reduce plant parasitic nematode abundance as nematicidal compounds accumulate in soil during decomposition (Akhtar and Malik, 2000); in NWD soil, herbivores were significantly inversely related to total N and weakly inversely related to total N with all crops irrespective of farm. Variation in pH was inversely related to omnivore, herbivore, fungivore and predator abundance in corn cropped soil irrespective of farm, although not significantly. At HF, omnivore, fungivore, predator, and bacterivore nematode abundances were weakly inversely related to pH. Omnivores and predators have been previously reported to be particularly sensitive to acidification (Ruess

et al., 1996). Predators and omnivores had the lowest abundance of all trophic groups, a result expected given their position in the trophic chain. Variation in percent sand was weakly inversely related to omnivore, fungivore, predator, and bacterivore abundance in HF soil; furthermore, a correlation analysis confirmed the lack of relationship between nematode population structure response and the interaction between sand and manuring status. However, the more dominant bacterivores and herbivores were significantly correlated to total N. The relationship was direct for the former and inverse for the latter. For manured lands there was a significant inverse correlation between pH and herbivore abundance but a significant positive correlation between pH and the abundance of fungivores and bacterivores. For the non-manured soil, there was a statistically significant inverse correlation between pH and predator abundance, a finding that too needs to be contextualised with the relative minor differences in pH.

Throughout these complex webs of correlations between environmental soil parameters and nematodes it was most evident that bacterivores dominated the soil nematode communities for all tested conditions. This may be interpreted as indicating that while N mineralisation is high, C is cycled very rapidly through the system via the bacterial community (Ingham et al., 1985; Ferris et al., 1997) and thus might not be available to higher trophic levels. High-input systems, like those found in Newfoundland, have low soil fauna diversity and employ preferentially bacterial driven decomposition pathways (Bardgett and Cook, 1998).

3.4.3 Western Newfoundland microarthropods

Discriminant analysis has shown that microarthropod profiles were notably distinct between the two management options, *Ag.* and *Nat.* A confusion matrix analysis was able to accurately classify 94% of Experiment 1 samples. All misclassified samples (4) were from *Nat.* soil for HF, misclassification may be observed as HF *Nat.* site was a long-term fallow location, not truly a natural system. Additionally, abundance of Acari, Collembola, Coleoptera, Diptera, and Pseudoscorpions were significantly different between *Nat.* and *Ag.* lands (Table 10) indicating that these microarthropod orders might be particularly sensitive to agriculture management.

The distinction between farm, crop, and manure treatment might have been more apparent in the exploratory results had outliers not been included. PERMANOVA results have shown farm, manure, and crop to impact arthropod composition; further analysis have shown no significant difference in arthropod community structure between oat & pea and soybean crops, thus pointing to the corn crop as having a distinct impact. This conclusion was in line with the results of the nematode survey.

While *Ag.* soil had more individuals at HF than NWD, HF *Nat.* soil had fewer individuals than NWD *Nat.* soil. These abundance trends were also found in Experiment 1.

At NWD, the manured soil had significantly more arthropods than non-manured soil, it is tempting to match this to the general differences in physicochemical properties between the manured and not manured experimental treatments. On the other hand, HF arthropod counts were not impacted by manure even though manured soil had lower pH,

less total N, less water at sampling, and was slightly finer textured than the not manured soil. The impact of the detailed history of manure application on soil faunal populations was consequently dependent on farm, this is clarified by the interaction between farm and manure treatment. Although not analysed, HF and NWD specific manures likely have different physicochemical properties, as partial digestion is involved in the manure treatment at NWD while at HF manure is simply stored, in a liquid form, until used. It is generally understood that the quality of litter and farm waste does affect soil microarthropods; microarthropods favour fungal activities and are thus important for recycling cellulosic organic matter.

NWD *Nat.* soil had significantly more arthropods than all cropped soils. When crop impact was analysed irrespective of farm, oat & pea and soybean soil had fewer arthropods than *Nat.* while soil under corn cropping had a microarthropod population similar to both other soils under crops and *Nat.* soil.

Although the best explanatory variables for microarthropod community structure were total N and pH, both parameters together only explained 6.6% of the variability in the system. This indicates that microarthropods in the tested soils are predominantly affected by variables and interactions not measured during this project. Again, SOC was not an explanatory parameter which might indicate that SOC concentrations across the tested conditions were within the acceptable range for arthropod survival.

In general, total microarthropod abundance was positively related to pH. Abundance profiles for all arthropod orders were weakly negatively related to total pH in cropped soil (pH= 5.05 to 6.56), but positively for *Nat.* soil (pH=3.26 to 4.19); these

perceived differences in response to pH might be due to the very different pH ranges of *Ag.* and *Nat.* soils (i.e. at low pH, raising pH might increases arthropod abundance and the reverse at higher pH). More exactly, Geophilomorpha was significantly positively related to pH in *Nat.* soil but not in *Ag.* soil and Acari was significantly positively related to pH in oat & pea soil. Arthropod groups showed a positive trend with pH for both farms with the exception of Diptera when *Nat.* and *Ag.* soils were analysed together. Collembola were significantly positively related to pH only at HF. The influence of *Nat.* soil on the analysis might have caused a shift in the results seen previous in crop treatment. Orthoptera, Geophilomorpha, and Aranea were inversely related to pH in manure soil (pH= 5.05 to 6.31) but positively in no manure soil (pH= 5.27 to 6.56), an indication that pH correlations were probably not the best explanatory variable in these particular contexts. This is expected as microarthropods within the same order classification can be influenced differently by soil acidity (Van Straalen and Verhoef, 1997).

Total N was inversely correlated to the abundance of arthropods for both corn and *Nat.* soil. However, the relationship to total N varied across experimental factors: a closer look at the trophic important Collembola has shown a significantly positive relationship to total N in corn cropped soil and for both manured and not manured soils irrespective of farm, for oat & pea, most groups were positively related to total N but not statistically significant. On the other hand, Orthoptera, Geophylomorpha, and Aranea were significantly negatively related to total N at NWD, Coleoptera was significantly negatively related to total N for manure soil, and Aranea was negatively related to total N for non-manured soil. This simply confirms that while various single parameter analyses

may offer an insight into microarthropod community structure they are likely not recommendable especially as many are partial proxies, of variable strengths, of management conditions.

4 Chapter 4: Experiment 3: Impact of biochar amendments on soil arthropods and nematodes for a land recently converted from forest to agricultural use under boreal conditions in central Labrador

4.1 Statement regarding the experimental setup

The experiment assessed the impact of biochar amendments on land newly converted from boreal forest to agriculture use on the abundance and diversity of micoarthropods and nematodes. Experimental site was located in central Labrador, and sampling was carried out in 2017. All experimental design and sampling was independent of the Newfoundland experiments (see Chapter 3) and thus results have been discussed separately.

4.2 Methodology for Experiment 3

4.2.1 Field site description

The experiment was carried out at the Agricultural Research Station in Happy Valley- Goose Bay, Labrador, Canada (53.3017° N, 60.3261° W) (Figure 46). Average monthly temperature ranges from -18.1 to 15.4°C with an average yearly precipitation of 949 mm, 458 of which is snow. The site was converted from boreal forest to agriculture land in 2012 and consists of alluvia deposits from the Churchill River. Beet (*Beta vulgaris*, cultivar Red Ace) was planted on all test plots and was harvested at maturity on September 3, 2017.

Figure 46 Experiment 3 field location (red marker) in Goose Bay, Labrador, Canada. The grey markers represent Newfoundland field locations for Experiment 1 and 2.

4.2.2 Biochar characteristics

The biochar used was hardwood with particle size <2.5 cm, from Basques Hard Wood Charcoal (*http://www.basquescharcoal.com/*). Biochar was approximately 70% carbon by mass (as per communication from the manufacturer).

4.2.3 Experimental design and soil sampling

The incorporation of biochar was accomplished in two stages. In 2013, biochar (BC) was added in 0 (*control*, C), 10, 20, and 40 t C ha^{-1} rates to 8x4 m plots. The plots were further divided in 2014 into two 4x4 m plots, one 4x4 m plot of each incorporation

rate then received an additional application of biochar, in the same rate, resulting in *new* (N) (biochar applied in 2013 and 2014) and *old* (O) (biochar applied only in 2013) treatments. One 4x4 m plot that did not receive BC in 2013 received 10 t C ha-1 BC in 2014. The plots thus had final BC application rates of 0, 10, 20, 40 and 80 t C ha^{-1} (Figure 47). Plots were then labeled based on their final BC application *rate* and biochar *age* (e.g. N20, O40, etc…). The sampling design was completely randomised with 4 replicates and 32 samples. Soil was sampled 14 days after harvest from 0-10 cm depth using a 3 cm corer. Several cores were taken from each 4x4m plot.

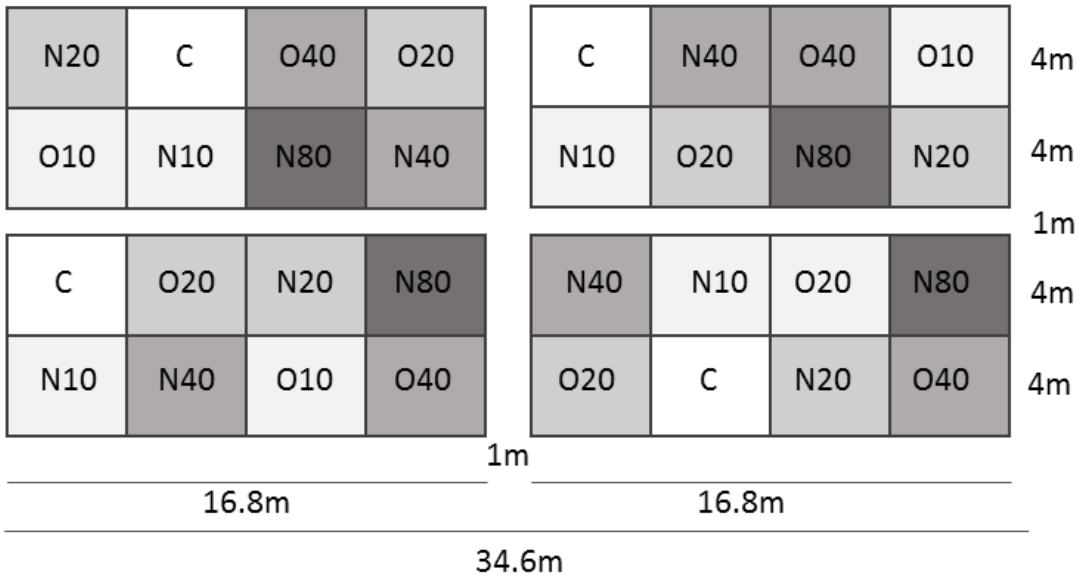


Figure 47 Experimental design for Experiment 3. Plots were labeled based on their final BC application *rate* (10, 20, 40, 80 tC ha^{-1}) and *age* (C=*control*, no biochar applied, N=*new*, biochar applied in 2013 and 2014, O=*old*, biochar added only in 2013). Diagram not to scale.

4.2.4 Soil handling and storage

Samples were prepared as described in section 3.3.1.4. A 175 g sub-sample for nematode extraction was collected by taking small scoops from different parts of the

sample. Another sub-sample, 150 g dry-weight equivalent, was taken from the remaining fresh sample in the same manner for microarthropod extraction.

Soil was stored in plastic containers with holes punched in the lids at 4 °C until extraction.

4.2.5 Soil physicochemical parameters

Soil texture, pH, cations (Ca, Mg, K, P, Fe, Cu, Mn, Zn, B, Na, Al, S), CEC, SOM, and SWC, and SAR were measured as described in Experiment 1 and 2 (section 3.2.1, 3.3.1).

4.2.6 Soil microarthropod extraction, preservation, counts, and identification.

Microarthropods were extracted as described in section 3.2.1.8.

4.2.7 Nematode extraction, cleaning, preservation, counts, and identification

Nematodes were extracted as described in Experiment 2 (section 3.3.1.9) with the following differences:

1. Nematodes were extracted from 175 g soil instead of 100 g, as test extraction showed significantly lower nematode abundance in Labrador soil than in Newfoundland soil.
2. After cleaning with the centrifuge flotation method (Gooris and D'Herde, 1972), the samples were gradually reduced to 2 mL, and preserved by adding 4 mL of 70 °C 5% formalin followed by 4 mL of cold (4 °C) 5% formalin. The final extract was 10 mL of 4% formalin.

4.2.8 Statistical analysis

All statistics were carried out as described in Experiment 1 and 2 (section 3.2.1.9, 3.3.1.10).

Exploratory statistics were done to assess the differences driven by community structure according to biochar *age*, *rate* or age and rate interaction (*full treatment*). Note that the Labrador nematode data was normalized per mass soil prior to analysis. For the RDA analyses, soil parameters were normalized in units of standard deviation around the mean to eliminate the impact of the different units commonly employed for various soil parameters.

4.3 Results for Experiment 3: Impact of biochar on soil fauna

4.3.1 Soil physicochemical properties

Texture

Soil texture was analysed for the top 15 cm of soil previous to this experiment and was loamy sand.

Bulk Density and Porosity

Mean BD for the plots was 1.21±0.14 g cm^{-3} prior to biochar application. Mean porosity was 0.54.

Soil Water Content at sampling

SWC was not significantly different between biochar age or rate treatments and did not differ between *control* and biochar treated soil. SWC ranged from 16.52 to 24.72% for *control* soil and 17.53 to 34.57% for biochar treated soil.

Acidity

Control (0 t C ha^{-1}) soil had significantly lower pH ($\bar{x}$=4.75) than 10, 40, and 80 t C ha^{-1} treatments ($\bar{x}$=5.40, 5.60, 6.35 respectively). 20 t C ha^{-1} pH ($\bar{x}$= 5.08) did not differ from *control* soil (Table A6.1). *New* treatments had significantly less acidic soil than *control* or *old* soil ($\bar{x}$=5.76 vs. 4.75, 5.15) (Table A6.2). *Control* soil pH ranged from 4.4 to 5.1, 10 t C ha^{-1} from 4.9 to 5.7, 20 t C ha^{-1} from 4.6 to 5.2, 40 t C ha^{-1} from 5.1 to 6.1, 80 t C ha^{-1} from 6.1 to 6.6. *New* soil ranged from 5 to 6.6, *old* from 4.6 to 5.7. Soil pH was significantly different between *control* and biochar treated soil.

