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Introduced earthworms (Lumbricidae) in restored and remnant tallgrass prairies of southern Ontario

Heather A. Cray[1,2], **Justin M. Gaudon**[1,3,*], and **Stephen D. Murphy**[1]

[1]School of Environment, Resources and Sustainability, University of Waterloo, 200 University Avenue West, Waterloo, Ontario N2L 3G1 Canada

[2]School for Resource and Environmental Studies, Dalhousie University, 6100 University Avenue, Halifax, Nova Scotia B3H 4R2 Canada

[3]*rare* Charitable Research Reserve, 1679 Blair Road, Cambridge, Ontario N3H 4R8 Canada

[*]Corresponding author: justin.gaudon@uwaterloo.ca

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Abstract

Introduced earthworms alter the trajectory and composition of plant communities, for example, through their feeding, burrowing behaviour, and interactions with seeds. High densities of several earthworm species may decrease native biodiversity and disrupt restoration efforts in tallgrass prairies. This affects efforts to conserve and restore such habitat, which is of high conservation and restoration priority in eastern North America and typically restored through seeding events. To date, *Lumbricus terrestris* (Lumbricidae) and other species have remained largely undocumented in tallgrass prairies. We surveyed 22 tallgrass prairie sites in southern Ontario, Canada, to document earthworm density and species. *Lumbricus terrestris* was found at all sites. The average density was 66 ± 91 (SD) earthworms/m^2 across our sampling plots, mostly juveniles (~94%). The number of all earthworms per plot significantly increased with the number of earthworm middens in each plot ($\chi^2_1 = 4.50$, $P = 0.034$). Prairies with a large number of middens had high earthworm density, but middens alone appear to explain little variation in our data (linear mixed-effects model, marginal $R^2 = 0.12$) meaning there are other biologically important factors that affect their density. However, we found no effects of soil pH, organic matter content, or texture on the number earthworms per plot suggesting that earthworms can invade a range of tallgrass prairie soils with pH values between 5.27 and 7.67.

Key words: Earthworm invasion; invasive species; *Lumbricus terrestris*; restoration ecology; tallgrass prairie restoration

Introduction

Agriculture, urban development, and woody encroachment have reduced the tallgrass prairie ecosystem in North America to less than 1% of its historical area (Bakowsky and Riley 1994; Samson and Knopf 1994). In southern Ontario, Canada, tallgrass prairie likely once covered 800–2000 km^2, but now typically exists as small, isolated parcels (Bakowsky and Riley 1994; Rodger 1998). These parcels are composed of plants that are unique to the tallgrass prairie ecosystem and provide rare habitat for native biodiversity (Morgan *et al*. 1995). Active restoration of tallgrass prairie is ongoing, often on former croplands, with the aim of re-establishing native vegetation communities through seeding (Kindscher and Tieszen 1998). Restoration sites vary in size and connectivity, but most are <0.03 km^2 and isolated (Bakowsky and Riley 1994). The success of tallgrass prairie restoration efforts has been mixed, as restoring historical, highly diverse vegetation communities may take a long time (Kindscher and Tieszen 1998). The richness of native plant species in restored tallgrass prairie is usually lower than in remnant parcels and often declines over time, whereas the richness of exotic plants is higher and increases with time (Leach and Givnish 1996; Sluis 2002; Camill *et al*. 2004; Martin *et al*. 2005; McLachlan and Knispel 2005).

Earthworms (Oligochaeta: Lumbricidae) are influential soil macro-organisms. As a result of their high consumption rates, burrowing activity, and large body sizes, they alter fundamental ecosystem processes, such as nutrient cycling, water infiltration, rates of decomposition, and seedbank conditions; this affects the availability of resources for other soil biota and influences vegetative communities (Brown 1995; Edwards and Bohlen 1996; Forey *et al.* 2011). In the context of tallgrass prairie restoration, which is typically initiated by a single seeding event, the impact of introduced earthworms on seed dispersal and consumption may be exacerbated. Earthworms are

