Effects of Jumping Worms on European Earthworms

and Soil Properties

By

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Abstract

Earthworms are ecosystem engineers which alter soil structure and impact other organisms and ecosystem functioning. In 2014, pheretimoid "jumping worms" (Megascolecidae spp.) were discovered in Ontario, Canada, with later discoveries in New Brunswick (2021), and Nova Scotia (2022). Jumping worms are having substantial impacts in the northeastern United States, including effects on nutrient cycling and other soil organisms. In Canada, little research has been done to examine spread or effects of jumping worms since they have established only recently. Thus, we sampled at a residential property in Oromocto, New Brunswick, which was the first location where jumping worms were found in the province. Our objectives were to evaluate: (1) how jumping worms impact soil properties (i.e., nitrogen, carbon); (2) how their presence impacts the abundance of European earthworms; and (3) the effectiveness of two jumping worm sampling methods. We found that jumping worms did not have significant impacts on European earthworm species or soil carbon, but they did have significant impacts on soil nitrogen levels. Our results suggest the existence of a positive relationship between jumping worm abundance and soil nitrogen levels when jumping worm abundance is low. Also, both sampling methods (i.e., mustard solution and wooden discs) were equally effective at detecting the presence of jumping worms at a site. Over the longer term, we hope to track the expansion of this population in order to determine rates of spread.

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1. Introduction

1.1 Invasive Ecosystem Engineers

Climate changes (e.g., extreme climatic events) and increased human activities (e.g., global trade, agricultural systems, and land degradation) are key factors involved in the spread of non-native species (Bellard et al. 2013). When species are introduced outside of their native range due to human activities, they are referred to as non-native species (Jeschke et al., 2014). The severity of this issue is increasing with time as the rate of introduction of non-native species is predicted to increase by 36% between 2005 and 2050 worldwide (Seebens et al., 2020). Invasive species refer to non-native organisms that rapidly spread once introduced to their new ecosystem (Ricciardi et al., 2013). However, their definition can vary, with other scholars defining invasive species as non-native species who have significant impacts on the environment (Young & Larson, 2011). Invasive species in and of themselves can have significant impacts on the habitats they invade, and these impacts can be amplified when these species are ecosystem engineers. Ecosystem engineers are organisms that create new habitat by directly or indirectly influencing the availability of resources to other species due to the effects they have on biotic or abiotic materials (Lawton & Shachak, 1994; Lawton & Shachak, 1997).

Soil ecosystem engineers include various organism groups such as plants, termites, ants, and earthworms, which play an important role in the functioning and structure of their ecosystem (Lavelle et al., 2016). When introduced outside of their native range, ecosystem engineers can alter their new habitat through the creation of microenvironments (Lavelle et al., 2016). These microenvironments are produced through the alteration of soil microbiological, chemical, and physical characteristics (Lavelle et al., 2016). Given the severity of the potential impacts associated with the introduction of these species to new regions, understanding the distribution and impacts of invasive soil-dwelling species, such as earthworms, is critical.

1.2 Invasive European Earthworm Species Ecosystem Impacts

During the last glaciation (i.e., the Wisconsin glaciation), which occurred nearly 12,000 years ago, native earthworms were almost entirely removed from Canadian soils (Gates, 1970; Gates, 1982). To our knowledge, eight native earthworm species occur in Canada, predominantly in Pacific coastal regions which were glacial refugia (Addison, 2008; Dymond et al., 1997). Therefore, the vast majority of contemporary Canadian soils and forests developed in the absence of earthworms. However, the arrival of European settlers to the continent approximately 400 years ago introduced non-native earthworm populations to Canada (Gates, 1982). Since their introduction, European earthworms have spread across the country, with range expansion being facilitated by recreational and industrial human development (e.g., fishing, vehicles, agriculture, etc.) (Cameron et al., 2007; Addison, 2008). Previous studies on European earthworm distribution in Canada have determined these invasive species to be well-established throughout Canada, as their presence has now been reported in all provinces and territories (Addison, 2008).

As previously mentioned, when found outside of their native range, earthworms can act as invasive ecosystem engineers. Non-native earthworm species can have a wide array of impacts on their environment such as altering soil physico-chemical properties, accelerating nutrient cycling, and altering the distribution of soil microbes (Bohlen et al., 2004a). However, these impacts vary depending on the species of earthworms that are invading. Earthworms are classified into defined ecological categories (i.e., anecic, epigeic, and endogeic) based on their morpho-anatomical traits, vertical distribution, and ecology (Bouché, 1972; Bouché, 1977). Earthworms belonging to epigeic functional groups (e.g., *Dendrobaena octaedra*) reside on the soil's surface, and are often found beneath litter layers (Edwards & Bohlen, 1972). Anecic earthworm species (e.g., *Lumbricus terrestris*) are classified based on behaviours that result in the formation of permanent vertical burrows in the soil (Edwards & Bohlen, 1972). Earthworm species belonging to the endogeic functional group (e.g., *Aporrectodea tuberculata*) reside in the mineral soil horizon (Edwards & Bohlen, 1972; Ferlian et al., 2019). Different earthworm ecological categories have varying degrees of influence on their habitat (Bouché, 1977). For example, in a meta-analysis, the presence of anecic earthworm species and endogeic species caused a significant decline of the diversity and density of soil invertebrates (Ferlian et al., 2019). Increased diversity of functional groups co-occurring at sites was also associated with greater soil carbon emissions and litter decay, (Huang et al., 2019). These impacts on soil characteristics have been linked to earthworms' feeding and burrowing habits, as they ingest organic matter and create more homogenous soils (Hale et al., 2005). Studies conducted in the Eastern United States and Canada revealed that invasive earthworm species decreased soil carbon storage in the upper soil layers (Bohlen et al., 2004b; Fahey et al., 2012; Lejoly et al., 2021). This reduction in soil carbon pools is believed to be caused by the elimination of the surface organic horizons (Fahey et al., 2012; Bohlen et al., 2004a).

On top of impacting soil carbon storage, the presence of European earthworms influences soil nitrogen levels. Studies revealed greater denitrification in quadrats where Lumbricidae spp. were present (Parkin & Berry, 1994; Burtelow et al., 1998). This decline in available nitrogen is believed to be the result of fresh litter mixing with mineral soils, thus redistributing nutrients among soil layers, and increasing soil nitrogen loss (Ferlian et al., 2019; Hale et al., 2005). Earthworms also create macropores in soils, which can increase the loss of gaseous nitrogen into the atmosphere from upper soil layers (Ferlian et al., 2019).

1.3 Jumping Worms' Introduction to Canada

Pheretimoid or "jumping worms" are a group of earthworms originating from eastern and southeastern Asia (i.e., Japan, Korea, and China) (Sims & Easton, 1972). The group contains over 1,000 described species that are divided into twelve genera (including *Amynthas, Metaphire, Pithemera*) within the family Megascolecidae (Sims & Easton, 1972; James, 2004). The first sighting of pheretimoid species in North America occurred near San Francisco, CA in the late 1860s (Kinberg, 1867). Since this initial discovery, there have been a total of sixteen species of pheretimoid earthworms identified in North America. It is believed that these species were introduced to the United States through horticultural practices that involve the transportation of soils (Gates, 1958; Görres & Melnichuk, 2012). Their resilient cocoons can survive for years in harsh weather conditions which can facilitate their spread through imported soils (Nuutinen et al., 1991). Since the time of their initial discovery, jumping worms have spread across the United States and have successfully established themselves in 38 states ranging from the Northeast to Midwest (Chang et al., 2021; Reynolds & McTavish, 2021). Their distribution within North America is concentrated around human establishments, with most reported sightings being observed in urban parks, residential yards, greenhouses, and compost piles (Chang et al., 2021). Although found in higher densities in urban settings, the introduction of these species to forest ecosystems is a major concern for conservationists and land managers (Chang et al., 2021; Moore et al., 2017; McCay et al., 2020). This marks the importance of acquiring a greater understanding of their current distribution, invasion potential, and predicted impacts on Canadian soils.