Soil Organic Matter

Mean soil organic matter (SOM) was 3.28% prior to biochar application. SOM was not significantly different between biochar age or rate treatments and did not differ between *control* and biochar treated soil. SOM ranged from 2.68 to 3.80% for *control* soil and 2.25 to 4.49% for biochar treated soil.

4.3.2 Soil elemental analysis

There were complex differences between soil treated with various biochar ages and rates (figure 48, 49).

Between biochar age and rate

TP, Cation Exchange Capacity (CEC) (Table 31, Table 32), K, Na, SAR (Table 33), Zn, B, Al, Fe, was not significantly different between biochar age or rate treatments and did not differ between *control* and biochar treated soil. Ca, Mg, Mn, and S were significantly lower in *control* than biochar treated soil. 40 and 80 t C ha^{-1} soil had significantly more Ca than 0 or 20 t C ha^{-1} treatments ($\overline{x}$=0.66, 1.01 vs. 0.23, 0.38 g kg^{-1} respectively). 10 t C ha^{-1} ($\overline{x}$=0.23 g kg^{-1}) was not different from 0, 20, or 40 t C ha^{-1}(Table A6.3).

There was significantly less Mg in *control* and 20 t C ha^{-1} than in 80 t C ha^{-1} soil ($\overline{x}$=25.50, 40.88 vs. 91.30 mg kg^{-1}). Mg concentration for 10 and 40 t C ha^{-1} ($\overline{x}$=53.50, 62.80 mg kg^{-1}) were not significantly different from each other or any other biochar rate (Table A6.5). There was significantly less Mn in *control* soil than in 80 t C ha^{-1} ($\overline{x}$=15.23 vs. 30.70 mg kg^{-1}). Mn concentrations in 10, 20, 40 t C ha^{-1} treatments ($\overline{x}$= 21.36, 22.81, 25.71 mg kg^{-1} respectively) were not significantly different from *control* or 80 t C ha^{-1}

(Table A6.7). There was significantly less S in *control* and 10 t C ha^{-1} treatments than in 40 or 80 t C ha^{-1} ($\bar{x}$=11.10, 12.28 vs. 14.54, 16.28 mg kg^{-1}) (Table A6.9).

There was significantly more Ca in *New* soil than *control* or *old* ($\bar{x}$=0.70, vs. 0.23, 0.38 g kg^{1}) (Table A6.4). *New* soil had significantly more Mg ($\bar{x}$=71.75 vs. 22.50, 39.50 mg kg^{-1}) (Table A6.6) and S ($\bar{x}$=14.36 vs. 11.10, 12.70 mg kg^{-1}) (Table A6.10). *New* soil had significantly more Mn than *control* soil ($\bar{x}$=25.33 vs. 15.23 mg kg^{-1}) while *old* soil ($\bar{x}$=23.05 mg kg^{-1}) did not differ from *control* or *new* (Table A6.8).

Elemental ranges

TP ranged from 107 to 170 mg kg^{-1} for *control* soil and 84 to 186 mg kg^{-1} for biochar treated soil. CEC ranged from 6.20 to 8.46 Cmol kg^{-1} for *control* soil and 6.01 to 10.14 Cmol kg^{-1} for biochar treated soil. K ranged from 90 to 145 mg kg^{-1} for *control* soil and 85 to 179 mg kg^{-1} for biochar treated soil. Na ranged from 7.7 to 9.20 mg $kg^{-}1$ for *control* soil and 5.2 to 12.40 mg kg^{-1} for biochar treated soil. SAR ranged from 0.04 to 0.05 in *control* soil and 0.03 to 0.05 in biochar treated soil. Cu ranged from 1.65 to 4.65 mg kg^{-1} for *control* soil and 1.86 to 12.30 mg kg^{-1} for biochar treated soil. Zn ranged from 3.13 to 5.12 mg kg^{-1} for *control* soil and 3.06 to 11.10 mg $kg^{-1}$for biochar treated soil. B ranged from 1.47 to 1.87 mg kg^{-1} for *control* soil and 1.14 to 2.26 mg kg^{-1} for biochar treated soil. Al ranged from 1.18 to 1.29 g kg^{-1} for *control* soil and 1.01 to 1.38 g kg^{-1} for biochar treated soil. Fe ranged from 0.53 to 0.65 g kg^{-1} for *control* soil and 0.41 to 0.68 g kg^{-1} for biochar treated soil.

Control soil Ca ranged from 0.16 to 0.34 g kg^{-1}, 10 t C ha^{-1} from 0.26 to 0.74, 20 t C ha^{-1} from 0.24 to 0.56, 40 t C ha^{-1} from 0.34 to 1.05, 80 t C ha^{-1} from 0.86 to 1.22. *New*

soil Ca ranged from 0.30 to 1.22 g kg^{-1}, *old* soil ranged from 0.24 to 0.54. *Control* soil Mg ranged from 8.35 to 35 mg $kg^{-1,}$ 10 t C ha^{-1} from 24.74 to 93.00, 20 t C ha^{-1} from 16.42 to 64.00, 40 t C ha^{-1} from 31.10 to 107.0, 80 t C ha^{-1} from 29.90 to 136.0. *New* soil ranged from 29 to136, *old* from 20 to 74 mg kg^{-1}. *Control* soil Mn ranged from 12.5 to 19.5 mg kg^{-1}, 10 t C ha^{-1} from 11.7 to 33.80, 20 t C ha^{-1} from 15.7 to 31.6, 40 t C ha^{-1} from 18.1 to 35, 80 t C ha^{-1} from 27.7 to 35.9. *New* soil ranged from 15.7 to 35.9 mg kg^{-1}, *old* from 11.7 to 35.0. *Control* soil ranged in S from 9.7 to 11.9 mg kg^{-1}, 10 t C ha^{-1} from 10.6 to 14.1, 20 t C ha^{-1} from 11.3 to 15.4, 40 t C ha^{-1} from 12.7 to 16.8, 80 t C ha^{-1} from 13.6 to 18.2. *New* soil ranged from 11.5 to 18.2 mg kg^{-1}, *old* from 10.6 to 14.8.

Table 31 Soil characteristics for Experiment 3 (Labrador) soil amended with biochar in 2013 (old) and 2014 (new). *Control* soil received no biochar (0 t C ha^{-1}). Error term is CI_{95}.

Biochar age	Soil pH	CEC (Cmol kg^{-1})	Organic matter (%)	Water content (%)
New (2014)	5.76±0.28	7.89±0.71	3.32±0.34	22.36±2.41
Old (2013)	5.15±0.178	8.08±0.84	3.23±0.34	22.80±2.46
Control	4.75±0.46	7.04±1.64	3.24±1.02	21.59±6.27

Table 32 Soil characteristics for Experiment 3 (Labrador) soil amended with various amounts of biochar (0, 10, 20, 40, 80 t C ha^{-1}). Error term is CI_{95}.

Biochar rate (t C ha^{-1})	Soil pH	CEC (Cmol kg^{-1})	Organic matter (%)	Water content (%)
0	4.75±0.46	7.04±1.64	3.24±1.02	21.59±6.27
10	5.4±0.28	7.76±1.20	2.95±0.45	20.12±2.07
20	5.06±0.25	7.89±1.19	3.2±0.43	22.76±2.26
40	5.6±0.41	8.16±0.75	3.57±0.46	23.85±5.05
80	6.35±0.33	8.19±3.00	3.53±1.07	24.37±6.56

Table 33 Sodium Adsorption Ratio (SAR) of Experiment 3 (Labrador) soil treated with various rates of biochar (10, 20, 40, 80 t C ha^{-1}) in 2013 (*old*) and 2014 (*new*). *Control* soil received no biochar (0 t C ha^{-1}) Error term is CI_{95}.

Age	Rate	SAR
Control	*0*	0.047±0.012
New	*10*	0.030±0.009
	20	0.032±0.015
	40	0.024±0.004
	80	0.018±0.005
Old	*10*	0.028±0.007
	20	0.041±0.012
	40	0.028±0.007

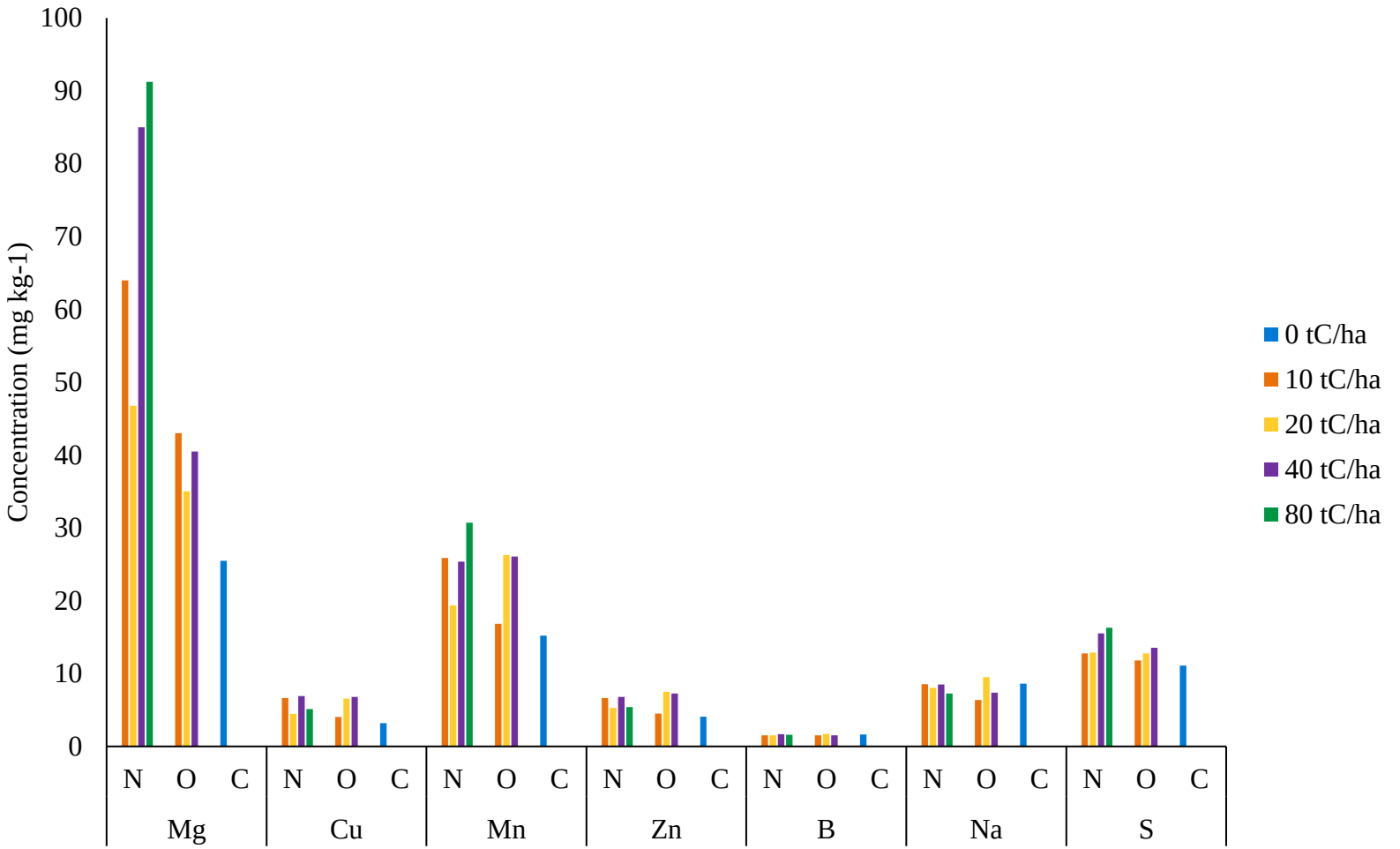


Figure 48 Elemental analysis of Experiment 3 (Labrador) soil amended with various rates of biochar (0 (*control*- C), 10, 20, 40, 80 t C ha^{-1}) in 2013 (*old*-O) and 2014 (*new*-N).

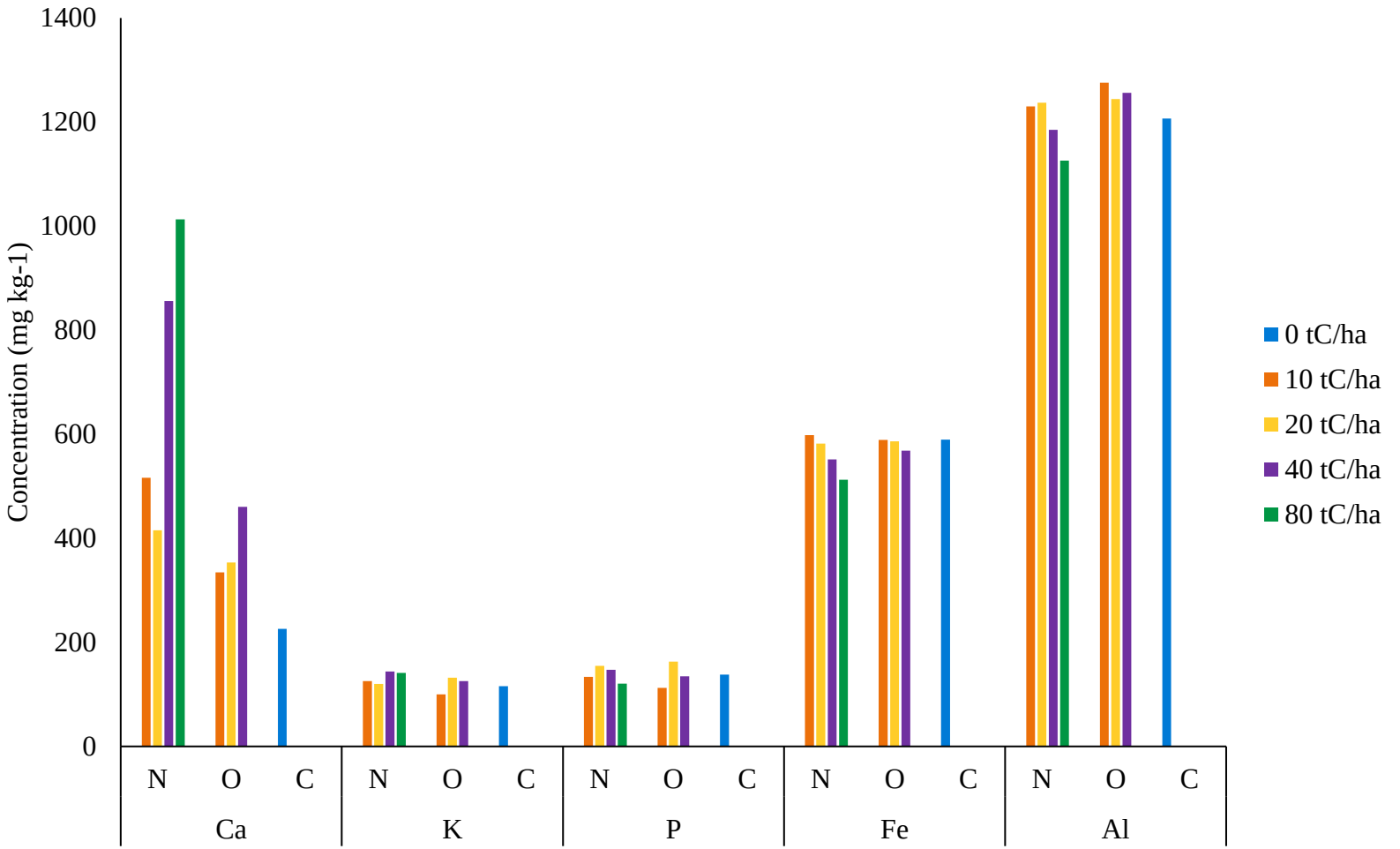


Figure 49 Elemental analysis of Experiment 3 (Labrador) soil amended with various rates of biochar (0 (*control*- C), 10, 20, 40, 80 t C ha^{-1}) in 2013 (*old*-O) and 2014 (*new*-N)