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increasingly recognized as important and under-studied seed predators (Eisenhauer *et al*. 2010; Forey *et al*. 2011; Drouin *et al*. 2014) that affect the dispersal, survival, and establishment of seeds through selection pressure (Forey *et al*. 2011; Clause *et al*. 2016). This pressure includes selective ingestion as well as digestion and egestion (Shumway and Koide 1994; Eisenhauer *et al*. 2009; Clause *et al*. 2016; McTavish and Murphy 2019), accelerated or inhibited germination (Decaëns *et al*. 2003; Clause *et al.* 2011), and transport of seeds (McRill and Sagar 1973; Thompson *et al.* 1994). Thus, earthworms have direct effects on the composition and function of plant communities, but these vary by ecosystem, and species–specific interactions are common (Shumway and Koide 1994; Eisenhauer *et al.* 2009; Clause *et al.* 2016; Craven *et al.* 2016). These effects compound other post-dispersal challenges to seed establishment, e.g., granivory by birds, rodents, and insects; competition with ruderal weeds; and water availability (Moles and Westoby 2006; Eisenhauer and Scheu 2008; Forey *et al.* 2011). Thus, it is critical to understand the distribution and density of earthworms to effectively manage and restore invaded ecosystems.

Although earthworms did not survive the Wisconsin glaciation that receded approximately 11 000 years ago in Canada and the northern United States (Gates 1982; Reynolds 1994; Edwards and Bohlen 1996), 21 species have been recorded in Ontario. Of these, 19 are introductions from Europe and Asia, while the other two suspected native species, *Bimastos parvus* Eisen and *Sparganophilus tamesis* Benham, are provincially rare and known exclusively from arboreta and aquatic or semi-aquatic mud, respectively (Reynolds 2014). Introduced earthworms can expand their range naturally by only 5–10 m/year (Marinissen and van den Bosch 1992); consistent with their origin, most introduced earthworms expand their range because humans move them (soil and bait movement; Callaham *et al.* 2006; Hale 2007). Despite human dispersal (Edwards and Bohlen 1996), the distribution of earthworms is limited by soil pH, texture, and moisture as well as food availability (i.e., leaf litter, vegetation, and consolidated organic matter) and temperature (Guild 1952; Murchie 1958).

Previous work on Ontario earthworms has focussed on compiling individual observations to create a province-wide map of distribution by species (Reynolds 1977, 2011a,b; Reynolds and Reynolds 1992) and earthworm-driven changes in forest ecosystems (Cassin and Kotanen 2016; Jennings and Watmough 2016; Choi *et al.* 2017). Although the negative effects of industrial tillage practices on earthworm populations in agricultural fields are well established (Clapperton *et al.* 1997; VandenBygaart *et al.* 1999; Simonsen *et al.* 2010; Briones and Schmidt 2017), there is neither an estimate of the average biomass of earthworms in Ontario soils nor a comprehensive survey of earthworm species, densities, and biomass. Such surveys can be difficult if earthworms in samples are mostly juveniles (e.g., as in surveys of deadwood in forests; Ashwood *et al.* 2019), which are difficult to identify to species.

Although the establishment and spread of non-native earthworm species in North America has been occurring for centuries, we are only beginning to understand their current distribution (Phillips *et al.* 2019). *Lumbricus terrestris* (Lumbricidae) appears to be widely distributed (Addison 2009), perhaps because it is commonly used as fishing bait (Keller *et al.* 2007). Research conducted in the midwestern United States (e.g., Callaham *et al.* 2001, 2003; Loss *et al.* 2017) can be relevant to Ontario because the two areas are part of the current northern range limit of tallgrass prairie. However, research is still needed in Ontario because Canada and the northern United States had few widespread native earthworm communities following glaciation (Reynolds 2014), the northern tallgrass prairie plant community of Ontario forms a distinct subtype (Rodger 1998), and Ontario tallgrass prairie conservation remnants and restorations occur on a small scale (e.g., <1 ha; Bakowsky and Riley 1994).

Introduced earthworms have severely impacted North American ecosystems and tallgrass prairies in southern Ontario may experience similar effects of earthworm invasion, specifically changes in plant composition and desired trajectory in restored sites. The objectives of this study were (1) to determine the densities of earthworms in tallgrass prairies of southern Ontario, (2) to document the species of earthworms found in tallgrass prairies, and (3) to summarize the relationship between earthworm numbers and soil properties to provide some direction on where and how to focus tallgrass prairie restoration efforts.

