

Nematodes require space: The relationship between nematode community assemblage and soil carbon across varying aggregate fractions

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ABSTRACT

Soil aggregates are critical for soil carbon (C) sequestration and serve as a habitat for microorganisms, including free-living nematodes. Yet, the interactions between nematode community composition and soil C cycling across aggregate fractions is rarely assessed. The objectives of this study were to 1) determine how soil type alters nematode community dispersal across aggregate fractions, 2) understand how soil type may alter the relationship between nematode communities and labile C pools, and 3) assess the impact that tillage intensity has on nematode community assemblage across aggregate fractions. Soil cores were taken at two identical long-term trials located in Ohio (silt loam vs. clay). Each trial compared two different tillage intensities (chisel till vs. no-till). Soils were fractionated into three aggregate fractions (>2000 μm , 2000–250 μm , and <250 μm). For each fraction, nematode communities were identified to feeding group and permanganate oxidizable carbon (POXC) was measured. Results indicated that silt loam soils had greater bacterivore nematodes in the >250 μm aggregate fraction ($p < 0.05$) and relatively lower predator-omnivore nematode proportions when compared to clay soils. Tillage did not have a significant effect on nematode feeding groups. Correlation analyses revealed that predator-omnivore nematodes and POXC were positively correlated in clay soils, but only in the largest aggregate fraction ($p < 0.05$). Bacterivore nematodes were positively correlated with POXC in clay soils but negatively correlated in silt loam soils ($p < 0.05$). These findings reveal that predator-prey dynamics likely drive soil C accumulation in larger sized aggregates. Moreover, shifts in nematode community composition by soil type demonstrates how inherent properties influence soil food web structure and ultimately, soil C cycling.

1. Introduction

Soil aggregates exist at the intersection of chemical, physical, and biological processes and strongly influence microbial community dynamics and soil carbon (C) sequestration (Wang et al., 2022b). Aggregates are largely formed via biological activity including plant root exploration, fungal hyphae, and polysaccharides produced by bacteria (Tisdall and Oades, 1982). The extent to which aggregates can physically protect soil C depends on aggregate size formation, and stability (Tiemann and Grandy, 2015). For example, unstable macroaggregates are often loosely held together by plant roots and fungal hyphae and contain labile C that is more exposed to microbial activity. Permanganate oxidizable carbon (POXC) can serve as an early indicator of soil C stabilization because it reflects a more processed pool of soil C (Hurisso et al., 2016; Sprunger et al., 2020; Martin and Sprunger, 2022). That

said, historically POXC has been used to reflect more labile or ‘Active’ pools of C.

Mineral organic associated C is stabilized within microaggregates that are strongly held together by chemical binding agents including polysaccharides and glomalin. These microaggregates are physically protected because they are further enmeshed within stable macroaggregates (Six et al., 2000). Aggregate stability is strongly maintained by sustainable management practices that reduce soil physical disturbance and foster increased organic matter, pore conductivity, and enhanced biological activity (Nunes et al., 2020). However, the destabilization of aggregates via physical and biological perturbation also re-orient aggregates, and impacts soil C stabilization potential due to exposure to microbial activity (von Haden et al., 2019; Baumhardt et al., 2015).

While the interaction between microbial communities and soil

Abbreviation: POXC, Permanganate oxidizable carbon.

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physical structure is a key component of soil C dynamics, much less attention has been placed on higher trophic level fauna that could also have significant impacts on soil C cycling. Aggregates serve as a habitat to much of the soil food web and the potential for soil C accumulation within aggregates could largely depend on trophic level interactions amongst various soil fauna. Predator-prey relationships significantly influence downstream impacts on soil C and nitrogen (N) cycling (Neher, 2001; Ferris, 2010). For instance, nematode communities could provide insight into C dynamics across aggregate fractions because of their trophic complexity and role in modulating soil organic matter decomposition (Zhang et al., 2013; Martin and Sprunger, 2021). Moreover, nematodes are, for the most part, specialists that consist of various sizes and traits that influence their ability to access resources across different sized aggregates. For example, with exception of certain genera, much of the nematode community consists of bacterivores that solely feed on bacteria, fungivores that solely feed on fungi, herbivorous nematodes that feed on plant roots, predatory nematodes that feed on other nematodes, and omnivores that change their feeding preferences based on environmental conditions and resource availability (Bongers, 1990; Ferris et al., 2001).

Nematode community composition can provide further insight into how soil C is processed through the soil food web because metabolic footprints vary by feeding groups. Systems dominated by bacterivores may indicate labile soil C cycling with low C:N ratios compared to a system dominated by fungivores, which may be associated with more processed pools of C with higher C:N ratios (Margenot and Hodson, 2016). Generally, nematodes are examined within the bulk soil, however, examining nematodes by aggregate fraction could provide further insight into how soil biota drive soil C accumulation (Martin and Sprunger, 2021). For example, bacterivorous nematodes suppress the soil metabolic quotient, thereby reducing mineralization processes and promoting soil C accumulation within macro-aggregates (Neher, 2010). A recent meta-analysis also found that greater abundance of bacterivorous nematodes was strongly correlated with soil organic carbon (SOC) stabilization in mid-sized aggregates (Martin and Sprunger, 2021). Further understanding soil fauna distribution across aggregate fraction, could shed light on key physio-biological interactions that shape soil C dynamics. Specifically, it is unknown how nematode trophic groups may alter labile C pools between varying aggregate fractions.