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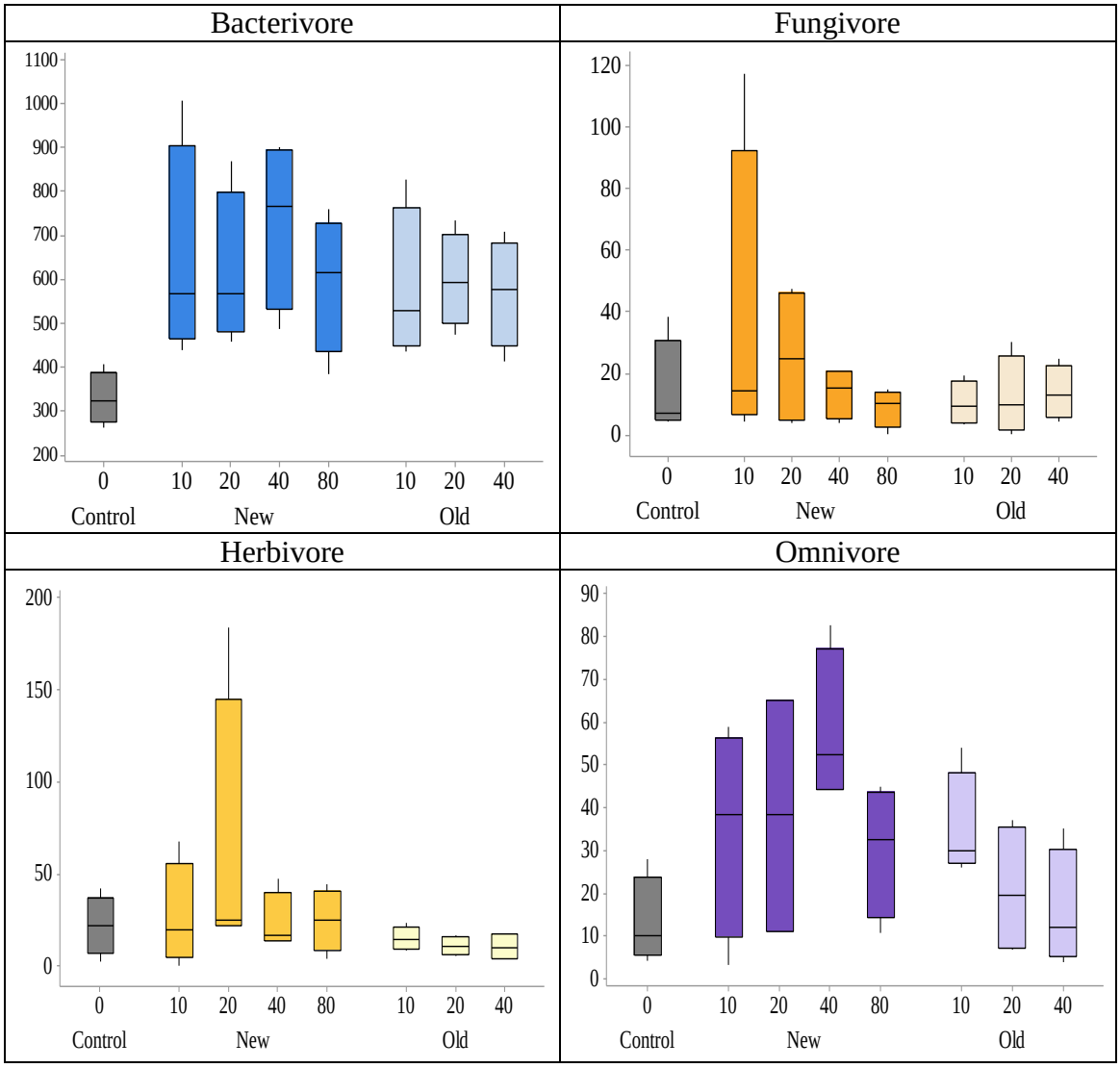
4.3.3 Nematode composition

There were significantly more nematodes in *new* soil than *control* ($\bar{x}$=750 vs. 380 individuals per 100 g dry soil) although nematode abundance in o*ld* soil ($\bar{x}$=639 individuals per 100 g dry soil) was not different than that in *control* or *new* treatments (Table A7.1). Nematode abundance was not significantly different with biochar rate or full treatment.

There were significantly more nematodes in biochar treated soil than in *control* soil (Figure 50, Figure 51).


Bacterivore
1100
1000
900
800
700
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0 10 20 40 80 10 20 40
Control New Old
Fungivore
120
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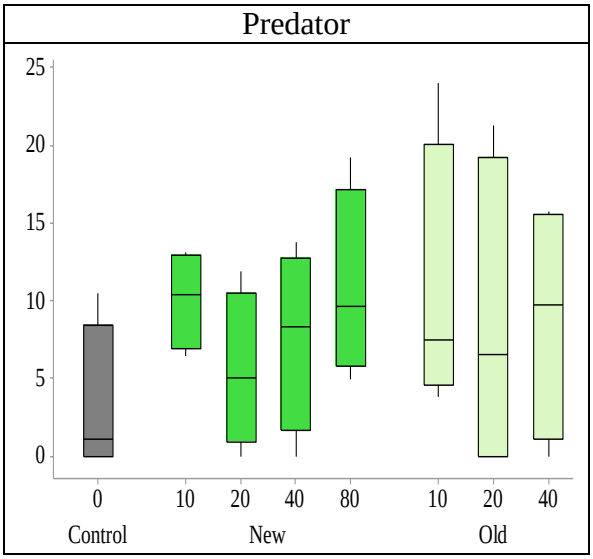

Figure 50 Boxplots of nematode trophic composition (number of individuals per 100 g dry soil) for Experiment 3 (Labrador) soil amended with various rates of biochar (0, 10, 20, 40, 80 t C ha^{-1}) in 2013 (*old*) and 2014 (*new*). *Control* soil received no biochar (0 t C ha^{-1}).

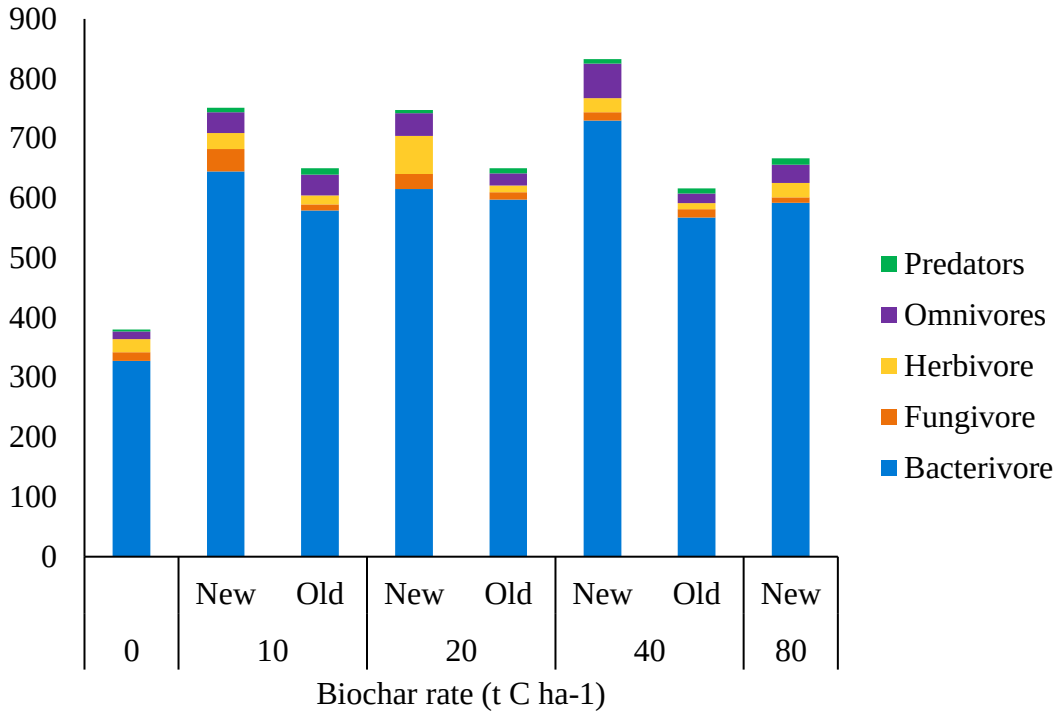

Figure 51 Nematode community composition for Experiment 3 (Labrador) soil amended with various rates of biochar (0, 10, 20, 40, 80 t C ha^{-1}) in 2013 (*old*) and 2014 (*new*). *Control* received no biochar (0 t C ha^{-1}).

4.3.4 Microarthropod composition

In general, the microarthropod counts were very low (Table 34, Table 36) without significantly different (α=0.05) abundances across the biochar age, rate, or age and rate interaction (*full treatment*).

Table 34 Arthropod counts (individuals per 100 g dry soil) for Experiment 3 (Labrador) biochar amended soil in 2013 (*old*) and 2014 (*new*). *Control* received no biochar. Counts were rounded to nearest individual. Error term is CI_{95}.

Biochar age	Colembola	Coleoptera	Total Count
New (2014)	0±0.8	0	0±0.8
Old (2013)	1±1.2	0	1±1.2
Control	0	0	0

Table 35 Arthropod counts (individuals per 100 g dry soil) for Experiment 3 (Labrador) soil amended with 0, 10, 20, 40, and 80 t C ha^{-1} biochar. Counts were rounded to nearest individual. Error term is CI_{95}.

Biochar rate (t C ha^{-1})	Colembola	Coleoptera	Total Count
0	0	0	0
10	0	0	0
20	2±2.6	0	2±2.6
40	0	0	0
80	0	2±2.6	2±2.6

Table 36 Arthropod counts (individuals per 100 g dry soil) for Experiment 3 (Labrador) soil amended with various biochar rates (0, 20, 40, 80 t C ha^{-1}) in 2013 (*old*-O) and 2014 (*new*-N). *Control* (C) received no biochar (0 t C ha^{-1}). Error term is CI_{95}.

Treatment	Colembola	Coleoptera	Total Count
C	0	0	0
N10	0	0	0
N20	2±5.3	0	2
N40	0	0	0
N80	0	2±5.3	2±5.3
O10	0	0	0
O20	2±5.3	0	2±5.3
O40	0	0	0

4.3.5 Exploratory and explanatory statistics

4.3.5.1 Central Labrador nematode analysis

Results show that biochar *age*, *rate*, and age and rate interaction (*full treatment*) had an impact on the population structure (Figure 52). Nematode structure for 0 and 20 t C ha^{-1} and for 10, 40, 80 t C ha^{-1} are grouped and appear to have similar behaviour (Figure 52). PERMANOVA analysis has confirmed that biochar *age*, *rate*, and *full treatment* had a statistically significant role (Table 37, Table 38). The interaction between biochar *age* and *rate* was not significant suggesting similar community structure behaviour for same application *rate* independent of biochar *age* (Table 37). The nematode populations in the *control* soil were not statistically different from *N10*, *N80*, and *O40* ($p=0.06$) but was significantly different for all other treatment with $p<0.05$ (Table 38). Nematode community structure was not statistically different between new and old biochar ages but new and old were different from the *control* soil (Table 39). *Control* soil

was significantly different from all other biochar application *rates* with the exception of 80 t C ha^{-1} ($p=0.06$) (Table 40).

Bacterivore abundance was significantly correlated to omnivore and predator abundance. Fungivore abundance was related to herbivore abundance and herbivore abundance was related to omnivore abundance (Table 41).

RDA analysis showed that all measured environmental parameter explained 67% of variation in nematode trophic abundance but a model was not strongly statistically significant ($p=0.099$). The best explanatory variables of nematode composition were pH ($p<0.001$) and CEC ($p=0.024$). Both parameters explained 27.5% of the variability but failed to explain 72.5% (Figure 53).

In general, all nematode trophic groups were weakly positively related to pH except for bacterivores in *control* soil (Figure 54, Figure 55, Figure 56). Total nematode abundance had an increasing trend with pH (Figure 57).While not significant, total nematode abundance appeared to be positively related to variation in CEC for biochar treated soil (Figure 54, Figure 55, Figure 56). There was an increasing trend of total nematode abundance with CEC when treatments were not considered (Figure 58).

Total number of nematodes was positively related to K and P in *control* soil but negatively to Fe (Figure 55). Total number of nematodes was significantly related to bacterivores (Figure 55, Figure 56).

