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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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Manuscripts

1   **Exotic earthworm (Oligochaeta: Lumbricidae) assemblages on a landscape-scale in central**

2   **Canadian woodlands: Importance of region and vegetation type**

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20   **Abstract:** A growing understanding about the impacts of earthworms (Oligochaeta:

21   Lumbricidae) on ecosystem processes and forest restoration necessitates an examination of their

22   role in Canadian forests where they have become invasive. Little is known about the landscape

23   scale responses of earthworm populations to different regional characteristics and vegetation

24   types within Canada's central woodlands. We examined the regional variation of earthworm

25   species richness, biomass, and assemblage composition across a range of four municipal regions

26   (from south to north: Halton, Wellington, York, and Simcoe) and four habitat types (deciduous

27   forest, mixed forest, tree plantation, and meadow) with varying soil characteristics in woodlands

28   of south-central Ontario, Canada. In general, earthworm communities differed by region but not

29   by habitat type. The most southern regions supported the highest earthworm species richness,

30   biomass (*i.e. Lumbricus* and *Octolasion*), and density, and this was associated with a south-north

31   gradient in soil characteristics. Assemblage composition differed by region but not by habitat

32   type. The observed south-north gradient suggests an underlying effect of invasion spread

33   associated with human settlement and density. Our results provide baseline information about

34   earthworm communities in south-central Ontario forests, and will enable managers to plan for

35   the increasing role of earthworms in Canada's future forests.

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

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Introduction

42

43   The southern extent of the last Wisconsonian 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).

64 Changes caused by the invasion of earthworms are also known to result in a loss of carbon (C)

65 storage due to increased respiration and microbial activity; however, in the long term,

66 earthworms may stabilize soil carbon by incorporating greater amounts of the litter layer into

67 their casts and stable aggregates (Bohlen et al. 2004).

68 The pattern of invasion and impact of earthworms is much dependent on land use history,

69 soil type, and functional traits of the invading species, amongst other factors (Frelich et al. 2006).

70 Whether a species is classified as epigeic, endogeic or anecic will determine the potential change

71 in soil characteristics. In general, as species richness increases, the thickness of the litter and duff

72 layer of the soil decreases (Gundale et al. 2005). McLean and Parkinson (1997) found that

73 epigeic species had effects on organic matter distribution and soil structure. Variation in the

74 effects of these functional groups are expected due to their differential impact on the litter 'LFH'

75 layers, where 'L' is the top organic horizon composed of accumulating leaves and twigs and still

76 easily recognized, 'F' is the layer of partially decomposed organic matter with a sometimes matt-

77 like appearance due to fungal hyphae and decaying litter, and 'H' is the lowest organic horizon

78 in which the original organic structures cannot be recognized and have been converted to humic

79 material (Denholm et al. 1993; Soil Classification Working Group 1998). Epigeic species mix

80 and consume the 'FH' layers, while the upper litter layer (L) remains intact (McLean and

81 Parkinson 1997; Hale et al. 2005). Endogeic and anecic species move deeper into the soil layers,

82 mixing the upper 25-30 cm of the mineral soil horizons (B or C) and converting the undisturbed

83 LFH and thin A horizon characteristic of native soils to a mull soil more similar to previously-

84 tilled agricultural soil (Frelich et al. 2006), and in turn impacting water infiltration (Capowiez et

85 al. 2014).

86   Understanding the biogeographic patterns of invasive species as they colonize new

87   regions will help predict patterns of spread and the relative vulnerability of particular regions and

88   ecosystems. At the landscape scale, the distribution of earthworms appears to be dependent on

89   habitat suitability (pH and litter quality), climatic conditions (temperature and moisture), human

90   activity, and land use practices (Tiunov et al. 2006). Among habitat types, earthworm abundance

91   and species richness are generally the highest in deciduous forests and the lowest in old

92   meadows and coniferous forests (Smith et al. 2008). In some cases, the dominant tree species

93   may be important; for example, earthworms occurred less frequently in forests dominated by

94   American beech (*Fagus grandifolia*) and hemlock (*Tsuga canadensis*) than in other hardwoods

95   (Suárez et al. 2006b). In areas where forests are transitioning from southern deciduous species to

96   northern coniferous species, earthworm invasion may progress more slowly due to either

97   combined or individual effects of a decrease in litter quality (changes in tree species

98   composition), decrease in soil pH, or decrease in temperature along a latitudinal gradient

99   (Addison 2009). Studies in Europe have shown earthworm distribution at the landscape level to

100   be dependent on climatic conditions, habitat suitability (pH and litter quality), and human

101   activity and land use practices (Tiunov et. al. 2006).

