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.

Résumé : Avec l'amélioration de notre compréhension des impacts des vers de terre (Oligochètes : Lumbricidés) sur les processus des écosystèmes et la restauration forestière, il devient nécessaire d'étudier leur rôle dans les forêts canadiennes où ils sont devenus envahissants. On ne sait pas comment les populations de vers de terre répondent, à l'échelle du paysage, aux caractéristiques régionales et aux types de végétation dans les boisés du centre du Canada. Nous avons étudié la variation régionale de la richesse spécifique, de la biomasse et de la composition des assemblages de vers de terre dans quatre régions municipales (du sud au nord : Halton, Wellington, York et Simcoe) et quatre types d'habitat (forêt feuillue, forêt mixte, plantation d'arbres et prairie) avec diverses caractéristiques du sol dans des boisés du centre-sud de l'Ontario, au Canada. En général, les communautés de vers de terre variaient selon les régions mais pas selon le type d'habitat. Les régions les plus au sud avaient les plus grandes richesses spécifiques, biomasses (i.e. *Lumbricus* et *Octolasion*) et densités de vers, reflétant un gradient sud–nord dans les caractéristiques des sols. La composition des assemblages variait selon les régions, mais pas selon les types d'habitats. Le gradient sud–nord que nous avons observé indique que la progression de l'invasion serait associée à l'établissement des populations humaines et à leur densité. Nos résultats, en fournissant de l'information de base sur les communautés de vers de terre dans les forêts du centre-sud de l'Ontario, vont permettre aux aménagistes de mieux prendre en compte le rôle croissant des vers de terre dans les futures forêts du Canada. [Traduit par la Rédaction]

Mots-clés : communautés de vers de terre, groupes fonctionnels, espèces envahissantes, utilisation du territoire, Lumbricidés.

Introduction

The southern extent of the last Wisconsonian glaciation eliminated most native earthworm species from Canada and the northern United States (Callaham et al. 2006; Addison 2009). Exotic earthworm species (Oligochaeta: Lumbricidae) were introduced during the late 1800s, presumably through the release of contaminated soil in ship ballasts from Europe (Reynolds 1977; Tiunov et al. 2006); they are now widely distributed in select locations across much of North America (Gates 1982; Reynolds 1994), where they re-engineer soil and site characteristics. Because soils, vegetation, and ecosystem processes in Canadian temperate forests have developed in the absence of earthworms following recent

glacial recession, such invasions cause major shifts in ecosystem functioning and services, impacting forest floor structure, soil biogeochemistry, and faunal and plant community composition (e.g., Alban and Berry 1994; Bohlen et al. 2004; Hale et al. 2005; Migge-Kleian et al. 2006; Holdsworth et al. 2007a, 2007b; Costello and Lamberti 2009; Szlavecz et al. 2011; Crumsey et al. 2013; Sackett et al. 2013; Craven et al. 2017).

It is now well documented that earthworm invasion results in significant changes to nutrient dynamics and soil structure in temperate forests (e.g., Hale et al. 2005; Sackett et al. 2013). For example, the feeding habits of *Lumbricus terrestris* Linnaeus, 1758 have been shown to increase the immobilization of nutrients leading to nitrification and leaching and an overall decrease in

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nutrient availability (Hale et al. 2005). Earthworms may also shift the soil decomposer community from one dominated by fungi and their interactions to one dominated by bacteria or fungi antagonistic to decomposing fungi, again changing the rate of nutrient cycling and 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, among 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* Ehrh.) and eastern hemlock (*Tsuga canadensis* (L.) Carrière) 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), 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 char-

