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Exotic earthworm (Oligochaeta: Lumbricidae) assemblages on a landscape-scale in central Canadian woodlands: Importance of region and vegetation type

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Abstract: A growing understanding about the impacts of earthworms (Oligochaeta: Lumbricidae) on ecosystem processes and forest restoration necessitates an examination of their role in Canadian forests where they have become invasive. Little is known about the landscape scale responses of earthworm populations to different regional characteristics and vegetation types within Canada's central woodlands. We examined the regional variation of earthworm species richness, biomass, and assemblage composition across a range of four municipal regions (from south to north: Halton, Wellington, York, and Simcoe) and four habitat types (deciduous forest, mixed forest, tree plantation, and meadow) with varying soil characteristics in woodlands of south-central Ontario, Canada. In general, earthworm communities differed by region but not by habitat type. The most southern regions supported the highest earthworm species richness, biomass (*i.e. Lumbricus* and *Octolasion*), and density, and this was associated with a south-north gradient in soil characteristics. Assemblage composition differed by region but not by habitat type. The observed south-north gradient suggests an underlying effect of invasion spread associated with human settlement and density. Our results provide baseline information about earthworm communities in south-central Ontario forests, and will enable managers to plan for the increasing role of earthworms in Canada's future forests.

Key words: earthworm communities, functional groups, invasive species, land use, *Lumbricidae*

42 Introduction

43 The southern extent of the last Wisconsinian glaciation eliminated most native
44 earthworm species from Canada and the northern United States (Callaham et al. 2006, Addison
45 2009). Exotic earthworm species (Oligochaeta: Lumbricidae) were introduced during the late
46 1800s, presumably through the release of contaminated soil in ship ballasts from Europe
47 (Reynolds 1977; Tiunov et al. 2006); they are now widely distributed in select locations across
48 much of North America (Gates 1982; Reynolds 1994), where they re-engineer soil and site
49 characteristics. Because soils, vegetation, and ecosystem processes in Canadian temperate forests
50 have developed in the absence of earthworms following recent glacial recession, such invasions
51 cause major shifts in ecosystem functioning and services, impacting forest floor structure, soil
52 biogeochemistry, and faunal and plant community composition (e.g., Alban and Berry 1994;
53 Hale et al. 2005; Bohlen et al. 2004; Migge-Kleian et al. 2006; Holdworth et al. 2007a and b;
54 Costello and Lamberti 2009; Szlavecz et al. 2011; Crumsey et al. 2013; Sackett et al. 2013,
55 Craven et al. in press).

56 It is now well documented that earthworm invasion results in significant changes to
57 nutrient dynamics and soil structure in temperate forests (e.g., Hale et al. 2005; Sackett et al.
58 2013). For example, the feeding habits of *Lumbricus terrestris* have been shown to increase the
59 immobilization of nutrients leading to nitrification and leaching and an overall decrease in
60 nutrient availability (Hale et al. 2005). Earthworms may also shift the soil decomposer
61 community from one dominated by fungi and their interactions to one dominated by bacteria or
62 fungi antagonistic to decomposing fungi, again changing the rate of nutrient cycling and
63 decomposition (Bohlen et al. 2004; Jayasinghe and Parkinson 2009; Ewing et al. 2015).

Changes caused by the invasion of earthworms are also known to result in a loss of carbon (C) storage due to increased respiration and microbial activity; however, in the long term, earthworms may stabilize soil carbon by incorporating greater amounts of the litter layer into their casts and stable aggregates (Bohlen et al. 2004).

The pattern of invasion and impact of earthworms is much dependent on land use history, soil type, and functional traits of the invading species, amongst other factors (Frelich et al. 2006). Whether a species is classified as epigeic, endogeic or anecic will determine the potential change in soil characteristics. In general, as species richness increases, the thickness of the litter and duff layer of the soil decreases (Gundale et al. 2005). McLean and Parkinson (1997) found that epigeic species had effects on organic matter distribution and soil structure. Variation in the effects of these functional groups are expected due to their differential impact on the litter 'LFH' layers, where 'L' is the top organic horizon composed of accumulating leaves and twigs and still easily recognized, 'F' is the layer of partially decomposed organic matter with a sometimes matt-like appearance due to fungal hyphae and decaying litter, and 'H' is the lowest organic horizon in which the original organic structures cannot be recognized and have been converted to humic material (Denholm et al. 1993; Soil Classification Working Group 1998). Epigeic species mix and consume the 'FH' layers, while the upper litter layer (L) remains intact (McLean and Parkinson 1997; Hale et al. 2005). Endogeic and anecic species move deeper into the soil layers, mixing the upper 25-30 cm of the mineral soil horizons (B or C) and converting the undisturbed LFH and thin A horizon characteristic of native soils to a mull soil more similar to previously-tilled agricultural soil (Frelich et al. 2006), and in turn impacting water infiltration (Capowiez et al. 2014).