Although Megascolecidae spp. have been present in North America since the late 1800s, they were only recently discovered in Canada. In 2014, the first pheretimoid jumping worm was discovered on the Ojibway Prairie in Essex County, Ontario, Canada (Reynolds, 2014). The collected specimens were later identified as *Amynthas hilgendorfi* and *Amynthas agrestis*. Since this time, a total of six pheretimoid species have been recorded in Canada (*A. hilgendorfi*, *A. agrestis*, *Amynthas tokioensis*, *Amynthas gracilis*, *Amynthas minimus*, and *Pithemera bicincta*) (Reynolds & McTavish, 2021; McAlpine et al., 2022; Dr. Erin Cameron, personal communication, February 26th, 2023). Currently identified Megascolecidae spp. in Canada are epi-endogeic species, implying that they live near the surface of the soil (McCay et al., 2020). Co-invasion dynamics have been hypothesized between *A. agrestis*, *A. hilgendorfi* and *A. tokioensis* as they have been shown to co-occur, with *A. tokioensis* being the dominant species observed in natural habitats (Chang et al., 2017; Schult et al., 2016). Recent distributional data places Megascolecidae spp. in three Canadian provinces: Ontario, Nova Scotia, and New Brunswick (McAlpine et al., 2022; Reynolds, 2022)

The first Atlantic Canadian jumping worm population was observed in the summer of 2021 in Oromocto, New Brunswick, on a residential property (McAlpine et al., 2022). The discovery took place near a woodpile, with populations present at relatively high densities compared to other invasive earthworm species populations found in Canada (McAlpine et al., 2022). Three species of jumping worms have since been discovered in New Brunswick: *Metaphire hilgendorfi*, *A. tokioensis* and *A. minimus* (McAlpine et al., 2022). Due to their potentially elevated invasion potential and rapid rate of spread, there is concern that these species will continue to expand their range and establish themselves throughout Atlantic Canada.

1.4 Megascolecidae spp. environmental impacts

Pheretimoid species are believed to have differing effects on soil properties when compared to European earthworms. The variation in impacts when compared to Lumbricidae spp. is thought to be associated with their larger body size, ecological category, high population densities, as well as their greater dietary flexibility (Greiner et al., 2012). Although invasive earthworm species have been present in Canada since the beginning of European settlement, jumping worms were only recently discovered in Canada. Due to the novelty of their discovery, little is known about their distribution throughout North America, their life-history or about their potential impacts on soils (McAlpine et al., 2022). Studies evaluating their impacts on soil in Canada have yet to be conducted, providing managers with little information on how they may impact Canadian ecosystems and spread throughout the country.

Although no studies have examined jumping worms' effects on Canadian soils, studies in the United States have evaluated their impact on deciduous and mixed wood forest soils (Bethke & Midgley, 2020; Chang et al., 2021; Greiner et al., 2012). These studies were conducted both in the field and in mesocosms, and they have shown that invasive jumping worms affect soil properties at rates greater than those of Lumbricidae spp. Jumping worms were shown to increase the concentrations of soil nitrogen and phosphorus mineral forms at rates faster than Lumbricidae spp. (Greiner et al., 2012). The presence of jumping worms at a site also increased mean soil aggregate sizes, an effect not observed in the presence of Lumbricidae spp. (Greiner et al., 2012). However, more studies are needed to better understand the differences in ecological impacts of jumping worms and European worms in North America (Chang et al., 2021).

As mentioned in the previous section, invasive pheretimoid earthworm populations possess traits that may favor their establishment in Canada. The invasion potential of jumping worms has been attributed to their life cycle characteristics and population ecology traits (Greiner et al., 2012; Zhang et al., 2010). These species have exhibited a wide dietary flexibility allowing them to acquire nutrients from a greater number of resources than European species (Greiner et al., 2012). These species also have larger body sizes and can reproduce at faster rates when compared to some European earthworms, giving them the ability to potentially competitively exclude Lumbricidae spp. from co-occurring sites (Zhang et al., 2010; Bethke & Midgley, 2020). In a study conducted in Tennessee, USA, the presence of Lumbricidae spp. had no impact on the abundance of jumping worms, which suggests that the occurrence of Lumbricidae spp. does not hinder the invasion potential of Amynthas spp. (Zhang et al., 2010). This is due to jumping worms' dietary flexibility allowing them to consume varying litter types, and other nutrient sources (e.g., soil gram positive bacteria and non-microbial soil fauna) (Zhang et al., 2010). On the other hand, the presence of pheretimoid earthworms negatively affects the ability of Lumbricidae spp. to consume litter and soil microbes, which ultimately leads to reduction in Lumbricidae abundance (Zhang et al., 2010). These findings suggest that Amynthas spp. may be able to outcompete Lumbricidae spp., leading to a reduction in Lumbricidae spp. abundance and limiting their range expansion. The ability of jumping worms to outcompete Lumbricidae spp. suggests that they may potentially pose risk to native earthworm populations in cohabitated regions and supports claims that they can lead to reductions in invertebrate populations.

Jumping worms have been associated with a decrease in litter horizon depth, increased pH levels, as well as fluxes in carbon and nitrogen availability (Bethke & Midgley, 2020). Jumping worms cause litter layers to decompose at faster rates when compared to *Lumbricus rubellus,* a

European invasive species (Greiner et al., 2012). Research suggests that jumping worms' impact on American soils is context dependent, with impacts being greater on soils least resembling their natural habitat (Bethke & Midgley, 2020). Alterations to soil carbon were dependent on forest soil type and earthworm density with soil carbon availability increasing with earthworm density in sugar maple forests but decreasing with increasing earthworm density in oak forests (Bethke & Midgley, 2020). Therefore, although studies show that the presence of jumping worms at a site influences soil carbon, there is little consensus on the direction of these interactions. *Amynthas* spp. were also shown to increase the concentrations of both NH_4^+ and NO_3^-, suggesting that they may have greater impacts on soil nitrogen concentrations compared to Lumbricidae spp. (Greiner et al., 2012). Although few studies have been conducted as yet on the effects of jumping worms on soil ecosystems, there is reason to believe that their introduction to Canada will alter soil nutrient regimes, and potentially at levels greater than those of Lumbricidae spp.

1.5 Community Science and Methods

The study of earthworm distributions can be relatively difficult, as sampling is time consuming and costly. Citizen science (hereafter referred to as community science), is one approach that can be used to address these issues, as it allows for a wide array of data collection at lower costs (Silvertown, 2009). Community science uses volunteers to collect and/or process data (Silvertown, 2009). The use of community science can help increase both the spatial scale and the sampling efforts of a project resulting in increased data acquisition (Roy-Dufresne et al., 2019; César de Sá et al., 2019).