Shifts in nematode community composition by aggregate fraction is likely influenced by inherent properties, such as soil type and external factors including agricultural management (Fig. 1). Soil type drastically alters the spatial distribution of soil aggregates and has been shown to subsequently alter microbial distribution (Tiemann and Grandy, 2015). For instance, bacteria and fungi are more highly concentrated in smaller pore sizes due to greater resources as well as protection from higher order fauna (Martin and Sprunger, 2021). Contrasting management practices also alters the spatial distribution of aggregates. For example, tillage intensity consistently reduces stable aggregates, leading to losses of soil C (Grandy and Robertson, 2007). Zhang et al., (2013) found that conservation tillage increased soil food web complexity across all aggregate fractions. However, nematode community composition shifted by aggregate fraction, whereby large macroaggregates had a greater abundance of predator and omnivorous nematodes relative to microaggregates.

Here we seek to assess the relationship between nematode community composition and soil C cycling in row-crop agriculture. To this end, we investigate nematode community composition by aggregate fraction and relate community structure to the labile pool of soil C. Moreover, we compare these relationships in agroecosystems under contrasting management and soil type. Our specific objectives are to 1) determine how soil type alters nematode community dispersal across aggregate fraction, 2) understand how soil type may alter the relationship between nematode communities and labile C pools, and 3) assess the impact that tillage intensity has on nematode community assemblage across aggregate fraction. We hypothesize that 1) clay soils will have greater aggregate fractions, more structured soil food webs, and overall greater C stabilization relative to silt loam soils and 2) no-till systems will have greater pore size and a more even distribution of the nematode community, leading to more structured soil food webs and greater labile soil C.

2. Methods

2.1. Site description and experimental design

This study was conducted at two identical long-term research experiments as a part of The Ohio State University Tripplet-Van Doren

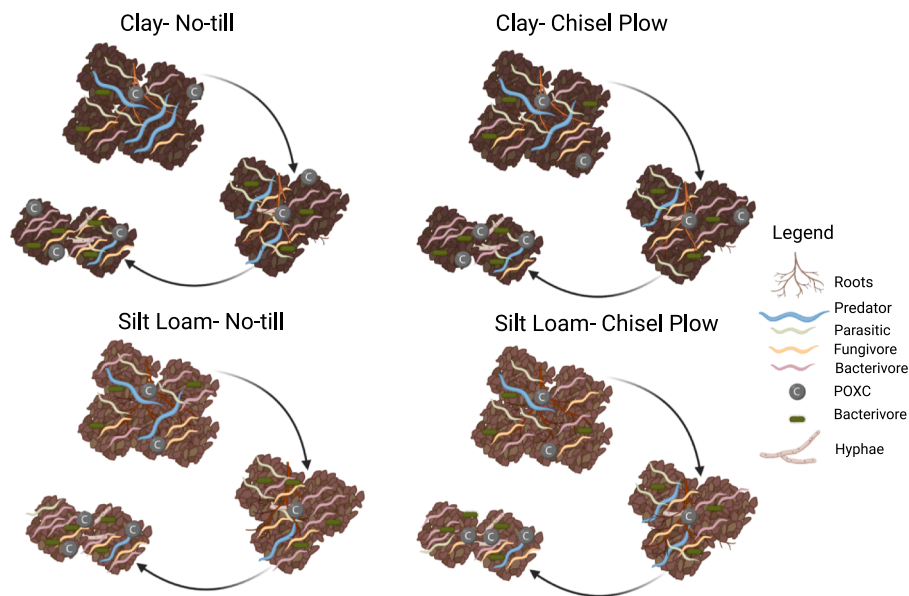


Fig. 1. Conceptual figure of the varying distribution of nematode communities and active carbon in clay and silt loam aggregates (>2000 μm , 2000–250 μm , and <250 μm) under no-till and chisel plow.

Long-Term Research trial. The Northwest Crop Rotation experiment is located in Hoytville, OH and was established in 1963 (Table 1). The Northwest site is dominated by clay soils and will be referred to as the “clay” site. The Wooster Site is in Wooster, OH and was established in 1962 (Table 1). The Wooster site will be referred to as the “silt loam” site. Both sites are full-factorial randomized complete block designs, however the trials differ in plot size. Specifically, the clay site has a 30.5 m × 6.4 m plot size, whereas the silt loam site has a 22.3 × 4.5 m plot size. The main factor within this experiment is tillage, whereby No-Till (NT) and Chisel Till (CT) treatments are compared. The NT treatment is never tilled. The CT treatment is tilled in the Spring with 30% of crop residue left on the field. Starter fertilizer was applied at 34–45 kg ha⁻¹ during pre-planting in the Spring of 2020. Samples were taken just prior to corn (*Zea Mays*) harvest. The seeding rate for corn was 84,014 seeds ha⁻¹. Each treatment has three replicate plots, which makes up six replicate plots total per site.

2.2. Soil sampling

Soil samples were collected in October 2020 two-weeks before corn harvest. Fifteen soil cores were collected at a 10 cm depth using a 1.91 cm diameter soil probe. Soil cores were collected using a stratified random sampling and then composited by treatment.