Fig. 1. Centroid locations of the 31 study sites for the four study regions (shaded in grey) in southern Ontario. Symbols: ○, deciduous forests (DF); △, mixed forests (MF); □, plantations (PP); +, meadows (M).
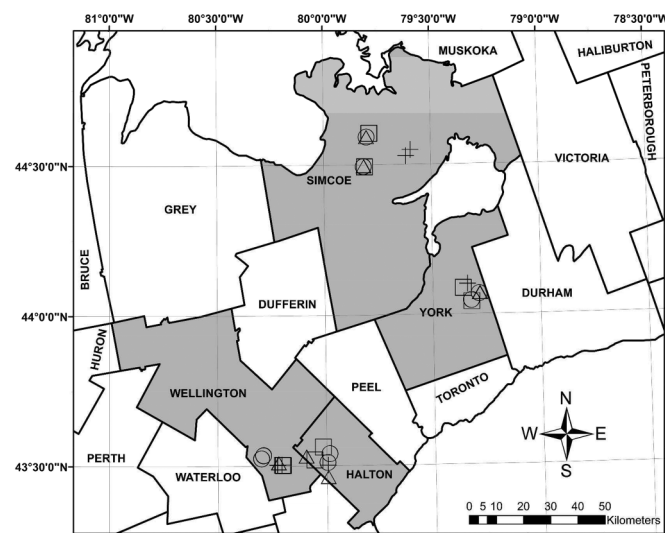
acteristics associated with attributes of earthworm communities. We predict that southern regional jurisdictions will have greater earthworm abundance and species richness, especially for *Lumbricus* and epigeic species, than the northern regions due to the history of European settlement in this region. 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 ~125 km 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). 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 to decrease variation (Table 2).

At each site, five plots (10 m × 10 m) were sampled for earthworms, soils, and vegetation during September to 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 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 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

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	Gray–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

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

Habitat and site code	Dominant species[a]	Age (years)	Previous land use	Centroid location[b] 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 (unknown)[d]	44.0877	−79.3596
YRPPB	Pr (85%)	55	Cleared (unknown)[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. For a list of species names and abbreviations, see Appendix A, Table A1.

[b]Centroid location of the five plots was determined using the "median center" function of the spatial statistics toolbox in the program ArcMap 10.0 (ESRI, Redlands, California; http://www.esri.com).

[c]Dominant vegetation is based on flowering herbaceous species and does not account for grasses or sedges.

[d]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.

species in the seven northern Carolinian forest sites included American beech, maples (*Acer* spp.), black walnut (*Juglans nigra* L.), 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* Aiton), white pine (*Pinus strobus* L.), and eastern hemlock, as well as by yellow birch (*Betula alleghaniensis* Britton), 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 were similar between the four regions, and all sites are below 400 m in elevation.

All meadow and plantation sites that we selected were historically used for agriculture, with the exception of the two plantation sites in York region for which there was no record of use for the cleared land prior to the plantation; agricultural use was likely in this case as well, 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: (*i*) Deciduous Forest, dominated by deciduous species such as sugar maple (*Acer saccharum* Marsh.) or ash (*Fraxinus* spp.), comprising more than 50% of the total tree cover with only a minor component of coniferous species, if any; (*ii*) 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; (*iii*) 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 (*Picea glauca* (Moench) Voss); and (*iv*) 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).

Earthworm sampling and identification

Earthworms were sampled using three 25 × 25 cm metal quadrats, spaced at least 2 m apart, in each of the 155 plots. Specimens were extracted using a mustard powder (allyl isothiocyanate, AITC) solution at a concentration of approximately 100 mg AITC·L^{-1} (Zaborski 2003; Čoja et al. 2008), appropriate for the range of soil types sampled here (Lawrence and Bowers 2002). The 25 × 25 cm quadrat was edged with a spade and pressed into the litter-cleared soil surface to a depth of approximately 2 cm. Two litres of mustard solution was slowly poured within the quadrat over the span of 10 min. All earthworms emerging within those 10 min were collected and killed in a solution of 70% isopropyl alcohol and water (Hale et al. 2008). Specimens collected from the three quadrats were pooled by plot and then by site (five plots per site). Earthworms were fixed using a 10% formalin solution for 24 h and then put back into 70% isopropyl alcohol for preservation.

