Exploring the influence of natural features on soil mesofaunal communities in agricultural landscapes through DNA metabarcoding

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Soil mesofauna critically support organic matter decomposition and nutrient cycling,
as well as regulating pests and diseases. Agricultural activities can physically and
chemically influence soil mesofauna communities, thereby impacting the ecosystem
services they provide. Conserving natural features in agricultural environments
is well known to support biodiversity and associated ecosystem services, yet soil
mesofauna communities and their functions in such features require further study.
We sampled the soil mesofaunal communities (focusing on Acari [mites] and
Collembola [springtails]) in agriculturally-dominated landscapes in southeastern
Ontario, Canada. Semi-natural features were represented by drainage ditch banks
with varying amounts of woody vegetation to different kinds of forested blocks.
Specimens were extracted using Berlese funnels, and COI metabarcoding was
conducted on the bulk specimen samples. A total of 585 operational taxonomic
units (OTUs; a proxy for species) were identifiable based on DNA sequences,
390 of which belonged to either Acari (273) or Collembola (117); however, it
was rarely possible to identify specimens to species-level. Mesofaunal richness
did not differ among habitat types but community composition varied between
low woody-vegetation drainage bank sites and forested blocks. Maintaining
heterogeneous natural and semi-natural features appears to increase the overall
diversity of mesofauna on the landscape, and even small forest patches support
forest-associated soil biodiversity in this agricultural region.

KEYWORDS

Acari, agricultural landscape, Collembola, metabarcoding, soil biodiversity

1 Introduction

Soil is home to a vast number of species, including microorganisms (e.g., bacteria, fungi, protists); microfauna (<200 μm in size, e.g., nematodes, rotifers); mesofauna (~0.2–2 mm, e.g., mites, springtails, enchytraeids); and macrofauna (>2 mm, e.g., earthworms, snails, many insects) (Lavelle et al., 2006; FAO et al., 2020). This fauna is critically important for maintaining essential ecosystem functions, such as soil formation, nutrient and carbon cycling, and pest and disease control in both natural and human-dominated landscapes (Lavelle et al., 2006; Barrios, 2007; Mishra and Singh, 2020; Guerra et al., 2021). Threats to soil biodiversity such

as habitat loss and fragmentation, agricultural intensification/ extensification, and soil erosion may consequently degrade the important functions that these communities provide (Gardi et al., 2013; Geisen et al., 2019; Tibbett et al., 2020; Prăvălie et al., 2021). However, the extreme complexity and sheer diversity of these communities make it especially challenging to monitor their taxa (Thakur et al., 2020; Gongalsky, 2021; FAO, 2022), which is a key step in identifying how their diversity (and its loss) will impact ecosystem services across the globe (Remelli et al., 2024).

Agricultural activities can cause large reductions in the abundance and richness of soil fauna by physically disrupting the soil habitats via tillage and field traffic (Ouellet et al., 2008; Müller et al., 2022), and through chemical influences like fertilizer and pesticide use (de Graaff et al., 2019). Recent efforts to conserve soil biodiversity and soil health have focused on within-field methods to conserve soil biodiversity, such as conservation tillage or cover cropping (van Capelle et al., 2012; Carmona et al., 2021). However, landscape-based approaches have the potential to benefit both agricultural productivity and local biodiversity (Haan et al., 2021). In particular, a mosaicked landscape that retains forest patches and linear features, such as hedgerows, fence lines, and watercourse margins, among agricultural plots can provide crucial habitat and refugia for soil biodiversity in otherwise depauperate zones (Rusch et al., 2010; Schirmel et al., 2016; Udawatta et al., 2019; Bennewicz and Barczak, 2020; Montgomery et al., 2020). While these features may reduce the amount of arable land, introduce shading influences on crops, and provide refugia for pests, such cases are context dependant (Mesman, 2016; Rempel et al., 2017; Marais et al., 2022). Increasingly, natural features are being lost at the expense of agricultural and urban development, as documented in the focal region of this study—eastern Ontario, Canada (Noteboom et al., 2021), and many other regions in the world (Hu et al., 2021; Pendrill et al., 2022). An additional yet often overlooked semi-natural feature that may act as effective refugia for soil biodiversity in agro-ecosystems includes uncultivated and semi-cultivated field margins (Clark et al., 2006; de Zwaan et al., 2024; Guo et al., 2024), but these features remain poorly documented in the context of soil mesofaunal communities.

Understanding how agricultural extensification or conversely, mosaicked landscapes affect soil mesofaunal diversity is a missing component in soil health surveys. A number of relevant soil health indices and models have been developed for soil physicochemical properties (Vereecken et al., 2016; Vogel et al., 2019; Maurya et al., 2020). Despite the increasing interest in developing similar indices and models for soil fauna (Parisi et al., 2005; Caoduro et al., 2014; Menta et al., 2018; Buchkowski and Lindo, 2020), there remain large gaps in our knowledge of how mesofauna interact with their physical and chemical environment (Deckmyn et al., 2020; Potapov et al., 2022). Collecting baseline data on soil faunal communities and the physical and chemical matrix in which they reside is an essential step in developing testable hypotheses about soil ecological processes, and ultimately, the development of more robust soil health models and indices.

The soil mesofauna is dominated by microarthropods, of which the most diverse and abundant taxa are mites (Acari) and springtails (Collembola) (Behan-Pelletier, 2003). Acari, in particular, are one of the most diverse and abundant arthropod taxa within soil communities and fill a wide range of ecological roles (Beaulieu et al., 2019). For example, predatory mites, such as Mesostigmata (Parasitiformes) and some Prostigmata (Trombidiformes), can

influence prey populations by exerting top-down effects on the abundance of prey taxa (e.g., nematodes, Collembola) (Koehler, 1999; Schneider and Maraun, 2009), and have even been used as biological control for arthropod pests, such as thrips and phytophagous mites (McMurtry et al., 2015; Knapp et al., 2018). Conversely, oribatid (Sarcoptiformes) mites are responsible for nutrient cycling within the soil by feeding on dead organic matter and microbial residues and contribute strongly to soil structural development by producing nutrient rich faecal pellets that act as a natural fertilizer (FAO et al., 2020; Potapov et al., 2022). While Collembola are often less diverse than Acari, they are frequently as abundant in soil systems and serve as an important prey source for other small arthropods, such as beetles, mites, and spiders (Bilde et al., 2000). They also contribute to nutrient-cycling by breaking down organic matter and impact soil food-webs by feeding on microorganisms (Coulibaly et al., 2019).

Due to the vast abundance and diversity of soil mesofauna, assessing species richness and composition of the entire community is rarely feasible using pure morphological methods; for this reason, most studies focus on selected taxa (Postma-Blaauw et al., 2012; Baumann, 2021), or else only identify organisms to high taxonomic levels (Ruf and Beck, 2005; Meehan et al., 2019). In contrast, DNA metabarcoding couples DNA taxonomy with high-throughput sequencing to identify multiple species within a given sample (Coissac et al., 2012). This method shows great promise for efficiently and accurately assessing soil communities (Yang et al., 2014), including for Acari (Arribas et al., 2016; Hoage, 2018; Young and Hebert, 2022) and Collembola (Saitoh et al., 2016). While metabarcoding has been used to assess soil arthropod communities within a variety of different ecosystems: i.e., Arctic, temperate, tropical, freshwater, and grassland habitats (Arribas et al., 2016; Oliverio et al., 2018; Schenk et al., 2020; Basset et al., 2022; Young and Hebert, 2022), its application in agricultural settings is very limited (Kestel et al., 2022).