2.3. Soil processing for bulk soil analyses

Bulk soil samples were subsampled for organic matter (5 g) and soil texture (50 g). Subsamples for organic matter analysis were sent to Spectrum Analytics (Wooster, OH), where soil organic matter was measured using the Walkley-Black chromic acid wet oxidation method (Heanes, 1984). Soil texture was measured by pre-treating the soil with sodium hexametaphosphate and using a hydrometer to ensure the density of soil particles in suspension (Gee and Bauder, 1986). The rest of the bulk soil was refrigerated at 4 °C until aggregate fractionation took place. Soils were maintained at this temperature to allow the soil to reach an approximate soil water content of 100 g H₂O kg⁻¹.

2.4. Aggregate fractionation

Soil aggregates were fractionated using dry sieving to reduce the stress caused to free-living nematodes (Schutter and Dick, 2002; Zhang et al., 2016). Each composite sample was hand sieved through a 7 mm screen. Soil aggregates were then separated by placing 250 g of soil onto two sieves (2000 µm and 250 µm) that would divide the soil into three aggregate fractions: >2000 µm, 2000–250 µm, and <250 µm (Jiang et al., 2017). Next, the two sieves were stacked and mechanically shaken using a W.S Tyler® Ro-Tap® RX-29 8 Inch Sieve Shaker at an amplitude of 1.5 mm for 2 min (Zhang et al., 2016). Next, each aggregate fraction weight was recorded to determine the proportion of aggregate fractions per sample. Aggregate proportions were calculated as shown in Eq. (1). Samples from each aggregate fraction were subsampled for nematode extraction (50 g) and POXC analysis (2.5 g).

Table 1

Site descriptions and soil characteristics for chisel and no-till systems at the clay and silt loam sites.

Soil Type	Location	Soil Series	Taxonomic Class	30-year monthly average precipitation (mm)	Tillage	Sand	Silt	Clay	Organic Matter
						(%)	(%)	(%)	(g kg ⁻¹)
Clay	Custar, Wood County, OH 41° 13' N, 83° 45' W	Hoytville Clay Loam	Fine, illitic mesic Mollic Epiaqualf	81	Chisel till	29	43	28	2.6
					No-till	28	25	47	3.7
SiltLoam	Wooster, Wayne County OH 40° 45' N, 81° 54' W	Wooster silt loam	Fine-loamy, mixed active, mesic Oxyaquic Fragiudalfs	83	Chisel till	50	30	21	2.2
					No-till	21	57	22	2.1

$$\frac{\text{Aggregate fraction weight (g)}}{250 \text{ (g)}} \times 100 = \text{Proportion of aggregate (100)} \quad (1)$$

2.5. Nematode extraction and identification

Nematodes were extracted from each aggregate fraction using the Baermann funnel extraction technique (Baermann, 1917; Cesarz et al., 2019). Nematodes were collected from the Baermann funnels after 72 h. Nematodes were then fixed in 4% paraformaldehyde solution. Nematode abundance was collected through counting using a dissecting microscope at 50x magnification and the eye piece magnification at 10x. Afterwards 100 nematodes from each sample were sorted into their correct feeding group (bacterivore, fungivore, plant parasitic, and predator/omnivore) using a compound microscope at 100–400x magnification (Bongers, 1988). Bacterivore to fungivore (B:F) ratios were calculated by dividing the absolute abundance of bacterivores in each sample by the absolute abundance of fungivores.

2.6. Permanganate oxidizable carbon

Permanganate oxidizable carbon was measured utilizing the protocol adapted from Culman et al., (2012). Specifically, 20 mL of 0.02 M potassium permanganate (KMnO₄) solution was added to 2.5 g of soil. This soil solution was shaken and then allowed to settle. Afterwards, the supernatant and deionized water were mixed and read on a 96 well spectrophotometer at 550 nm. Permanganate oxidizable carbon was calculating use the equation adapted from Hurriso et al., (2016) (Eq. (2)). Specifically, 0.02 mol L⁻¹ is the initial concentration of KMnO₄, *a* is the intercept of the standard curve, and *b* is the slope of the standard curve.

$$POXC = [0.02 \text{ mol L}^{-1} - (a + bAbs)] \times (9000 \text{ mg C mol}^{-1}) \times (0.02 \text{ L solution Wt}^{-1}) \quad (2)$$

2.7. Statistical analyses

Data was assessed for normality using {MASS} in R 4.1.0 (R Core Team, 2021; Venables and Ripley, 2002). Analysis of variance (ANOVA) was conducted using the *lme* function in the {lme4} package, where tillage, aggregate fraction, site, the interaction between tillage and aggregate fraction, the interaction between site and tillage, the interaction between site and aggregate fraction, and the interaction between site, tillage, and aggregate fraction were treated as fixed effects (Bates et al., 2015). Block was treated as a random effect. Means separation was obtained using the Tukey's pairwise comparisons in the {multcomp} package in R (Hothorn et al., 2008). Significance was determined at $\alpha = 0.05$. All graphing was performed with {ggplot2} in R (Wickham, 2016).

Correlations were run between nematode feeding group and POXC to determine the relationship between these two variables in each aggregate fraction. Pearson's correlation coefficients were calculated using the *cor.mat* function in the {rstatix} package (Kassambara, 2022).