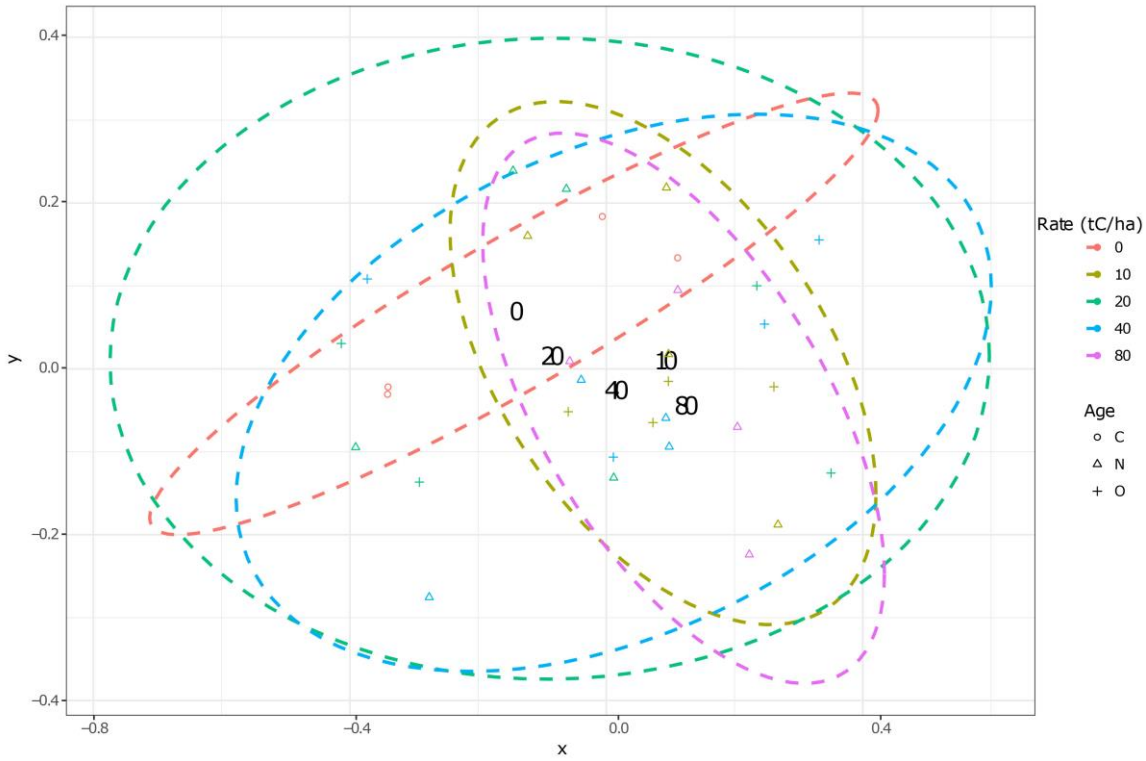

Figure 52 Non-metric multi-dimensional scaling (NMDS) ordination displaying the impact of age and rate of biochar application on nematode population structure similarities for Experiment 3 (Labrador) soil.

Table 37 Two way PERMANOVA of nematode community composition for age and rate of biochar amended Experiment 3 (Labrador) soil (9999 permutations.

	SS	DF	MS	F	P-value
Age	0.33	2	0.16	2.60	**<0.001**
Rate	0.31	4	0.08	1.23	**0.009**
Interaction	-0.81	8	-0.10	-1.60	0.99
Residual	1.07	17	0.06		
Total	0.90	31			

Table 38 One way PERMANOVA of biochar age and rate combination (full treatment) in Experiment 3 (Labrador) soil.

Permutation N	9999
Total SS	0.90
Within-group SS	0.54
F	2.34
p (same)	**0.025**

Bray Curtis dissimilarity matrix

	0	N10	N20	N40	N80	O10	O20	O40
0		0.06	**0.029**	**0.029**	0.06	**0.027**	**0.032**	0.06
N10	0.06		0.97	0.60	1.00	0.88	0.91	0.77
N20	**0.029**	0.97		0.37	0.91	0.66	0.65	0.63
N40	**0.029**	0.60	0.37		0.43	0.28	0.26	0.19
N80	0.06	1.00	0.91	0.43		0.97	0.91	0.69
O10	**0.027**	0.88	0.66	0.28	0.97		0.80	0.74
O20	**0.032**	0.91	0.65	0.26	0.91	0.80		0.88
O40	0.06	0.77	0.63	0.19	0.69	0.74	0.88	

Table 39 One way PERMANOVA of biochar age in Experiment 3 (Labrador) soil.

Permutation N	9999
Total SS	0.90
Within-group SS	0.57
F	8.28
p (same)	**<0.001**

Bray Curtis dissimilarity matrix

	Control	*New*	*Old*
Control		**<0.001**	**0.001**
New	**<0.001**		0.22
Old	**0.001**	0.22	

Table 40 One way PERMANOVA of biochar rate in Experimetn 3 (Labrador) soil.

Permutation N	9999
Total SS	0.90
Within-group SS	0.59
F	3.55
p (same)	**0.007**

Bray Curtis dissimilarity matrix

	0 t C ha^{-1}	10 t C ha^{-1}	20 t C ha^{-1}	40 t C ha^{-1}	80 t C ha^{-1}
0 t C ha^{-1}		**0.009**	**0.002**	**0.004**	0.058
10 t C ha^{-1}	**0.009**		0.86	0.80	0.986
20 t C ha^{-1}	**0.002**	0.86		0.74	0.957
40 t C ha^{-1}	**0.004**	0.80	0.74		0.884
80 t C ha^{-1}	0.06	0.99	0.96	0.88	

Table 41 Correlation matrix for nematode community composition.

	Bacterivores	Fungivores	Herbivores	Omnivores	Predators
Bacterivores		0.07	0.47	**<0.001**	**0.012**
Fungivores	0.07		**0.002**	0.12	0.88
Herbivores	0.47	**0.002**		**0.021**	0.99
Omnivores	**<0.001**	0.12	**0.021**		0.18
Predators	**0.012**	0.88	0.99	0.18	

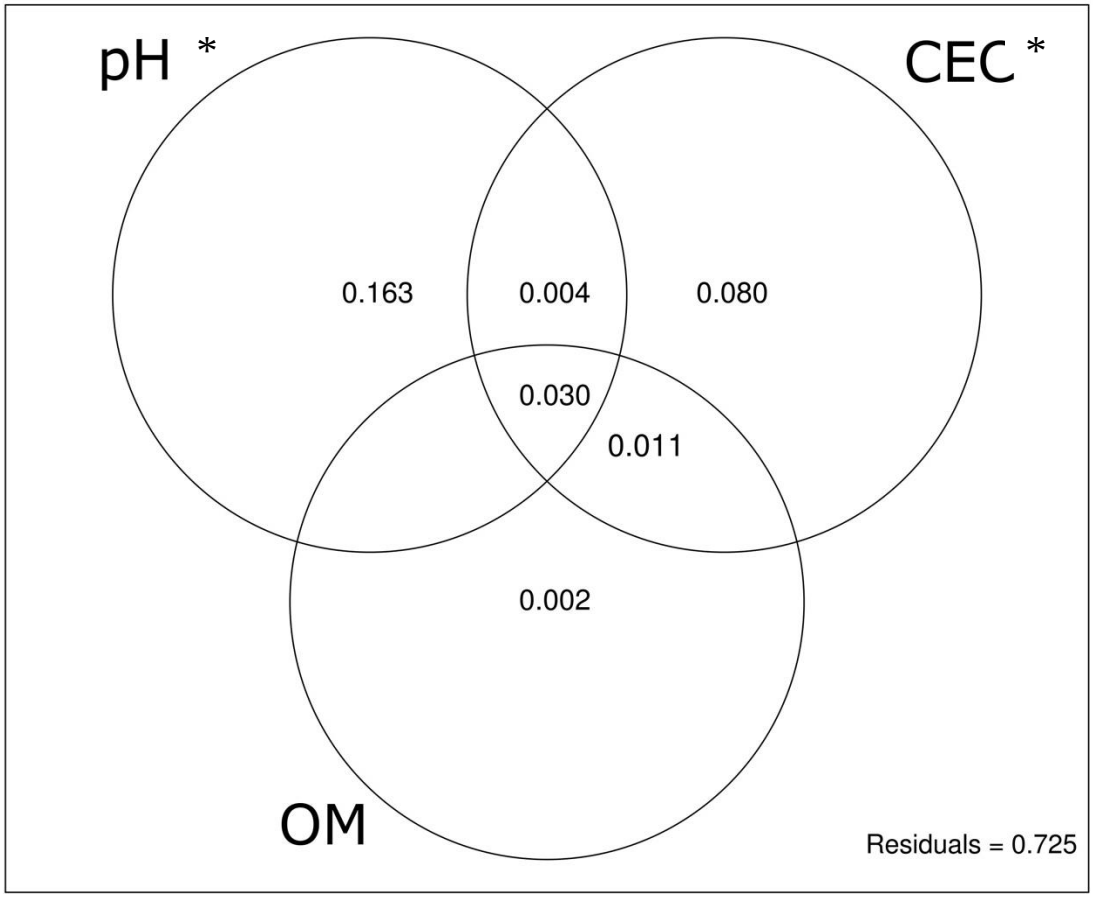

Figure 53 Impact of abiotic environmental parameters on the partition of the variation in nematode composition in Experiment 3 (Labrador) soil.

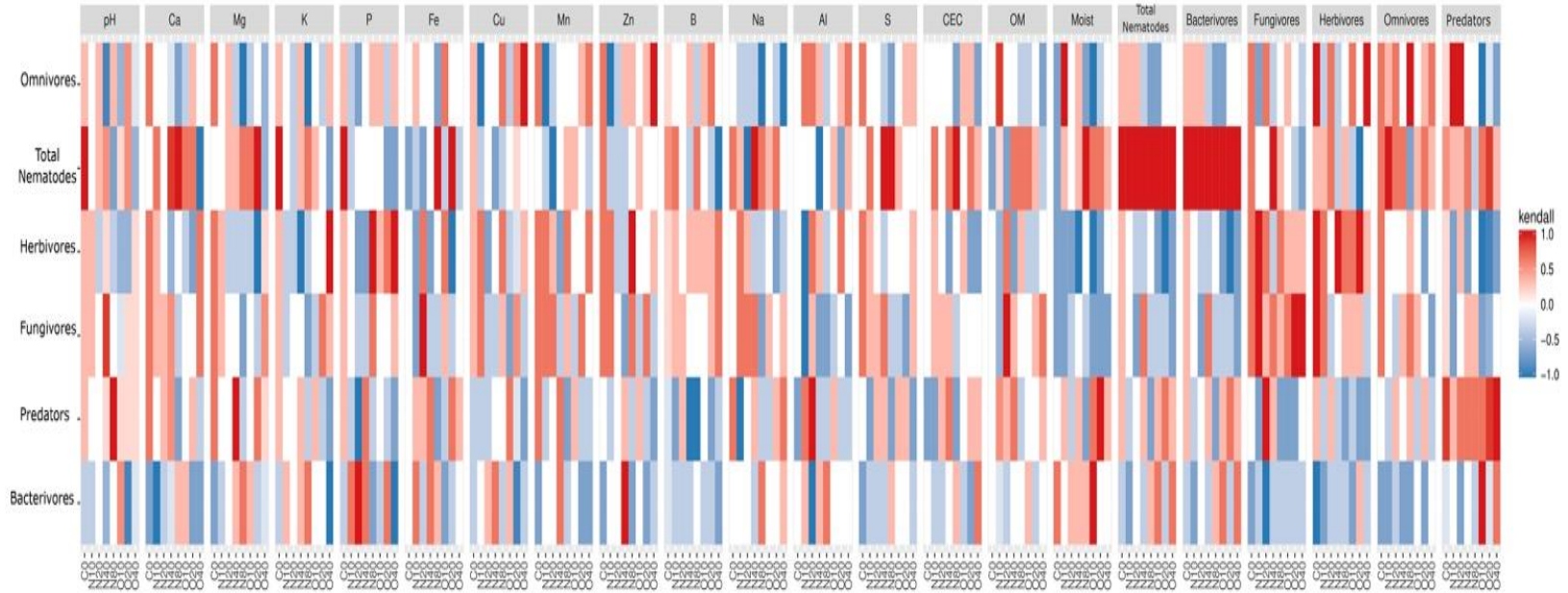

Figure 54 Correlation matrices for nematode composition and environmental variables of biochar treatments (C, N10, N20, N40, N80, 010, 020, 040) in Experiment 3 (Labrador) soil.

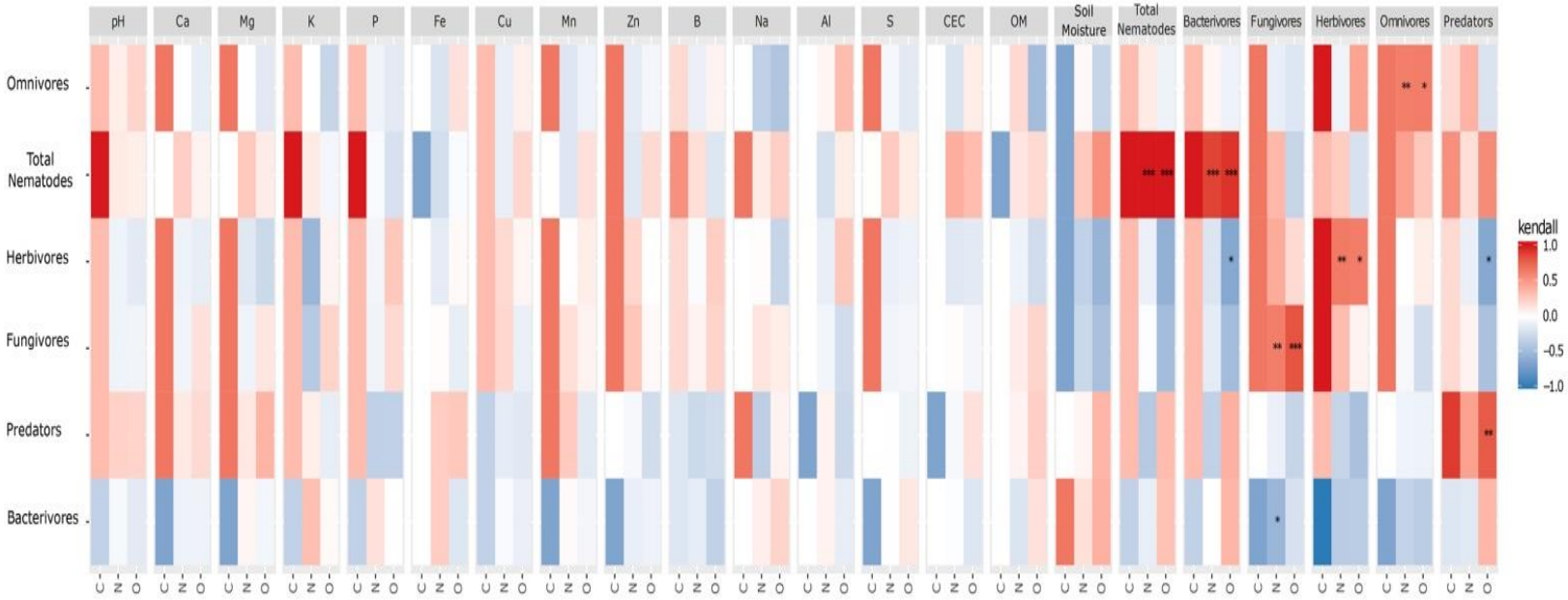

Figure 55 Correlation matrices for nematode composition and environmental variables of biochar age (*control*-C, *New* (2014)-N, *Old* (2013)-O) in Experiment 3 (Labrador) soil. *P<0.05, **P< 0.01, ***P<0.001.