Jumping worms are well suited for study via community science, due to their large body size, easy to identify traits, and thrashing behaviours (Ziter et al., 2021). The existence of well-developed and simplified taxonomic keys has eased the process of identification for jumping worms to the family level. These taxonomic keys rely on two key features to differentiate jumping worms from other earthworm groups: their clitellum and their thrashing behaviour (Chang et al., 2021). Unlike European earthworm species whose clitellum is saddle-shaped, jumping worms' have an annular clitellum which means that it encircles the entirety of their body (Chang et al., 2021). However, it is important to note that the clitellum is a reproductive organ and therefore is not present on juvenile specimens. Therefore, the clitellum will only allow for the identification of adult jumping worms (Chang et al., 2021). However, both adult and juvenile jumping worms exhibit thrashing behaviors, which allows for the identification of juvenile earthworms (Chang et al., 2021). Given the ease through which jumping worms can be identified, community science represents an effective way by which the presence of jumping worms at a site can be identified and allows for more data to be collected on jumping worm distributions.

Simple and cost-effective sampling methods that meet the standards of academic science are essential to community science (Freitag et al., 2016). Standard methods for sampling epi-endogeic earthworm populations include hand sampling and liquid extraction methods. Mustard solution, a form of liquid extraction, is a chemical expulsion method that irritates the mucus membrane of earthworms and causes them to emerge on the surface (Iannone III et al., 2012). This method is a standard method of sampling earthworms as it is non-toxic and is effective under a wide range of environmental conditions making it optimal for community science (Gunn 1992; Lawrence and Bowers 2002; Heneghan et al. 2007). However, this method can be difficult to prepare and requires greater search effort in comparison to other methods. Therefore, finding alternatives to this method could benefit the use of community science in the detection of jumping worms. Seeing that jumping worms migrate towards areas of greater moisture (Snyder et al., 2010), along with sightings of jumping worms near wooden surfaces, we envisioned the use of wooden discs to sample the presence of jumping worms. A wooden disc method would require less material when compared to liquid extraction and use a simpler protocol involving no mixing or measuring of ingredients. The use of wooden discs to detect the presence of jumping worms at a site also is a non-destructive sampling method, which is preferable when sampling urban areas. In order to develop the best sampling methods to detect the presence of jumping worms, we evaluated the differences in efficiency between the two methods to detect the presence of jumping worms at a site. With jumping worm populations spreading across Canada, it is important for managers to obtain a greater grasp on the distribution of jumping worms, an objective that can be easily achieved through the use of community science.

1.6 Objectives

With increasing rates of commercial globalization and greater movement of soil, mulch, and plants, there is greater potential for the introduction of non-native earthworms across Canada (Klein et al., 2019). Given the impacts that pheretimoid earthworms have had in invaded ecosystems, it is important to evaluate their impacts in Atlantic Canada. This project offers an opportunity to study the early stages of jumping earthworm invasion in Canada as well as their interactions with European invasive earthworms.

In this research, we evaluated the ecological impacts of jumping worms on the abundance of European earthworms (Lumbricidae spp.) and soil nitrogen and carbon concentrations. We hypothesized that the presence of jumping worms would be associated with lower abundances of European earthworms and lower soil carbon and nitrogen content. In order to acquire a better understanding of these dynamics, we posed the following questions: (1) Does the presence of jumping worms impact Lumbricidae spp. biomass and abundance? (2) Does the presence of jumping worms impact soil properties (i.e., carbon and nitrogen)? (3) Is the wooden disc method as effective as a sampling method as mustard solution at detecting the presence of jumping worms?

2. The Study Area

This study took place in a residential neighborhood in Oromocto, New Brunswick, Canada. Jumping worms were first discovered in the Maritimes in 2021 near the woodpile found at the center of the study site (McAlpine et al., 2022). The observation of unusually elevated densities of earthworms as well as a noted thrashing behaviour led to the initial sampling of this site in 2021 (McAlpine et al., 2022). The introduction of substantial amounts of soils in 2019-2021 are believed to be linked to the introduction of jumping worms at the site (McAlpine et al., 2022). The site spanned across three neighbouring properties (Figure 1), all of which have been subjected to various degrees of landscaping activities including activities such as gardening, altering ground cover, and introducing soils and plants from other locations. This introduction of soils, vegetation and wood may have acted as an important vector explaining the introduction of Megascolecidae species at the site. Given the recent discovery of jumping earthworms on these properties, this study area is ideal for examining the impact of invasive jumping earthworms on soil properties as well as their interactions with invasive European earthworms.

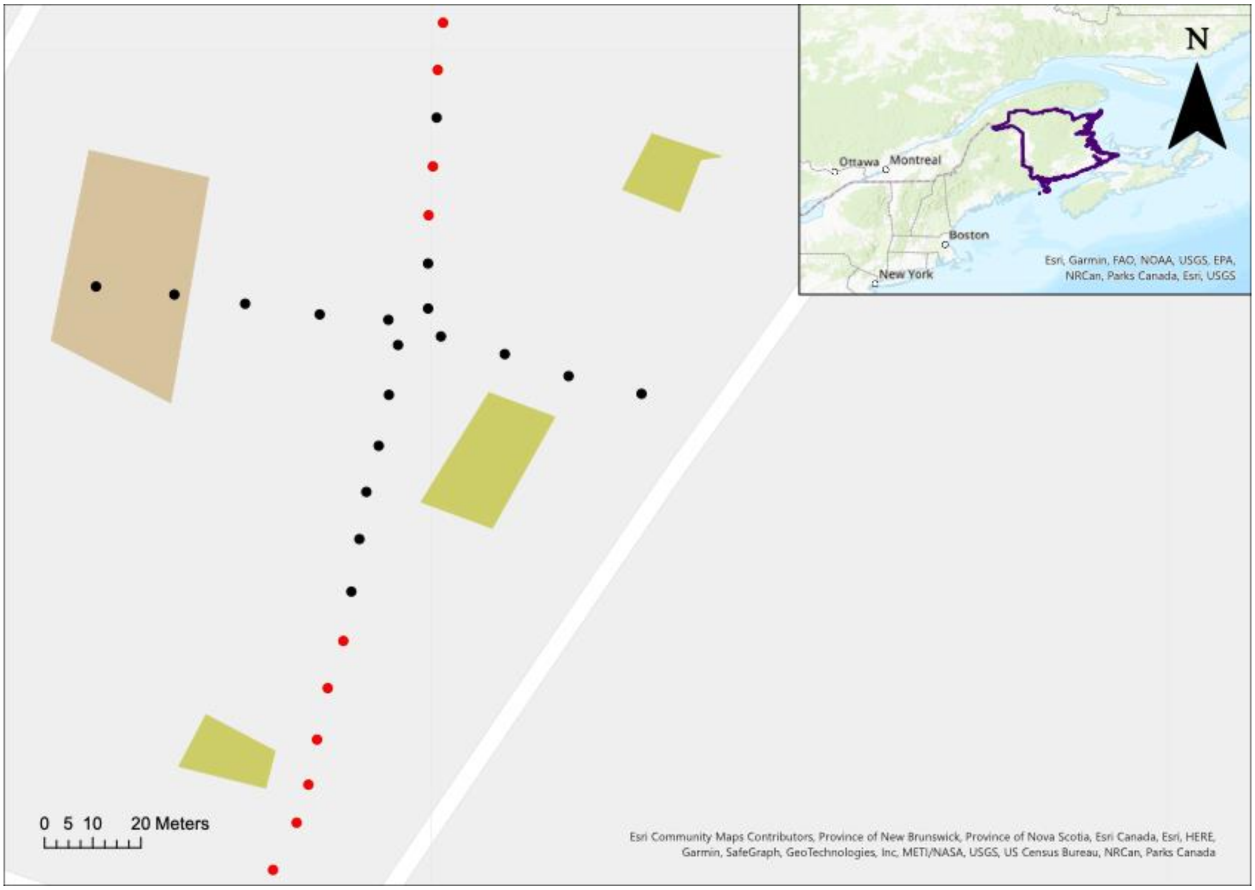

Figure 1. This map depicts the study boundaries of this project and illustrates the four transects along which jumping worms were sampled. The red points illustrate all quadrats where jumping worms were absent, while the black points represent all quadrats containing jumping worms. The green polygons represent the three neighbouring houses present at the study site while the brown polygon represents an area of significant disturbance resulting from land management practices. The inset map present in the upper right corner delineates the provincial boundaries of New Brunswick.