102   Little is known about the distribution, impact or drivers of earthworm expansion into

103   central Canadian forests. Here, we conduct a landscape-scale study to compare earthworm

104   communities in different regions and habitat types in central Canadian woodlands. Specifically,

105   we examine the regional variation in earthworm community attributes (species richness, density,

106   biomass, composition) across a range of municipal counties and habitat types in south-central

107   Ontario, and identify habitat characteristics associated with attributes of earthworm communities.

108   We predict that southern regional jurisdictions will have greater earthworm abundance and

109   species richness, especially for *Lumbricus* and epigeic species, than the northern regions, due to

110   the history of European settlement in this region. We also expected to find similar earthworm

111   species among similar habitat types due to the local biotic and abiotic conditions associated with

112   each, and higher biomass in deciduous forests than in other habitat types (Smith et al. 2008).

113

114   **Methods**

115   **Study design**

116   To analyze whether earthworm communities differed between regions and habitat type,

117   we selected four regional municipalities distributed along a ~125km transect in the south-north

118   direction of south-central Ontario: Simcoe County, York Region, Wellington County, and Halton

119   Region (Fig. 1; Table 1). These regions encompass the northern edge of the Carolinian forest

120   ecotone (Halton) as well as the Great-Lakes-St. Lawrence forest ecotone (Wellington, York, and

121   Simcoe) (NRCan 2012). We included four habitat types: deciduous forest, mixed forest, tree

122   plantation, and meadow. Within each of the four regions, two subsamples (sites) of each habitat

123   type were selected (with the exception of Halton Region where only one suitable meadow site

124   could be found) for a total of eight (seven in Halton) sites per region (total sites=31) (Fig. 1).

125   Sites were selected of similar age, history, and estimated recreational use wherever possible in

126   order to decrease variation (Table 2).

127   At each site, five plots (10 m x 10 m) were sampled for earthworms, soils, and vegetation

128   during September-October, for a total of 155 plots. The plots were systematically selected

129   without preconceived bias at 30-50 m towards the interior of the forest but within 100 m from

130   any road or trail. This approach to sampling was used in order to account for the degree of

131   uncertainty about differential invasion in each region and the potential for correlation between

132   earthworm abundance and proximity to roads (Cameron et al. 2007; Sackett et al. 2012).

133   Sampling was done at least 50 m away from a forest edge in order to ensure interior habitat.

134   Each plot was sampled once.

135

Study area and site description

137   The four study regions were similar in topography and representative of their respective

138   forest ecozones. Predominant tree species in the seven northern Carolinian forest sites included

139   American beech, maples (*Acer* spp.), black walnut (*Juglans nigra*), hickories (*Carya* spp.), and

140   oaks (*Quercus* spp.), while the 24 sites in the Great Lakes-St. Lawrence forest were

141   characterized by a greater percentage of conifers, such as red pine (*Pinus resinosa*), eastern white

142   pine (*Pinus strobus*), and eastern hemlock (*Tsuga canadensis*), as well as by yellow birch (*Betula*

143   *alleghaniensis*), maples, and oaks. The soils were variable among the regions, with podzolic soils

144   generally found in the north, andgleysols, luvisols, and brunisols in the south (Table 1). Average

145   annual precipitation and temperature was similar between the four regions, and all sites are

146   below 400 m in altitude.

147   All meadow and plantation sites we selected were historically used for agriculture, with

148   the exception of the two plantation sites in York region where there was no record of use for the

149   cleared land prior to the plantation; agricultural use was likely in this case too, considering the

150   historical patterns of cleared land across southern Ontario (Table 2). The deciduous and mixed

151   forest sites in the two southerly regions, Halton and Wellington, were also historically used for

152   agriculture, with the exception of one mixed forest site in Halton, which had always remained as

153   forest. The deciduous and mixed forest sites in the two northern regions, York and Simcoe, had

154   never been cleared, and had no agricultural record. Forest management activities in these regions

155   are presently overseen by their respective regional foresters, with the exception of Wellington

156   County, where management is conducted by the City of Guelph and the Grand River

157   Conservation Authority. These forested areas are currently open to the public for a range of

158   recreational activities, including hiking, horseback riding, and cycling.