Table 2

Proportion of aggregates and total abundance of nematodes in micro (<250 μm), mid (250–2000 μm), and macro (>2000 μm) aggregates under chisel plow and no-till treatments in clay and silt loam soils. Columns with letters indicate significant differences at $p < 0.05$ after Tukey’s adjustment within each aggregate size.

Soil Type	Tillage	Aggregate Proportion (%)	Nematode Abundance (#)
>2000 μm Clay	Chisel Plow	39.8 (7.6) ab	330 (20)
	No-till	48.7 (14.9) ab	119.3 (86.9)
Silt Loam	Chisel Plow	51.12 (6.1) ab	278.3 (124.9)
	No-till	61.0 (3.9) b	587.7 (140.7)
2000–250 μm Clay	Chisel Plow	20.4 (1.7) ab	207.0 (175)
	No-till	16.1 (1.1) a	321.3 (145.6)
Silt Loam	Chisel Plow	29.3 (4.5) ab	224.3 (173.3)
	No-till	25.9 (4) ab	603 (177.1)
<250 μm Clay	Chisel Plow	26.0 (3.2) ab	26 (18)
	No-till	33.6 (14.4) ab	41 (25.4)
Silt Loam	Chisel Plow	17.2 (10.4) a	144.7 (63.8)
	No-till	10 (7.5) a	109.7 (15.6)

Correlations were significant at $p < 0.05$. All correlation graphs were conducted in {ggplot2} using the *geom_smooth* function.

3. Results

3.1. Site characteristics and aggregate proportion

Tillage appeared to have slim to no effect on percent clay and organic matter at the silt loam and clay site (Table 1). Aggregate proportion was

Table 3

Proportion of bacterivore, fungivore, plant parasitic, predator omnivore nematodes, and the bacterivore:fungivore (B:F) ratio in micro (<250 μm), mid (250–2000 μm), and macro (>2000 μm) aggregates under chisel plow and no-till till treatments in clay and silt loam soils. Columns with different letters indicate significant differences at $p < 0.05$ after Tukey’s adjustment within each aggregate size.

Soil Type	Tillage	Bacterivore	Fungivore	Plant Parasitic	Predator-omnivore	B:F
>2000 μm Clay	Chisel Plow	37.86 (2.14) ab	24.29 (4.29)	29.73 (0)	8.08 (2.18)	1.63 (0.38)
	No-till	15.48 (1.19) a	13.1 (3.57)	39.29 (22.62)	32.14 (17.86)	1.25 (0.25)
Silt Loam	Chisel Plow	48.69 (8.84) ab	19.53 (3.48)	21.27 (3.44)	10.51 (4.23)	2.89 (1.14)
	No-till	36.73 (6.48) ab	32.26 (6.96)	11.73 (0.62)	19.28 (14.05)	1.15 (0.05)
2000–250 μm Clay	Chisel Plow	37.5 (7.5) ab	25 (5.00)	26 (4.00)	6.5 (3.50)	1.63 (0.63)
	No-till	44.31 (11.08) ab	19.17 (3.63)	25.83 (14.46)	10.69 (1.45)	2.28 (0.36)
Silt Loam	Chisel Plow	54.94 (11.17) ab	20.69 (0.32)	17.51 (4.94)	6.87 (6.44)	2.67 (0.56)
	No-till	58.41 (7.18) ab	27.62 (7.62)	7.46 (4.89)	6.51 (5.15)	2.41 (0.65)
<250 μm Clay	Chisel Plow	48.19 (6.09) ab	12.09 (1.09)	27.5 (7.4)	11.71 (0.29)	3.97 (0.14)
	No-till	47.13 (2.87) ab	33.52 (9.46)	10.15 (5.14)	9.2 (9.2)	1.63 (0.41)
Silt Loam	Chisel Plow	67.7 (11.2) b	19.71 (2.25)	8.26 (6.03)	4.33 (4.33)	3.61 (0.91)
	No-till	62.18 (12.18) ab	19.87 (4.49)	16.67 (8.98)	1.28 (1.28)	3.44 (1.39)

not significantly affected by tillage or soil type. Not surprisingly, aggregate fraction did have a significant effect on aggregate proportion ($p < 0.01$; Table S1; Table 2). Specifically, silt loam NT treatments had a significantly greater aggregate proportion in >2000 μm fractions than compared to NT clay 2000–250 μm fractions and silt loam CT and NT <205 μm fractions.

3.2. Nematode abundance

Soil type had a significant effect on nematode abundance where silt loam soils had significantly greater nematode abundance than the clay soils ($p < 0.05$; Table S1). Additionally, aggregate fraction had a significant effect on total nematode abundance ($p < 0.01$). Nematode abundance was 48%, 11%, and 47% greater in the >2000 μm, 2000–250 μm, and the <250 μm fractions at the silt loam site, respectively compared to the clay site (Table 2). In addition, total nematode abundance in the >2000 μm and 2000–250 μm fraction was on average 3 and 7 times greater than in the <250 μm fraction, respectively (Table S2).