Preserved earthworms were separated into three age classes based on clitellum development: (*i*) juveniles (lacking clitellum), (*ii*) adults (clitellate), and (*iii*) pre- or post-clitellate adults (clitellum not fully developed or clitellum absent but scar visible, respectively) (Reynolds 1977). Adult earthworms were then identified to species using Reynolds' (1977) key, counted, and recorded. Juveniles could only be recorded to genus, unless the species were *Dendrobaena octaedra* (Savigny, 1826) or *Dendrodrilus rubidus* (Savigny, 1826), in which case, they could be identified to species even as juveniles. *Octolasion* juveniles and adults were grouped for analyses even though their juveniles could not be confidently identified to species because only *Octolasion tyrtaeum* (Savigny, 1826) adults were found. Earthworms were weighed to ±0.001 g, grouped by genus, with the exception of *D. octaedra* and *D. rubidus* because they could be identified to species as juveniles.

Vegetation sampling

Tree species composition, size class distribution, and basal area were assessed using a standard stand analysis field sheet (Ontario Ministry of Natural Resources 2004) and a BAF2 prism at each of the 155 plots. Trees were divided into four size classes by diameter at breast height (dbh): polewood (10–25 cm), small (26–36 cm), medium (38–48 cm), and large (>50 cm). Values obtained from the

five plots were pooled for each variable to characterize the site. Presence of understory shrub and herbaceous species were recorded for all plant species observed within the 10 × 10 m plots. The presence and species of tree seedlings were also determined and included in the analysis. In Meadow plots, only forb species were recorded. Values from the five plots were pooled for each site. To determine the approximate stand age and management history, we consulted the forest managers in each region. A record of previous harvesting practices and land uses was maintained for all regions.

Soil sampling and analysis

Approximately 10 cm^3 of soil was collected from the top 10 cm of each plot, pooled by site (five plots per site), and analyzed for gravimetric soil moisture content, organic matter, texture, pH, and bulk density. Organic matter was measured through loss of ignition at 500 °C for 4 h; initial tests for calcareousness using 10% HCl determined that most of the soils were non- to weakly calcareous (Denholm et al. 1993). Soil texture was measured using an adapted pipette 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 diameter × 4.4 cm height) 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, personal observation).

We compared the biomass of earthworms among habitat types and regions and considered both total biomass and biomass of earthworm genera for all samples in which there were more than two observations (*Aporrectodea*, *Dendrobaena*, *Lumbricus*, and *Octolasion*). Differences in total earthworm biomass among habitat types and regions were 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 nonrandom 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.

Nonmetric multidimensional scaling (NMDS) was used to visually explore differences in earthworm community composition among sites, after which, we used permutational multivariate analysis of variance (ANOVA) (pMANOVA) to test for differences in earthworm assemblage composition among habitats and regions (Anderson 2001; McArdle and Anderson 2001). This analysis is a nonparametric ANOVA 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)

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

Functional group and species	Mean density (individuals·m⁻²)				
	Simcoe (n = 40)	York (n = 40)	Wellington (n = 40)	Halton (n = 35)	Average density
Epigeic					
Dendrobaena octaedra	0.47 (0–3.75)	0.91 (0–10.00)	0	0.25 (0–3.75)	0.41 (0–10.00)
Dendrodrilus rubidus	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					
Aporrectodea 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)
A. rosea	0	0	0.09 (0–1.25)	0.29 (0–2.50)	0.09 (0–2.50)
A. trapezoides	0.06 (0–2.50)	0	0	0	0.02 (0–2.50)
A. tuberculata	0	0.5 (0–5.00)	0.40 (0–5.00)	0.82 (0–3.75)	0.42 (0–5.00)
Aporrectodea turgida (Eisen)	0.03 (0–1.25)	0	0.13 (0–3.75)	0.86 (0–5.00)	0.23 (0–5.00)
Octolasion 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)
***Lumbricus* group**					
Lumbricus 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)
L. rubellus	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)
L. terrestris	0	0.03 (0–1.25)	0.09 (0–1.25)	0.64 (0–5.00)	0.18 (0–5.00)
Total *Lumbricus* 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 (individuals·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	