Understanding the biogeographic patterns of invasive species as they colonize new regions will help predict patterns of spread and the relative vulnerability of particular regions and ecosystems. At the landscape scale, the distribution of earthworms appears to be dependent on habitat suitability (pH and litter quality), climatic conditions (temperature and moisture), human activity, and land use practices (Tiunov et al. 2006). Among habitat types, earthworm abundance and species richness are generally the highest in deciduous forests and the lowest in old meadows and coniferous forests (Smith et al. 2008). In some cases, the dominant tree species may be important; for example, earthworms occurred less frequently in forests dominated by American beech (*Fagus grandifolia*) and hemlock (*Tsuga canadensis*) than in other hardwoods (Suárez et al. 2006b). In areas where forests are transitioning from southern deciduous species to northern coniferous species, earthworm invasion may progress more slowly due to either combined or individual effects of a decrease in litter quality (changes in tree species composition), decrease in soil pH, or decrease in temperature along a latitudinal gradient (Addison 2009). Studies in Europe have shown earthworm distribution at the landscape level to be dependent on climatic conditions, habitat suitability (pH and litter quality), and human activity and land use practices (Tiunov et. al. 2006).

Little is known about the distribution, impact or drivers of earthworm expansion into central Canadian forests. Here, we conduct a landscape-scale study to compare earthworm communities in different regions and habitat types in central Canadian woodlands. Specifically, we examine the regional variation in earthworm community attributes (species richness, density, biomass, composition) across a range of municipal counties and habitat types in south-central Ontario, and identify habitat characteristics associated with attributes of earthworm communities. We predict that southern regional jurisdictions will have greater earthworm abundance and

species richness, especially for *Lumbricus* and epigeic species, than the northern regions, due to the history of European settlement in this region. We also expected to find similar earthworm species among similar habitat types due to the local biotic and abiotic conditions associated with each, and higher biomass in deciduous forests than in other habitat types (Smith et al. 2008).

Methods

Study design

To analyze whether earthworm communities differed between regions and habitat type, we selected four regional municipalities distributed along a ~125km transect in the south-north direction of south-central Ontario: Simcoe County, York Region, Wellington County, and Halton Region (Fig. 1; Table 1). These regions encompass the northern edge of the Carolinian forest ecotone (Halton) as well as the Great-Lakes-St. Lawrence forest ecotone (Wellington, York, and Simcoe) (NRCan 2012). We included four habitat types: deciduous forest, mixed forest, tree plantation, and meadow. Within each of the four regions, two subsamples (sites) of each habitat type were selected (with the exception of Halton Region where only one suitable meadow site could be found) for a total of eight (seven in Halton) sites per region (total sites=31) (Fig. 1). Sites were selected of similar age, history, and estimated recreational use wherever possible in order to decrease variation (Table 2).

At each site, five plots (10 m x 10 m) were sampled for earthworms, soils, and vegetation during September-October, for a total of 155 plots. The plots were systematically selected without preconceived bias at 30-50 m towards the interior of the forest but within 100 m from

any road or trail. This approach to sampling was used in order to account for the degree of uncertainty about differential invasion in each region and the potential for correlation between earthworm abundance and proximity to roads (Cameron et al. 2007; Sackett et al. 2012). Sampling was done at least 50 m away from a forest edge in order to ensure interior habitat. Each plot was sampled once.

Study area and site description

The four study regions were similar in topography and representative of their respective forest ecozones. Predominant tree species in the seven northern Carolinian forest sites included American beech, maples (*Acer* spp.), black walnut (*Juglans nigra*), hickories (*Carya* spp.), and oaks (*Quercus* spp.), while the 24 sites in the Great Lakes-St. Lawrence forest were characterized by a greater percentage of conifers, such as red pine (*Pinus resinosa*), eastern white pine (*Pinus strobus*), and eastern hemlock (*Tsuga canadensis*), as well as by yellow birch (*Betula alleghaniensis*), maples, and oaks. The soils were variable among the regions, with podzolic soils generally found in the north, and gleysols, luvisols, and brunisols in the south (Table 1). Average annual precipitation and temperature was similar between the four regions, and all sites are below 400 m in altitude.

All meadow and plantation sites we selected were historically used for agriculture, with the exception of the two plantation sites in York region where there was no record of use for the cleared land prior to the plantation; agricultural use was likely in this case too, considering the historical patterns of cleared land across southern Ontario (Table 2). The deciduous and mixed forest sites in the two southerly regions, Halton and Wellington, were also historically used for

agriculture, with the exception of one mixed forest site in Halton, which had always remained as forest. The deciduous and mixed forest sites in the two northern regions, York and Simcoe, had never been cleared, and had no agricultural record. Forest management activities in these regions are presently overseen by their respective regional foresters, with the exception of Wellington County, where management is conducted by the City of Guelph and the Grand River Conservation Authority. These forested areas are currently open to the public for a range of recreational activities, including hiking, horseback riding, and cycling.

Habitat types

We defined the four habitat types based on the following criteria: (a) Deciduous Forest, dominated by deciduous species such as sugar maple (*Acer saccharum*) or ash (*Fraxinus* spp) comprising more than 50% of the total tree cover, and with only a minor component of coniferous species, if any; (b) Mixed Forest, with more than 25% of the total canopy cover consisting of coniferous species such as hemlock, and usually having a higher diversity of tree species than deciduous forests; (c) Plantation Forest, originally planted (usually in rows) as a monoculture of one or a few conifer species such as white pine, red pine or white spruce (*Piceaglauca*); and (d) Meadow, an area with less than 25% cover of tree and shrub species, composed largely of grasses and forbs such as goldenrods (*Solidago* spp.), and not actively maintained for agricultural use (Lee et al. 1998) (Table 2).