3.3. Nematode feeding groups

Soil type had a significant effect on bacterivore nematodes where silt loam nematode abundances were 77% greater in <250 μm fractions compared to clay NT nematode abundances in >2000 μm fractions ($p < 0.01$; Table S2; Table 3). Soil type also had a significant effect on plant parasitic nematodes. When averaging across tillage, abundances were greatest in the clay soil by up to 62% relative to silt loam when considering all three aggregate fractions (Table 3).

Aggregate fraction also substantially influenced the distribution of bacterivore nematodes ($p < 0.01$; Table S2). At the clay site, bacterivore nematodes abundances were significantly reduced in the clay NT treatment at the >2000 μm aggregate fraction (Fig. 2; Table 3). In addition, silt loam CT treatments had the greatest bacterivore abundances in the <250 μm aggregate fraction when compared to bacterivore abundances in the clay NT >2000 μm aggregate fraction.

Surprisingly, tillage did not influence nematode feeding groups (Table S2; $p > 0.05$). However, both fungivores and predator/omnivores trended higher in NT relative CT (Table 3). In contrast, plant parasitic

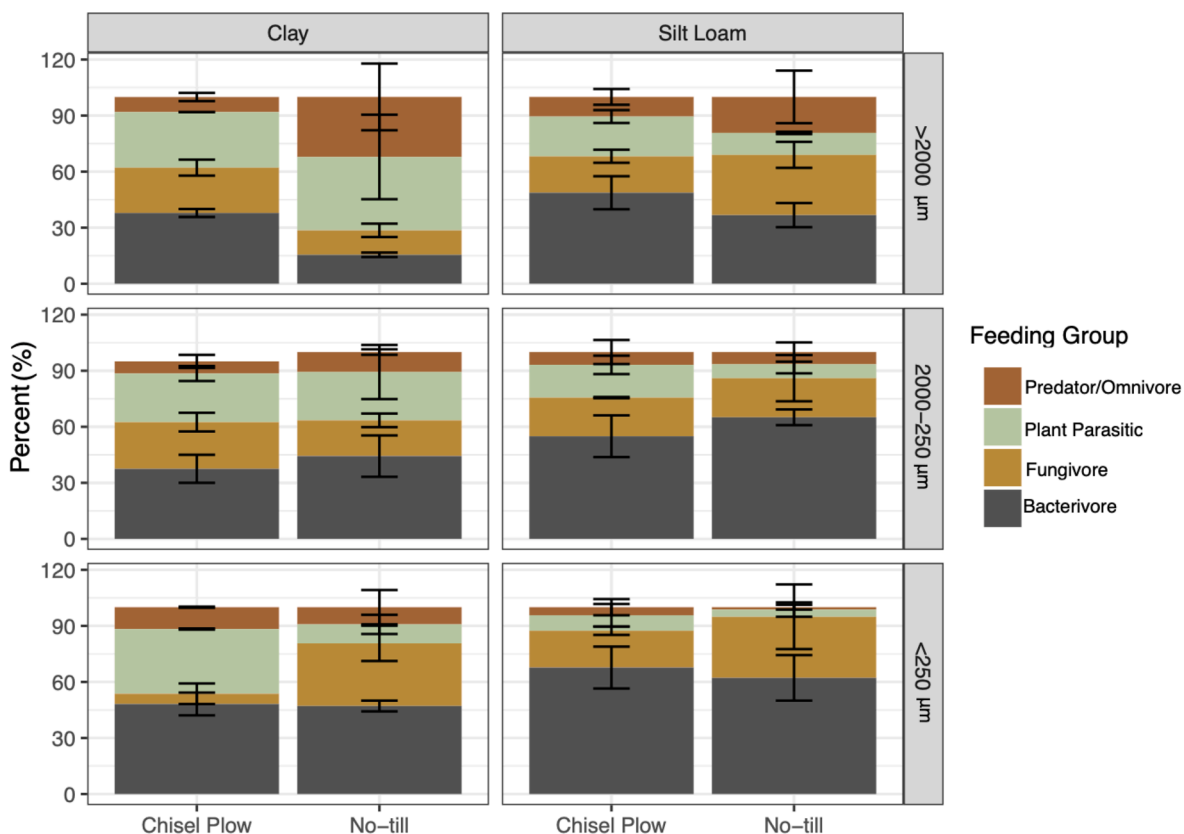


Fig. 2. Proportion of predator-omnivore, plant parasitic, fungivore, and bacterivore nematodes in >2000 μm, 2000–250 μm, and <250 μm aggregate fractions under chisel plow and no-till treatments in clay and silt loam soils. Error bars represent one standard error of the mean.