159

160   **Habitat types**

161   We defined the four habitat types based on the following criteria: (a) Deciduous Forest,

162   dominated by deciduous species such as sugar maple (*Acer saccharum*) or ash (*Fraxinus* spp)

163   comprising more than 50% of the total tree cover, and with only a minor component of

164   coniferous species, if any; (b) Mixed Forest, with more than 25% of the total canopy cover

165   consisting of coniferous species such as hemlock, and usually having a higher diversity of tree

166   species than deciduous forests; (c) Plantation Forest, originally planted (usually in rows) as a

167   monoculture of one or a few conifer species such as white pine, red pine or white spruce

168   (*Piceaglauca*); and (d) Meadow, an area with less than 25% cover of tree and shrub species,

169   composed largely of grasses and forbs such as goldenrods (*Solidago* spp.), and not actively

170   maintained for agricultural use (Lee et al. 1998) (Table 2).

171

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 ±0.001g, grouped by genus, with the exception of *D. octaedra* and

193   *D. rubidus,* since they could be identified to species as juveniles.

194

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

Soil sampling and analysis

209   Approximately 10 cm^3 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),

215   silt (2–50 μm), and clay (0–2 μm) based on Stoke's law. Soil pH was measured from a well-

216   mixed slurry of 10 g of fresh soil and 40 mL of distilled water (Hendershot et al. 2008).A

217   separate soil sample was taken from the top 10 cm of soil of each plot using a metal ring of fixed

218   dimensions (7.6 cm D x 4.4 cm H) and thus, a fixed volume (199.7 cm^3) to determine bulk

219   density.

220   **Statistical analyses**

221   For statistical analysis, earthworm data from the two habitat subsamples per region were

222   pooled, and environmental data (soil and vegetation data) were averaged over the two habitat

223   subsamples per region. We decided to pool data rather than analyze subsample data in nested

224   analyses due to the high dissimilarity in earthworm biomass between many subsample pairs. This

225   high earthworm spatial heterogeneity on a local scale is not unusual based on repeated sampling

226   in other sites across this region of southern Ontario (T. Sackett, pers.obs.).

227   We compared the biomass of earthworms among habitat types and regions, and

228   considered both total biomass and biomass of earthworm genera for all samples where there were

229   more than two observations (*Aporrectodea, Dendrobaena, Lumbricus,* and *Octolasion*).

230   Differences in total earthworm biomass among habitat types and regions was tested using a

231   general linear model (function lm in R); earthworm biomasses were (log+1) transformed before

232   analysis to achieve normality of residuals and homogenous variances. For each separate

233   earthworm genus, the error residuals and variances could not meet model assumptions even after

234   transformation. Therefore, we used a permutation (i.e. randomization) test to detect non-random

235   patterns of earthworm biomass with habitat and regions. For each genus, we randomized the

236   biomass data over all cells (habitat and region) and calculated the F-statistic for habitat and

237   region effects using the lm function. We repeated this for 5000 iterations, creating a random

238   distribution of F-statistics for these data. We then compared our actual F-statistic with this

239   distribution to find the probability that the actual statistic was obtained from the random

240   distribution.

241   Non-metric multidimensional scaling (NMDS) was used to visually explore differences

242   in earthworm community composition among sites, after which, we used permutational

243   multivariate analysis of variance (pMANOVA) to test for differences in earthworm assemblage

244   composition among habitats and regions (Anderson 2001; McArdle and Anderson 2001). This

245   analysis is a non-parametric analysis of variance of distance matrices among groups. If any

246   factors in the overall pMANOVAmodel were significant, we performed pairwise tests. For both

247   the ordination and pMANOVA, we included only those earthworms identified to species (i.e.,

248   specimens only identified to genus were eliminated for the analysis), and also removed species

249   where less than five individuals were collected (*D. rubidus, A. trapezoides*) in order to reduce the

250   effect of these rare species on the analysis. Sites where no earthworms were identified to species

251   were also removed; this included the Plantation Forests in the Wellington region, and all

252   Meadow sites because only one of the seven Meadow sites had individuals identified to species.

253   For the remaining sites, we pooled the data from the five collections per sub-sample and two

254   sub-samples per habitat to yield 11 samples in the data set.

255   For the NMDS, we used a scree plot of dimensions versus stress to choose the number of

256   dimensions for the ordination (2 dimensions were adequate).The metaMDS function from the R

257   package {vegan} was then used to perform the NMDS of species composition among sites,

258   building a Bray-Curtis distance matrix. We were particularly interested in exploring whether any

259   measured environmental or geographical (i.e., latitude) variables were correlated with

260   differences in species composition among the sites, as represented in the NMDS configuration.