172 Earthworm sampling and identification

173 Earthworms were sampled using three, 25x25-cm metal quadrats, spaced at least 2 m
174 apart, in each of the 155 plots. Specimens were extracted using a mustard powder
175 (allylisothiocyanate) solution at a concentration of approximately 100 mg l⁻¹ AITC (Zaborski
176 2003, Coja et al. 2008), appropriate for the range of soil types sampled here (Lawrence and
177 Bowers 2002). The 25x25-cm quadrat was edged with a spade and pressed into the litter-cleared
178 soil surface to a depth of approximately 2 cm. Two liters of mustard solution was slowly poured
179 within the quadrat over the span of 10 min. All earthworms emerging within those 10 min were
180 collected and killed in a solution of 70% isopropyl alcohol and water (Hale et al. 2008).
181 Specimens collected from the three quadrats were pooled by plot and then by site (5 plots per
182 site). Earthworms were fixed using a 10% formalin solution for 24 h, and then put back into 70%
183 isopropyl alcohol for preservation.

184 Preserved earthworms were separated into three age classes based on clitellum
185 development; 1) juveniles (lacking clitellum), 2) adults (clitellate), and 3) pre-/post- clitellate
186 adults (clitellum not fully developed or clitellum absent but scar visible, respectively) (Reynolds
187 1977). Adult earthworms were then identified to species using Reynolds' (1977) key, counted,
188 and recorded. Juveniles could only be recorded to genus, unless the species were *Dendrobaena*
189 *octaedra* or *Dendrodrilus rubidus*, in which case they could be identified to species even as
190 juveniles. *Octolasion* juveniles and adults were grouped for analyses even though their juveniles
191 could not be confidently identified to species because only *O. tyrtaeum* adults were found.
192 Earthworms were weighed to ± 0.001 g, grouped by genus, with the exception of *D. octaedra* and
193 *D. rubidus*, since they could be identified to species as juveniles.

194

195 **Vegetation sampling**

196 Tree species composition, size class distribution, and basal area were assessed using a
197 standard stand analysis field sheet (Ontario Ministry of Natural Resources 2004) and a BAF2
198 prism at each of the 155 plots. Trees were divided into four size classes by diameter at breast
199 height (dbh); polewood (10-25 cm), small (26-36 cm), medium (38-48 cm), and large (>50 cm).
200 Values obtained from the five plots were pooled for each variable to characterize the site.
201 Presence of understory shrub and herbaceous species were recorded for all plant species
202 observed within the 10x10-m plots. The presence and species of tree seedlings were also
203 determined and included in the analysis. In Meadow plots, only forb species were recorded.
204 Values from the five plots were pooled for each site. To determine the approximate stand age
205 and management history, we consulted the forest managers in each region. A record of previous
206 harvesting practices and land uses was maintained for all regions.

207

208 **Soil sampling and analysis**

209 Approximately 10 cm³ of soil was collected from the top 10cm of each plot, pooled by
210 site (5 plots per site), and analyzed for gravimetric soil moisture content, organic matter, texture,
211 pH, and bulk density. Organic matter was measured through loss of ignition at 500°C for 4 h;
212 initial tests for calcareousness using 10% HCl determined that most of the soils were non- to
213 weakly calcareous (Denholm et al. 1993). Soil texture was measured using an adapted pipette
214 method (Kroetsch and Wang 2008), that measures sedimentation rates of sand (50 µm– 2 mm),

silt (2–50 μm), and clay (0–2 μm) based on Stoke's law. Soil pH was measured from a well-mixed slurry of 10 g of fresh soil and 40 mL of distilled water (Hendershot et al. 2008). A separate soil sample was taken from the top 10 cm of soil of each plot using a metal ring of fixed dimensions (7.6 cm D x 4.4 cm H) and thus, a fixed volume (199.7 cm^3) to determine bulk density.

Statistical analyses

For statistical analysis, earthworm data from the two habitat subsamples per region were pooled, and environmental data (soil and vegetation data) were averaged over the two habitat subsamples per region. We decided to pool data rather than analyze subsample data in nested analyses due to the high dissimilarity in earthworm biomass between many subsample pairs. This high earthworm spatial heterogeneity on a local scale is not unusual based on repeated sampling in other sites across this region of southern Ontario (T. Sackett, pers.obs.).

We compared the biomass of earthworms among habitat types and regions, and considered both total biomass and biomass of earthworm genera for all samples where there were more than two observations (*Aporrectodea*, *Dendrobaena*, *Lumbricus*, and *Octolasion*). Differences in total earthworm biomass among habitat types and regions was tested using a general linear model (function `lm` in R); earthworm biomasses were ($\log+1$) transformed before analysis to achieve normality of residuals and homogenous variances. For each separate earthworm genus, the error residuals and variances could not meet model assumptions even after transformation. Therefore, we used a permutation (i.e. randomization) test to detect non-random patterns of earthworm biomass with habitat and regions. For each genus, we randomized the biomass data over all cells (habitat and region) and calculated the F-statistic for habitat and

region effects using the `lm` function. We repeated this for 5000 iterations, creating a random distribution of F-statistics for these data. We then compared our actual F-statistic with this distribution to find the probability that the actual statistic was obtained from the random distribution.