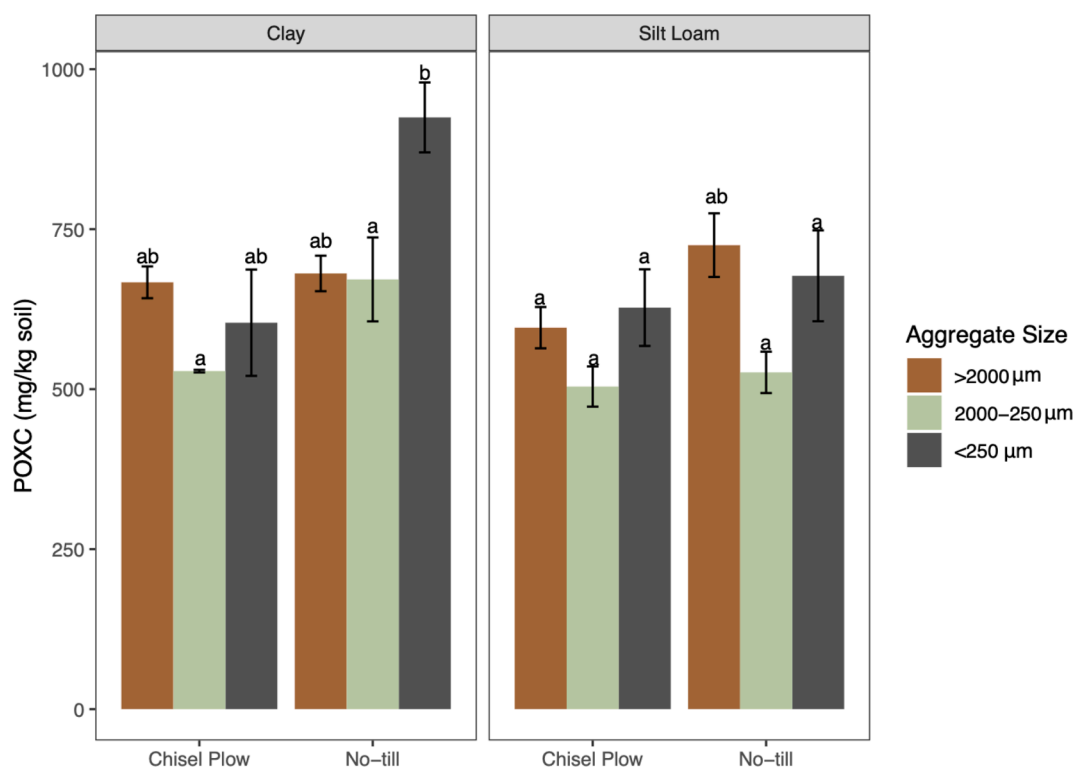


Fig. 3. Permanganate Oxidizable Carbon (PMNOC) (mg/kg soil) in >2000 μm, 2000–250 μm, and <250 μm aggregate size under chisel plow and no-till treatment in clay and silt loam sites. Error bars represent one standard error of the mean. Means separation represent significant differences at p < 0.05 after Tukey's adjustment.

nematodes abundances appeared to be greater in CT treatments at the silt loam site (Table 3).

3.4. Permanganate oxidizable carbon

Soil type ($p < 0.05$), tillage ($p < 0.001$), and aggregate fraction ($p < 0.001$) all had a significant effect on POXC (Table S1). These significant effects were primarily driven by the substantially greater POXC values evident within the NT $< 250 \mu\text{m}$ at the clay site. This fraction had greater POXC values compared to all other treatments and fractions at the silt loam site, except that of the silt loam NT $> 20000 \mu\text{m}$ aggregate fraction (Fig. 3).

3.5. Correlations between Permanganate oxidizable carbon and nematode feeding groups.

Bacterivores and predator/omnivores were the only feeding groups significantly correlated with POXC (Table S3; Fig. 4). Furthermore, these correlations were only apparent within specific aggregate fractions and soil types (Fig. 4). Predator/omnivore feeding groups were significantly correlated with POXC in the $> 2000 \mu\text{m}$ aggregate fraction at the clay site ($p < 0.05$; Fig. 4). This same relationship was also positive at the silt loam site; however, it was not significant ($p > 0.05$). Additionally, in the aggregate fraction $2000\text{--}250 \mu\text{m}$, bacterivore nematodes were positively and significantly correlated with POXC at the clay site, whereas bacterivores at the silt loam site were significantly and negatively correlated with POXC ($p < 0.05$; Fig. 4).

4. Discussion

4.1. Aggregate fraction affected nematode community distribution

The results of this study indicate that nematode abundance and nematode community assemblage shift by aggregate fraction. Total nematode abundance was greatest in the larger aggregate fraction and corroborates findings from several previous studies (Jiang et al., 2017; Zhang et al., 2016). Greater nematode abundance in the $> 2000 \mu\text{m}$ aggregate fraction is not surprising given that large aggregates provide a more expansive habitat, equipped with the necessary resources to support a larger and more diverse nematode community (Neher, 2010). Community composition also shifted by aggregate fraction, whereby the proportion of bacterivores increased with decreasing aggregate fraction. Although shifts of predator/omnivore nematodes within aggregate fractions were not significant it is noticeable that they were most highly concentrated in the largest aggregate fraction. These findings follow expected trends in soil food web trophic level interactions. For instance, bacterivores are often most concentrated in the smaller aggregate fractions to escape predator/omnivore nematode grazing. In addition, Zhang et al., (2013) reported that smaller aggregates have greater microbial diversity and biomass, which may drive an increase in the proportion of bacterivores in $< 250 \mu\text{m}$ aggregate fractions. The differences in bacterivore proportionality between aggregate size may also be altered by the availability of water filled pores. Specifically, bacterivore nematodes have been found to be able to reproduce and colonize water filled pores that were $1 \mu\text{m}$ (Yeates et al., 2002). Thus, bacterivore nematodes had an additional advantage over other nematode feeding groups for reproducing in smaller size aggregates where pore size is smaller. Overall, these findings indicate that if soil is not carefully