3. Methods

3.1 Experimental Design

In the spring of 2022, four transects were created using the woodpile as the centre point. This woodpile is believed to be the initial point of introduction of jumping worms at the site. These transects were delineated by laying out 28 wooden discs with a diameter of 25 cm. All wooden discs were placed along the transects by June 28, 2022. The discs were made either from birch or spruce wood. The wooden discs were 25 cm in diameter and 10 cm in height. Wooden discs were used as they create a microhabitat that is preferred by earthworms due to increased moisture levels, thus creating a preferred habitat for earthworms to migrate towards (Snyder et al., 2010). Due to their discovery near the woodpile and the noted affinity earthworms demonstrate towards areas of greater moisture levels, we believed that wooden discs would represent a convenient sampling method to use when trying to detect the presence of jumping worms at a site. These transects ran at 90-degree angles from one another facing in the four cardinal directions. Each transect spanned the greatest distance permissible by the limitation of the yard with the longest being the South-facing transect that spanned 120 m and the shortest being the East-facing transect that spanned 40 m. For each of the four transects, the first disc was placed at the edge of the woodpile. Each following disc along the transect was placed 10 m apart. The distance between each disc was selected due to the average yearly spread of earthworm populations being estimated at 10 m (Marinissen & van den Bosch, 1992). The South-facing transect contained twelve discs, the East-facing transect contained four discs, the North-facing transect had seven discs and the West-facing transect had five discs. Once the discs were placed along the transects, they were not disturbed until the fall sampling period.

The fall sampling period lasted a total of three days, spanning from August 23 to August 25, 2022. For each point/disc, the GPS coordinates were recorded using ESRI Fieldmaps and a GNSS device (Eos Arrow 100+). A 50 cm x 50 cm quadrat was then placed 30 cm to the right of the disc. A 2 cm diameter and 5 cm depth soil core was taken in the corners and center of each quadrat, resulting in five soil cores per quadrat. The five soil cores from each quadrat were homogenized and placed in a cooler to keep samples cold while in the field. Following this, the wooden disc was overturned, and all visible earthworms were collected. As jumping worms are epi-endogeic species, implying that they reside in upper soil layers, a liquid extraction method (i.e., mustard solution) was used to sample quadrats (McCay et al., 2022). In order to do so, a mustard solution consisting of 80 g of mustard powder combined with 8L of water was prepared. Half of this solution was poured in the quadrat, causing earthworms to emerge from the soil's surface. During a 10-minute period, all emerging earthworms found within the quadrat boundaries were collected. If any earthworms appeared outside of the quadrat, they were collected and placed in a separate vial. After the 10-minute period had elapsed, the remaining 4 L of mustard solution was added to the quadrat. Over a 20-minute period, emerging earthworms were collected. Following this 30-minute search period, the collected earthworms were stored in 70% ethanol. Two additional quadrats were added to the West-facing transect beyond the last wooden disc, as ongoing yard work taking place in the spring did not initially allow for discs to be placed in this area. Once all of the data was collected, the soil and earthworm samples were transported back to Nova Scotia.

3.2 Soil Properties Analyses

Soil analyses were conducted on the samples gathered in the field. Soil samples were air-dried for a 96h period. Once dried, the soil samples were sieved through a 2 mm sieve allowing us to analyze the carbon, nitrogen, and pH values of the samples.

Each sample's respective nitrogen and carbon values were determined by running the samples on the Elemental Analyzer Perkin Elmer CHN, 2400 Series II. This process utilized dry combustion mechanisms to determine the concentrations of nitrogen and carbon. For each sample, we weighed10 mg of soil and placed it in a capsule. After every five soil samples, a LECO soil standard was processed to determine the expected accuracy of the analysis.

Soil pH was measured in 0.01 M $CaCl_2$ using a benchtop electrode pH meter (OrionStar A215). For this process, 10 g of soil was placed in a beaker alongside 20 mL of 0.01 M $CaCl_2$. The solution was mixed for 10 seconds and then left to stand for 30 minutes while stirring occasionally. The samples were left to settle for a one-hour period after which the respective electrodes were placed in the solutions and the values recorded.

3.3 Earthworm Identification

In the laboratory, European earthworms were identified to the species level under a microscope using taxonomic keys from Sherlock (2018). Earthworms belonging to the Megascolecidae family were identified under a microscope to the family level using the key of Chang et al. (2016a). Specimens' body length was measured, allowing us to calculate biomass. Earthworm biomass was calculated using allometric equations for Lumbricidae spp. and Megascolecidae spp. to determine the ash-free dry mass from lengths (Greiner et al., 2010).

3.4 Statistical Analysis

Statistical analyses were conducted through R 4.2.2 (R Core Team, 2021) using lmerTEST package designed for mixed linear models (Kuznetsova et al., 2017), and glmer (lme4) package for generalized linear mixed-effect models (Bates et al., 2015). Although pH values were collected with the intent of including the data as a covariate, they were excluded from models due to the complexity of the models and the small sample sizes. The results obtained from these models were considered significant when the model's P-value < 0.05.

Four linear mixed effect models were created to determine the effects of jumping worms on European earthworm species. Jumping worm abundance and biomass were used as the predictor variables to determine their effects on both European earthworm species' abundance and biomass. The response variables (abundance and biomass of Lumbricidae spp.) were square root transformed as the data was skewed, resulting in non-normal distributed residuals. The quadrat's distance from center was included in models as a covariate, with transect direction (i.e., N, S, E, W) included as a random effect variable.

To determine the effects of jumping worms on soil properties, four linear mixed effects models were created using soil carbon and nitrogen percentages as the response variables and jumping worm abundance and biomass as the predictor variables. Due to the potential for non-linearity, data transformations on the predictor variables were tested. Quadratic terms were then added, through a polynomial transformation, to the predictors of jumping worm abundance and biomass. However, they were removed if the model was not significantly different from a model without the polynomial term. In the model evaluating the effect of jumping worms on soil carbon, jumping worm abundance was transformed into a quadratic variable through the addition of a polynomial term. For the effect of jumping worm biomass, the polynomial term was not significant, and instead the log of the jumping worm biomass was used to evaluate this variable's effect on soil carbon. The polynomial term of both jumping worm abundance and biomass was used in models evaluating the effects of jumping worms on soil nitrogen. Once again, quadrat distance from center was used as a covariate and transect direction as a random effect variable in all four models.

A generalized linear effect model was used to compare the effectiveness of the two sampling methods in question (i.e., wooden disc and mustard solution). In this model, presence-absence of jumping worms was the response variable and the sampling method used was the predictor variable. The model also included the transect direction as a random effect variable and used a binomial error distribution.