The objectives of this study were: (a) to conduct a baseline survey of soil mesofauna (Acari and Collembola) in natural and semi-natural features in an agricultural landscape in eastern Ontario, Canada; (b) to analyze soil mesofaunal diversity and community composition in a variety of semi-natural habitats (ranging from grassy un-treed field margins to larger forested blocks), using specimen-based metabarcoding; and (c) to explore relationships between soil physicochemical parameters and the mesofaunal community at each site.

2 Methods

2.1 Study area and experimental design

This study was conducted east of Ottawa, ON, Canada in the South Nation river watershed (Figure 1). This region has a temperate climate, categorized as Dfb (cold winter, no dry season, warm summer) in the Köppen-Geiger climate classification system (Beck et al., 2018). The area is primarily agricultural and has experienced a notable loss of natural features, as 4.1% of its forest was lost in just 6 years (Mesman, 2016). The region's main agricultural land uses are livestock/cash corn, soybean, and forage (i.e., hay and alfalfa for livestock feed) (Ontario Data Catalogue, 2024). The average field size in the study area is approximately 8.3 ha, based on calculations in ArcGIS, and fields are typically bordered by linear features, such as
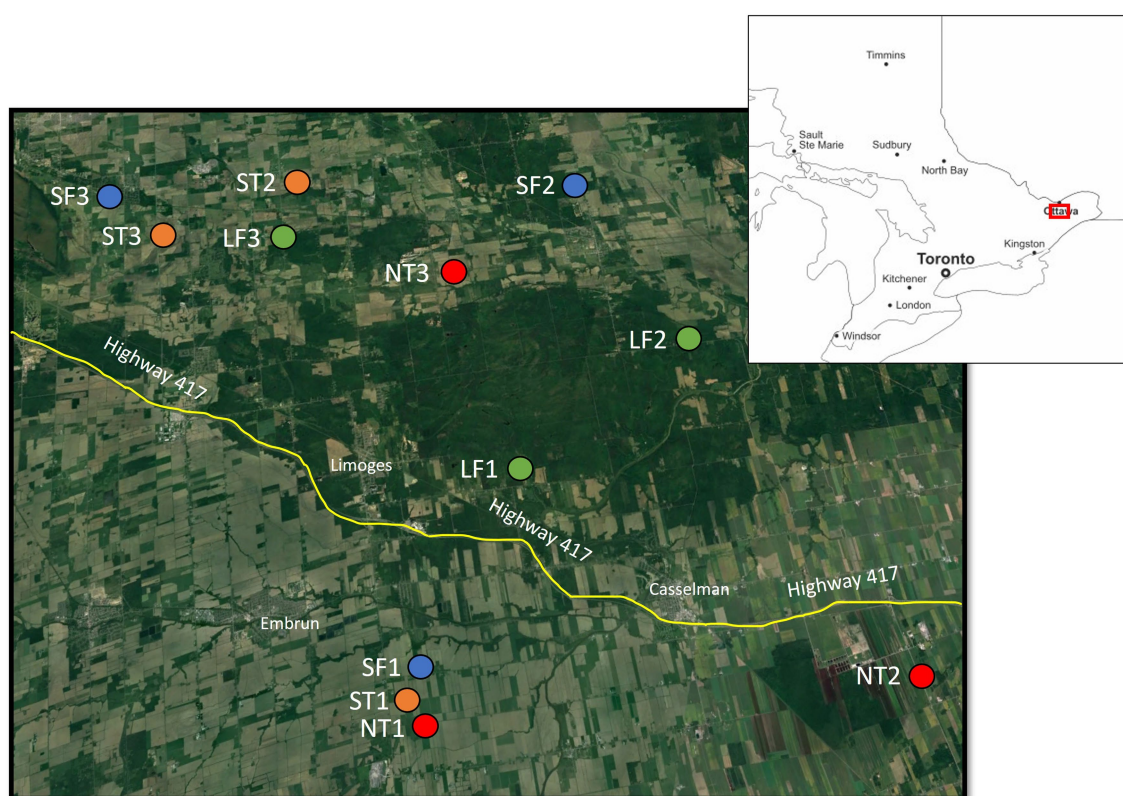
FIGURE 1
Map showing the location of the 12 sites that were sampled in this study. Source: Main map modified from https://www.google.com/earth (accessed August 20, 2023), inset map modified from https://d-maps.com/ (accessed February 18, 2025).

hedgerows, vegetated fencelines, and agricultural drainage ditches (de Zwaan et al., 2024; Guo et al., 2024). The banks of these drainage ditches are generally semi-natural, uncultivated habitats, ranging from narrow strips of herbaceous vegetation, which may or may not include scattered trees and shrubs, to (less commonly) wider, forested areas. The study region is dominated by soils classified as Orthic Humic Gleysols and Gleyed Melanic Brunisols (Canadian System of Soil Classification).

We collected soil samples along agricultural drainage ditch margins and forested areas along waterways in this agricultural matrix in June 2022 (Figure 1). Twelve sites were selected and were categorized as one of four habitat types, with three replicates of each type. Two site categories were classified as "unforested," including "No trees" = agricultural ditches brushed of woody vegetation (as per Guo et al., 2024) and "Scattered trees" = agricultural ditches with a few scattered shrubs and trees (less than 20% tree canopy cover). These uncultivated strips, including the ditch proper, were less than 10 m in width and were dominated by grasses, particularly smooth brome grass (*Bromus inermis*), and included both native herbaceous plants (e.g., *Solidago canadensis* [Canada goldenrod]) and invasive alien species (primarily *Pastinaca sativa* [wild parsnip]) (Supplementary Table S1). At most ditch sites, the litter layer was predominantly a dense cover of dead brome grass that protected the soil surface from excessive soil desiccation during drier periods. The other two categories were classified as "forested" and included "Small forest" = small patches of forest ranging from 30 to 300 m in width and either isolated, or narrowly connected to larger forested areas, and

"Large forest" = large patches of forest ranging from 400 to 2,000 m in width, and connected to more extensive forested areas (Figure 2). Due to the availability of suitable sites, there was limited ability to standardize the vegetation composition of these forested stands. However, they were mostly deciduous-dominated (one site was mixed-wood), with the most common native trees being *Fraxinus americana* (white ash), *Acer saccharum* (sugar maple), *Fagus grandifolia* (American beech) and *Tsuga canadensis* (Eastern hemlock); the most common non-native tree was *Rhamnus cathartica* (European buckthorn), which was particularly abundant in the small forest sites (Supplementary Table S1). The forests had distinct canopy, shrub, and herb layers; at many sites, the herb layer (most commonly fern-dominated) was sparse, with the moist forest floor primarily covered in deciduous leaf litter. Soil texture across all sites was highly variable, though clay soils were more common in the ditch sites compared to the forested sites (Supplementary Table S1).

Six sampling points were randomly selected at each of the 12 sites by first overlaying each site with a 10 m × 10 m grid in Google Earth. A random number generator was then used to select six cells of the grid to be sampled, and a GPS point was arbitrarily selected in the approximate centre of each cell. Sampling points were evenly divided across the ditch/waterway (three per side) and were at least 20 m apart. The ditch samples were collected at the mid-slope of the bank. Paired samples (immediately adjacent to each other) were collected at each sampling point; one for mesofaunal metabarcoding, and the other for soil physicochemical analysis. Each of these two samples was collected by using a tulip bulb planter to extract two soil cores/sample

FIGURE 2
Representative satellite images and photographs of each of the four habitat types.

(each approximately 6 cm in diameter and 11 cm deep) that were combined in a Ziploc bag, totaling approximately 2.5 L of soil per sample. Tree canopy cover was also measured at each sampling point using a convex spherical crown densiometer; four measurements were taken, one at each cardinal direction, and the mean of these measurements was then calculated.