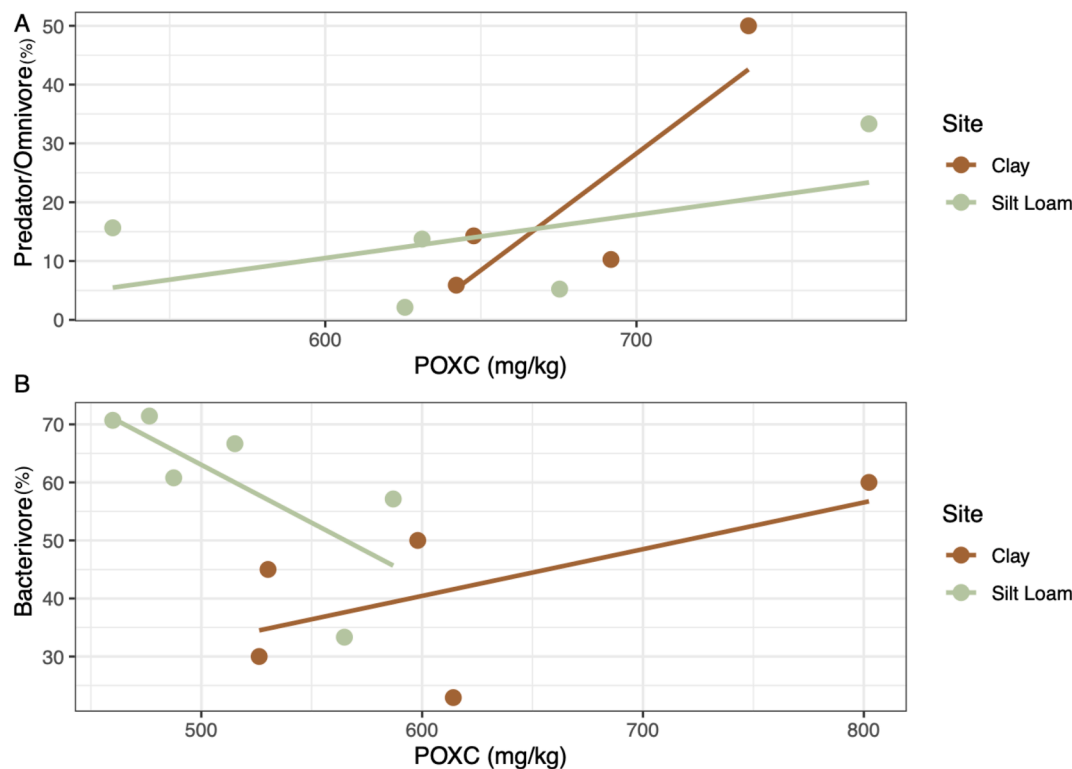


Fig. 4. Correlations between A) Permanganate Oxidizable Carbon (POXC) (mg/kg soil) and Predator/Omnivore nematodes at aggregate fraction $> 2000 \mu\text{m}$. Correlations were significant at $p < 0.05$ for the clay site but not the silt loam site and B) Permanganate Oxidizable Carbon (POXC) (mg/kg soil) and bacterivore nematodes at aggregate fraction $2000\text{--}250 \mu\text{m}$. Both the clay and silt loam sites had a significant correlation at $p < 0.05$. Color represents the clay and silt loam site.

maintained and larger aggregates are not protected, a system may start selecting for nematode populations dominated by r-strategists, which could reduce ecosystem functioning (Ferris et al., 2001; Culman et al., 2010; Sprunger et al., 2019).

4.2. Soil type and nematode community composition across aggregate fraction

Our study confirmed our first hypothesis that clay soils would foster a more structured soil food web relative to silt loam soils. Findings from this study corroborate this hypothesis as clay soils had a lower percentage of bacterivores and greater percentage of predator-omnivores compared to silt loam soils. Reduced populations of bacterivores may also indicate sustained ecosystem functioning, as greater abundance of bacterivore nematodes have been found to suppress the bacteria and archaeal community (Neilson et al., 2020). Additionally, lower predator-omnivore populations and greater bacterivores has been reportedly linked to disturbed food webs (Ferris et al., 2001; Dupont et al., 2014). The greater abundance of bacterivores in silt loam soils was most likely linked to the reduced predator/omnivore abundances, where bacterivore populations were larger due to limited predator/omnivore predation on the community (Wardle and Yeates 1993; Neher 2001). Furthermore, reduced protection of organic matter at the silt loam site, indicated through more evenly distributed POXC values across aggregates, likely primed the microbial community and created an abundance of prey for the bacterivore community (Christensen, 2002; Jiang et al., 2018; Hassink et al., 1993).

Our results suggest that it is even more important to implement conservation tillage practices on silt loam soils given that they are already predisposed to a less structured soil food web relative to clay soils. Thus, it is important to consider management practices, that foster aggregation and improved overall soil physical health. For example, adoption of no-till strategies may enhance nematode community structure in sandier soils. Although not statistically significant, our study found that no-till management has the potential to increase abundances of fungivores, and predator/omnivores and decrease abundance of plant parasitic nematodes in silt loam soils. Moreover, previous studies have found that no-till systems can enhance the structure and function of nematode communities in bulk soils (Burkhardt et al., 2022; Zhang et al., 2016; Martin and Sprunger, 2022).