Non-metric multidimensional scaling (NMDS) was used to visually explore differences in earthworm community composition among sites, after which, we used permutational multivariate analysis of variance (pMANOVA) to test for differences in earthworm assemblage composition among habitats and regions (Anderson 2001; McArdle and Anderson 2001). This analysis is a non-parametric analysis of variance of distance matrices among groups. If any factors in the overall pMANOVA model were significant, we performed pairwise tests. For both the ordination and pMANOVA, we included only those earthworms identified to species (i.e., specimens only identified to genus were eliminated for the analysis), and also removed species where less than five individuals were collected (*D. rubidus*, *A. trapezoides*) in order to reduce the effect of these rare species on the analysis. Sites where no earthworms were identified to species were also removed; this included the Plantation Forests in the Wellington region, and all Meadow sites because only one of the seven Meadow sites had individuals identified to species. For the remaining sites, we pooled the data from the five collections per sub-sample and two sub-samples per habitat to yield 11 samples in the data set.

For the NMDS, we used a scree plot of dimensions versus stress to choose the number of dimensions for the ordination (2 dimensions were adequate). The `metaMDS` function from the R package `{vegan}` was then used to perform the NMDS of species composition among sites, building a Bray-Curtis distance matrix. We were particularly interested in exploring whether any measured environmental or geographical (i.e., latitude) variables were correlated with

differences in species composition among the sites, as represented in the NMDS configuration. To evaluate this, we used the `envfit` function from the `{vegan}` package to test the strength and direction of the relationship between the environmental variables with the NMDS sample coordinates. Values from environmental variables were log-transformed before overlaying because `envfit` assumes a linear relationship between the environmental vectors and the ordination coordinates. For the two-way pMANOVA model (habitat and region as predictors), F-statistics were estimated from 1000 permutations of the Bray-Curtis distance matrix using the `adonis` function in the R library `vegan`.

We tested for significant relationships between environmental (edaphic: soil pH, moisture, organic matter, bulk density; vegetation: [basal area conifer and deciduous?]) variables and the predictors of region and habitat using general linear models (least squares estimation method) (function `lm` in R).

Results

Earthworm richness and biomass

A total of 806 earthworms from nine species and 5 genera (*Dendrobaena*, *Dendrodrilus*, *Aporrectodea*, *Octolasion*, *Lumbricus*) were collected in our study (Tables 3 and 4). Two specimens of unknown and unidentifiable species were not included in the analyses. The highest number of earthworm species was found in Halton Region (Table 3). *Aporrectodea rosea*, *A. tuberculata*, *Octolasion* sp., and *Lumbricus terrestris* were absent from Simcoe County samples,

whereas *A. rosea* and *A. turgida* were not found in York County. *Dendrobaena octaedra* and *Dendrodrilus rubidus* were absent in Wellington County samples and *D. rubidus* absent in Halton Region. All nine species were collected with our sampling system in the Deciduous Forests, and *Aporrectodea trapezoides* was found only in Deciduous Forests (2 specimens) (Table 4). Meadows held the lowest species richness among the studied habitat types, where only *A. tuberculata*, *A. turgida*, *Octolasion* sp. and *L. rubellus* were collected. Earthworms sampled in the most southerly sites of Halton Region comprised 58% of the total number of earthworms collected; York, Wellington, and Simcoe Regions represented 21%, 16% and 5% of the total, respectively (Table 3). Throughout the four regions, the total density of earthworms was the highest in the Deciduous Forests (50%), followed by the Mixed Forests (32%), the Plantation Forests (15%), and finally the Meadows (3%) (Table 4).

Earthworm biomass ranged from 0 to 47 g per m² (0 to 153 individuals per m²) in the samples. The general linear model showed that total earthworm biomass decreased with increasing latitude ($F_{1,11}=6.60$; $p=0.026$) (Fig. 2), indicating regional differences in biomass with the highest being in Halton Region followed by Wellington, York, and Simcoe Regions. Permutation tests on each earthworm genus indicated that the biomass of *Lumbricus* ($p=0.036$) and *Octolasion* ($p=0.044$) genera both decreased with increasing latitude, but there was no relationship between the biomass of either *Dendrobaena* or *Aporrectodea* with latitude ($p>0.050$). Neither total earthworm biomass, nor the biomass of a particular genus, differed significantly among the four habitats ($p>0.050$).

Earthworm community composition

The NMDS ordination (Fig. 3) showed species composition and assemblages to be grouped similarly by region (Final stress = 0.06; probability of achieving observed stress through randomization = 0.04). Sites in the Simcoe and York Regions grouped at the left of the biplot (upper and lower part, respectively) while those from Wellington and Halton Regions grouped together on the right of the biplot. The ordination coordinates for the samples were significantly associated with region and soil pH ($p < 0.050$). *Dendrodrilus rubidus* and *Dendrobaena octaedra* were associated with Simcoe and York Regions, respectively, whereas *Lumbricus* sp and *Aporrectodea turgida* were associated with the southern regions. The pMANOVA indicated that the species composition in the earthworm assemblages differed among regions ($F_{3,5}=3.05$, $p=0.020$) but not among habitats ($F_{2,5}=0.82$, $p=0.640$). Pairwise comparisons showed that assemblage composition differed between the northernmost Simcoe Region and the two southerly regions, Wellington and Halton ($p<0.050$). Differences in functional groups were not clear between habitat types. Mixed Forests and Plantation Forests had the highest density of epigeic earthworm species, which were absent from in the Meadow samples (Table 4). Both endogeic earthworms and the *Lumbricus* group were most abundant in Deciduous Forest and Mixed Forest types.