The mesofaunal samples were kept in the fridge overnight, and then specimens were extracted on Berlese funnels lined with cheesecloth for 4 days using heat from a 60 W bulb, with specimens collected into 95% ethanol. Specimens were then filtered through a 45-µm sieve into 2 mL tubes filled with 95% ethanol and stored at −20°C until DNA extraction. Specimens were not cleaned prior to DNA extraction, resulting in some sediment being included in the extraction.

The soil samples collected for physicochemical analysis were stored at −20°C prior to processing. The samples were thawed at 20°C for 1 h, sieved through a 2 mm mesh, and then subsampled for downstream analyses. Gravimetric moisture was measured by drying a 20 g field moist subsample at 105°C for 48 h. A separate 100 g subsample was then air dried at 20°C for 1 week. These air-dried subsamples were sent to A&L Canada Laboratories Inc., London, ON, for physicochemical analysis, which included measurements of

organic matter (loss on ignition), phosphorus (Bray-P1), total and percent base saturation of potassium, magnesium, and calcium (ammonium acetate extraction), and cation exchange capacity (CEC).

2.2 Sequencing

DNA was extracted non-destructively from the mesofaunal specimens with a Qiagen DNeasy Blood and Tissue kit (Qiagen), modified to allow for the recovery of voucher specimens. Specifically, the ethanol was first evaporated from the tubes containing specimens using a vacuum centrifuge (Vacufuge, V-AL, ambient temperature, 10 min). 270 µL of buffer ATL and 30 µL of proteinase K were then added to the tubes without grinding the specimens, and tubes were incubated overnight at 56°C. Following incubation, 200 µL of solution was withdrawn from the top and placed into a 1.5 mL tube, being careful not to disturb or pipette up any specimens. The tubes containing the voucher specimens were then refilled with 95% ethanol for storage. The remainder of the DNA extraction steps followed the standard kit protocol, with the final elution step repeated once to maximize DNA yield, for a total elution volume of 200 µL. Following the extraction, the spin column filters were rinsed with 95% ethanol

and inspected under a microscope to check for any specimens that were drawn up with the extraction buffer; any specimens found during this step were added to the voucher specimen tube. Initial PCR tests had high failure rates, possibly due to PCR inhibitors from soil residue that was extracted with the specimens. Diluting the DNA improved PCR results, and it was determined that a 20% dilution was most successful across samples; consequently, all DNA extracts were diluted with water to 20% prior to sequencing.

Based on the recommendation of Krehenwinkel et al. (2016), we sequenced a 313 bp region of COI, using the mICOIintF (Brandon-Mong et al., 2015) and FoldR (Arribas et al., 2016) primer pair, with modified adapters that had 0–6 "N" bases between the amplicon-specific primer and the adapter sequence to increase base diversity. PCR was replicated 3 times for each DNA extract; therefore, a total of 216 samples were sequenced (12 sites × 6 sampling points × 3 PCR replicates). Two extraction negatives × 3 PCR replicates and 10 PCR negatives were also sequenced. Each PCR reaction consisted of 16.75 μL nuclease-free water, 2.3 μL 10× Titanium buffer, 2.3 μL of 2 mM dNTPs, 0.575 μL each of the forward and reverse primers, 0.5 μL Titanium Taq, and 2 μL of DNA. Cycling was conducted in an Eppendorf thermocycler, with the following program: 95°C for 3 min, 40 cycles of 94°C for 30 s, 45°C for 90 s, 72°C for 90 s, and a final extension of 72°C for 3 min, before being held at 4°C. The remaining steps in the protocol were conducted at the Molecular Technologies Laboratory of the Ottawa Research and Development Centre. In brief, samples were purified and normalized using an NGS Normalization 96-Well Kit (Norgen Biotek Corporation, Canada). A second round of PCR was then performed wherein samples were tagged using i3 and i5 indices of the Nextera Index Kit (Illumina, San Diego, CA, USA), followed by PCR clean-up and normalization as above. The samples were then pooled, quality was assessed using a Tapestation (Agilent, Santa Clara, CA, USA), and quantification was done using a KAPA Library Quantification Kit (KAPA Biosystems, Wilmington, MA, USA). Finally, next-generation sequencing was performed using a single MiSeq v3 600 Kit (Illumina, San Diego, CA, USA). Raw sequences were submitted to NCBI Sequence Read Archives (BioProject ID PRJNA1197472).

2.3 Bioinformatics and taxon assignment

The raw sequence data were analyzed and grouped into operational taxonomic units (OTUs) using the 'JAMP' package available in R (Elbrecht, 2018). Paired end merging was conducted with USEARCH (Edgar, 2010), with a minimum 75% alignment match. Primer trimming (maximum error of 0.2) and length filtering (301–319 bp) were done using Cutadapt (Martin, 2011). Error filtering was conducted with USEARCH (Emax = 1). Finally, singleton reads were discarded and OTUs were clustered with 3% similarity using VSEARCH (Rognes et al., 2016). In addition, any replicates that contained less than 5 mesofaunal OTUs were considered a failed replicate and were excluded from the dataset. By these criteria, a total of 34 PCR replicates failed and were removed from the dataset; this resulted in the complete removal of some samples (two samples from Site NT3 and one sample from each of Sites LF1, SF3, and LF3). Two sampling points (Sites NT2-3 and NT2-6) were also excluded from the final dataset, as they were outliers with far more than the expected number of OTUs; examination of the voucher specimens confirmed that the sequencing

data greatly overestimated the number of mesofaunal OTUs. All voucher specimens were databased and submitted to the Canadian National Collection of Insects, Arachnids, and Nematodes.

Following OTU clustering, abundance filtering was performed on all OTUs, using a dynamic approach; any OTU occurrence with less than 0.01% of the total read count in a sample was removed, as well as occurrences totaling <0.01% of the total read count for that OTU. To reduce the possible impact of tag switching or other sources of contamination, the maximum read count for each OTU in the negative controls was subtracted from all other instances of that OTU. PCR replicates were then pooled for each sample, only including OTUs that were present in at least two out of three PCR replicates; however, in instances where one or two PCR replicates failed, OTUs were kept if they were in a single replicate. OTU read counts were then converted to presence-absence data. Finally, taxonomy was assigned to each OTU using the BOLDigger program (Buchner and Leese, 2020) and the Barcode of Life Database (BOLD; Ratnasingham and Hebert, 2007); accessed June 2023. OTUs were designated as classified (at least to kingdom level) or unclassified (no taxon assigned). Taxonomy was assigned according to the best hit selected by BOLDigger, using the BOLD API correction tool (Buchner and Leese, 2020). All figures and the majority of statistical analyses were conducted in R (v4.2.3; R Core Team, 2023).

2.4 Statistical analyses

All statistical analyses were conducted on mesofauna taxa only, defined for the purposes of this study as those taxa assigned to Acari or Collembola. We compared the total mesofaunal OTU richness between habitats, as well as the richness of each order of Acari and Collembola using generalized linear mixed models (GLMM). Analyses were conducted in R, using the lme4 (Bates et al., 2015) and glmmTMB packages (Brooks et al., 2017), with the diagnostics of the model (uniformity, dispersion, heteroscedasticity) tested using the DHARMa package (Hartig, 2024). Data were initially analyzed using the Poisson distribution model, as recommended for count data. However, data that were overdispersed (a dispersion ratio of >1.2; Payne et al., 2018), or otherwise failed to meet the assumptions of the model, were analyzed using negative binomial distribution models, following the same diagnostic testing as above. In these cases, the two models were compared using AIC, to confirm the better fit of the negative binomial model. Each model included fixed effects for "habitat" and random intercepts for "site" (to control for sample interdependence among sites). The "No Trees" habitat type was selected as the intercept for the model. Post-hoc tests were conducted using Estimated Marginal Means from the emmeans package in R (Lenth, 2025).