4.3. Nematodes drive soil carbon stabilization in larger aggregates

Permanganate oxidizable carbon was chosen as a measure of soil C in this study to assess how nematode communities are related to a more processed pool of labile C (Andrews et al., 2004; Moebius-Clune et al., 2016). While POXC is often referred to as 'active C' this may in fact be a misnomer as numerous studies have demonstrated that POXC is more closely associated with smaller and heavier particulate organic C fractions and can therefore serve as an early indicator of C stabilization (Culman et al., 2012; Hurisso et al. 2016; Sprunger et al., 2020).

To our knowledge, this is the first study to link nematode community assemblage and POXC within varying aggregate fractions. Interestingly, our measures of POXC were elevated in smaller aggregate fractions in clay soils, like that of the soil organic C pool (Zhou et al., 2020). When assessing the relationships between nematodes and POXC we found that soil C and nematode communities were related, but only in larger sized aggregates. Relationships between nematode communities and POXC may have only been apparent in larger sized aggregates as low microbial biomass in smaller aggregate fractions may not support the development of the nematode community (Zhang et al., 2016). In addition, reduced nematode abundance due to pore size may restrict nematode access to smaller aggregates, hindering nematodes from playing a role in C stabilization within smaller aggregate fractions. In the large sized aggregates, predator/omnivore nematodes were positively correlated with POXC at >2000 μm . This relationship indicates that predator/omnivore

nematodes may aid in stabilizing C in larger aggregate fractions. Zhang et al., (2016) reported similar results where nematode functional metabolic footprints were greatest in larger sized aggregates, which indicates that nematodes have a high C utilization in larger aggregate fractions and greater potential for C to be stabilized as C flows up the food chain.

Our study demonstrates that the relationship between POXC and bacterivores is dependent upon soil type. We found that bacterivore nematodes were positively related to POXC at the 2000–250 μm aggregate fraction, however a negative relationship was found between POXC and bacterivores for the silt loam site. In contrast, bacterivores were found to be negatively correlated to POXC in the silt loam soil. This relationship is expected as C may not be physically protected in silt loam soils and is therefore more likely to be mineralized by the bacterial communities, causing an increased presence of bacterivores across all aggregate fractions (Christensen, 2002). In contrast, bacterivores were positively correlated to POXC in clay soils at the 2000–250 μm aggregate fraction. Jiang et al., (2013) has reported a similar trend and speculates that selective feeding on active bacteria by the bacterivore community may suppress the metabolic quotient and stimulate soil C accumulation. However, as our study demonstrates, the relationship between POXC and bacterivores is clearly dependent on soil type.

This study explores the interactions between nematode assemblage and labile C across different sized aggregates in contrasting soils. The results are important and novel as the literature has largely focused on microbial communities across aggregate fractions, and higher trophic level soil fauna are often ignored (Tiemann and Grandy, 2015; Wang et al., 2022b). To be clear, research efforts focus on microbial communities because they are critical drivers of soil C stabilization via multiple pathways including aggregate formation and the persistent necromass pool (Tiemann and Grandy, 2015; Kästner et al., 2021; Wang et al., 2022a). However, microbial communities exist as part of a larger soil food web, where top-down trophic interactions mediate the fate of soil C (Grandy et al., 2016). For instance, Mielke et al., (2022) reports that increased nematode grazing led to greater C transfer via bacterial and fungal pathways. Moreover, the study found that enhanced bacterivore grazing further stimulated bacterial growth and soil organic matter formation via microbial necromass. In our study, we found that while POXC values increased with decreasing aggregate fractions, nematodes were most strongly correlated with POXC in larger sized aggregates. Our results, demonstrate that nematodes have the capacity to drive soil C accrual in larger sized aggregates. This is a potentially novel mechanism and key pathway for SOC accrual in larger sized aggregates. As scientists continue to make linkages between soil biodiversity and ecosystem function, it will be imperative to assess the relationship between soil fauna and soil C across spatial and temporal scales (Martin and Sprunger, 2021).

4.4. Limitations and future directions

Although this study was the first to link POXC to nematode communities within aggregate fractions there were limitations in this study. Most notably is that microbial communities were not assessed in conjunction with nematodes by aggregate fractions. However, others have reported that large macroaggregates have greater microbial biomass, gram-positive bacteria, and microbial diversity (Potapov et al., 2021; Wang et al., 2022b). In addition, the identification of the nematodes to family or genus would have allowed us to calculate ecological indexes to further aid in interpreting the relationship between soil food web processing and soil C cycling within varying aggregate fractions (Jiang et al., 2017; Jiang et al., 2018). Incorporating an additional labile C measure, such as soil respiration, would have allowed us to infer nematode relationships to soil C trajectories. For instance, more concrete inferences on mineralization versus stabilization processes could have been made (Hurisso et al., 2016; Sprunger and Martin, 2022). Future research should assess how C is assimilated into

the aggregates through the nematode community through use of stable isotope tracing (Mielke et al., 2022). Moreover, the use of steady state C-labelling could track the flow of organic matter through the soil food web and C incorporation into aggregates (Paterson et al., 2011). This could be done in conjunction with long-term incubation experiments to understand the mechanisms that govern interactions between nematodes and nutrients within varying aggregates fractions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2023.116536>.

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