Methods

For sampling earthworm populations, we selected 22 tallgrass prairie sites, including five remnant, two restored-remnant, and 15 restored sites in southern Ontario, Canada (Table 1, Figure 1). Restored-remnant sites describe prairie that has re-established unexpectedly from the seedbank following accidental fire or large-scale brush cutting. To represent the diversity of tallgrass prairie sampling sites across southern Ontario, we selected sites that varied in geographic range, management history, restoration age, adjacent land use, parcel size, and soil characteristics. Study site vegetation communities included ruderal weeds,

TABLE 1. Site characteristics and management history of restored and remnant tallgrass prairies sampled for earthworms in southern Ontario, Canada.

Site no.	Location*	Area, ha	Status	Adjacent land use†	Year restoration started	Method of restoration	Most recent burn	Site management practice		
								Herbicide	Removal of woody plants	Grazed
1	Windsor	17.5	Remnant	P, H	—	—	2010	—	—	—
2	Cambridge	1.2	Remnant	P, H, A	—	—	2010	—	Yes	—
3	Windsor	1.3	Remnant	I, P, H	—	—	2012	Yes	Yes	—
4	Windsor	1.9	Remnant	I, P, H	—	—	2014	Yes	Yes	—
5	East Gwillimbury	3.5	Remnant	P, H	—	—	—	—	Yes	—
6	Brantford	3.3	Restored-remnant	P, H	2006	Seeded	2015	—	Yes	—
7	East Gwillimbury	0.6	Restored-remnant	P, H	2015	—	—	Yes	—	—
8	Oakville	3.3	Restored	P, A, H	2015	Seeded	—	Yes	Yes	—
9	Windsor	1.6	Restored	I, H, A, E	2013	Seeded	—	—	—	—
10	Windsor	0.3	Restored	I, H	2013	Planted	—	Yes	—	—
11	Windsor	2.1	Restored	I, H	2013	Seeded + planted	—	Yes	—	—
12	Cambridge	1.2	Restored	P, E, H, A	2006	Seeded + planted	2010	Yes	Yes	—
13	Chatham-Kent	21.5	Restored	A, I	2010	Seeded	—	Yes	Yes	—
14	Middlesex County	2.0	Restored	A, P	2011	Seeded	—	Yes	Yes	Yes
15	Norfolk County	36.0	Restored	P, A	2013	Seeded	—	Yes	Yes	—
16	Norfolk County	14.5	Restored	P, A	2012	Seeded	—	Yes	Yes	—
17	Norfolk County	14.0	Restored	P, A	2011	Seeded	—	Yes	Yes	—
18	Oakville	6.1	Restored	P, I, H	2012	Seeded	—	Yes	—	—
19	Oakville	6.0	Restored	P, I, H	2013	Seeded	—	Yes	—	—
20	Oakville	6.3	Restored	P, I, H	2014	Seeded	—	Yes	—	—
21	Cambridge	16.0	Restored	P, H	2010	Seeded	2015	—	—	—
22	North Dumfries	23.5	Restored	P, A	2011	Seeded	2015	—	Yes	—

*Specific latitude and longitude of sample sites are not provided because of data sensitivity and research permit requirements.
†H = suburban housing, P = protected area, E = resource extraction, A = agriculture, I = major infrastructure.

invasive plant species, and expected southern Ontario tallgrass prairie plants including grasses (Poaceae), such as Big Bluestem (*Andropogon gerardi* Vitman), Yellow Indiangrass (*Sorghastrum nutans* (L.) Nash), Switchgrass (*Panicum virgatum* L.), Little Bluestem (*Schizachyrium scoparium* (Michaux) Nash), and Canada Wildrye (*Elymus canadensis* L.) as well as forbs, such as Wild Bergamot (*Monarda fistulosa* L.; Lamiaceae), Virginia Mountain-mint (*Pycnanthemum virginianum* (L.) B.L. Robinson & Fernald; Lamiaceae), Black-eyed Susan (*Rudbeckia hirta* L; Asteraceae), Grey-headed Prairie Coneflower (*Ratibida pinnata* (Ventenat) Barnhart; Asteraceae), *Asclepias* spp. L. (Apocynaceae), Beardtongue (*Penstemon* spp. Schmidel; Plantaginaceae), Round-headed Bush-clover (*Lespedeza capitata* Michaux; Fabaceae), Dense Blazing-start (*Liatris spicata* (L.) Willdenow; Asteraceae), *Symphyotrichum* spp. Nees (Asteraceae), *Solidago* spp. L. (Asteraceae), and *Desmodium* spp. Desvaux (Fabaceae).