4. Results

European earthworms were found at a total of 26 out of the 28 quadrats, and 18 quadrats were also occupied by jumping worms. Six European earthworm species were identified at the site, with *Dendrobaena octaedra* and *Lumbricus rubellus* being present in the highest abundance (Table 1). Average soil carbon concentrations across the site were 2.56% (ranging from 0.57% to 5.8%). Average soil nitrogen concentration across the site was 0.20% (range = 0.04% to 0.37%). The average soil pH across the site was 5.27, with the highest pH values (of 7.24 and 7.27) recorded at quadrat W4 and W5 where clay-rich soils were brought in by landowners, and the lowest pH (of 4.19) recorded at quadrat N6.

Table 1. European earthworm species present at the site, including their mean abundance and biomass per m^2, standard error, as well as the number of quadrats where they were present.

Species	Mean Abundance per m^2	Standard Error	Mean Biomass (g) per m^2	Standard Error	Percent of Quadrats where Present (%)
Dendrobaena octaedra	9.76	3.44	14.14	25.67	57
Lumbricus rubellus	5.85	2.12	40.25	76.64	50
Aporrectodea rosea	0.15	0.19	17.16	0.5	3.6
Lumbricus terrestris	0.62	0.46	161.21	149.8	11
Octolasion tyrtaeum	1.23	1.20	49.72	41.96	7.1
Aporrectodea tuberculata	0.77	0.62	45.07	48.77	11

Objective 1: Impacts on European Earthworms

European earthworm species abundance (Fig. 2; t-value = 0.95, P = 0.35) and biomass (Fig. 2; t-value = 0.25, P = 0.81) were not significantly affected by the abundance of jumping worms. Similarly, European earthworm abundance (Fig. 3; t-value = 0.65, P = 0.52) and biomass (Fig. 3; t-value = 0.43, P = 0.67) were not significantly affected by jumping worm biomass.

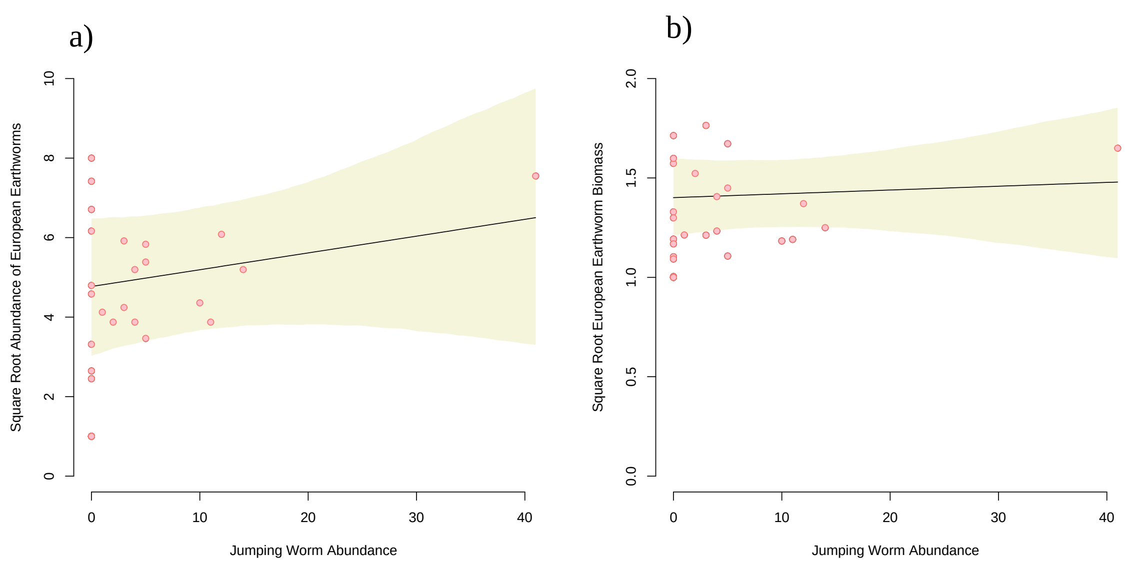

Figure 2. Effect of jumping worm abundance on European earthworms (n=28). ***(a)*** *Square root value of European earthworms' abundance in relation to the number of jumping worms present at the quadrats (P=0.35; S.E. = 0.04).* ***(b)*** Square root biomass *of European earthworms in relation to the number of jumping worms present at the quadrats (P=0.81; S.E. = 0.33). Black lines are the predicted trend lines based on the models, with the confidence intervals represented by the beige polygons.*

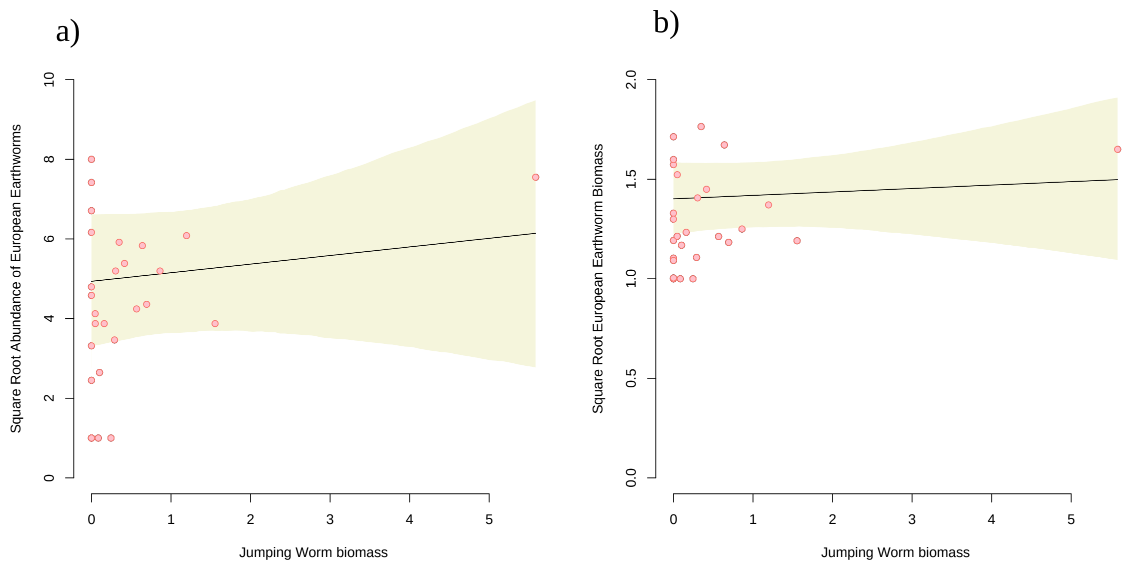

Figure 3. Effect of jumping worm biomass on European earthworms (n=28). ***(a)*** *Square root value of European earthworms' abundance in relation to the jumping worm biomass at each quadrat (P= 0.52; S.E. = 0.01).* ***(b)*** Square root biomass *of European earthworms in relation to jumping worm biomass at each quadrat (P= 0.67; S.E. = 0.04). Black lines are the predicted trend line based on the models, with the confidence intervals represented by the beige polygons.*

Objective 2: Impacts on Soil Carbon and Nitrogen

The percentage of carbon in quadrats with jumping worms had a mean value of 2.39% (S.E. = 1.26) whereas carbon levels in quadrats without jumping worms had a mean value of 2.87% (S.E. = 0.77). The soil carbon concentration was not significantly affected by jumping worm abundance (Fig. 4; t-value = -2.0, P = 0.06) or biomass (Fig. 4; t-value = -0.24, P = 0.81). In quadrats where jumping worms were present, percent soil nitrogen composition had a mean value of 0.19% (S.E. = 0.09) whereas nitrogen levels in quadrats without jumping worms had a mean value of 0.21% (S.E. = 0.04). Similarly, to soil carbon, the percent composition of soil nitrogen did not vary significantly in relation to jumping worm biomass (Fig. 5; t-value = -1.79, P = 0.10).