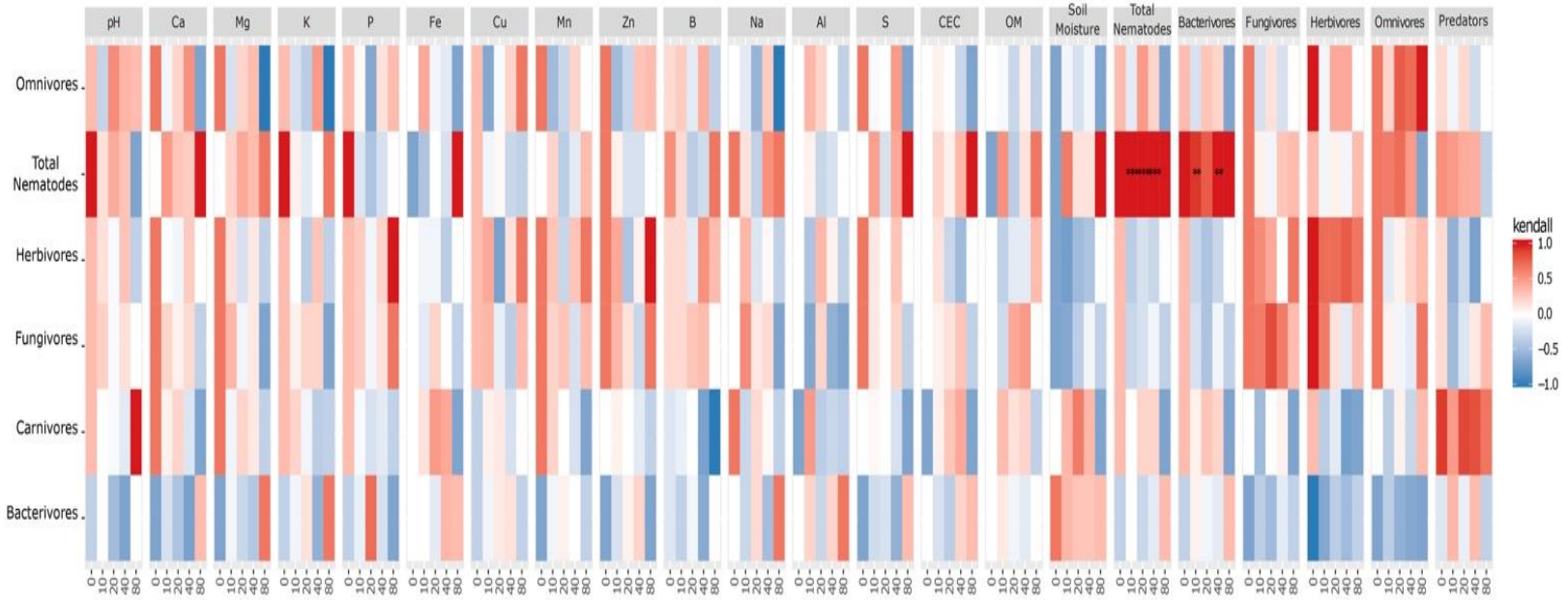

Figure 56 Correlation matrices for nematode composition and environmental variables of biochar rates (0, 10, 20, 40, 80 t C ha^{-1}) in Experiment 3 (Labrador) soil. *P<0.05, **P< 0.01, ***P<0.001.

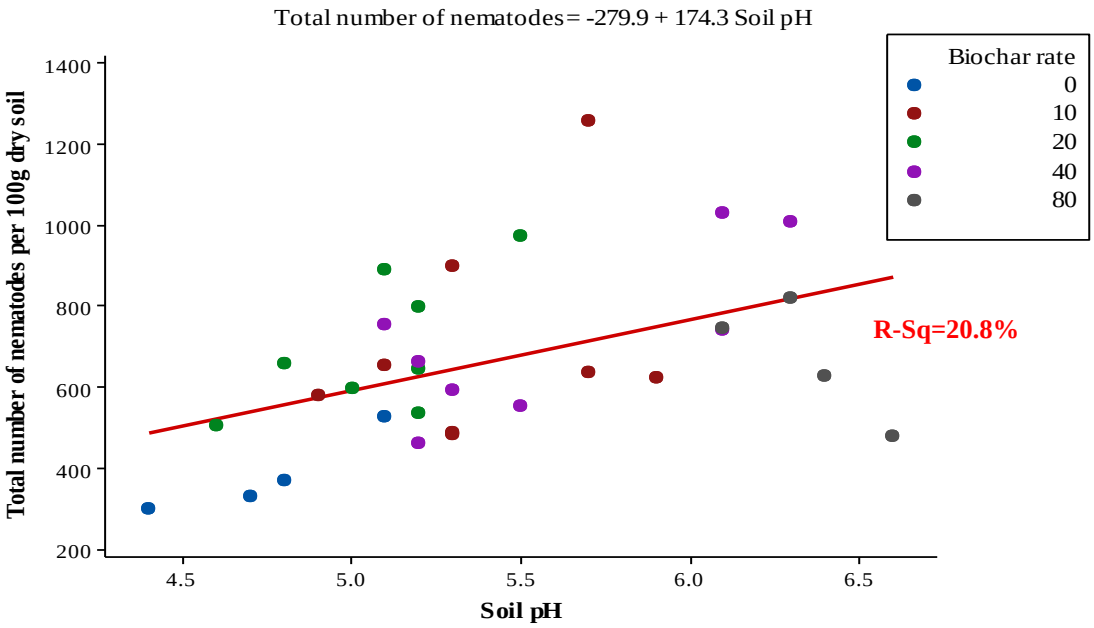

Figure 57 Regression analysis for Experiment 3 (Labrador) nematode abundance (individuals per 100 g dry soil) for New World Dairy (NWD) and Hammond Farms (HF) with pH.

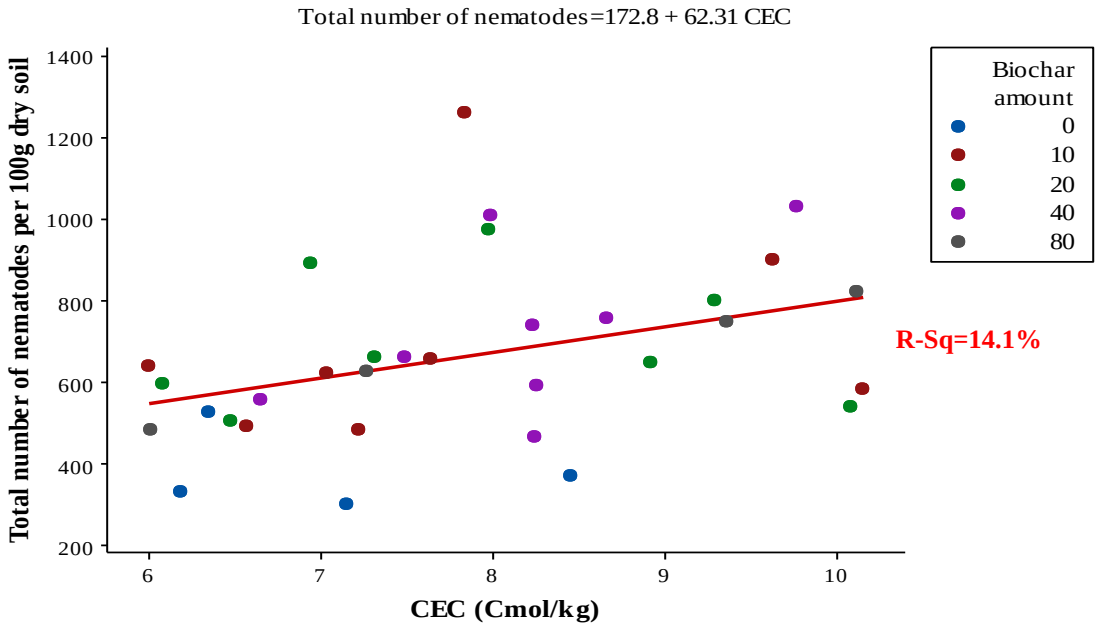

Figure 58 Regression analysis for Experiment 3 (Labrador) nematode abundance (individuals per 100 g dry soil) for New World Dairy (NWD) and Hammond Farms (HF) with cation Exchange Capacity (CEC) (Cmol kg^{-1}).

4.3.5.2 Central Labrador microarthropod analysis

Microarthropod abundances in Labrador soil were too low to perform exploratory and explanatory statistics.

4.4 Discussion for Experiment 3: Impact of land conversion and biochar use on soil arthropods and nematodes under boreal conditions in central Labrador

4.4.1 Soil characterisation

Biochar is reported to increase soil pH (Yuan et al., 2011; Shaaban et al., 2018) due to cation retention (Glaser et al., 2002; Novak et al., 2009); all Labrador soil that received biochar had higher pH than *control* soil except for the 20 t C ha^{-1} treatment. Soil cations (Ca, Mg, Mn) were significantly higher in concentration in biochar treated soil than *control* with the exception of the 20 t C ha^{-1} treatment. *New* soil received twice as much biochar than the corresponding *old* soil and had higher pH, Ca and Mg concentrations than *control* and *old* treatments therefore confirming the role of the biochar and the value of higher application rates. Although there was no statistically significant relationship between Al concentration with biochar application, measured Al was nevertheless proportionally lower in 80 t C ha^{-1} treatment versus the untreated *control*.

Though other studies have described increases in K, N, P, CEC, organic matter, and water content with biochar application (e.g. Glaser et al., 2002; Novak et al., 2009; Zheng et al., 2013) this was not obvious for the Labrador site. There was a significant increase in S with biochar application, Novak et al. (2009) reported a decrease in S with biochar application to agricultural Ultisols. A decrease in S could be an indication of biochar quality.

4.4.2 Central Labrador nematodes

While not entirely clear, an initial analysis indicated that biochar application had a rate related impact on the structure of the soil nematodes although not perfectly linear

along rate increase. Nevertheless, the same non-linear grouping was similar to the one observed in the abiotic parameters. The non-linearity was driven by the 20 t C ha^{-1} treatment which was more similar to control than the 10 t C ha^{-1} treatment. A PERMANOVA analysis indicated that nematode communities were impacted by biochar *age* (as defined in the methodology), *rate* and the interaction. Surprisingly, a dissimilarity index analysis indicated that the nematode structure in the *control* soil was not statistically different from the 80 t C ha^{-1}. This might indicate a rate related response up to 40 t C ha^{-1} but followed by a negative impact for excessive rates of biochar (e.g. Gul et al., 2015). While the interaction between *rate* and *age* (*full treatment*) had a significant impact on nematode community composition nematode abundance was not statistically different across biochar application rates. This may be interpreted as an indication that the effect of biochar amendment is best assessed once biochar has reached an equilibrium state with the soil (Mia et al., 2017b). Nematode population structure and overall abundance in the *control* soil was significantly different from the *new* and *old* soil but no difference was observed between *new* and *old* treatments. Nematodes were most abundant in the *new* treatments. These results clearly confirm that biochar application to these newly converted soils had an impact on soil nematode populations, both on overall community structure and abundances. These findings are in apparent contradiction with other reports that found no influence of natural or man-made biochar on nematode populations (Matlack, 2001; Pressler et al., 2017; Soong et al., 2017). Xiao-Ke et al. (2013) reported significant changes in fungivore and bacterivore abundance (as well as changes in soil organic carbon, total nitrogen, and C/N ratio) with biochar addition, observations again not confirmed for the Labrador soil.

However, these contradictions must be assessed in the context of the soils tested by various studies. All other studies were carried out on soils other than podzols and with various concentrations and, critically, types of biochar, all factors known to modify biochar effectiveness (Atkinson et al., 2010). Moreover, the Labrador test site was converted from boreal forest to agriculture use only within 4 years; these soils are of extremely poor quality, are acid, as may be seen in the soil parameters reported here, and are thus more sensitive to any quality improvement than soils of better overall quality, located in less harsh climates.

Nematode populations reflect the micro-faunal populations of the system (Parmelee and Alston, 1986), and are affected by the quality and quantity of degradable organic matter (Mcsorley and Frederick, 1999). Results have confirmed that for the Labrador site, bacterivores were significantly correlated to omnivores and predators. The relationship to predators is quite expected given the dominance in abundance of bacterivorous nematodes; omnivores are likewise expected to be driven by the dominant food source which, for the tested conditions seem to be bacteria. Fungivores were correlated to herbivores; increased plant density and thus root presence in soil provides opportunities for mycorrhizal fungi (Smith and Read, 2008). Omnivores were similarly correlated with herbivorous nematodes; as herbivores and omnivores share a common food source they may be expected to respond to vegetative inputs in similar ways.