Fieldwork was conducted 10–25 October in 2015 and 3–30 October in 2016. Five plots per site in 2015 and ten plots per site in 2016 were pre-assigned using satellite imagery to distribute sampling plots evenly across the entire prairie area and not within 10 m of any edge. Because of a severe flooding event that led to standing water on the sampling area at six sites (three restored, three remnant) in 2016, the data presented for these sites are from 2015 only. Field sampling was conducted during the day when soil temperatures were above 10°C and no rain had fallen in the previous 24 h.

At each site, earthworms were collected from one 20 × 20 cm plot using a mustard liquid extraction technique (Lawrence and Bowers 2002). Plot boundaries were marked with a plastic frame. At plots

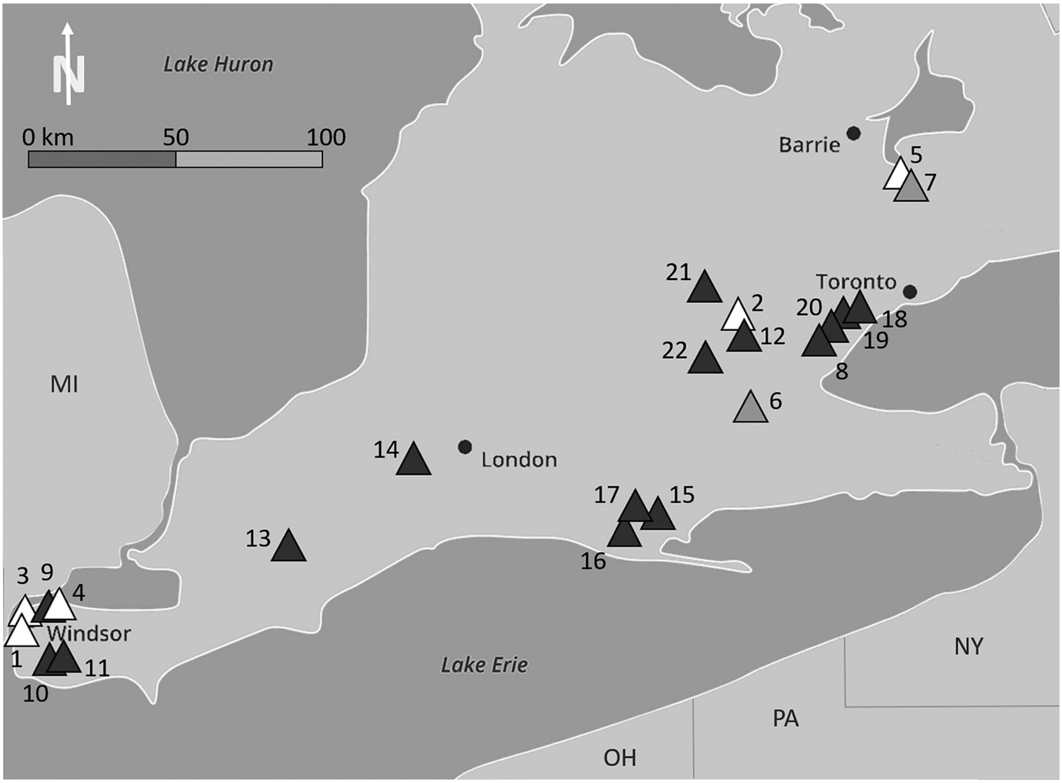

FIGURE 1. Earthworm sampling locations in restored (▲), remnant (△), and restored-remnant (▲) tallgrass prairies in southern Ontario, Canada.

with litter cover, the surface litter was first removed and searched for earthworms. Then, 2 L of mustard solution (10 g of hot mustard powder [Weston Inc., Bulk Barn, Aurora, Ontario, Canada] per litre of distilled water) was applied to the plot over 10 min, and emerging earthworms were collected for the following 15 min. As we were unable to reliably identify juveniles (i.e., sub-adult but ≥2 cm long) to species level based on physical traits alone, body length and counts were used to characterize the earthworm populations. Each earthworm was allowed to become active in a collection container before its length was measured. We assumed that annelids <2 cm long and white were not earthworms but rather Enchytraeidae (i.e., microdriles, Oligochaeta: Annelida) and, thus, they were not counted.