261   To evaluate this, we used the envfit function from the {vegan} package to test the strength and

262   direction of the relationship between the environmental variables with the NMDS sample

263   coordinates. Values from environmental variables were log-transformed before overlaying

264   because envfit assumes a linear relationship between the environmental vectors and the

265   ordination coordinates. For the two-way pMANOVA model (habitat and region as predictors), F-

266   statistics were estimated from 1000 permutations of the Bray-Curtis distance matrix using the

267   adonis function in the R library vegan.

268   We tested for significant relationships between environmental (edaphic: soil pH,

269   moisture, organic matter, bulk density; vegetation: [basal area conifer and deciduous?]) variables

270   and the predictors of region and habitat using general linear models (least squares estimation

271   method) (function lm in R).

272

273   **Results**

274

275   **Earthworm richness and biomass**

276   A total of 806 earthworms from nine species and 5 genera (*Dendrobaena, Dendrodrilus,*

277   *Aporrectodea, Octolasion, Lumbricus)* were collected in our study (Tables 3 and 4). Two

278   specimens of unknown and unidentifiable species were not included in the analyses. The highest

279   number of earthworm species was found in Halton Region (Table 3). *Aporrectodea rosea*, *A.*

280   *tuberculata*, *Octolasion* sp., and *Lumbricus terrestris* were absent from Simcoe County samples,

281   whereas *A. rosea* and *A. turgida* were not found in York County. *Dendrobaena octaedra* and

282   *Dendrodrilus rubidus* were absent in Wellington County samples and *D. rubidus* absent in

283   Halton Region. All nine species were collected with our sampling system in the Deciduous

284   Forests, and *Aporrectodea trapezoides* was found only in Deciduous Forests (2 specimens)

285   (Table 4). Meadows held the lowest species richness among the studied habitat types, where only

286   *A. tuberculata, A. turgida, Octolasion* sp. and *L. rubellus* were collected. Earthworms sampled in

287   the most southerly sites of Halton Region comprised 58% of the total number of earthworms

288   collected; York, Wellington, and Simcoe Regions represented 21%, 16% and 5% of the total,

289   respectively (Table 3). Throughout the four regions, the total density of earthworms was the

290   highest in the Deciduous Forests (50%), followed by the Mixed Forests (32%), the Plantation

291   Forests (15%), and finally the Meadows (3%) (Table 4).

292   Earthworm biomass ranged from 0 to 47 g per m^2 (0 to 153 individuals per m^2) in the

293   samples. The general linear model showed that total earthworm biomass decreased with

294   increasing latitude ($F_{1,11}$=6.60; p=0.026) (Fig. 2), indicating regional differences in biomass with

295   the highest being in Halton Region followed by Wellington, York, and Simcoe Regions.

296   Permutation tests on each earthworm genus indicated that the biomass of *Lumbricus* (p=0.036)

297   and *Octolasion* (p=0.044) genera both decreased with increasing latitude, but there was no

298   relationship between the biomass of either *Dendrobaena* or *Aporrectodea* with latitude

299   (p>0.050). Neither total earthworm biomass, nor the biomass of a particular genus, differed

300   significantly among the four habitats (p>0.050).

301

302   **Earthworm community composition**

303   The NMDS ordination (Fig. 3) showed species composition and assemblages to be

304   grouped similarly by region (Final stress = 0.06; probability of achieving observed stress through

305   randomization = 0.04). Sites in the Simcoe and York Regions grouped at the left of the biplot

306   (upper and lower part, respectively) while those from Wellington and Halton Regions grouped

307   together on the right of the biplot. The ordination coordinates for the samples were significantly

308   associated with region and soil pH ($p < 0.050$). *Dendrodrilus rubidus* and *Dendrobaena*

309   *octaedra* were associated with Simcoe and York Regions, respectively, whereas *Lumbricus* sp

310   and *Aporrectodea turgida* were associated with the southern regions. The pMANOVA indicated

311   that the species composition in the earthworm assemblages differed among regions ($F_{3,5}=3.05$,

312    $p=0.020$) but not among habitats ($F_{2,5}=0.82$, $p=0.640$). Pairwise comparisons showed that

313   assemblage composition differed between the northernmost Simcoe Region and the two

314   southerly regions, Wellington and Halton ($p<0.050$). Differences in functional groups were not

315   clear between habitat types. Mixed Forests and Plantation Forests had the highest density of

316   epigeic earthworm species, which were absent from in the Meadow samples (Table 4). Both

317   endogeic earthworms and the *Lumbricus* group were most abundant in Deciduous Forest and

318   Mixed Forest types.

319

320   **Regional and habitat variations in soil**

321   The general linear models indicated that latitude was associated with differences in soil

322   characteristics such as pH, gravimetric soil moisture, organic matter, and proportion of two size