The RDA indicated that the best explanatory variables for nematode population composition were pH and CEC but both parameters only explained 27.5% of the variability in the system. The pH was highest in 80 t C ha^{-1} followed by 40 t C ha^{-1} and

control, CEC was not different between age or rate of biochar application. Total nematode abundance increased with pH and CEC indicating that nematodes were most impacted by variation in soil acidity and nutrient availability than by absolute rate of biochar application. Organic matter (SOM) did not impact nematode composition; SOM ranged from 2.25 to 4.49% with no difference between *control* and biochar amendment soils. Stable, complex SOM is expected to support fungivorous nematodes (Matlack, 2001). However, in the converted podzols the low pH and general low fertility did not support accumulation of stable OM and consequently fungivore abundance was low for all Labrador soil. Podzols' OM is expected to be dominated either by un-degraded plant litter, in the top LFH horizon, or by fulvic acids in the low pH subsoil. Given that these soils have lost the LFH horizon during conversion, the litter expected to support fungal growth was just not there, thus helping to explain the observation of low abundance of fungivorous nematodes.

In general, all nematode trophic groups were weakly positively related to pH except for bacterivores in *control* soil where it had a relatively small range (pH 4.4 to 5.1). Total number of nematodes was significantly driven by the dominant bacterivores. Predators and omnivores, those particularly sensitive to disturbance (Ferris et al., 2001), had the lowest abundance.

It is of note that bacterivores dominated both the Labrador and the Newfoundland soils. Both are acid soils, converted from podzols either recently or within a few decades, respectively. It is of interest to understand the soil organic carbon speciation in these soils

and how such speciation may explain the dominant role of bacterially driven trophic chains across management options.

4.4.3 Central Labrador microarthropods

Only two microarthropod orders were found, at low abundance, in the Labrador experiment: *Collembola* and *Coleoptera*. *Collembola* was found in *new* and *old* plots with 20 t C ha^{-1} while *Coleoptera* was found in the *new* plot with 80 t C ha^{-1}. No arthropods were found in *control* soil. Total microarthropod abundance was not significantly different between biochar *age*, *rate*, or *full treatment*. As the literature suggests (Bedano et al., 2006; Postma-Blaauw et al., 2010), disturbance might have played a substantial role in low arthropod abundance; these soils do not appear to support arthropod communities once the LFH layer is removed during conversion from boreal forest to agriculture use. It was suggested that the impact of disturbance far outweighs the impact of biochar on arthropod populations (Castracani et al., 2015).

5 Chapter 5: Conclusions

The pH was an explanatory variable for Newfoundland nematode, Newfoundland arthropod, and Labrador nematode composition indicating that in our particular soil the wide range of pH, and especially the contrast between strongly acid and nearly neutral ranges, was most strongly reflected in the status of the soil food webs out of all measured parameters. Soil organic carbon did not influence nematode or arthropod composition for either the Newfoundland or Labrador locations. This was surprising as the literature suggests (Bongers and Ferris, 1999; Matlack, 2001; Bedano et al., 2006) that soil organic matter is important to composition, distribution, and abundance. These results are likely a reflection of several factors that determine soil organic matter at the tested sites. Dairy farms in Newfoundland apply large amounts of manures to lands and, in several decades of farming, can bring the organic matter content of converted lands to similar or greater levels than in natural soils.

In future work, it would be advantageous to analyse organic matter speciation to determine if humic/fulvic acid ratios have an impact on nematode populations in boreal podzols. Nevertheless, management impact on soil parameters could be putatively linked to nematode population structure in Newfoundland; the latter was correlated to pH and total N. For the newly converted soil of Labrador, the nematode composition was correlated to pH and CEC. These results confirm that nematode composition reflects available resources in soil and the management induced status of soils, albeit differently for converted soil of different ages. Nonetheless, all soil had a high bacterivore/fungivore ratio indicating that nutrients are being quickly cycled through all systems.

The hypothesis that conversion to agriculture has a negative effect on the nematode populations was not supported by the results of Experiments 1 and 2; the *Ag.* soil had higher nematode abundance than *Nat.* soil. As nematode populations reflect microfaunal populations in the soil (Parmelee and Alston, 1986) one can propose that regular manure and crop residue inputs influenced the soil food web. Though, more microarthropods were found in *Nat.* soil than *Ag.*, indicating that agriculture intensification and disturbance likely impacted microarthropod community structure (Osler and Murphy, 2005; Postma-Blaauw et al., 2010).

The second hypothesis that manure application will result in higher biotic abundance was only partially corroborated by the Newfoundland nematode data; the manure treatments were of no relevance to nematode community structure. It is likely that the recent manure and no manure managements for this experiment had a minimal effect above the impact of long-term excessive manuring. It is thus quite possible that any differences in amounts of organic matter were not significant once an acceptable range for nematode survival was reached. Microarthropods, on the other hand, seemed to have responded to the newly added manure at only one farm. This emphasises that biotic response to manure is likely dependent on the physicochemical properties of the manure and hence can vary with location.

The third hypothesis, that biochar application will provide increased opportunity for biotic abundance and diversity by ameliorating soil physicochemical parameters was supported by Experiment 3. There was a clear increase in total nematode abundance with biochar application in the Labrador soil. Given the young age of the biochar amendment

and the low fertility status of a newly converted podzol, biochar did not improve soil organic matter or soil water content but increased soil pH; these changes were clearly correlated to changes in the nematode population but had no noticeable effect on microarthropods. The newly converted soil was not a suitable for microarthropod survival and was not sufficiently improved by biochar application as indicated by low abundance in all treatments. Low soil fauna abundance is related to poor ecosystem function (Wagg et al., 2014) emphasising that Labrador soil was of lower quality than that of Newfoundland.

Overall, the Newfoundland and Labrador soils have distinct food webs. The produced information has confirmed the distinctiveness is driven by the unique properties of converted acid podzols. The most obvious observation was the dominant role of bacterivorous nematodes, a probable indication of bacterially driven soil metabolism, independent of the differences in the scope of the management for soil organic matter and pH control.

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Appendix 1 Experiment 1 (2016) Physicochemical analysis

Soil Texture

Table A1.1 Table comparing 2016 *farmed* soil texture (% sand, silt, clay) from New World Dairy (NWD) and Hammond Farms (HF) irrespective of depth. Error term is CI_{95}. N=2.

	Mean			P-value		
	Sand	Silt	Clay	Sand	Silt	Clay
NWD	63.87±0.97	27.53±0.93	8.6±0.40	<0.001	<0.001	<0.001
HF	68.91±1.21	24.07±0.97	7.03±0.57			

Model Summaries

S			R-sq			R-sq(adj)		
Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
6.13	5.58	2.63	13.38%	8.09%	7.55%	13.03%	7.73%	7.18%

Table A1.2 Table comparing 2016 *natural* soil texture (% sand, silt, clay) from New World Dairy (NWD) and Hammond Farms (HF) irrespective of depth. Error term is CI_{95}. N=2.

	Mean			P-value		
	Sand	Silt	Clay	Sand	Silt	Clay
NWD	53.59±3.04	35.15±3.24	11.26±0.96	<0.001	<0.001	0.008
HF	68.49±5.81	22.66±4.61	8.85±1.54			

Model Summaries

S			R-sq			R-sq(adj)		
Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
8.03	6.95	2.26	47.99%	46.39%	23.31%	46.07%	44.41%	20.47%

Table A1.3 Table comparing 2016 *farmed* soil texture (% sand, silt, clay) of three depths of New World Dairy. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=3.

	Mean			P-value		
	Sand	Silt	Clay	Sand	Silt	Clay
0-10 cm	61.53±1.28	28.79±1.49	9.68±0.59	<0.001	0.012	<0.001
10-20 cm	62.81±1.44	28.22±1.44	8.97±0.63			
20-30 cm	67.27±1.96	25.59±1.84	7.14±0.70			

Model Summaries

S			R-sq			R-sq(adj)		
Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
5.87	5.92	2.36	15.17%	5.34%	17.21%	14.12%	4.17%	16.18%

	Groupings		
	Sand	Silt	Clay
0-10 cm	a	a	a
10-20 cm	b	ab	a
20-30 cm	b	b	b

Table A1.4 Table comparing 2016 *natural* soil texture (% sand, silt, clay) of three depths of New World Dairy. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=3.

	Mean			**P-value**		
	Sand	Silt	Clay	Sand	Silt	Clay
0-10 cm	50.81±7.02	39.47±7.13	9.71±1.81	0.393	0.124	0.043
10-20 cm	54.46±2.73	33.22±4.25	12.31±2.22			
20-30 cm	55.50±9.06	32.76±7.82	11.75±1.51			

Model Summaries

S			**R-sq**			**R-sq(adj)**		
Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
5.48	5.302	1.50	14.41%	29.42%	40.71%	0.15%	17.66%	30.83%

	Grouping		
	Sand	Silt	Clay
0-10 cm	a	a	a
10-20 cm	a	a	ab
20-30 cm	a	a	b

Table A1.5 Table comparing 2016 *farmed* soil texture (% sand, silt, clay) of three depths of Hammond Farms. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=3.

	Mean			**P-value**		
	Sand	Silt	Clay	Sand	Silt	Clay
0-10 cm	66.00±1.84	26.23±1.38	7.78±1.07	<0.001	<0.001	0.101
10-20 cm	68.28±1.34	24.78±0.91	7.02±0.80			
20-30 cm	72.62±2.38	21.12±2.10	6.26±1.12			

Model Summaries

S			**R-sq**			**R-sq(adj)**		
Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
5.08	4.08	2.68	23.33%	22.29%	5.24%	21.52%	20.46%	3.01%

	Grouping		
	Sand	Silt	Clay
0-10 cm	a	a	a
10-20 cm	b	a	a
20-30 cm	b	b	a

Bulk Density

Table A1.6 Table comparing 2016 *farmed* soil bulk density (g cm^{-3}) from New World Dairy (NWD) and Hammond Farms (HF) irrespective of depth. Error term is CI_{95}. N=2.

	Mean	**P-value**
NWD	1.08±0.003	<0.001
HF	1.25±0.018	

Model Summary

S	**R-sq**	**R-sq(adj)**
0.03	87.86%	87.75%

Table A1.7 Table comparing 2016 *natural* soil bulk density (g cm^{-3}) from New World Dairy (NWD) and Hammond Farms (HF) irrespective of depth. Error term is CI_{95}. N=2.

	Mean	**P-value**
NWD	0.97±0.099	<0.001
HF	1.29±0.052	

Model Summary

S	**R-sq**	**R-sq(adj)**
0.14	57.80%	56.24%

Table A1.8 Table comparing 2016 *farmed* and *natural* bulk density (g cm^{-3}) of New World Dairy soil. Error term is CI_{95}. N=2.

	Mean	**P-value**
Farmed	1.08±0.003	<0.001
Natural	0.97±0.099	

Model Summary

S	**R-sq**	**R-sq(adj)**
0.07	25.46%	24.69%

Soil Water Content at Sampling

Table A1.9 Table comparing 2016 *farmed* soil water content (%) from New World Dairy (NWD) and Hammond Farms (HF) irrespective of depth. Error term is CI_{95}. N=2.

	Mean	P-value
HF	24.98±1.53	<0.001
NWD	33.84±0.89	

Model Summary

S	R-sq	R-sq(adj)
7.15	22.94%	22.71%

Table A1.10 Table comparing 2016 *natural* soil water content (%) from New World Dairy (NWD) and Hammond Farms (HF) irrespective of depth. Error term is CI_{95}. N=2.

	Mean	P-value
HF	15.06±4.13	<0.001
NWD	39.53±6.49	

Model Summary

S	R-sq	R-sq(adj)
9.79	62.65%	61.26%

Table A1.11 Table comparing 2016 *farmed* soil water content (%) of three depths of New World Dairy. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=3.

	Mean	P-value
0-10 cm	34.94±1.43	0.049
10-20 cm	34.25±1.24	
20-30 cm	32.32±1.90	

Model Summary

S	R-sq	R-sq(adj)
7.07	2.43%	1.64%

	Grouping
0-10 cm	a
10-20 cm	ab
20-30 cm	b

Table A1.12 Table comparing 2016 *natural* soil water content (%) of three depths of New World Dairy. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=3.

	Mean	P-value
0-10 cm	50.31±11.78	0.018
10-20 cm	36.71±10.98	
20-30 cm	31.57±13.95	

Model Summary

S	R-sq	R-sq(adj)
9.05	48.80%	40.27%

	Grouping
0-10 cm	a
10-20 cm	ab
20-30 cm	b

Table A1.13 table comparing 2016 *farmed* soil water content (%) of three depths of Hammond Farms. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=3.

	Mean	P-value
0-10 cm	29.83±2.60	<0.001
10-20 cm	25.67±1.86	
20-30 cm	19.28±2.00	

Model Summary

S	R-sq	R-sq(adj)
5.79	36.85%	35.36%

	Grouping
0-10 cm	a
10-20 cm	b
20-30 cm	c

pH

Table A1.14 Table comparing 2016 *farmed* soil pH from New World Dairy (NWD) and Hammond Farms (HF) irrespective of depth. Error term is CI_{95}. N=2.

	Mean	P-value
NWD	5.68±0.07	<0.001
Hammond	6.31±0.07	

Model Summary

S	R-sq	R-sq(adj)
0.41	35.10%	34.84%

Table A1.15 Table comparing 2016 *natural* soil pH from New World Dairy (NWD) and Hammond Farms (HF) irrespective of depth. Error term is CI_{95}. N=2.

	Mean	P-value
NWD	4.41±0.09	<0.001
Hammond	4.82±0.09	

Model Summary

S	R-sq	R-sq(adj)
0.16	63.86%	61.22%

Table A1.16 Table comparing 2016 *farmed* and *natural* pH of New World Dairy soil. Error term is CI_{95}. N=2.

	Mean	P-value
Natural	4.41±0.09	<0.001
Farmed	5.68±0.07	

Model Summary

S	R-sq	R-sq(adj)
0.44	39.05%	38.71%

Table A1.17 Table comparing 2016 *farmed* and *natural* pH of Hammond Farms soil. Error term is CI_{95}. N=2.

	Mean	P-value
Natural	4.82±0.09	<0.001
Farmed	6.31±0.07	

Model Summary

S	R-sq	R-sq(adj)
0.30	74.90%	74.65%

Table A1.18 table comparing 2016 *farmed* soil pH of three depths of New World Dairy. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=3.

	Mean	P-value
0-10 cm	5.80±0.10	<0.001
10-20 cm	5.77±0.10	
20-30 cm	5.47±0.13	

Model Summary

S	R-sq	R-sq(adj)
0.43	10.66%	9.55%

	Grouping
0-10 cm	a
10-20 cm	a
20-30 cm	b

Appendix 2 Experiment 1 (2016) microarthropod analysis

Table A2.1 Table comparing 2016 *farmed* soil microarthropod abundance (individuals per 1 kg dry soil) from New World Dairy (NWD) and Hammond Farms (HF). Error term is CI_{95}. N=2.