Regional and habitat variations in soil

The general linear models indicated that latitude was associated with differences in soil characteristics such as pH, gravimetric soil moisture, organic matter, and proportion of two size classes of sand. Soil pH, proportion of organic matter, and percentage moisture all decreased with increasing latitude, from Simcoe to Halton Region (Table 5). The linear models indicated

that pH decreased by 1.1 units with each (decimal) unit of latitude ($F_{1,11}=40.6$; $p<0.001$), ranging from average value of 6.8 in the southern Wellington and Halton Regions, to 5.7 in the northernmost region, Simcoe. Organic matter decreased by 6% with each unit of latitude ($F_{1,11}=8.4$; $p=0.014$), ranging from 12-14 % in Wellington and Halton Regions to 7% in Simcoe Region. Soil moisture decreased by 22% with each unit of latitude ($F_{1,11}=15.2$; $p=0.002$), ranging from 37-44% in Wellington and Halton Regions to 16% in Simcoe Region. Habitat types were less associated with differences in soil characteristics than latitude. Only soil pH varied significantly across all habitats ($F_{3,11}=6.2$, $p=0.010$), and pairwise tests indicated that Meadows were significantly drier than Mixed Forests ($p<0.050$).

Discussion

Nine of the 17 known species of exotic earthworms in Ontario were collected in this regional study across south-central Ontario, representing the most common species known to be established in the province (Addison 2009). This is the first study since Reynold's (1977) to examine specific site and habitat information for earthworm assemblages across regions in south-central Canada.

Earthworm biomass varied significantly among the four municipal regions we studied, and this was correlated with soil characteristics. As expected based on visible signs of invasion (patches of missing leaf litter and earthworm middens, casts, and burrow entrances from Suárez et al. 2006a), the southern Halton Region had the highest richness, total biomass, and density of earthworms, followed in decreasing order by Wellington, York, and Simcoe Regions for biomass and density. In contrast, the opposite pattern was seen when only epigeic species were

considered, with the more northerly Simcoe and York Regions having the highest densities of this functional group. Although there are inherent differences in the ecological function of various epigeic species such as between *L. rubellus* and *D. octaedra*, we expected that epigeic species in general would be more dominant in sites with sandier soils since work by others has shown a relationship between their abundance and litter depth (McLean and Parkinson 1997), especially in sites where earthworms had been introduced more recently (Hale et al. 2005). In northwestern Canada, Cameron et al. (2007) found that epigeic species such as *D. octaedra* were more abundant in areas with deeper litter, but they considered this result might be due to unexplained environmental factors such as time since invasion.

Soil characteristics, litter quality, and litter quantity are known to influence earthworm abundance and composition. Increased deciduous regeneration provides litter for many earthworm species, but this is not equally palatable to all. The Plantation Forests in Wellington Region contained saplings of only one tree species whereas those in Halton Region had up to 10 species of deciduous tree saplings. In our study, the number of palatable tree species affecting leaf litter, soil pH, and temperature all decreased from south to north, and this would likely slow the rate of earthworm invasion and changes in the forest (Addison 2009) and help explain the differences we observed in Simcoe Region, where the lowest density of earthworms was found together with the lowest soil pH, moisture, and organic matter. In laboratory studies, *L. terrestris* biomass and growth was greatest at 20°C and 30% soil moisture and reproductive structures developed slower at cooler temperatures (Berry and Jordan 2001). Moreover, York and Simcoe Regions both had high amounts of fine and coarse sand respectively, which corresponded with low earthworm densities. In the national forests of Minnesota and Wisconsin (USA), Holdsworth et al. (2007b) also found increased sandiness to be correlated with decreased earthworm

abundance. Thus, it is not surprising that we found epigeic species to be in higher proportion in the two northerly regions as they appear to tolerate lower pH and more sandy sites.

The low density and biomass of earthworms in the most northerly regions of our study could also possibly be explained by the differential rate of invasion over this latitudinal gradient, with the northern sites likely being invaded more recently than the southern ones. According to Hale et al. (2005), newly invaded areas would be expected to have a higher abundance of epigeic species compared to anecic and endogeic species, and this is consistent with what we observed in the northern Simcoe Region where there was a high proportion of epigeic and *Lumbricus* species but a small proportion of endogeic species. In addition, because *L. terrestris* adults were not found in this region, it is likely that the *Lumbricus* component (adults and immatures) was largely composed of the epi-endogeic species *L. rubellus*, which is also capable of tolerating low pH and functions similarly to the other two epigeic species found. In contrast, the most southern regions of Halton and Wellington both had very low densities of epigeics and were dominated by endogeic species. The epigeic species that we saw in the northern regions, *Dendrobaena octaedra* and *D. rubidus*, were not found in the two southerly regions, while *A. rosea* was not found in the northerly regions. The lack of *Octolasion* species and *L. terrestris* in the northern Simcoe Region was somewhat surprising given that it was in close proximity to large bodies of water where frequent introductions of these species would be thought to occur from sport fishing. *Lumbricus terrestris* has a relatively slow rate of spread among the species considered here, so its absence in the north could be explained if the original introduction was in the southern sites. Although these variations in functional group composition and earthworm abundance are consistent with predictions from the theory of invasion succession (Hale et al.