We tested whether the mesofaunal community composition differed between habitat types with a nested permutational multivariate analysis of variance (PERMANOVA, 1,000 randomizations) using the incidence-based Sorensen's dissimilarity metric as implemented by the vegan package (Oksanen et al., 2015) in R. We conducted post-hoc testing using a nested pairwise PERMANOVA test with the pairwiseAdonis package (Martinez Arbizu, 2020). Overlap in mesofaunal OTUs between the three sites for each habitat type were compared using Venn diagrams constructed with the BioVenn package (Hulsen, 2021). In order to visualize differences in mesofaunal community composition, as well as the level of correlation with the environmental variables, non-metric

multidimensional scaling (NMDS) using Sorenson's dissimilarity calculations for community data (i.e., Acari + Collembola OTUs), and correlation vectors of the environmental variables, were constructed with the metaMDS function using *vegan* (Oksanen et al., 2015). The number of dimensions for the final NMDS analysis was selected by repeating the NMDS analysis with increasing numbers of dimensions (from 1 to 10), graphing the stress of each analysis, and determining at what number of dimensions a plateau was reached.

To determine whether soil physicochemical properties differed between habitats, we used linear mixed models (LMM), implemented in R, using the lmerTest package (Kuznetsova et al., 2017). To test the assumptions of the model, the Shapiro-Wilks test for normality was conducted using base R (R Core Team, 2023), while the Levene test for homogeneity of variance was conducted using the DHARMa package (Hartig, 2024). Initial analyses used a Gaussian distribution model with untransformed values; if the assumptions were not met, the data were log-transformed and re-tested with the same model and diagnostics as above. Post-hoc tests were conducted using Estimated Marginal Means from the emmeans package in R (Lenth, 2025). Data were only included for those sites that were successfully sequenced for mesofauna ($n = 65$), however one sample from the large forest sites was not analyzed successfully by the external laboratory, and is thus missing for all parameters except gravimetric moisture.

Mantel's test (10,000 randomizations) was used to assess for correlations between geographical distance matrices or soil physicochemical properties distance matrices and community composition distance matrices (using Sorenson's dissimilarity based on mesofaunal OTU presence-absence) with *vegan* (Oksanen et al., 2015). Possible correlations between pairs of soil parameters were also examined by calculating the Pearson correlation coefficients for each pair of soil parameters using R, with the strength of the relationship based on Evan's table (Evans, 1996).

3 Results

3.1 Overall diversity and community composition

After abundance-filtering, a total of 1,036 OTUs were detected from over 600,000 reads. Once reads were removed from the PCR and extraction negatives, and replicates were pooled, a total of 844 OTUs were recovered from all 12 sites; however, only 585 of these could be assigned to any taxon (Table 1; Supplementary Table S2). These classified OTUs consisted of 19 classes, 37 orders, and 100 families (Supplementary Table S2). Of the animal taxa found (551 OTUs), only 24% (131 OTUs) were assigned to the species level, and many OTUs (36%) had no identifications below the family level (Supplementary Tables S2, S3).

A total of 390 mesofauna OTUs were detected, of which 70% (273 OTUs) were Acari and 30% (117 OTUs) were Collembola (Supplementary Table S3). Within Acari, Trombidiformes (Prostigmata) and Sarcoptiformes (primarily Oribatida) were each far more OTU-rich than Mesostigmata (Figure 3). The most OTU-rich families were Eupodidae (44 OTUs), Tydeidae (20 OTUs), and Oppiidae (16 OTUs), while 14 families were only represented by a single OTU (Supplementary Table S3). Twenty-eight Acari OTUs (10%) were assigned to species, 57 (31%) were assigned only to the genus level, while the majority (47%) could only be assigned to family (129 OTUs) or order (59 OTUs) (Supplementary Table S3). For the Collembola, the orders Entomobryomorpha and Poduromorpha contained the majority of OTUs (52 and 36, respectively) in comparison with the other orders (Figure 3; Supplementary Table S3). The most OTU-rich families were Isotomidae (32 OTUs) and Entomobryidae (11 OTUs), while 2 families were only represented by a single OTU. Twenty-six springtail OTUs (22%) were assigned to species, 28 OTUs (24%) were assigned only to the genus level, 46 OTUs (39%) could only be assigned to family, and 17 OTUs (15%) were assigned only to order (Supplementary Table S3).
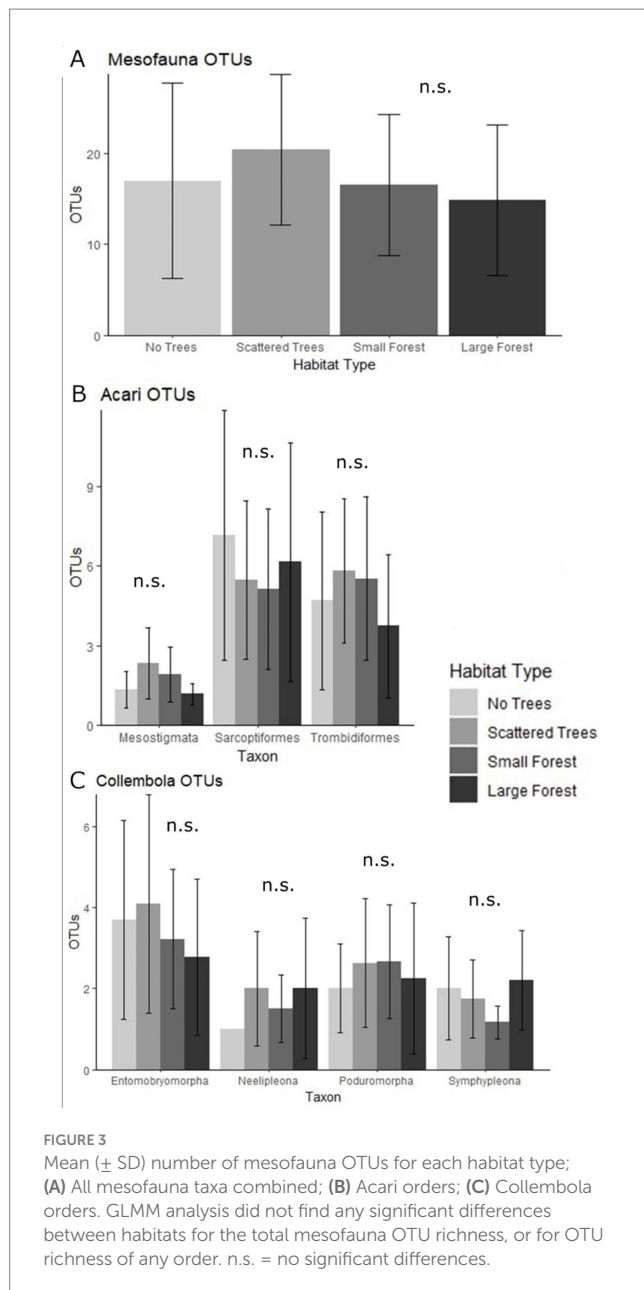
3.2 Habitat comparison

The total mesofauna OTU richness did not differ significantly between habitats (Figure 3A; Supplementary Tables S4, S5). At the ordinal level, OTU richness was highly variable within each habitat (Figures 3B,C), however there were no significant differences between the habitats (Supplementary Tables S4, S5).