Adult earthworms were identified by the presence of the clitellum. At each sampling site, a voucher specimen of any adult earthworm that could not be identified in the field was collected and immediately placed in a 75% isopropyl alcohol solution to obtain minimum species counts (i.e., the number of identifiable species) for each site. After being identified using physical attributes (Hale 2007), adult specimens were donated to The Barcode of Life project at the University of Guelph and are curated at that institution. The adult earthworms identified were used to create a minimum species list, which represents the lowest number of species that have been verified to occur at our sampling sites.

Lumbricus terrestris creates a permanent or semi-permanent vertical burrow system that may extend several metres into the soil profile and is likely to be under-sampled using extraction methods appropriate for most other earthworm species (Hamilton and Sillman 1989; Edwards and Bohlen 1996). To achieve a representative sampling of this species, we counted the number of middens that were contained wholly or in part within each 20 × 20 cm sampling plot. Middens are unique to this species in southern Ontario and occur as distinctive piles of cast, organic, and inorganic materials that an individual *L. terrestris* creates around the opening to its vertical burrow (Butt and Grigoropoulou 2010; Stroud *et al.* 2016).

To quantify soil characteristics at each site, three soil samples were collected within 20 cm of each sampling plot using a 3-cm diameter soil corer to a depth of 20 cm after the application of 2 L of mustard solution. Soil samples were stored in a sample bag (Whirl-Pak, Madison, Wisconsin, USA) and frozen until processing. Soil cores from each plot were homogenized and subsampled for analysis of pH, organic matter content, and texture following protocols by McKeague (1978).

Data were analyzed using R version 4.0.3 (R Core Development Team 2020). We tested the effects of soil pH, soil texture, soil organic matter content, and midden area on the number of all earthworms per plot (i.e., density, including juveniles) using a linear mixed-effects model (LMM). In the LMM, soil pH, soil texture, soil organic matter content, and midden area were fixed effects, and site was used as a random effect to account for the repeated measures within each tallgrass prairie site. Model fit was determined by assessing constancy of variance and normality of residuals using graphical methods. This model did not meet our assumptions of constancy of variance and normal residuals, so we $\log(x + 1)$ transformed the number of all earthworms (including juveniles) per plot, which accounted for heteroscedastic and non-normal residuals. Marginal and conditional R^2 values were calculated using the *r.squaredGLMM* function in the "MuMIn" package (Bartoń 2020). We used the *ggpredict* function in the "ggeffects" package (Lüdecke 2018) to compute marginal effects of the number of middens per plot on the number of all earthworms per plot. Data were then back-transformed for graphical representation and graphed using the package "ggplot2" (Wickham 2016). All means are presented with ± 1 SD.

Results

Soil properties varied across the sampling plots: soil pH 5.27–7.67, mean 6.27 ± 0.68. Soil textures across our study sites ranged from sand to silty clay. Organic matter content was 1.7–4.3% and averaged 3.0 ± 1.0% across our sampling plots. The percentage of sand, clay, or silt had no significant effect on the number of all earthworms (including juveniles) per plot in tallgrass prairie soils (sand: $\chi^2_1 = 1.96$, $P = 0.161$; clay: $\chi^2_1 = 1.95$, $P = 0.163$; silt: $\chi^2_1 = 1.96$, $P = 0.161$). There were also no significant effects of soil pH ($\chi^2_1 = 0.12$, $P = 0.728$) or soil organic matter content on the number of all earthworms per plot ($\chi^2_1 = 2.49$, $P = 0.115$).