and also removed species where less than five individuals were collected (*D. rubidus*, *Aporrectodea trapezoides* (Duges, 1828)) to reduce the effect of these rare species on the analysis. Sites in which 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 subsample and two subsamples 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 (two 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 of conifer and deciduous trees) 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 five 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* (Savigny, 1826), *Aporrectodea tuberculate* (Eisen, 1874), *Octolasion* spp., and *L. terrestris* were absent from Simcoe County samples, whereas *A. rosea* and *Aporrectodea turgida* (Eisen, 1873) were not found in York County. *Dendrobaena octaedra* and *D. rubidus* were absent in Wellington County samples and *D. rubidus* was absent in Halton Region. All nine species were collected with our sampling system in the Deciduous Forests, and *A. trapezoides* was found only in Deciduous Forests (two specimens) (Table 4). Meadows held the lowest species richness among the studied habitat types, where only *A. tuberculata*, *A. turgida*, *Octolasion* sp., and *Lumbricus rubellus* Hoffmeister, 1843 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·m⁻² (0 to 153 individuals·m⁻²) 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 both *Lumbricus* (p = 0.036) and *Octolasion* (p = 0.044) genera 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).
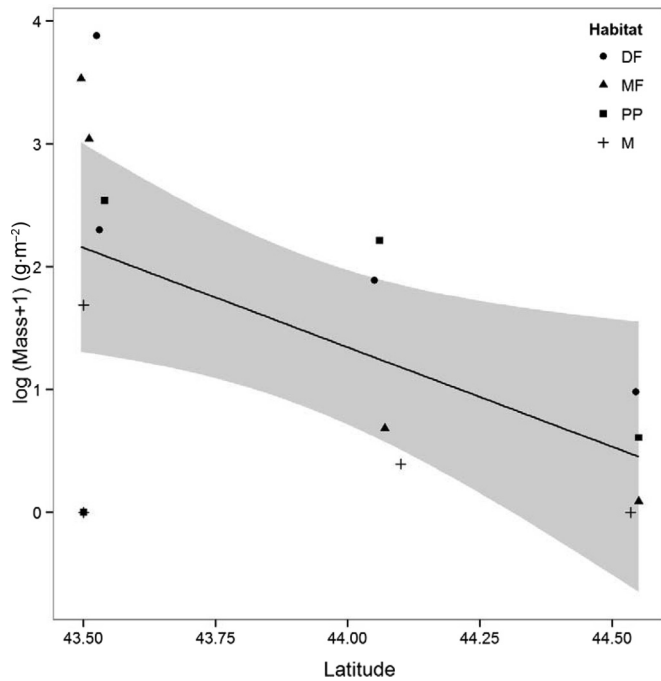
Earthworm community composition

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 parts, respectively), while those from

Table 4. Mean densities and range of earthworm species collected from the four habitat types in south-central Ontario during 2011.
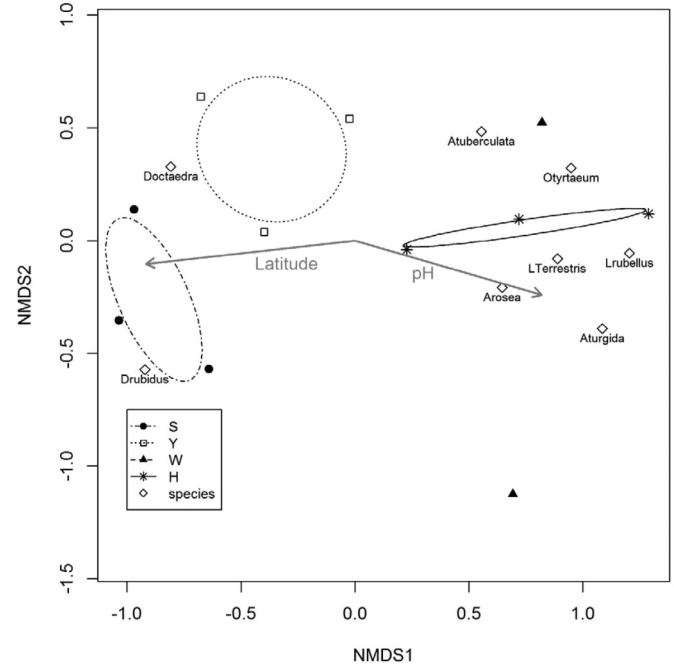
Functional group and species	Mean density (individuals·m⁻²)			
	Deciduous forest ($n = 40$)	Mixed forest ($n = 40$)	Plantation forest ($n = 40$)	Meadow ($n = 35$)
Epigeic				
Dendrobaena octaedra	0.34 (0–3.75)	0.66 (0–10.00)	0.59 (0–3.75)	0
Dendrodrilus rubidus	0.03 (0–1.25)	0.03 (0–1.25)	0.03 (0–1.25)	0
Total epigeic	0.37 (0–5.00)	0.69 (0–11.25)	0.62 (0–5.00)	0
Endogeic				
Aporrectodea juveniles	3.16 (0–16.25)	1.69 (0–6.25)	1.41 (0–11.25)	0.32 (0–7.50)
A. rosea	0.06 (0–1.25)	0.16 (0–1.25)	0.13 (0–2.50)	0
A. trapezoides	0.06 (0–2.50)	0	0	0
A. tuberculata	0.28 (0–3.75)	0.72 (0–5.00)	0.59 (0–5.00)	0.04 (0–1.25)
A. turgida	0.59 (0–5.00)	0.22 (0–2.50)	0.06 (0–2.50)	0.04 (0–1.25)
Octolasion 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**				
Lumbricus juveniles	4.47 (0–25.00)	1.94 (0–11.25)	0.47 (0–2.50)	0.29 (0–5.00)
L. rubellus	0.63 (0–8.75)	0.03 (0–1.25)	0.03 (0–1.25)	0.04 (0–1.25)
L. terrestris	0.28 (0–2.50)	0.31 (0–5.00)	0.09 (0–2.50)	0
Total Lumbricus group	5.38 (36.25)	2.28 (0–17.50)	0.59 (0–6.25)	0.33 (1.75)
Average density of all species (individuals·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