The PERMANOVA results indicated significantly different mesofaunal community composition between habitats (df = 3, $F = 2.45$, $R^2 = 0.10$, $p < 0.001$), as well as between sites within a habitat type (df = 8, $F = 1.95$, $R^2 = 0.21$, $p < 0.001$). Post-hoc pairwise testing indicated that all habitat types differed

TABLE 1 Summary of OTUs and reads (following abundance-filtering and pooling) for each habitat type.

Habitat	Samples/ PCR replicates	All taxa						Mesofauna	
		Total OTUs	Total reads	Identified OTUs	Identified reads	Unident. OTUs	Unident. reads	Total OTUs	Total reads
No trees	14/34	220	135,490	162	134,426	58	1,064	120	41,496
Scattered trees	17/49	276	174,828	236	172,365	40	2,463	175	40,212
Small forest	17/47	363	179,843	241	174,264	122	5,579	167	77,337
Large forest	16/46	294	144,034	212	125,441	82	18,593	152	65,040
Negatives	–/16	–	–	–	–	–	–	–	–
All sites	64/192	844	634,195	585	606,496	259	27,699	390	224,085

FIGURE 3
Mean (± SD) number of mesofauna OTUs for each habitat type;
(A) All mesofauna taxa combined; **(B)** Acari orders; **(C)** Collembola
orders. GLMM analysis did not find any significant differences
between habitats for the total mesofauna OTU richness, or for OTU
richness of any order. n.s. = no significant differences.

significantly in their mesofaunal community composition, as well as all sites within each pair of habitats ($p < 0.001$; Supplementary Table S6). This is supported by how few taxa overlap between different sites, even within the same habitat type (Figure 4).

Based on preliminary testing, a 3-dimensional solution was selected as the best NMDS result; however, the analysis failed to converge, even with running up to 100 iterations, and the final stress (0.19) was only marginally acceptable (Clarke, 1993). The NMDS analysis showed extensive overlap between the sites and habitat types, despite the results of the PERMANOVA; there was however an apparent distinction between unforested (no trees and scattered trees) and forested (large forest and small forest) sites along the NMDS2 axis (Figure 5). We did not observe a strong ecological signal with respect to habitat across the other axes (NMDS1 or NMDS3; Figure 5).

Samples within each site mostly clustered together, however there were several exceptions (Figure 5). For example, the samples from Sites NT2, SF2, and LF1 were widely dispersed across the ordination. The samples from Site NT1 formed two distinct clusters, with four samples in one cluster among the unforested sites and two samples clustering toward the forested sites. The samples from Site ST1 form three clusters, two among the unforested sites, and one sample among the forested sites. Samples from Site LF3 also formed two distinct clusters, with three samples in one cluster and two samples in the other. One sample from Site SF3 was also highly distinct from the rest of the site, and the six samples from Site LF2 formed three distinct clusters.
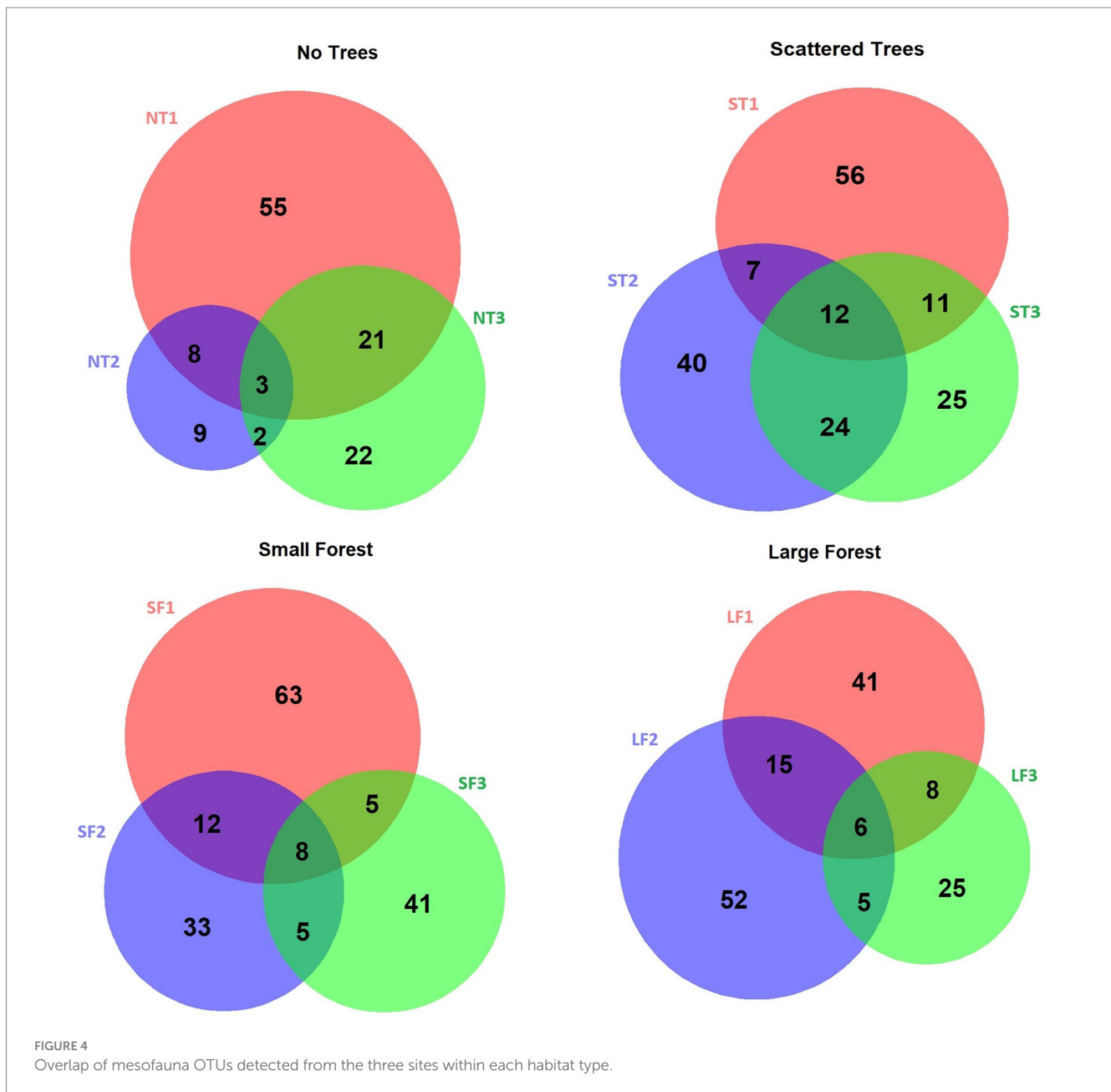
3.3 Environmental factors

Significant differences between habitats were only found for Potassium. It was lower in forested than unforested habitats, however the only significant differences were between the large forests and the two unforested habitats (scattered trees and no trees) (Figure 6C, Supplementary Tables S7, S8). Although not statistically significant, there was an observable pattern of increasing gravimetric moisture with increasing tree cover/forest patch size (Figure 6G).

Based on the Mantel tests, there was a significant correlation between community composition and organic matter ($p = 0.002$), cation exchange capacity (CEC) ($p = 0.008$), percent base saturation of magnesium ($p = 0.004$), and calcium ($p = 0.005$) (Table 2). As well, community composition was significantly correlated with the geographic distance between samples ($p < 0.001$) and with tree cover ($p = 0.001$; Table 2). These patterns were also apparent in the NMDS graph, which depicts tree cover, gravimetric moisture, and organic matter correlation vectors being associated with NMDS2, with all three increasing toward the forested sites (Figure 5). Pearson coefficients showed a strong correlation between CEC and organic matter; correlations were moderate or weak for all other pairs of parameters (Supplementary Figure S1).