2005), there may also be other factors, especially habitat, soil characteristics, and the relatively low sample size that contribute to the observed patterns and must also be considered.

In contrast to our predictions, earthworm biomass and the species assemblage composition did not differ significantly among the four habitat types. Based on soil pH, it was predicted that the Plantation Forests would have the highest proportion of epigeic species, followed by the Mixed Forests and Deciduous Forests. Epigeic species were not expected to be abundant in the Meadow sites because the litter layer there would have been reduced by regular agricultural tilling, leaving them unsuitable for earthworm habitat (Nuutinen et al. 1998). Plantation Forests had five times fewer *Lumbricus* species than endogeic species, which was surprising because endogeic species are not usually found in forest plantations whereas *L. rubellus* is an acid-tolerant species frequently collected from them (Ammer et al. 2006). The relatively high pH of the Plantation Forest sites, especially in the more southerly regions (Table 5), might account for this unexpected result, while the relatively high bulk density of the Meadow sites might account for the low overall abundance of earthworms found there. Smetak et al. (2007) showed that younger urban habitats had increased bulk density due to compaction than more natural habitats, and that this resulted in lower overall earthworm abundance.

The stage of succession in the conversion of Plantation Forests to more natural Deciduous Forests potentially had an influence on the earthworm communities we observed in this study. Two of the conifer plantations in the Wellington Region never thinned since being planted in 1982 (M. Neumann 2012, pers. comm.) had no earthworms present whereas the Plantation Forests in Halton Region that had been thinned to promote deciduous regeneration (Gartner Lee Ltd. et al. 2002) averaged 7.38 earthworms per m⁻². Thus, variations in forest

management history and practices between the different regions are important in helping to explain local earthworm abundance and assemblages.

Based on the general lack of historical information for the timing of earthworm invasion in our study areas, it is difficult to determine definitively the cause of variation in their earthworm communities across the four regions. We do know that the southern sites were settled for agriculture slightly earlier (e.g. late 1700s to early 1800s) than the northern sites (e.g. late 1800s to early 1900s). In addition, agricultural activities have tended to be more intensive in the southern than in the northern regions, and past land use and management have likely had a profound influence on any associated soil characteristics. Thus, it is likely that our results identify the presence of a latitudinal gradient in earthworm invasion for this region as soil variables were well within the range for survival and likely had little effect on historical movement and introduction patterns. The spread of earthworms is primarily anthropochorous, and species distributions in forests differ depending on the type of human activity (e.g., settlement, roads, fishing) and land-use history (e.g., Bohlen et al. 2004; Gundale et al. 2005; Cameron et al. 2007; Cameron and Bayne 2009; Sackett et al. 2012). Human population estimates in the northern region were 92/km² whereas those in the southern region averaged 550/km² (Statistics Canada 2011) supporting the likelihood that human density is an important explanatory variable for differences in the earthworm communities we observed here, and this suggests further investigation is warranted into the effects of human settlement. Our results provide new baseline information on the status, distribution, and relationship of functional earthworm communities with respect to habitat characteristics in south-central Ontario. Armed with this improved understanding, forest managers will be better able to plan for these soil invaders as they continue to advance throughout Canada's forests.

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Table 1 –Location, soil order, precipitation, and temperature (1982-2012) of the four study regions in south-central Ontario where earthworm populations were sampled in 2011.

Region	Latitudinal range (°N)	Soil order(s)	Average annual precipitation (mm)	Average annual temperature (°C)
Simcoe	44.49-44.60	Podzols	938	6.6
York	44.04-44.10	Grey brown Podzols	857	7.4
Wellington	43.50-43.54	Podzols and gleysols	905	6.6
Halton	43.46-43.56	Gleysols, luvisols, brunisols	884	6.8

577 Table 2. Location and characteristics of the 31 study sites in south-central Ontario, Canada where
 578 earthworm populations were sampled in 2011.