Earthworms were found at every tallgrass prairie site in this study. Species included *Allolobophora chlorotica* (process ID: HCOEW026-17, sample ID: BIOUG32056-C02), *Aporrectodea longa* (process ID: HCOEW012-17, sample ID: BIOUG32056-A12), *Aporrectodea rosea* (process ID: HCOEW005-17, sample ID: BIOUG32056-A05), *Aporrectodea tuberculata* (process ID: HCOEW009-17, sample ID: BIOUG32056-A09), *Dendrobaena octaedra* (process ID: HCOEW015-17, sample ID: BIOUG32056-B03), *Lumbricus rubellus* (process ID: HCOEW001-17, sample ID: BIOUG32056-A01), *L. terrestris* (process ID: HCOEW029-17, sample ID: BIOUG32056-C05), *Octolasion tyrtaeum* (process ID: HCOEW003-17, sample ID: BIOUG32056-A03), and the *Aporrectodea caliginosa* species complex (process ID: HCOEW019-17, sample ID: BIOUG32056-B07). DNA barcoding analysis could not distinguish between several species in the *A. caliginosa* species complex, so we list this species here. We consider *A. longa* and *A. tuberculata*, as well as *Aporrectodea turgida*, to be part of the *A. caliginosa* species complex. *Lumbricus terrestris* was the only species observed at every site. We report the first record of *D. octaedra* in Waterloo Region, Ontario, Canada, and *L. rubellus* in Halton Region, Ontario, Canada. Voucher specimens were deposited at the Biodiversity Institute of Ontario, University of Guelph. No native earthworms were identified in this study. Earthworm species richness (based on adults) at each site varied between one and five species per site. On average, we found 3 ± 1 earthworm species in each tallgrass prairie.

The total number of all earthworms per site (including juveniles) varied between five and 108 (Table 2), with a mean count of 37 ± 29 earthworms across all sites. The earthworm density across our tallgrass prairie sites was 8–346 earthworms/m^2 (average 66 ± 91 earthworms/m^2). Most of the earthworms found were juveniles (94.0 ± 6.5%). The highest percentage of adult earthworms (17%) was found in site 11, a restored tallgrass prairie with clay loam soil adjacent to suburban housing and other major infrastructure (Table 1). At eight sites, no adult earthworms were collected (Table 2).

The distribution of earthworm size classes varied considerably among sampling sites. Earthworms 5.0–9.9 cm were the most abundant overall (39.3 ± 15.7%, absent from four sites), followed by 1.0–4.9 cm (32.5 ± 25.0%, absent from one site), 10.0–15.0 cm (23.4 ± 24.6%, absent from three sites), and >15 cm (4.9 ± 6.3%, absent from 10 sites; Figure 2).

The number of middens per plot varied from zero to 10, with an average of 3 ± 2 middens per plot. The number of all earthworms per plot (including juveniles) significantly increased with the number of middens ($\chi^2_1 = 4.50$, $P = 0.034$; Figure 3). However, fixed effects in the LMM, such as the number of middens per plot, explained little variation in our data (marginal $R^2 = 0.12$). Most of the variance was explained by the full model (i.e., both fixed and random effects; conditional $R^2 = 0.63$).

Table 2. Number of earthworms and species distribution in tallgrass prairies sampled in southern Ontario, Canada.

Site no. (plots)	No. earthworms*		% adults	Species present†								
	Total	Mean ± SD		*Allobophora chlorotica*	*Allobophora* sp.	*Aporrectodea longa*	*Aporrectodea rosea*	*Aporrectodea tuberculata*	*Dendrobaena octaedra*	*Lumbricus rubellus*	*Lumbricus terrestris*	*Octolasion tyrtaeum*
1 (10)	35	4 ± 3	2	—	—	—	—	—	—	P	P	—
2 (10)	28	3 ± 3	5	—	—	—	—	P	P	—	P	—
3 (5)	31	6 ± 2	15	—	—	—	—	—	—	—	P	—
4 (5)	35	7 ± 5	4	—	—	—	—	—	—	—	P	—
5 (10)	9	1 ± 1	0	—	—	—	—	—	—	—	P	—
6 (10)	5	1 ± 1	0	—	P	—	—	—	—	—	P	—
7 (10)	18	2 ± 1	4	—	—	—	—	—	—	—	P	—
8 (10)	9	1 ± 1	0	—	—	—	—	—	—	—	P	—
9 (5)	37	7 ± 6	16	—	—	P	—	—	—	—	P	—
10 (5)	70	14 ± 8	0	—	—	—	—	—	—	—	P	—
11 (5)	108	22 ± 18	17	—	P	—	—	—	—	—	P	—
12 (10)	44	4 ± 3	11	—	—	—	P	P	—	P	P	P
13 (10)	5	1 ± 1	0	—	—	—	—	P	—	—	P	—
14 (10)	7	1 ± 1	0	—	—	—	—	—	—	—	P	—
15 (10)	79	8 ± 5	13	—	P	—	—	—	—	P	P	—
16 (10)	49	5 ± 4	3	—	—	—	—	P	—	—	P	—
17 (10)	7	1 ± 2	0	—	—	—	—	—	—	—	P	—
18 (10)	45	5 ± 3	9	P	—	—	—	P	—	P	P	—
19 (10)	68	7 ± 4	16	P	—	—	—	P	—	P	P	—
20 (10)	81	8 ± 3	15	P	—	—	—	P	—	P	P	—
21 (10)	32	3 ± 1	8	P	—	—	—	—	—	P	P	P
22 (10)	6	1 ± 1	0	—	P	—	—	—	—	P	P	—