4 Discussion

4.1 Soil mesofaunal diversity in an agricultural landscape

Agricultural ecosystems are home to a large diversity of soil organisms, particularly along field edges or other semi-natural habitats (Morris et al., 2010; Bennewicz and Barczak, 2020). Gathering baseline information on these soil organisms is essential in order to monitor and mitigate threats to soil biodiversity (FAO et al., 2020) and retain the ecosystem services they provide. However, surveys of soil mesofauna are still relatively rare in Canadian agroecosystems, particularly with identifications beyond the family level (exceptions being Berg and Pawluk, 1984; Osler et al., 2008; Walter et al., 2014; Walter and Lumley, 2021). Taxonomic richness is thus more often explored at the family or subfamily level, which may mask more fine-grained patterns of diversity (e.g., Reeleder et al., 2006; Carter and Noronha, 2007; Carter et al., 2009; Miller et al., 2014; Miller et al., 2017; Lupardus et al., 2021). For the habitats sampled in this

FIGURE 4
Overlap of mesofauna OTUs detected from the three sites within each habitat type.

agro-ecosystem, metabarcoding enabled the detection of over 1,000 OTUs (as a proxy for species) belonging to a wide range of taxa, including both the target mesofauna, as well as many other soil dwelling fauna. From this, 500 arthropod OTUs were detected, of which almost 400 OTUs were mesofauna.

The majority of mesofaunal taxa (75%) could not be assigned to the species level, and 44% could not be assigned past family. This lack of taxonomic resolution is unsurprising considering the number of undescribed mesofaunal species; for example, the majority of Acari species in Canada have yet to be described (Lumley et al., 2013; Beaulieu et al., 2019) and it is estimated that only 13% of Canadian Collembola have been described (Turnbull and Stebaeva, 2019). A lack of species designation for an OTU can also stem from incomplete reference sequence libraries, particularly for mesofauna (Young and Hebert, 2022; Recuero et al., 2023). For example, it is estimated that only 10% of the described Acari species have been assigned Barcode

Index Numbers (BINs; a formal OTU designation) in BOLD's COI reference library (Beaulieu et al., 2019). Although many of these species may be represented in BOLD, given the >16,000 Acari BINs from Canada alone (accessed November 2024), many BINs have yet to be identified beyond the family or genus level. Consequently, even OTUs that closely match a record in the reference library may not gain a lower-level identification. Taxonomic biases likely also exist in the database, as noted for Collembola, whose records in BOLD are skewed toward larger, surface-dwelling taxa, leaving the smaller soil-dwelling taxa far less complete (Turnbull and Stebaeva, 2019).

These taxonomic issues are further exacerbated by the possibility of incongruence between OTUs (or BINs in the BOLD database) and true biological species. While COI has been used extensively to distinguish arthropod species (Hebert et al., 2003; Virgilio et al., 2010; Wilson et al., 2017), it is not always successful for closely related species with low interspecific divergences (Kaila and Ståhls, 2006;
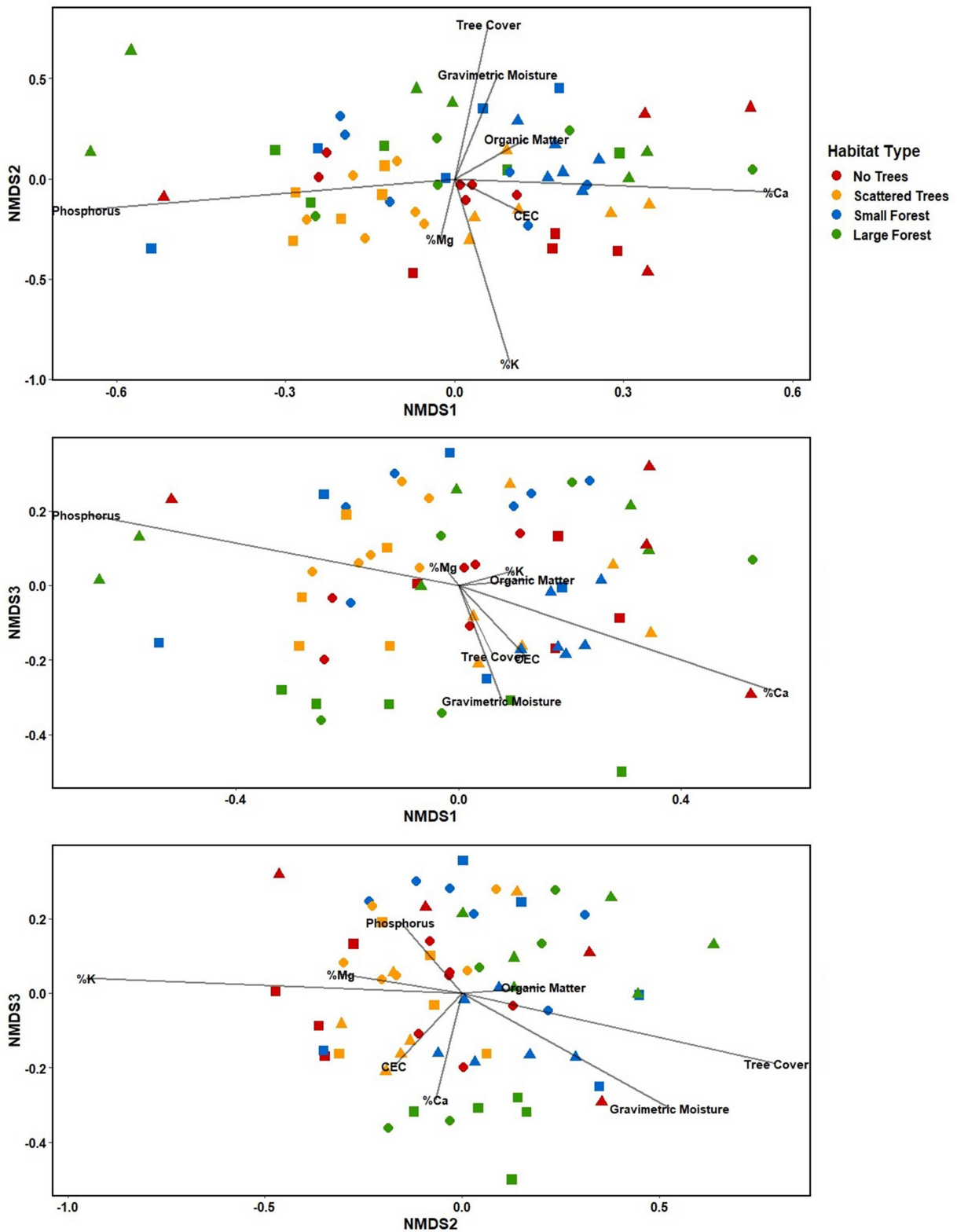
FIGURE 5
Three-dimensional non-metric multidimensional scaling (NMDS) of mesofaunal community composition at each site, with soil parameters represented as vectors (stress value = 0.19). Red = No trees: circles = NT1, triangles = NT2, squares = NT3. Yellow = Scattered trees: circles = ST2, triangles = ST1, and squares = ST3. Blue = Small forest: circles = SF2, triangles = SF1, and squares = SF3. Green = Large forest: circles = LF1, triangles = LF2, and squares = LF3.