Fig. 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 Fig. 1.

Fig. 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. Diamonds (◇) with species names indicate the relative distribution of species among sites.

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 *D. octaedra* were associated with Simcoe and York regions, respectively, whereas *Lumbricus* spp. and *A. turgida* were associated with the southern regions. The pMANOVA indicated that the species composition in the earthworm assemblages dif-

fered 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

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.

	pH	GSM (%)	OM (%)	BD (g·cm^{-3})	% Fine sand (<250 μm)	% Coarse sand (>250 μm)
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	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

Note: GSM, gravimetric soil moisture; OM, organic matter; BD, bulk density.

($p < 0.050$). 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 Region 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, representing the most common species known to be established in the province (Addison 2009), were collected in this regional study across south-central Ontario. This is the first study since Reynolds (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 that 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 as 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 that 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 that 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 with 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, *D. 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 surpris-

ing 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 that we observed in this study. Two of the conifer plantations in the Wellington Region that had never been thinned since being planted in 1982 (M. Neumann 2012, personal communication) had no earthworms present, whereas the Plantation Forests in Halton Region that had been thinned to promote deciduous regeneration (Gartner Lee Limited et al. 2002) averaged 7.38 earthworms·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 regions than in the northern ones, 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·km^{-2}, whereas those in the southern region averaged 550·km^{-2} (Statistics Canada 2011), supporting the likelihood that human density is an important explanatory variable for differences in the earthworm communities that we observed here, and this suggests that further investigation into the

effects of human settlement is warranted. 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.

Acknowledgements

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Appendix A

Table A1. List of tree and herbaceous species found with respective acronyms.

Common name	Scientific name	Acronym
Trees		
American beech	*Fagus gradifolia* Ehrh.	Be
Balsam fir	*Abies balsamea* L.	Bf
Basswood	*Tilia americana* L.	Bd
Bitternut hickory	*Carya cordiformis* (Wangenheim) Koch	Hb
Black ash	*Fraxinus nigra* Marsh.	Ab
Black cherry	*Prunus serotina* Ehrh.	Cb
Black walnut	*Juglans nigra* L.	Wb
Blue beech	*Carpinus caroliniana* Walter	Bb
Butternut	*Juglans cinerea* L.	Bn
Eastern hemlock	*Tsuga canadensis* (L.) Carrière	He
Eastern white cedar	*Thuja occidentalis* L.	Ce
Green ash	*Fraxinus pennsylvanica* Marsh.	Ag
Red oak	*Quercus rubra* L.	Or
Red pine	*Pinus resinosa* Aiton	Pr
Ironwood	*Ostrya virginiana* (Miller) Koch	Iw
Large-toothed aspen	*Populus grandidentata* Michaux	Alt
Red maple	*Acer rubrum* L.	Mr
Silver maple	*Acer saccharinum* L.	Msi
Sugar maple	*Acer saccharum* Marsh.	Mh
Trembling aspen	*Populus tremuloides* Mich.	At
White ash	*Fraxinus americana* L.	Aw