*Includes juveniles ≥2 cm long.
†Only presence (P) or not detected (—) is available at the species level.

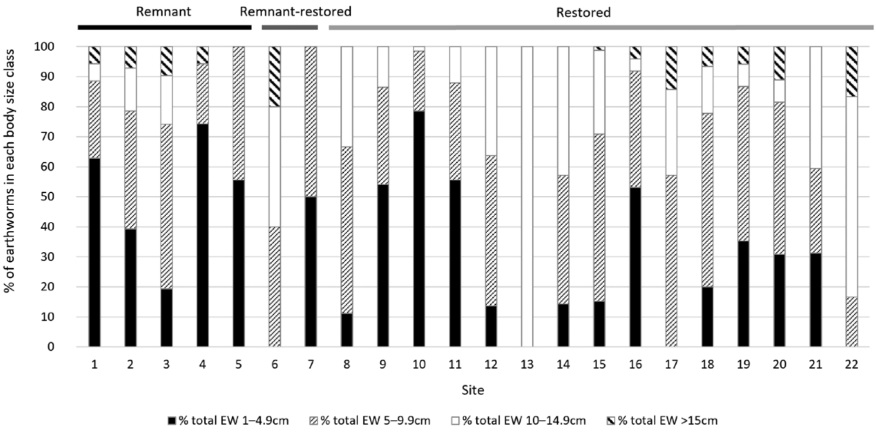

FIGURE 2. Percentage of earthworms (EW) in each body size class in tallgrass prairies sampled across southern Ontario, Canada.

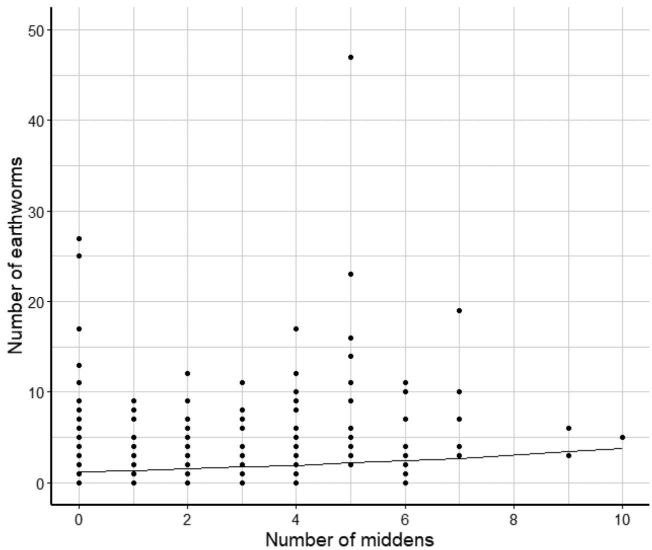

FIGURE 3. Relationship between the number of all earthworms (including juveniles ≥2 cm long) per plot and number of middens per plot in tallgrass prairies in southern Ontario. The solid line shows the predicted values computed using the R function *ggpredict*.

Discussion

Introduced earthworms were found in all the tallgrass prairie sites that we examined in southern Ontario, Canada. The earthworms we found likely underrepresent the number of endogeic and anecic species in particular because of the vertical stratification of earthworm communities, their phenology, and our choice of sampling method (Edwards and Bohlen 1996). Because we found earthworms at all sites and in all plots at an average density of 66 ± 91 earthworms/m^2, we suspect that earthworms are now important macrofauna in southern Ontario tallgrass prairie soils compared to before their introduction (Forey *et al.* 2011).