Habitat	Dominant species ^a	Age	Previous land	Centroid location ^b	
Site code		(years)	use	Northing	Easting
Deciduous					
SCDFA	Mh (57%), Aw (1%)	99	Natural	44.4943	-79.8148
SCDFB	Mh (91%)	107	Natural	44.5927	-79.7997
YRDFA	Mh (60%), Aw(19%)	80	Natural	44.0460	-79.3227
YRDfB	Mh (55%)	102	Natural	44.0682	-79.2864
CGDFA	Ag (53%), Msi (31%)	>40	Agriculture	43.5249	-80.3005
CGDFB	Ag (48%), Msi (48%)	>40	Agriculture	43.5358	-80.2914
HRDFA	Mh (82%)	65	Pasture	43.5403	-79.9865
HRDFB	Mh (52%)	75	Agriculture	43.5112	-79.9960
Mixed					
SCMFA	He (39%), Mh (29%)	80	Natural	44.4965	-79.8137
SCMFB	Mh (48%), He (36%)	98	Natural	44.5935	-79.8002
YRMFA	He (51%), Mh (19%)	132	Natural	44.0706	-79.2836
YRMFB	He (50%), Mh (18%)	132	Natural	44.0704	-79.2819
CGMFA	He (30%), Ag (28%)	>40	Agriculture	43.5102	-80.2209
CGMFB	Ag (33%), He (17%)	>40	Agriculture	43.5107	-80.2218
HRMFA	Mh (25%), He (14%)	57	Forestry	43.5320	-80.0940
HRMFB	Pw (23%), Bw 19%)	75	Agriculture	43.4632	-79.9934
Plantation					
SCPPA	Pr (67%)	52	Agriculture	44.4937	-79.8096
SCPPB	Pr (86%)	71	Agriculture	44.6054	-79.7865
YRPPA	Pr (81%)	53	Cleared (unk) ^d	44.0877	-79.3596
YRPPB	Pr (85%)	55	Cleared (unk) ^d	44.0428	-79.3209
CGPPA	Pw (100%)	30	Agriculture	43.5028	-80.2009
CGPPB	Pw (100%)	30	Agriculture	43.5037	-80.2049
HRPPA	Sw (48%), Pw (28%)	45	Agriculture	43.5203	-80.0558
HRPPB	Sw (48%), Pr (23%)	55	Agriculture	43.5630	-80.0166
Meadow ^c					
SCMA	Solispp, Asclsyri	unknown	Agriculture	44.5287	-79.6164
SCMB	Solispp, Asclsyri	unknown	Agriculture	44.5491	-79.5953
YRMA	Old crop	unknown	Agriculture	44.1013	-79.3388
YRMB	Old crop	unknown	Agriculture	44.1001	-79.3394
CGMA	Solispp, Dauccaro	unknown	Agriculture	43.5039	-80.2071
CGMB	Solispp, Corovari	unknown	Agriculture	43.5036	-80.2276
HRMA	Solispp, Rubuidae	unknown	Agriculture	43.5024	-79.9947

^a(%) Percentage composition of the dominant species based on proportion of total number of trees; See Appendix 1 for a list of species name and abbreviations.

^bCentroid location of the five plots was determined using the 'median center' function of the spatial statistics toolbox in the program ArcMap 10.0 (ESRI 2010).

^cDominant vegetation is based on flowering herbaceous species and does not account for grasses or sedges.

^dunk = Records of previous land use could not be found; however, it was most likely cleared for agriculture and replanted as a plantation based on the history of the two northerly regions.

579 Table 3 - Mean densities (and range) of earthworm species collected from the four regions of
 580 south-central Ontario, Canada during 2011.

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Functional group and species	Mean density (individuals per m ²)				Average density
	Simcoe (n=40)	York (n=40)	Wellington (n=40)	Halton (n=35)	
Epigeic					
<i>Dendrobaena octaedra</i>	0.47 (0-3.75)	0.91 (0-10.00)	0	0.25 (0-3.75)	0.41 (0-10.00)
<i>Dendrodrilus rubidus</i>	0.06 (0-1.25)	0.03 (0-1.25)	0	0	0.02 (0-1.25)
Total epigeic	0.53 (0-5.00)	0.94 (0-5.00)	0	0.25 (0-3.75)	0.43 (0-10.00)
Endogeic					
<i>Aporrectodea</i> juveniles	0.06 (0-1.25)	0.94 (0-5.00)	1.31 (0-7.50)	4.82 (0-16.25)	1.69 (0-16.25)
<i>A. rosea</i>	0	0	0.09 (0-1.25)	0.29 (0-2.50)	0.09 (0-2.50)
<i>A. trapezoides</i>	0.06 (0-2.50)	0	0	0	0.02 (0-2.50)
<i>A. tuberculata</i>	0	0.5 (0-5.00)	0.40 (0-5.00)	0.82 (0-3.75)	0.42 (0-5.00)
<i>Aporrectodea turgida</i> (Eisen)	0.03 (0-1.25)	0	0.13 (0-3.75)	0.86 (0-5.00)	0.23 (0-5.00)
<i>Octolasion</i> species	0	0.75 (0-7.50)	1.81 (0-22.5)	3.11 (0-21.25)	1.36 (0-22.50)
Total endogeic	0.15 (0-2.50)	2.19 (0-10.00)	3.74 (0-30.00)	9.90 (0-32.50)	3.81 (0-32.50)
<i>Lumbricus</i> group					
<i>Lumbricus</i> juveniles	0.44 (0-8.75)	0.75 (0-6.25)	1.5 (0-8.75)	5.07 (0-25.00)	1.84 (0-25.00)
<i>L. rubellus</i>	0.06 (0-1.25)	0.03 (0-1.25)	0.03 (0-1.25)	0.68 (0-8.75)	0.16 (0-8.75)
<i>L. terrestris</i>	0	0.03 (0-1.25)	0.09 (0-1.25)	0.64 (0-5.00)	0.18 (0-5.00)
Total <i>Lumbricus</i> group	0.50 (0-10.00)	0.81 (0-6.25)	1.62 (0-8.75)	6.39 (0-32.50)	2.18 (0-32.50)
Average density of all species (m ⁻²)	1.18 (0-11.25)	3.94 (0-13.75)	5.38 (0-38.75)	16.54 (0-65.0)	6.17 (0-65.00)
Number of species	5	6	5	7	

Table 4. Mean densities and range of earthworm species collected from the four habitat types in south-central Ontario during 2011.