Table A1 (*continued*).

Common name	Scientific name	Acronym
While birch	*Betula papyrifera* Marsh.	Bw
White elm	*Ulmus americana* L.	Ew
White oak	*Quercus alba* L.	Ow
White pine	*Pinus strobus* L.	Pw
White spruce	*Picea glauca* (Moench) Voss	Sw
Yellow birch	*Betula alleghaniensis* Britton	By
Forest herbaceous species		
White baneberry	*Actaea pachypoda*	Actapach
Red baneberry	*Actaea rubra*	Actarubr
Hooked Agrimonia	*Agrimonia gyposepala*	Agrigypo
Wild columbine	*Aquilegia canadensis*	Aquicana
Spikenard	*Aralia racemosa*	Aralrace
Burdock	*Arctium* species	Arctspp
Jack-in-the-pulpit	*Arisaema triphyllum*	Aristrip
Wild ginger	*Asarum canadense*	Asarcana
Blue cohosh	*Caulophyllum thalictroides*	Caulthal
Enchanter's nightshade	*Circaea lutetiana*	Circlute
Wild basil	*Clinopodium vulgare*	Clinvulg
Goldthread	*Coptis trifolia*	Copttrif
Woodland strawberry	*Fragaria vesca*	Fragvesc
Rough bedstraw	*Gallium asprellum*	Gallaspr
Fragrant bedstraw	*Galium triflorum*	Galitrif
Yellow avens	*Geum aleppicum*	Geumalep
Large-leaved avens	*Geum macrophyllum*	Geummacr
Trout lily	*Erythronium americanum*	Erytamer
Blunt lobed Hepatica	*Hepatica americana*	Hepaamer
Virginia waterleaf	*Hydrophyllum virginianum*	Hydrvirg
Canada mayflower	*Maianthemum canadense*	Maiacana
False Solomon's seal	*Maianthemum racemosum*	Maiarace
Virginia creeper	*Parthenocissus quinquefolia*	Partquin
True Solomon's seal	*Polygonatum pubescens*	Polypube
Bloodroot	*Sanguinaria canadensis*	Sangcana
Sweet sarsaparilla	*Smilax glyciphylla*	Smilglyc
Zig zag goldenrod	*Solidago flexicaulis*	Soliflex
White mandarin	*Streptopus amplexifolius*	Strepamp
Rose twisted stalk	*Streptopus roseus*	Streprose
Meadow rue	*Thalictrum* species	Thalispp
Tall meadow rue	*Thalictrum pubescens*	Thalpube
Foamflower	*Tiarella cordifolia*	Tiarcord
Trillium	*Trillium* species	Trillspp
Red trillium	*Trillium erectum*	Trilerec
White trillium	*Trillium grandiflorum*	Trilgran
Painted trillium	*Trillium undulatum*	Trilundu
Large bellflower	*Uvularia grandifolia*	Uvulgran
Common speedwell	*Veronica officinalis*	Verooffi
Violets	*Violet* species	Violspp
Downy yellow violet	*Violette pubescens*	
Barren ground strawberries	*Waldsteinia fragariodes*	Waldfrag
Ferns and allies		
Maidenhair fern	*Adiantum pedatum*	Adiapeda
Rattlesnake fern	*Botrychium virginianum*	Botrvirg
Spinulose wood fern	*Dryopteris carthusiana*	Dryocart
Ostrich fern	*Matteuchia struthiopteris*	Mattstru
Sensitive fern	*Onoclea sensibilis*	Onocsens
Christmas fern	*Polystichum acrostichoides*	Polyacro
Horsetails	*Equisetum* species	Equisspp
Meadow forbs		
Yarrow	*Achillea millefolium*	Achimill
Ragweed	*Ambrosia artemisiifolia*	Ambrarte
Common milkweed	*Asclepias syriaca*	Asclsyri
Thistles	*Cirsium* species	Cirsspp
Canada thistle	*Cirsium arvense*	Cirsarve
Queen Anne's lace	*Daucus carota*	Dauccaro
Common strawberry	*Fragaria virginiana*	Fragvirg
Northern bedstraw	*Galium boreale*	Galibore