FIGURE 6
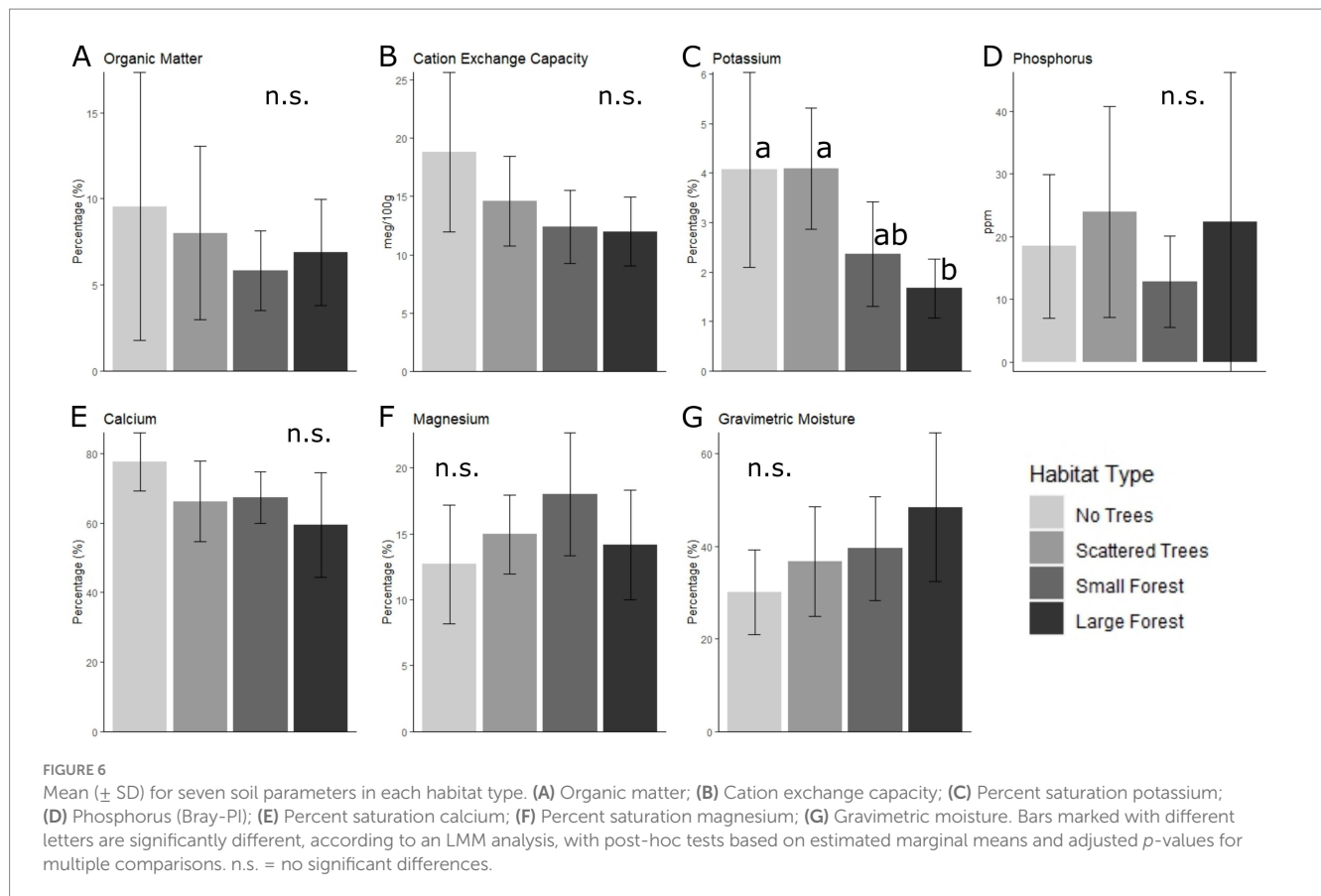Mean (± SD) for seven soil parameters in each habitat type. **(A)** Organic matter; **(B)** Cation exchange capacity; **(C)** Percent saturation potassium; **(D)** Phosphorus (Bray-PI); **(E)** Percent saturation calcium; **(F)** Percent saturation magnesium; **(G)** Gravimetric moisture. Bars marked with different letters are significantly different, according to an LMM analysis, with post-hoc tests based on estimated marginal means and adjusted *p*-values for multiple comparisons. n.s. = no significant differences.

TABLE 2 Correlation of community similarity with distance, tree cover, and soil physicochemical properties, based on Mantel tests.

Property	Test statistic	*p*-value
Distance	$r = 0.16$	<0.001*
Tree Cover	$r = 0.08$	0.001*
Organic Matter	$r = 0.17$	0.002*
Gravimetric Moisture	$r = 0.07$	0.08
Cation Exchange Capacity (CEC)	$r = 0.14$	0.008*
% of K	$r = -0.003$	0.50
% of Mg	$r = 0.14$	0.004*
% of Ca	$r = 0.14$	0.005*
Phosphorus	$r = 0.06$	0.13

*Significant *p*-values.

Meier et al., 2006; Elias et al., 2007; Carew and Hoffmann, 2015; Keck et al., 2023). Alternatively, species with high intraspecific COI variability can be over-split by this method (Lukic et al., 2021; Keck et al., 2023), artificially inflating estimates of species richness (Song et al., 2008). Although in some cases these may represent cryptic species that have not yet been described (Beaulieu et al., 2019; Zhang et al., 2019), disentangling these alternatives can be challenging without detailed taxonomic work. Clearly, increased efforts are needed to update mesofaunal reference libraries, through faunistic surveys, assessing concordance between BINs and biological species,

describing new taxa, and by morphologically identifying BINs that are already present in BOLD.

Despite the challenges in obtaining species-level data, evidence suggests that higher taxonomic levels can still be highly informative from an ecological lens (Bacci et al., 2009; George et al., 2017; Meehan et al., 2019). On top of this, OTU richness (even without named taxa) can be used as an effective proxy for species richness, allowing detailed comparisons of biological diversity (Ji et al., 2013), thus enabling a fine-grained understanding of ecological relationships within the soil.

4.2 Mesofaunal OTU richness in forested and unforested field margins

Maintaining natural areas in agricultural landscapes has been shown to benefit soil biodiversity (Bennewicz and Barczak, 2020) and forested habitats are known to contain more soil mesofaunal biodiversity than do unforested areas (Tomlin and Miller, 1987). This difference has been attributed not only to increased soil moisture and organic matter in forested sites (Arroyo et al., 2013; George et al., 2017; Szigeti et al., 2022), but also to the less disturbed soil environments in forests (Kuznetsova, 2006), compared to arable land that is, for example, tilled seasonally. Even the increased amount of vegetation found in shrub habitats, relative to grasslands, was found to be advantageous for soil arthropod diversity, as it increased soil moisture and provided microclimates more suitable for these

organisms (Ferguson, 2001). It was therefore surprising that we did not find significant differences in OTU richness between the unforested and forested sites in this study.

While the overall patterns of mesofaunal diversity may have masked more pronounced patterns among specific taxonomic groups, this was not observed, at least at the ordinal level, in our data. For example, the three orders of Acari collected in this study (Sarcoptiformes [primarily Oribatida], Trombidiformes [Prostigmata] and Mesostigmata have widely varying life strategies that should respond differently to the habitats we sampled). More specifically, Prostigmata and Mesostigmata are primarily made up of "r-selected" species, with high fecundity and shorter lifespans (Behan-Pelletier, 2003); because of this adaptability, it is less surprising that these groups had no differences in diversity between habitat types. Oribatids, conversely, are "K-selected" soil organisms that reproduce relatively slowly, have low fecundity, and have longer lifespans (Behan-Pelletier, 2003). Consequently, oribatids are often associated with more stable and less disturbed environments (Maraun and Scheu, 2000), but in this study we found no differences in their richness between seemingly very different habitat types (e.g., un-treed field ditches vs. forested habitats). Kokořovă and Starý (2017) found an increase in more tolerant and resilient oribatid species in disturbed forested ecosystems; further analysis at a finer taxonomic resolution would be necessary to test this hypothesis in this system. However, while at an earlier successional stage than the forested habitats, soils in the ditch habitats were nonetheless relatively undisturbed and often protected by a thick layer of brome grass litter. This observation may explain the suitability of ditches as habitat for oribatid mites which are known to be abundant and diverse in natural grassland habitats as well (Behan-Pelletier and Kanashiro, 2010). Future work comparing the ditch and forest mesofaunal communities with those of the adjacent cultivated fields would help determine the impact of disturbance on these soil communities and illuminate the extent to which adjacent habitats influence the in-field taxa.