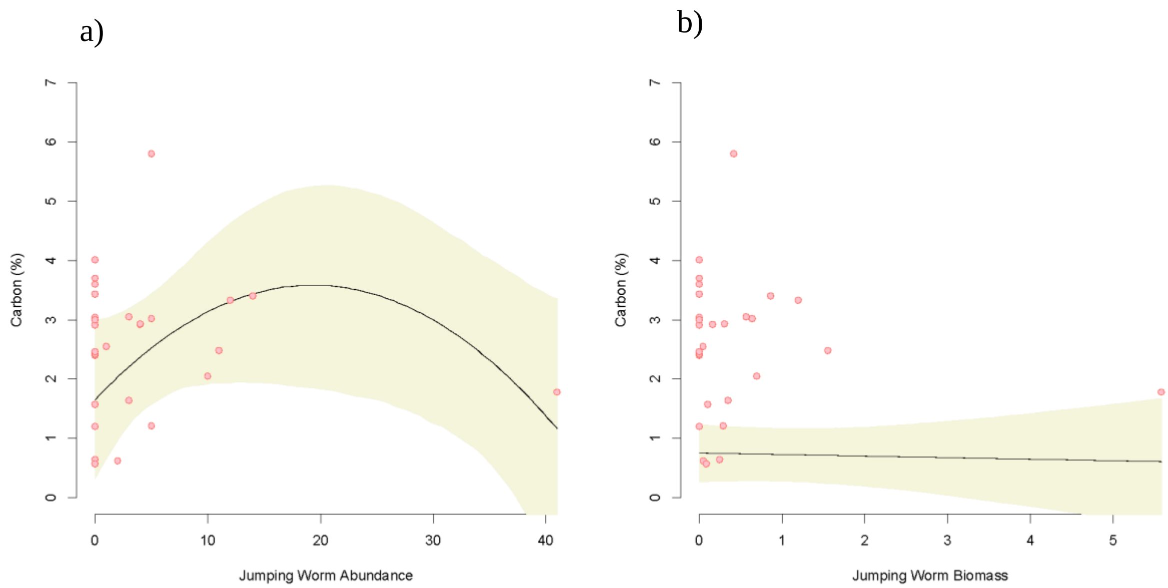

Figure 4. Jumping worm biomass effect on soil carbon percent (n=28). ***(a)*** *Soil carbon percent in relation to the quadratic transformation of jumping worm abundance (P= 0.06; S.E. = 1.55).* ***(b)*** *Soil carbon percent recorded at each quadrat in relation to the log of jumping worm biomass (P= 0.81; S.E. = 0.11). Black lines are the predicted trend lines based on the models, with the confidence intervals represented by the beige polygons.*

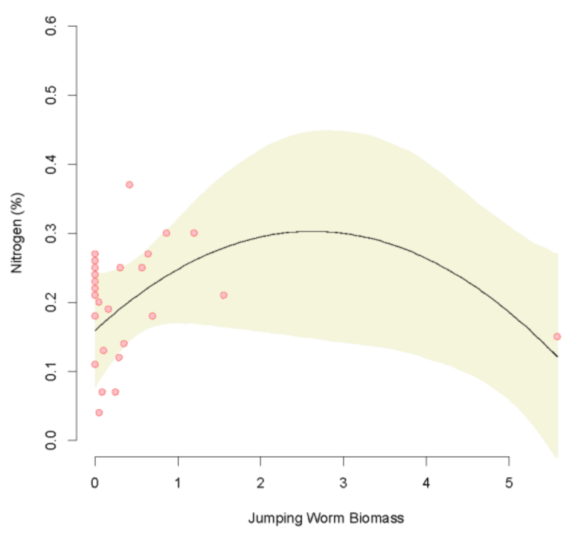

Figure 5. Jumping worm biomass effect on soil nitrogen percent (n=28). This figure presents the soil nitrogen percentage recorded at each quadrat in relation to the quadratic transformation of jumping worm biomass (P=0.10; S.E. = 0.10). The model's predicted trend line is illustrated by the black line with the confidence interval represented by the beige polygon.

Jumping worm abundance did have a significant effect on soil nitrogen concentrations. The model revealed a positive non-linear relation between the percent soil nitrogen and jumping worm abundance. This non-linear relationship shows that an increase in the number of jumping worms is associated with an increase in soil nitrogen concentrations (Fig. 6; t-value = -2.36, P = 0.03). But, once a certain threshold is met, higher jumping worm abundance could lead to decreased soil nitrogen concentrations. However, this non-linear relationship is only driven by a single quadrat, quadrat N1. This quadrat, closest to the woodpile, had the greatest jumping worm abundance and lower nitrogen concentrations compared to other sampled quadrats.

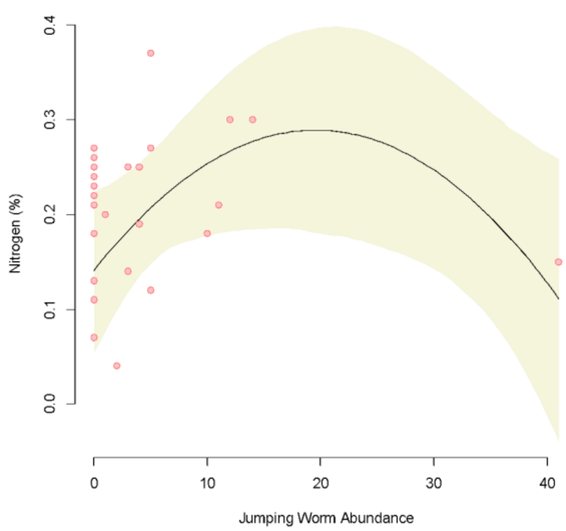

Figure 6. The effect of jumping worm abundance on the percent nitrogen composition in soil (P= 0.03; S.E. = 0.10). Pink dots show each quadrat's individual soil nitrogen percentage as well as its respective jumping worm abundance (n=28). The calculated confidence interval is represented by the beige polygon. The quadratic trend line shows the relationship of soil nitrogen percent to the number of jumping worms found at each quadrat.

Objective 3: Wooden Disc vs. Liquid Extraction Methods Comparison

Combining results from both methods revealed that jumping worms were present at a total of 18 of the 28 sampled quadrats (Fig. 7). Sampling methods using mustard solution had a detection rate of 78%, with detections of jumping worms at 14 of the 18 quadrats (Fig. 7). Wooden discs successfully detected the presence of jumping worms at 13 of the 18 quadrats (Fig. 7), resulting in a detection rate of 72%. The jumping worm detection rate did not differ significantly between the two sampling methods (Z-value = -0.28, P=0.78, S.E. = 0.57).