	Mean	P-value
HF	54.7±35.70	0.002
NWD	6.04±3.20	

Model Summary

S	R-sq	R-sq(adj)
55.61	16.29%	14.74%

Table A2.2 Table comparing 2016 *natural* soil microarthropod abundance (individuals per 1 kg dry soil) from New World Dairy (NWD) and Hammond Farms (HF). Error term is CI_{95}. N=2.

	Mean	P-value
HF	2.67±3.89	0.022
NWD	39.60±32.40	

Model Summary

S	R-sq	R-sq(adj)
41.72	17.32%	14.36%

Table A2.3 Table comparing 2016 0-10cm *Farmed* and 0-10cm *natural* soil microarthropod abundance (individuals per 1 kg dry soil) from New World Dairy. Error term is CI_{95}. N=2.

	Mean	P-value
Farmed	6.04±3.20	<0.001
Natural	94.7±93.10	

Model Summary

S	R-sq	R-sq(adj)
26.7	57.64%	56.43%

Table A2.4 Table comparing 2016 *natural* soil microarthropod abundance (individuals per 1 kg dry soil) for three depths from New World Dairy. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=3.

	Mean	P-value
0-10 cm	94.70±93.10	0.018
10-20 cm	20.00±27.45	
20-30 cm	4.00±7.40	

Model Summary

S	R-sq	R-sq(adj)
45.3	48.75%	40.21%

	Grouping
0-10 cm	a
10-20 cm	ab
20-30 cm	b

Appendix 3 Experiment 2 (2017) physicochemical analysis

Soil Texture

Table A3.1 table comparing 2017 *farmed* soil texture (% sand, silt, clay) from New World Dairy (NWD) and Hammond Farms (HF). Error term is CI_{95}. N=2.

	Mean			**P-value**		
	Sand	Silt	Clay	Sand	Silt	Clay
NWD	45.19±5.07	45.06±4.76	9.75±0.90	<0.001	<0.001	<0.001
HF	66.06±1.34	26.19±1.14	7.75±0.55			

Model Summaries

S			**R-sq**			**R-sq(adj)**		
Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
7.93	7.4	1.59	64.56%	63.09%	29.57%	63.63%	62.12%	27.72%

Table A3.2. Table comparing 2017 *natural* soil texture (% sand, silt, clay) from New World Dairy (NWD) and Hammond Farms (HF). Error term is CI_{95}. N=2.

	Mean			**P-value**		
	Sand	Silt	Clay	Sand	Silt	Clay
NWD	22.80±7.21	69.17±7.51	8.03±2.85	<0.001	<0.001	0.002
HF	56.46±1.80	30.38±2.35	13.17±1.12			

Model Summaries

S			**R-sq**			**R-sq(adj)**		
Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
4.23	4.48	1.74	95.18%	95.91%	73.08%	94.58%	95.40%	69.71%

Table A3.3. Table comparing 2017 *farmed* and *natural* soil texture (% sand, silt, clay) from New World Dairy irrespective of depth. Error term is CI_{95}. N=2.

	Mean			**P-value**		
	Sand	Silt	Clay	Sand	Silt	Clay
Farmed	45.19±5.07	45.06±4.76	9.75±0.90	<0.001	<0.001	0.096
Natural	22.80±7.21	69.17±7.51	8.03±2.85			

Model Summaries

S			**R-sq**			**R-sq(adj)**		
Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
10.14	9.59	1.99	**45.90%**	52.37%	**11.55%**	43.55%	50.30%	7.70%

Table A3.4. Table comparing 2017 *farmed* and *natural* soil texture (% sand, silt, clay) from Hammond Farms irrespective of depth. Error term is CI_{95}. N= 2.

	Mean			P-value		
	Sand	Silt	Clay	Sand	Silt	Clay
Farmed	66.06±1.37	26.19±1.14	7.75±0.55	<0.001	0.002	<0.001
Natural	56.46±1.80	30.38±2.35	13.17±1.12			

Model Summaries

S			R-sq			R-sq(adj)		
Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
2.72	2.35	1.13	68.38%	35.61%	80.02%	67.00%	32.81%	79.15%

Table A3.5 Table 2017 soil texture (% sand, silt, clay) for crop treatment irrespective of farm. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=4.

	Mean			P-value		
	Sand	Silt	Clay	Sand	Silt	Clay
Corn	60.04±4.78	31.52±4.25	8.45±0.76	0.001	0.003	0.044
Oat & pea	51.22±17.03	40.24±15.75	8.54±2.51			
Soybean	44.58±5.79	45.53±6.21	9.90±0.68			
Natural	39.63±13.00	49.78±14.93	10.60±2.26			

Model Summaries

S			R-sq			R-sq(adj)		
Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
13.29	13.36	2.15	30.98%	26.44%	15.95%	26.48%	21.65%	10.47%

	Grouping		
	Sand	Silt	Clay
Corn	a	a	a
Oat & pea	ab	ab	ab
Soybean	b	ab	ab
Natural	b	b	b

Soil Water Content at Sampling

Table A3.6 Table comparing 2017 *natural* soil water content (%) from New World Dairy (NWD) and Hammond Farms (HF). Error term is CI_{95}. N=2.

	Mean	P-value
NWD	54.04±6.93	<0.001
HF	26.61±5.43	

Model Summary

S	R-sq	R-sq(adj)
5.01	90.35%	89.14%

Table A3.7 Table comparing 2017 *farmed* and *natural* fresh soil water content (%) from New World Dairy irrespective of depth. Error term is CI_{95}. N=2.

	Mean	P-value
Farmed	34.58±3.38	<0.001
Natural	54.04±6.93	

Model Summary

S	R-sq	R-sq(adj)
6.97	57.54%	55.69%

Table A3.8 Table comparing 2017 *farmed* and *natural* fresh soil water content (%) from Hammond Farms irrespective of depth. Error term is CI_{95}. N=2.

	Mean	P-value
Farmed	31.18±1.61	0.019
Natural	26.61±5.43	

Model Summary

S	R-sq	R-sq(adj)
3.62	21.77%	18.37%

Table A3.9 Table comparing 2017 fresh soil water content (%) for crop treatment from New World Dairy soil. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=4.

	Mean	P-value
Corn	38.45±3.70	<0.001
Oat & pea	26.36±7.07	
Soybean	37.83±3.54	
Natural	54.04±6.93	

Model Summary

S	R-sq	R-sq(adj)
5.06	79.55%	76.63%

	Grouping
Corn	a
Oat & pea	c
Soybean	a
Natural	b

Table A3.10 Table comparing 2017 fresh soil water content (%) for crop treatment irrespective of farm. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=4.

	Mean	P-value
Corn	32.86±1.86	0.007
Oat & pea	26.36±7.07	
Soybean	37.83±3.54	
Natural	40.33±10.88	

Model Summary

S	R-sq	R-sq(adj)
8.03	23.17%	18.16%

	Grouping
Corn	ab
Oat & pea	a
Soybean	ab
Natural	b

pH

Table A3.11 Table comparing 2017 *farmed* soil pH from New World Dairy (NWD) and Hammond Farms (HF). Error term is CI_{95}. N=2.

	Mean	P-value
NWD	5.65±0.14	<0.001
HF	6.14±0.07	

Model Summary

S	R-sq	R-sq(adj)
24	51.43%	50.15%

Table A3.12 Table comparing 2017 *natural* soil pH from New World Dairy (NWD) and Hammond Farms (HF). Error term is CI_{95}. N=2.

	Mean	P-value
NWD	3.55±0.25	0.001
HF	4.08±0.09	

Model Summary

S	R-sq	R-sq(adj)
0.16	78.84%	76.19%

Table A3.13 Table comparing 2017 *farmed* and *natural* soil pH from New World Dairy soil. Error term is CI_{95}. N=2.

	Mean	P-value
Farmed	5.65±0.14	<0.001
Natural	3.55±0.25	

Model summary

S	R-sq	R-sq(adj)
0.29	89.97%	89.54%

Table A3.14 Table comparing 2017 *farmed* and *natural* soil pH from Hammond Farms. Error term is CI_{95}. N=2.

	Mean	P-value
Farmed	6.14±0.07	<0.001
Natural	4.08±0.09	

Model summary

S	R-sq	R-sq(adj)
0.15	97.18%	97.06%

Table A3.15 Table comparing 2017 soil pH for crop treatment irrespective of farm. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=4.

	Mean	P-value
Corn	6.01±0.12	<0.001
Oat & pea	5.65±0.59	
Soybean	5.70±.09	
Natural	3.81±0.23	

Model Summary

S	R-sq	R-sq(adj)
0.31	88.73%	87.99%

	Grouping
Corn	a
Oat & pea	a
Soybean	a
Natural	b

Soil Organic Carbon

Table A3.16 Table comparing 2017 *farmed* soil organic carbon (%) from New World Dairy (NWD) and Hammond Farms (HF). Error term is CI_{95}. N=2.

	Mean	P-value
NWD	3.75±0.47	0.047
HF	4.29±0.30	

Model Summary

S	R-sq	R-sq(adj)
0.83	9.99%	7.63%

Table A3.17 Table comparing 2017 *natural* soil organic carbon (%) from New World Dairy (NWD) and Hammond Farms (HF). Error term is CI_{95}. N=2.

	Mean	P-value
NWD	15.30±9.45	0.015
HF	4.82±0.71	

Model Summary

S	R-sq	R-sq(adj)
5.40	54.06%	48.32%

Table A3.18 Table comparing 2017 *farmed* and *natural* soil organic carbon (%) from New World Dairy. Error term is CI_{95}. N=2.

	Mean	P-value
Farmed	3.75±0.47	<0.001
Natural	15.30±9.45	

Model Summary

S	R-sq	R-sq(adj)
3.3	68.03%	66.64

Total Carbon

Table A3.19 Table comparing 2017 *farmed* soil total carbon (mg kg^{-1}) from New World Dairy (NWD) and Hammond Farms (HF). Error term is CI_{95}. N=2.

	Mean	P-value
NWD	32050±3869	<0.001
HF	42280±3681	

Model Summary

S	R-sq	R-sq(adj)
8068.49	29.73%	27.88%

Table A3.20 Table comparing 2017 *farmed* and *natural* soil total carbon (mg kg^{-1}) from New World Dairy. Error term is CI_{95}. N=2.

	Mean	P-value
Farmed	32050±3869	<0.001
Natural	88280±63611	

Model Summary

S	R-sq	R-sq(adj)
22647.2	51.74%	49.64

Total Nitrogen

Table A3.21 Table comparing 2017 *farmed* soil total nitrogen (mg kg^{-1}) from New World Dairy (NWD) and Hammond Farms (HF). Error term is CI_{95}. N=2.

	Mean	P-value
NWD	2020±225	<0.001
HF	3725±411	

Model Summary

S	R-sq	R-sq(adj)
706.91	60.49%	59.45%

Table A3.22 Table comparing 2017 *farmed* and *natural* soil total nitrogen (mg kg^{-1}) from New World Dairy. Error term is CI_{95}. N=2.

	Mean	P-value
Farmed	2020±225	<0.001
Natural	5100±2873	

Model Summary

S	R-sq	R-sq(adj)
1058.96	59.53%	57.77

Table A3.23 Table comparing 2017 *farmed* and *natural* soil total nitrogen (mg kg^{-1}) from Hammond Farms. Error term is CI95. N=2.

	Mean	P-value
Farmed	3725±411	0.011
Natural	2580±710	

Model summary

S	R-sq	R-sq(adj)
832.11	24.77%	21.50%

Table A3.24 Table comparing 2017 soil total nitrogen (mg kg^{-1}) for crop treatment irrespective of farm. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=4.

	Mean	P-value
Corn	3335±433	0.004
Oat & pea	1867±744	
Soybean	2125±256	
Natural	3840±1482	

Model Summary

S	R-sq	R-sq(adj)
1238.61	25.05%	20.16%

	Grouping
Corn	ab
Oat & pea	a
Soybean	a
Natural	b

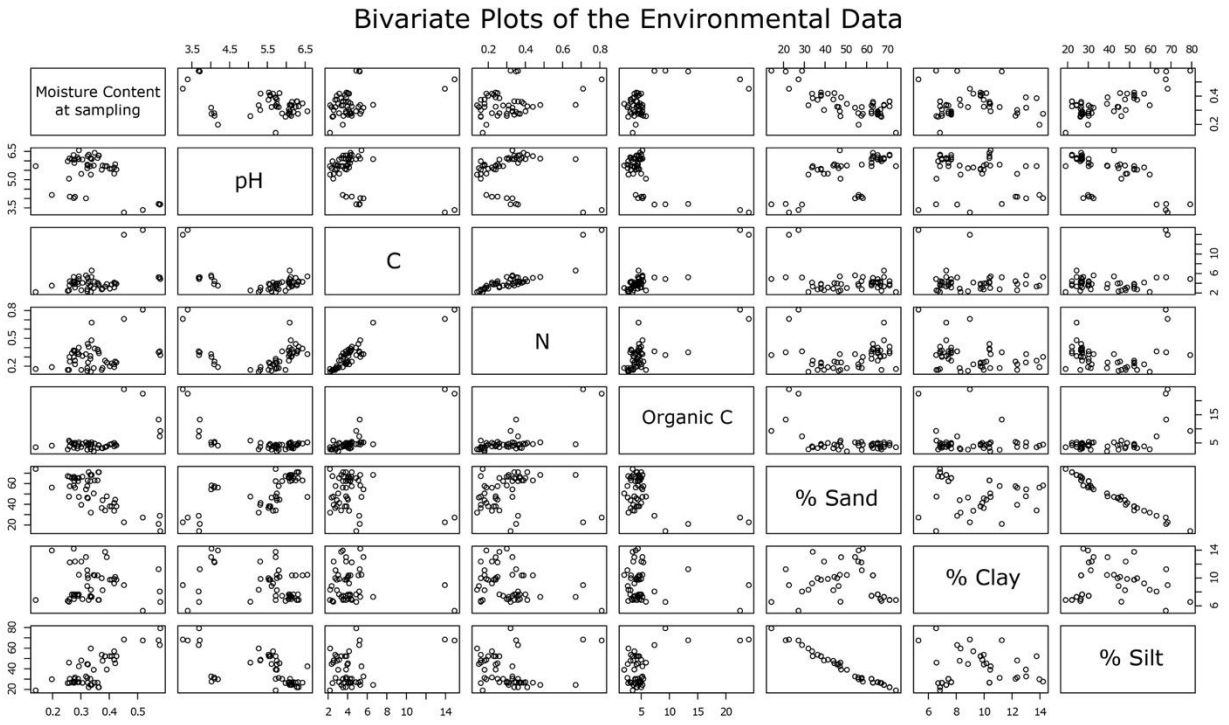

Figure 11.3.25 bivariate plots for 2017 abiotic factors.