Despite these observations, the possibility remains that this pattern of unchanged OTU richness between habitat types was simply due to high sample variability and insufficient sampling effort. Because of logistical constraints, we were only able to sample three sites per habitat type, and significant variability was found in the number of OTUs detected even between the sampling points within each site. Increasing the number of sites within each habitat type, as well as the number of replicates within each site, would likely improve our understanding of species richness patterns in these settings. In addition, we were only able to include a single time point in early summer for this study. Since Acari and Collembola abundance and richness have been shown to change over the year (Ferguson and Joly, 2002; Rochefort et al., 2006; Kaczmarek et al., 2011), increasing the temporal range of sampling would have improved our understanding of the species richness at these sites.

4.3 Mesofaunal community composition in forested and unforested field margins

Significant differences in mesofaunal community composition were detected by PERMANOVA, both between habitat types and between sites within a habitat type. However, the NMDS ordination revealed considerable overlap in community composition between sites and habitats. This discrepancy is likely because PERMANOVA methods consider both the centroid and the spread of samples (Anderson, 2017). As noted in our OTU richness analysis, these samples were also characterized by highly variable community composition data. Therefore, the significant differences observed by PERMANOVA may reflect changes in the variability within groups, in addition to overall community dissimilarity. As well, the high stress of the ordination indicates that the pattern of points in the ordination space should be considered cautiously; including more dimensions in the analysis would likely have improved the fit, but would also become increasingly challenging to interpret (McCune and Grace, 2002). This further emphasizes the high variability within and between each site.

While we expected microhabitats (and consequently sampling points) in forested sites to be heterogeneous, and thus to contain more heterogeneous groups of mesofauna (Kuznetsova, 2006), unforested sites appeared more homogeneous at the macro-scale, and we expected to find a more homogeneous array of fauna in these samples. In fact, we found little overlap in species presence between sites in any of the habitats, and no distinct clustering of the communities. This indicates that the scale at which the communities are being structured is more fine-grained than the large-scale habitats defined here. For example, despite relative homogeneity on a landscape scale, the ditch sites varied in terms of grass density, herbaceous species present, and distance to the nearest shrub or tree; soil moisture and other physicochemical traits also varied across a very small scale. In forested sites, these small-scale differences are even more pronounced, with overstory trees, understory vegetation, litter layer quality and quantity, and dead wood features all varying throughout the habitat. In all habitats, therefore, differences in community composition between samples could be attributed to small-scale habitat features or associated vegetation of each sampling location (Hansen, 2000; Heydari et al., 2020), or in differences in the underlying soil layer (George et al., 2017). Geographical distance can also impact mesofaunal species composition (Lehmitz et al., 2012; Arribas et al., 2020; Andújar et al., 2022), with the similarity of these communities decreasing as spatial distance increases (Gao et al., 2014); an observation that was supported in our study.

One factor that limited our ability to detect differences in community composition is the lack of abundance data for the collected taxa. Read counts are likely a poor indicator of abundance due to issues arising from primer bias and specimen biomass (Elbrecht and Leese, 2015). The very large specimen diversity detected here limited the capacity to identify and count all voucher specimens, which would have revealed details about richness and evenness at each site, as well as specifically which taxa were dominant. Using presence-absence data treats all taxa equally, which excludes consideration of abundance or strength of association with particular habitats. As well, it is possible to detect gut contents of predatory taxa, potentially resulting in higher numbers of OTUs for prey species, such as Collembola (Eitzinger et al., 2013). Finally, these methods can detect DNA from dead organisms or their remains that are not actively living in that habitat (Deiner et al., 2017; van der Heyde et al., 2022). These factors may have resulted in more diffuse patterns of community compositions within and between sites.

Despite high sample to sample, and site to site variability, we nonetheless found that forested sites (regardless of size) contained apparently distinct mesofaunal communities than did

the unforested sites (whether with or without scattered trees). These communities are strongly associated with increasing tree cover, unsurprisingly associated with increased moisture, and to a lesser extent, organic matter. This indicates that even small, fragmented forest patches are maintaining a forest-like community, reinforcing the important ecological role that forest patches play in supporting biodiversity in agricultural systems (Hendrickx et al., 2007; Vanbergen et al., 2007; Decocq et al., 2016). However, even in the larger forest patches, our samples were collected relatively near edges, due to logistical constraints. Sampling in the interior of large forest patches may have resulted in more distinct forest specialist communities (Vanbergen et al., 2007). Consequently, our data do not reveal whether small forest patches are similar to larger ones in terms of the mesofaunal communities, but rather that they support communities that are distinct from unforested field margins. Further work to explore the connectedness of habitat patches would be beneficial to understand the soil biodiversity patterns in these ecosystems (Rantalainen et al., 2005).

Understanding the relationship between soil physicochemical parameters and soil mesofauna communities is valuable to predict when environmental changes may create shifts in soil community composition that alter ecosystem function for both agroecosystems and adjacent semi-natural (or natural) areas. Here, we found that ecological characteristics associated with forest areas (e.g., high canopy cover, elevated soil moisture, and soil organic matter) help to structure mesofaunal communities observed from forested sites. However, these trends were less pronounced than we predicted, further emphasizing the high variability between samples within sites, and sites within habitats, both in terms of environmental parameters and community composition. The thick layer of grass litter in many of the non-forested sites may also have resulted in more protected soils than would otherwise be expected in such exposed habitats. More detailed work at a finer scale would help shed additional light on the mechanistic relationships between soil mesofauna and their environment. Expanding sample sizes would also be valuable, but challenges remain regarding the identification of organisms within soil communities, whether future work relies on morphological or molecular techniques, or a combination of both.

5 Conclusion

This study examined soil mesofaunal OTU richness and community composition from a range of natural features within an agriculturally dominated landscape. Gathering this baseline information helps fill a knowledge gap in our understanding of mesofauna function and habitat in agroecosystems within Canada, and perhaps even more broadly across other humid temperate regions in the world. This study also highlighted a need for more robust taxonomic efforts to be made in order to update mesofaunal reference libraries for future metabarcoding work. Differences in OTU richness were not detected between the four habitat types or between most sites within habitats; however, community composition differences were found to be significant. Due to the small scale at which the mesofauna operate, community patterns were probably influenced more strongly by microhabitats within sites, rather than

by the macrohabitat scale of this study. Nevertheless, it is clear that uncultivated natural features in otherwise arable cropland environments are home to a vast diversity of soil organisms. We also demonstrated that even small, forested patches can contain mesofaunal communities similar to those of larger forested patches, highlighting the importance of preserving these features as small yet robust sources of biodiversity within an agricultural matrix.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

AS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. MY: Formal analysis, Methodology, Writing – review & editing. LP: Methodology, Investigation, Resources, Writing – review & editing. NA: Data curation, Methodology, Investigation, Writing – review & editing. DL: Conceptualization, Methodology, Project administration, Funding acquisition, Resources, Writing – review & editing. JK: Conceptualization, Supervision, Writing – original draft, Writing – review & editing. MS: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2025.1443215/full#supplementary-material

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