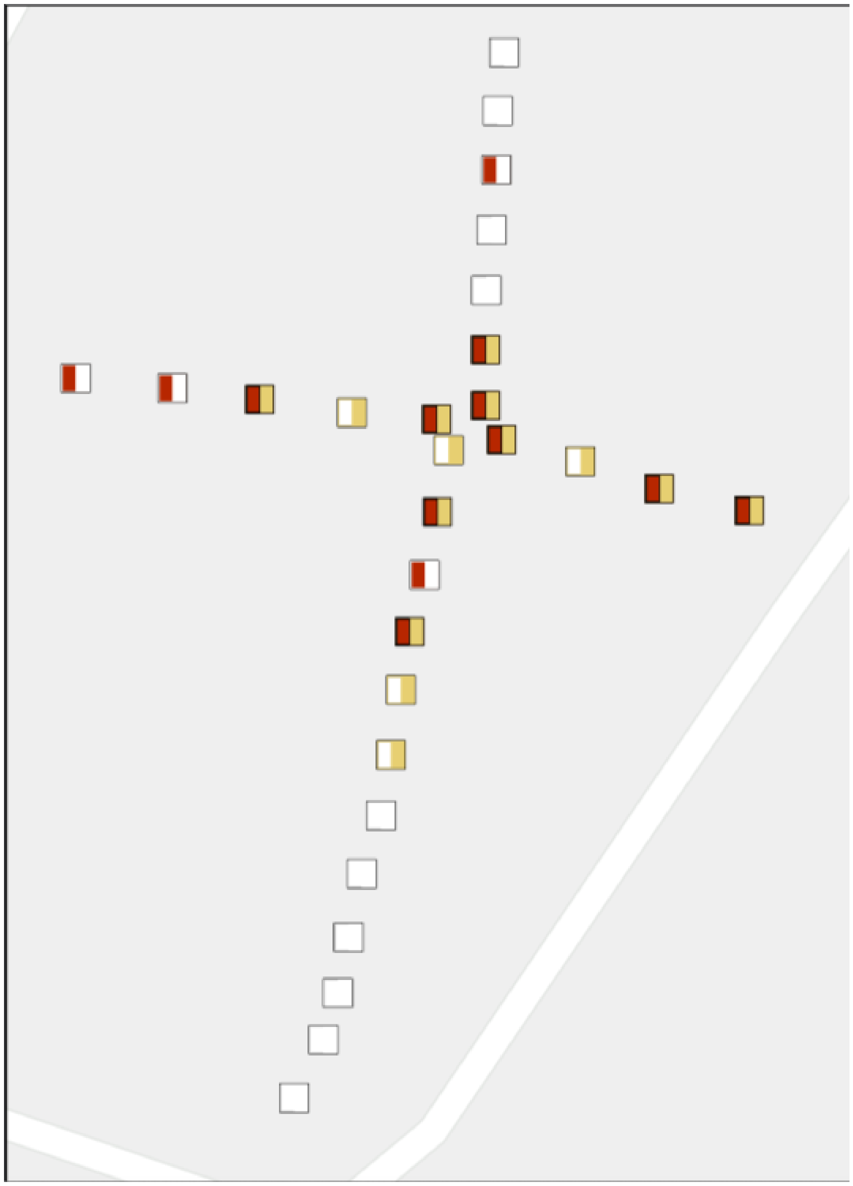

Figure 7. The detection of jumping worms at quadrats using two sampling methods (i.e., Mustard solution, Wooden disc). Quadrats are represented by square symbols, fully white symbols ( ) indicate that no jumping worms were detected at the quadrat using either method. When the right-hand half of the symbol is mustard color ( ), jumping worms were detected at the quadrat using mustard solution. When the left-hand half of the symbol is cherry-brown ( ), jumping worms were detected using wooden discs. When both sides of the symbol are colored ( ), both methods detected the presence of jumping worms at the quadrat.

5. Discussion

Results from our study did not find a significant effect of jumping worms on European earthworm abundance and biomass, or soil carbon concentrations. However, although jumping worm biomass did not yield significant impacts on soil nitrogen, jumping worm abundance was positively related to soil nitrogen levels when jumping worm population densities were low, where an increase in jumping worm abundance was related to an increase in soil nitrogen percent concentrations.. Results also hinted towards a possible negative linear or a quadratic relation between the two variables when jumping worm population densities surpassed a certain threshold. In this scenario, an increase in jumping worm abundance is followed by a decrease in nitrogen levels. The study also concluded that jumping worm detection rates did not differ between the two sampling methods (i.e., wooden disc, mustard solution).

5.1 Effects of Jumping Worms on European Earthworm Species

In this study, we hypothesized that greater abundance and biomass of jumping worm populations would lead to a decrease in European earthworm species abundance and biomass. However, jumping worm abundance and biomass had no significant effects on European earthworm abundance or biomass.

Prior research discovered that the presence of jumping worms at a site reduces European earthworms' consumption rates of leaf litter and soil microbes. These effects limit available resources, ultimately impacting the abundance and biomass of European earthworm species (Zhang et al., 2010). The absence of tree cover and thus the absence of leaf litter cover at the site suggests that earthworms present at the site would depend on soil microbes as their primary food source. This would therefore suggest that the presence of jumping worms would increase interspecific competition. However, jumping worm density at the site was possibly too low to outcompete European earthworm species and cause a reduction in their biomass and abundance. Jumping worm populations can reach densities greater than 200 individuals per square meter (Ziter et al., 2021), however, the mean jumping worm population density at our study site ranged from its highest value of 160 jumping worms per square meter to 0 jumping worms per square meter. The preliminary study conducted in 2021 suggested that jumping worms had not been present at the site for an extensive period of time (McAlpine et al., 2022), and therefore it is possible that with increased time since invasion and increased density, jumping worms will cause greater effects on European earthworm species abundance and biomass. It was also shown that the presence of jumping worms at a site for periods surpassing a year creates a shift in the species composition of soil microbial communities (Price-Christenson et al., 2020). Soil microbes are an important food source for European earthworm species (Zhang et al., 2010). As European earthworm species do not exhibit the same dietary flexibility as jumping worms, this shift in soil microbial species composition could lead to a reduction of European earthworm species (Zhang et al., 2010). Therefore, it is possible that jumping worms have not been present at this site for sufficient time to cause significant shifts in soil microbial communities, explaining why they had little impact on European earthworm abundance or biomass. The possible role of temporal scale on the degree to which jumping worms' impact interspecific earthworm interactions represents an important question to address in future research.

5.2 Effect of Jumping Worms on Soil Properties

We found no significant effects of jumping worms' abundance and biomass on soil carbon. The biomass of jumping worms also did not significantly affect soil nitrogen; however, jumping worm abundance did reveal a possible linear relationship between jumping worm abundance and soil nitrogen concentrations, where an increase in jumping worm abundance would cause an increase in soil nitrogen percent concentrations when jumping worms are present at lower population densities.

Prior studies revealed that the presence of jumping worms significantly impacts soil carbon, however there is little consensus on whether these impacts result in an increase or decrease of soil carbon (Bethke & Midgley, 2020; Greiner et al., 2012; Qiu & Turner, 2016). Therefore, we hypothesized that jumping worms would have significant impacts on soil carbon levels. However, results from our study did not align with those from previous research and the presence of jumping worms at the site yielded no significant effects on soil carbon. These contrasting results may be related to one of two reasons: (1) jumping worms are not present at the site in high densities and (2) the effects of jumping worms' species on soil carbon are dependent on habitat type.

Prior research showed that increased jumping worm density leads to effects of greater significance on soil carbon (Bethke & Midgley, 2020). Jumping worm castings and their burrowing habits increase carbon mineralisation rates ultimately leading to greater carbon loss (Chang et al., 2021). Therefore, with greater earthworm densities there are greater amounts of earthworm castings and greater disturbance from burrowing activities, possibly resulting in greater effects on soil carbon levels. Studies have determined that jumping worm populations can reach densities greater than 200 individuals per square meter (Ziter et al., 2021). However, at our sites, the average jumping worm density was 18 individuals per square meter. The recent introduction of jumping worms at the study site may explain our results as populations may not have been present at the site over sufficiently large time scales. This would imply that populations have not had sufficient time to achieve densities elevated enough to cause significant impacts on soil carbon percent concentration.