Functional group and species	Mean density (individuals per m ²)			
	Deciduous forest (n=40)	Mixed forest (n=40)	Plantation forest (n=40)	Meadow (n=35)
Epigeic				
<i>Dendrobaena octaedra</i>	0.34 (0-3.75)	0.66 (0-10.00)	0.59 (0-3.75)	0
<i>Dendrodrilus rubidus</i>	0.03 (0-1.25)	0.03 (0-1.25)	0.03 (0-1.25)	0
Total epigeic	0.37 (0-5.00)	0.69 (0-11.25)	0.62 (0-5.00)	0
Endogeic				
<i>Aporrectodea</i> juveniles	3.16 (0-16.25)	1.69 (0-6.25)	1.41 (0-11.25)	0.32 (0-7.50)
<i>A. rosea</i>	0.06 (0-1.25)	0.16 (0-1.25)	0.13 (0-2.50)	0
<i>A. trapezoides</i>	0.06 (0-2.50)	0	0	0
<i>A. tuberculata</i>	0.28 (0-3.75)	0.72 (0-5.00)	0.59 (0-5.00)	0.04 (0-1.25)
<i>A. turgida</i>	0.59 (0-5.00)	0.22 (0-2.50)	0.06 (0-2.50)	0.04 (0-1.25)
<i>Octolasion</i> species	2.66 (0-21.25)	2.19 (0-22.50)	0.31 (0-5.00)	0.14 (0-2.50)
Total endogeic	6.81 (0-50.00)	4.98 (0-37.50)	2.50 (0-26.25)	0.54 (0-5.00)
Lumbricus group				
<i>Lumbricus</i> juveniles	4.47 (0-25.00)	1.94 (0-11.25)	0.47 (0-2.50)	0.29 (0-5.00)
<i>L. rubellus</i>	0.63 (0-8.75)	0.03 (0-1.25)	0.03 (0-1.25)	0.04 (0-1.25)
<i>L. terrestris</i>	0.28 (0-2.50)	0.31 (0-5.00)	0.09 (0-2.50)	0
Total <i>Lumbricus</i> group	5.38 (36.25)	2.28 (0-17.50)	0.59 (0-6.25)	0.33 (1.75)
Average density of all species (m ⁻²)	12.56 (0-65.00)	8.00 (0-38.75)	3.72 (0-22.50)	0.86 (0-13.8)
Number of species	9	8	8	4

Table 5. Mean values of soil variables and statistical variation between regions or habitat types based on the top 10 cm of soil from the four sites sampled in southern Ontario. GSM: gravimetric soil moisture, OM: organic matter, BD: bulk density

	pH	GSM (%)	OM (%)	BD (g/cm ³)	% fine sand (<250µm)	% coarse sand (>250µm)
Region						
Simcoe	5.70±0.16	16.4±2.8	7.23±1.1	0.67±0.10	43.9±6.9	50.1±6.4
York	6.41±0.25	34.0±4.4	9.35±1.8	0.69±0.09	68.5±5.0	10.1±5.1
Wellington	6.80±0.10	37.1±5.0	13.9±2.2	0.76±0.03	42.8±3.0	14.1±3.6
Halton	6.89±0.09	44.3±1.8	12.6±0.9	0.69±0.02	43.0±1.9	14.8±1.1
Habitat type						
Deciduous	6.65±0.16	36.9±5.3	13.4±2.1 ¹	0.64±0.04	41.8±4.8	31.3±8.0
Mixed	6.03±0.24	37.0±5.7	12.5±1.3	0.53±0.07	49.8±6.4	19.4±8.8
Plantation	6.28±0.26	28.3±5.3	10.0±1.3	0.69±0.03	55.1±7.6	18.6±6.6
Meadow	6.82±0.16	27.9±4.1	6.29±1.3	0.93±0.06	51.6±4.9	20.1±6.3

Figure captions

Figure 1. Centroid locations of the 31 study sites for the four study regions (shaded in grey) in southern Ontario (shaded). ○=deciduous forests (DF), Δ=mixed forests (MF), □= plantations (PP), +=meadows (M).

Figure 2. Total earthworm biomass at 15 sites in four different habitat types distributed latitudinally across southern Ontario, Canada. The solid line is the linear regression line and the shaded area is the 95% confidence region ($r^2=0.55$; $p=0.048$). Habitat abbreviations are as in Figure 1.

Figure 3. Ordination (NMDS) plots of earthworm species assemblage composition from 15 collections across southern Ontario. Points are individual observations and ellipses enclose 1 SD of the data for samples within the Halton (H), York (Y) and Simcoe (S) Regions. No ellipse is drawn for Wellington (W) Region as only two samples are plotted. Gray arrows indicate the direction of significantly correlated environmental or geographical variables with differences in species composition among sites. Hollow diamonds with species names indicate the relative distribution of species among sites.

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