Comparisons among studies of earthworm populations are complicated by variations in timing, method of collection, and their uneven distribution. Hand sorting is usually considered superior to other methods for quantifying earthworm populations; in comparison, the mustard extraction method will tend to underestimate numbers (Pelosi *et al.* 2009). The main argument against hand extraction is that it necessitates digging up, breaking apart, and sieving an entire column of soil for each sampling plot (Nordström and Rundgren 1972). This has consequences for the sampling plot, including homogenization of the soil profile and disturbance of plant root networks, fungal hyphae, and soil-dwelling organisms. In contrast, mustard extraction is a low-disturbance method particularly suitable for use in sensitive, conservation-focussed habitats. Although we anticipated low earthworm densities as a result of using the mustard solution extraction method, we found densities similar to those documented in other ecosystems (Shakir and Dindal 1997; Price and Gordon 1998; Bohlen *et al.* 2004).

It was not surprising to find that the number of middens was related to the number of all earthworms per plot (including juveniles). Although the number of earthworms was poorly correlated with the number of middens in our tallgrass prairie plots (i.e., little variation in our data was explained), middens may be centres of activity for other earthworm species, meaning that the invasion of *L. terrestris* may facilitate introductions of other species (Butt and Lowe 2007). Therefore, plots with more middens could be expected to contain higher numbers of earthworms, including species other than *L. terrestris*. Middens may provide some indication of earthworm density in tallgrass prairies as observed in forests ecosystems (Loss *et al.* 2013); thus, assessing midden prevalence may be a cost-effective and low-impact approach to determining whether an alternative planting method is needed for tallgrass prairie restoration (e.g., plugging in addition to seeding).

If there was temporal bias from sampling only in October, we would have expected to see earthworms that were similar in size and of the same species. The high percentage of juvenile earthworms of varying size recorded in this study suggests that the populations we sampled are persistent and successfully reproducing. Whereas some species can only breed sexually (e.g., earthworms in the genera *Lumbricus*), many others can reproduce parthenogenetically (e.g., *Octolasion* and *Dendrobaena* spp.; Edwards and Bohlen 1996). With this reproductive flexibility, we suspect that the earthworm populations we found are either resilient to stochastic disturbances, such as the prolonged flooding or fire events that have occurred in our tallgrass prairie sites (e.g., by escaping flooded or burned areas), and/or have recolonized from nearby areas post-disturbance. If this is the case, earthworms now represent a persistent and dominant soil fauna in tallgrass prairies in southern Ontario, which complicates our capacity to manage and restore these ecosystems, especially because of earthworms' potential to damage seeds.

We found that *L. terrestris* density was similar across a range of tallgrass prairie sites. Although we specifically analyzed site history (i.e., remnant versus restored tallgrass prairie), that does not appear to influence susceptibility to invasion based on our data. As such, restoration efforts in all sites may require high-density broadcasting of seeds to account for the relatively high density of earthworms that will ingest and transport seeds. Because we did not observe effects of soil texture or soil pH on earthworm density, our work suggests that ecosystems previously considered resistant to earthworm invasion (e.g., sandy and acidic soils; Frelich *et al.* 2006) should be monitored for earthworm introductions, and proactive planning may be a necessary component of restoration efforts in ecosystem management plans.

New research using nested polymerase chain reaction to improve detection of earthworm DNA is promising for early detection and rapid response to introduced earthworms, but has yet to be widely implemented (Jackson *et al.* 2017). If viable, this approach would be effective in generating a comprehensive survey of earthworm distribution and anticipating future earthworm spread. This is particularly important in the context of tallgrass prairie restoration because plant community trajectory and composition are affected by earthworm species-specific interactions with seeds, including ingestion and digestion, accelerated or inhibited germination, and seed transport through the soil profile (McRill and Sagar 1973; Shumway and Koide 1994; Thompson *et al.* 1994; Decaëns *et al.* 2003; Eisenhauer *et al.* 2009; Clause *et al.* 2011, 2016).

Author Contributions

Conceptualization and Design: H.A.C. and S.D.M.; Investigation: H.A.C.; Formal Analysis: H.A.C. and J.M.G.; Writing: H.A.C., J.M.G., and S.D.M.

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