Table A1 (*concluded*).

Common name	Scientific name	Acronym
St. John's wort	*Hypericum perforatum*	Hypeperf
Black medick	*Medicago lupulina*	Medilupu
Alfalfa	*Medicago sativa*	Medisati
White sweet clover	*Melilotus albus*	Melialbu
Evening primrose	*Oenothera biennis*	Oenobien
Broad-leaved plantain	*Platago major*	Platmaj
Goldenrods	*Solidago* species	Solispp
Yellow goat's beard	*Tragopogon dubium*	Tragdubi
Common mullein	*Verbascum thapsus*	Verbthap
Cow vetch	*Vicia cracca*	Vicicrac
Shrubs and woody vines		
Speckled alder	*Alnus rugosa*	Alnurugo
Alternate-leaved dogwood	*Cornus alternifolia*	Cornalte
Round-leaved dogwood	*Cornus rugosa*	Cornrugo
Red osier dogwood	*Cornus sericea*	Cornseri
Hawthorns	*Crataegus* species	Crataspp
Partridgeberry	*Mitchella repens*	Mitcrepe
Pin cherry	*Prunus pensylavanica*	Prunpens
Chokecherry	*Prunus virginiana*	Prunvirg
Sumac	*Rhus* species	Rhusspp
Wild black currant	*Ribes americanum*	Ribeamer
Prickly gooseberry	*Ribes cynosbatic*	Ribecyno
Wild red currant	*Ribes triste*	Ribetris
Common blackberry	*Rubus allegheniensis*	Rubualle
Wild Red raspberry	*Rubus idaeus*	Rubuidae
Flowering raspberry	*Rubus odoratus*	Rubuodor
Red elderberry	*Sambucus racemosa*	Sambrace
Mountain ashes	*Sorbus* species	Sorbspp
Poison ivy	*Toxicodendron radicans*	Toxiradi
Maple leaf Viburnum	*Viburnum acerifolium*	Vibuacer
Non-natives		
Garlic mustard	*Alliaria petiolata*	Allipeti
Deadly nightshade	*Atropa belladonna*	Atrobell
Lily of the valley	*Convallaria majalis*	Convmaja
Crown vetch	*Coronilla varia*	Corovari
Queen Anne's lace	*Daucus carota*	Dauccaro
Viper's bugloss	*Echium vulgare*	Echivulg
Helleborine	*Epipactus helleborine*	Epiphell
Euonymus	*Euonymus* species	Euonspp
Herb Roberts	*Geranium robertianum*	Gerarobe
St. John's wort	*Hypericum perforatum*	Hypeperf
Butter and eggs	*Linaria vulgaris*	Linavulg
Sweet whie clover	*Melilotus albus*	Melialbu
Common plantain	*Plantago major*	Planmajo
Heal-all	*Prunella vulgaris*	Prunvulg
Buckthorn	*Rhamnus cathartica*	Rhamcath
Wood betony	*Stachys officinalis*	Stacoffi
Lilac	*Syringa vulgaris*	Syrivulg
Dandelion	*Taracaxum officinale*	Taraoffi
Alsike clover	*Trifolium hybridum*	Trifhybr
Red clover	*Trifolium pretense*	Trifprat
Coltsfoot	*Tussilago farfara*	Tussfarf
Common mullein	*Verbascum thapsus*	Verbthap
Highbush cranberry	*Viburnum opulus*	Vibuopul
Cow vetch	*Viccia cracca*	Vicccrac
Wild grape	*Vitis* species	Vitisspp