Effects of jumping worms on soil carbon properties may vary depending on habitat types, although the mechanisms responsible for this are not well understood (Bethke & Midgley, 2020). Jumping worms have been shown to exhibit different impacts on forest ecosystems compared to turfgrass ecosystems, with their invasion having lesser impacts on prairie ecosystems than on forest ecosystems (Maddi, 2019; Qiu & Turner, 2016). Studies suggest that leaf litter quality, soil moisture levels, soil bulk densities, and soil microbial life may influence the magnitude and direction in which jumping worms impact soil carbon (Bethke & Midgley, 2020; Greiner et al., 2012; Qiu & Turner, 2016). Jumping worms alter soil carbon levels through the reduction of leaf litter layers, as the consumption of leaf litter causes greater amounts of CO_2 gas to be emitted into the atmosphere (Chang et al., 2021). The low quantities of leaf litter present at the site, as well as the urban setting of this study may explain why results revealed no significant impacts of jumping worms on soil carbon. Future research should focus on the sampling of soils over varying temporal scales to evaluate the effects of jumping worm population densities on soil carbon levels as well as aim to develop a better understanding of how ecosystem types can influence these effects.

Another objective of this study was to evaluate the impacts of invasive jumping worms on soil nitrogen. Soil nitrogen plays an important role in ecosystem functioning as nitrogen availability has been shown to impact plant growth in natural environments (Marschner, 1995). Studies have revealed that soil nitrogen is an important limiting factor when looking at vegetation growth (Tamm, 1991; Vitousek & Howarth, 1991), and therefore changes in soil nitrogen concentrations may result in a loss of productivity in Canadian vegetative ecosystems (Duran et al., 2016). Results showed that jumping worm abundance had significant effects on soil nitrogen percent composition, where an increase in abundance resulted in an increase in soil nitrogen percentage. This trend occurred until a certain population density was met resulting in a decrease in soil nitrogen levels. This initially observed trend of increasing nitrogen levels being associated with increasing jumping worm abundance was similar to other studies (Greiner et al., 2012; Qiu & Turner, 2016; Bethke & Midgley, 2020). Similar to trends noted with soil carbon, it was expected that soil nitrogen would change more substantially with increasing jumping worm density (Bethke & Midgley, 2020). As was observed in our study, higher jumping worm densities could result in decreased soil nitrogen levels. However, this observation was based on data collected at a singular data point (N1). This data point was recorded from a quadrat closest to the woodpile, where the highest density of jumping worms was recorded. Therefore, it is unclear how robust this non-linear relationship is, and further data points at higher jumping abundance would be needed to ascertain whether the relationship remained. The mechanisms driving these shifting soil nitrogen dynamics are still poorly understood and are believed to be dependent on the ecosystem type and forest type (Bethke & Midgley, 2020). However, it has been suggested that once nitrogen levels reach high enough concentrations, accelerated nitrogen leaching and the downward movement of nutrients may occur (Resner et al., 2014). This may explain why higher densities of jumping worm abundance eventually led to a decrease in soil nitrogen levels. These contrasting study results as well as the lack of proper understanding of the mechanisms responsible for these changes in soil properties highlights the need for long-term studies on jumping worm impacts on soil properties.

5.3 Methods Comparison

Liquid mustard solution is a commonly used sampling method that irritates the mucus membrane of earthworms and causes them to emerge on the surface (Iannone III et al., 2012). However, previous studies have shown that the migration of jumping worms is moisture dependent, as populations shift towards areas of greater moisture during times of drought (Snyder et al., 2010). With previous jumping worm sightings suggesting their presence under wooden surfaces, it was hypothesized that jumping worm detection rates would be very similar between the liquid extraction method (i.e., mustard solution) and wooden discs. This hypothesis was supported, as our findings revealed that there is no significant difference between the jumping worm detection rate of both methods. These findings indicate that both methods can be considered when evaluating the presence of jumping worms at a site.

This finding is particularly important due to its implication for community science projects that aim to detect the presence of jumping worms at a site. Although the wooden disc method may not be used to collect density related or quantitative data, it is an effective way to detect whether or not jumping worms are present at a site. Jumping worm sampling using wooden discs meets the criteria for usefulness in community science projects, as it is simple, requires relatively low time investment, and is safe. Therefore, as our findings suggest, the use of a wooden disc sampling method offers a means through which the community can participate in the collection of jumping worm distribution data. Through this active participation, individuals can learn more about the spread of invasive species, the scientific method, and help reduce costs of jumping worm distribution research. Considering its potential use in community science, further research investigating how the size, thickness, and wood type of these discs would impact detection rates would be beneficial.

5.4 Limitations

Discrepancies between previously published results and those obtained in this study may be partially explained by limitations of our study. Varying degrees of soil compaction across the site may have influenced both European earthworm and jumping worm abundance results. The inclusion of soil compaction measurements as a covariate in the models evaluating the impacts of jumping worms on European earthworm species may have therefore helped mitigate this issue. Quadrats S7 and S8 represent two points where soil compaction was greater compared to other sampled quadrats due to higher degrees of foot traffic. While sampling this area, it was evident that the sampling method selected, liquid mustard solution, was not as effective at extracting earthworms. The elevated soil compaction prevented the mixture from penetrating soils, resulting in greater runoff and in the failure to detect the presence of earthworms. Previous landscaping work being performed near quadrats W4 and W5 may have also impacted the results. Clay-rich soils were imported near these quadrats, resulting in soil types that were visibly different from those at other quadrats, and soils of greater compaction. As was seen at quadrats S7 and S8, this compaction inhibited the mustard solution's ability to effectively extract earthworms from the soil. This may explain why lower densities of earthworms were found at these quadrats. The small sample size (n=28) may have also limited the power of the study. However, this was the maximal sample size possible due to the presence of multiple disturbances at the study site (e.g., property boundaries, roads) and the recent nature of this invasion.

6. Conclusion

The study's results suggest that the presence of jumping worms in Oromocto, NB have yet to yield significant impacts on their ecosystem with the exception of their effects on soil nitrogen percent composition. Results from prior studies suggest that low population densities characteristic of recent invasion dynamics as well as the urban setting of this study may be behind these inconclusive results (Bethke & Midgley, 2020; Maddi, 2019). Therefore, these findings may support claims that jumping worm density plays an important role in determining the significance of their impacts on their environment (Bethke & Midgley, 2020). This highlights the need to continue studying these effects at the site. The data collected in this project offer future research the opportunity to evaluate jumping worm spread rates, as well as compare soil characteristics prior to and post jumping worm invasion. These findings could aid in distinguishing differences in impacts resulting from the presence of jumping worms and European earthworms at co-invaded sites. Therefore, this research lays an important foundation for future research projects evaluating the impacts and spread of jumping worms in Atlantic Canada. Once the implications of jumping worm invasion on urban Canadian soil ecosystems are better understood, improved management strategies may be developed to help mitigate potential damages. A lack of conclusive published literature regarding this topic reinforces the need for future research on the invasion of jumping worms in Canada. Although the invasion of jumping worms in New Brunswick was detected early in their invasion process, it will be vital to develop greater understanding of their impacts on these ecosystems, and potentially develop strategies limiting their spread into neighboring environments. If we wish to mitigate damages and achieve soil conservation and management goals, it will be crucial to further explore the ecosystem impacts and invasion dynamics of jumping worms in Canada.

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