Appendix 4 Experiment 2 (2017) Nematode analysis

Table A4.1 Table comparing nematode abundance (individuals per 100 g dry soil) for *farmed* and *natural* soil from Hammond Farms (HF) and New World Dairy (NWD). Error term is CI_{95}. N=2.

		Mean	P-value
HF	Farmed	2323±336	0.008
	Natural	1375±180	
NWD	Farmed	2010±306	0.013
	Natural	1208±148	

Model Summaries

	S	R-sq	R-sq(adj)
HF	656.5	26.59%	23.40%
NWD	595.7	23.97%	20.66%

Table A4.2 Table comparing nematode abundance (individuals per 100 g dry soil) for crop treatment for New World Dairy. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=4.

	Mean	P-value
Corn	1964±336	0.002
Oat & pea	2552±822	
Soybean	1638±405	
Natural	1208±148	

Model Summary

S	R-sq	R-sq(adj)
501.4	50.82%	43.80%

	Grouping
Corn	ab
Oat & pea	a
Soybean	b
Natural	b

Table A4.3 Table comparing nematode abundance (individuals per 100 g dry soil) for crop treatment irrespective of farm. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=4.

	Mean	P-value
Corn	2240±267	<0.001
Oat & pea	2552±822	
Soybean	1638±405	
Natural	1292±109	

Model Summary

S	R-sq	R-sq(adj)
5871	37.19%	33.09%

	Grouping
Corn	ab
Oat & pea	a
Soybean	bc
Natural	c

Appendix 5 Experiment 2 (2017) Microarthropod analysis

Table A5.1 Table comparing 2017 microarthropod abundance for *farmed* and *natural* soil (individuals per 1 kg dry soil) from New World Dairy (NWD) and Hammond Farms (HF). Error term is CI_{95}. N=2.

		Mean	P-value
Farmed	NWD	11.36±2.88	<0.001
	HF	29.63±7.50	
Natural	NWD	57.80±29.40	0.024
	HF	22.96±12.84	

Model Summaries

	S	R-sq	R-sq(adj)
Farmed	20.81	16.42%	15.63%
Natural	29.54	28.09%	23.59%

Table A5.2 Table comparing 2017 *farmed* and *natural* soil microarthropod abundance (individuals per 1 kg dry soil) from New World Dairy. Error term is CI_{95}. N=2.

	Mean	P-value
Farmed	11.36±2.88	<0.001
Natural	57.80±29.40	

Model summary

S	R-sq	R-sq(adj)
17.01	48.49	47.65

Table A5.3 Table comparing 2017 microarthropod abundance (individuals per 1 kg dry soil) for manure treatment from New World Dairy. Error term is CI_{95}. N=2.

	Mean	P-value
Manure	8.40±3.70	0.038
No manure	14.32±4.37	

Model Summary

S	R-sq	R-sq(adj)
10.24	8.00%	6.24%

Table A5.4 Table comparing 2017 microarthropod abundance (individuals per 1 kg dry soil) for crop treatment from New World Dairy. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=4.

	Mean	P-value
Corn	11.85±5.52	<0.001
Oat & pea	9.26±5.10	
Soybean	12.96±5.27	
Natural	57.80±29.40	

Model Summary

S	R-sq	R-sq(adj)
17.24	48.87%	46.27%

	Grouping
Corn	a
Oat & pea	a
Soybean	a
Natural	b

Table A5.5 Table comparing 2017 microarthropod abundance (individuals per 1 kg dry soil) for crop treatment irrespective of farm. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=4.

	Mean	P-value
Corn	25.19±6.00	<0.001
Oat & pea	9.26±5.10	
Soybean	12.96±5.27	
Natural	40.37±16.81	

Model Summary

S	R-sq	R-sq(adj)
23.86	13.64%	11.52%

	Grouping
Corn	ab
Oat & pea	a
Soybean	a
Natural	b

Appendix 6 Experiment 3 (Labrador) Physicochemical analysis

pH

Table A6.1 Table comparing Labrador soil pH with biochar *rates*. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=5.

Rate (t C ha^{-1})	Mean	P-value
0	4.75±0.46	<0.001
10	5.40±0.28	
20	5.08±0.23	
40	5.60±0.41	
80	6.35±0.33	

Model Summary

S	R-sq	R-sq(adj)
0.35	65.70%	60.61%

	Grouping
0	a
10	bc
20	ab
40	c
80	d

Table A6.2 Table comparing Labrador soil pH with biochar *age*. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=3.

	Mean	P-value
Control	4.75±0.46	<0.001
New	5.76±0.28	
Old	5.15±0.18	

Model Summary

S	R-sq	R-sq(adj)
0.43	46.16%	42.44%

	Grouping
Control	a
New	b
Old	a

Calcium

Table A6.3 Table comparing Labrador soil Ca concentration (mg kg^{-1}) with biochar *rates*. Groupings were evaluated using a post-hoc Tukey test (95% confidence).

Rate (t C ha^{-1})	Mean	P-value
0	226.0±123.7	<0.001
10	425.4±128.2	
20	384.4±83.5	
40	658.4±219.2	
80	1013.0±253.2	

Model Summary

S	R-sq	R-sq(adj)
173.19	67.60%	62.80%

	Grouping
0	a
10	ab
20	a
40	b
80	c

Table A6.4 Table comparing Labrador soil Ca concentration (mg kg^{-1}) with biochar *age*. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=3.

	Mean	P-value
Control	226.0±123.7	<0.001
New	700.2±156.6	
Old	382.8±59.2	

Model Summary

S	R-sq	R-sq(adj)
220.4	43.64%	39.75%

	Grouping
Control	a
New	b
Old	a

Magnesium

Table A6.5 Table comparing Labrador soil Mg concentration (mg kg^{-1}) with biochar *rates*. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=5.

Rate (t C ha^{-1})	Mean	P-value
0	25.50±13.28	0.006
10	53.50±20.69	
20	40.88±13.72	
40	62.80±25.90	
80	91.30±47.50	

Model Summary

S	R-sq	R-sq(adj)
24.21	40.54%	31.73%

	Grouping
0	a
10	ab
20	a
40	ab
80	b

Table A6.6 Table comparing Labrador soil Mg concentration (mg kg^{-1}) with biochar *age*. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=3.

	Mean	P-value
Control	22.50±16.28	0.001
New	71.75±15.89	
Old	39.50±9.09	

Model Summary

S	R-sq	R-sq(adj)
23.35	40.62%	36.53%

	Grouping
Control	a
New	b
Old	a

Manganese

Table A6.7 ANOVA table comparing Labrador soil Mn concentration (mg kg^{-1}) with biochar *rates*. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=5.

Rate (t C ha^{-1})	Mean	P-value
0	15.23±4.95	0.018
10	21.36±5.88	
20	22.81±5.12	
40	25.71±6.07	
80	30.70±5.75	

Model Summary

S	R-sq	R-sq(adj)
6.22	34.82%	25.17%

	Grouping
0	a
10	ab
20	ab
40	ab
80	b

Table A6.8 Table comparing Labrador soil Mn concentration with biochar *age*. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=3.

	Mean	P-value
Control	15.23±4.95	0.036
New	25.33±3.33	
Old	23.05±4.93	

Model Summary

S	R-sq	R-sq(adj)
6.63	20.42%	14.94%

	Grouping
Control	a
New	b
Old	ab

Sulfur

Table A6.9 Table comparing Labrador soil S concentration (mg kg^{-1}) with biochar *rates*. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=5.

Rate (t C ha^{-1})	Mean	P-value
0	11.10±1.62	<0.001
10	12.28±0.83	
20	12.81±1.12	
40	14.54±1.22	
80	16.28±3.06	

Model Summary

S	R-sq	R-sq(adj)
1.34	61.29%	55.56%

	Grouping
0	a
10	a
20	ab
40	bc
80	c

Table A6.10 Table comparing Labrador soil S concentration (mg kg^{-1}) with biochar *age*. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=3.

	Mean	P-value
Control	11.10±1.62	0.003
New	14.36±1.12	
Old	12.70±0.74	

Model Summary

S	R-sq	R-sq(adj)
1.71	32.91%	28.29%

	Grouping
Control	a
New	b
Old	a

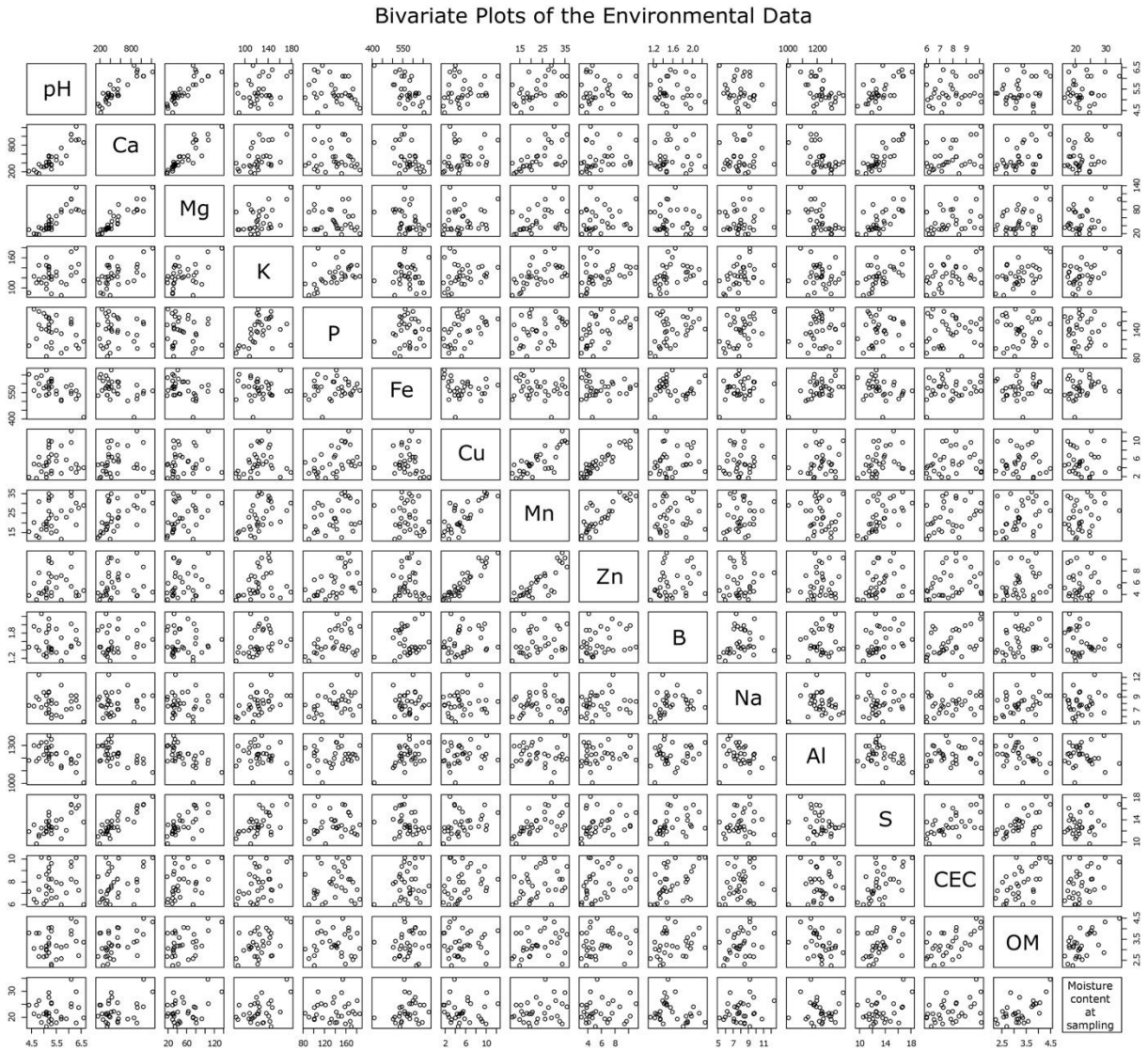

Figure A6.1 Bivariate plots for Labrador abiotic factors

Appendix 7 Experiment 3 (Labrador) Nematode analysis

Table A7.1 Table comparing Labrador soil nematode abundance (individuals per 100 g dry soil) with biochar *age* at 95% confidence. Groupings were evaluated using a post-hoc Tukey test (95% confidence). Error term is CI_{95}. N=3.

	Mean	P-value
Control	380.4±159.0	0.004
New	750.2±121.0	
Old	639.1±82.1	

Model Summary

S	R-sq	R-sq(adj)
184.44	31.24%	26.49%

	Grouping
Control	a
New	b
Old	ab

Appendix 8 Additional methodology

Table A8.1 ICP-MS Instrument Detection Limit (IDL) for the measured cations.

Cations	0.5 (mg kg^{-1})	1 (mg kg^{-1})	Sample range (NL soil) (mg kg^{-1})
P	3135.86	2159.09	322.82 - 3418.75
K	838.30	559.67	1876.25 - 2338.34
Ca	1339.49	2003.30	737.83 - 11579.08
Mn	597.41	585.92	48.36 - 2889.94
Cu	669.84	661.97	0.00 – 601.80
Zn	412.12	288.27	0.00 – 250.80
Na	1602.01	942.65	0.00 -792.96
Al	1400.24	2231.34	20720.86 - 39965.33
Mg	966.12	1393.96	10457.12 -28348.31
Fe	1826.75	2893.13	15751.04 - 208368.02

$$\boldsymbol{IDL} = 3SD_{blk} x \frac{STD_{cone}}{STD_x - BLK_x}$$

SD_{blk} Std dev of the intensities of the multiple blank measurements

STD_{Conc} Concentration of the standard

STD_X Average signal for the standard

BLK_X Average signal for the blank