323   classes of sand. Soil pH, proportion of organic matter, and percentage moisture all decreased

324   with increasing latitude, from Simcoe to Halton Region (Table 5). The linear models indicated

325　　that pH decreased by 1.1 units with each (decimal) unit of latitude ($F_{1,11}$=40.6; p<0.001), ranging

326　　from average value of 6.8 in the southern Wellington and Halton Regions, to 5.7 in the

327　　northernmost region, Simcoe. Organic matter decreased by 6% with each unit of latitude

328　　($F_{1,11}$=8.4; p=0.014), ranging from 12-14 % in Wellington and Halton Regions to 7% in Simcoe

329　　Region. Soil moisture decreased by 22% with each unit of latitude ($F_{1,11}$=15.2; p=0.002), ranging

330　　from 37-44% in Wellington and Halton Regions to 16% in Simcoe Region. Habitat types were

331　　less associated with differences in soil characteristics than latitude. Only soil pH varied

332　　significantly across all habitats ($F_{3,11}$=6.2, p=0.010), and pairwise tests indicated that Meadows

333　　were significantly drier than Mixed Forests (p<0.050).

334

Discussion

336　　　　Nine of the 17 known species of exotic earthworms in Ontario were collected in this

337　　regional study across south-central Ontario, representing the most common species known to be

338　　established in the province (Addison 2009). This is the first study since Reynold´s (1977) to

339　　examine specific site and habitat information for earthworm assemblages across regions in south-

340　　central Canada.

341　　　　Earthworm biomass varied significantly among the four municipal regions we studied,

342　　and this was correlated with soil characteristics. As expected based on visible signs of invasion

343　　(patches of missing leaf litter and earthworm middens, casts, and burrow entrances from Suárez

344　　et al. 2006a), the southern Halton Region had the highest richness, total biomass, and density of

345　　earthworms, followed in decreasing order by Wellington, York, and Simcoe Regions for biomass

346　　and density. In contrast, the opposite pattern was seen when only epigeic species were

347 considered, with the more northerly Simcoe and York Regions having the highest densities of

348 this functional group. Although there are inherent differences in the ecological function of

349 various epigeic species such as between *L. rubellus* and *D. octaedra*, we expected that epigeic

350 species in general would be more dominant in sites with sandier soils since work by others has

351 shown a relationship between their abundance and litter depth (McLean and Parkinson 1997),

352 especially in sites where earthworms had been introduced more recently (Hale et al. 2005). In

353 northwestern Canada, Cameron et al. (2007) found that epigeic species such as *D. octaedra* were

354 more abundant in areas with deeper litter, but they considered this result might be due to

355 unexplained environmental factors such as time since invasion.

356   Soil characteristics, litter quality, and litter quantity are known to influence earthworm

357 abundance and composition. Increased deciduous regeneration provides litter for many

358 earthworm species, but this is not equally palatable to all. The Plantation Forests in Wellington

359 Region contained saplings of only one tree species whereas those in Halton Region had up to 10

360 species of deciduous tree saplings. In our study, the number of palatable tree species affecting

361 leaf litter, soil pH, and temperature all decreased from south to north, and this would likely slow

362 the rate of earthworm invasion and changes in the forest (Addison 2009) and help explain the

363 differences we observed in Simcoe Region, where the lowest density of earthworms was found

364 together with the lowest soil pH, moisture, and organic matter. In laboratory studies, *L. terrestris*

365 biomass and growth was greatest at 20ºC and 30% soil moisture and reproductive structures

366 developed slower at cooler temperatures (Berry and Jordan 2001). Moreover, York and Simcoe

367 Regions both had high amounts of fine and coarse sand respectively, which corresponded with

368 low earthworm densities. In the national forests of Minnesota and Wisconsin (USA), Holdsworth

369 et al. (2007b) also found increased sandiness to be correlated with decreased earthworm

17

370   abundance. Thus, it is not surprising that we found epigeic species to be in higher proportion in

371   the two northerly regions as they appear to tolerate lower pH and more sandy sites.

372   The low density and biomass of earthworms in the most northerly regions of our study

373   could also possibly be explained by the differential rate of invasion over this latitudinal gradient,

374   with the northern sites likely being invaded more recently than the southern ones. According to

375   Hale et al. (2005), newly invaded areas would be expected to have a higher abundance of epigeic

376   species compared to anecic and endogeic species, and this is consistent with what we observed in

377   the northern Simcoe Region where there was a high proportion of epigeic and *Lumbricus* species

378   but a small proportion of endogeic species. In addition, because *L. terrestris* adults were not

379   found in this region, it is likely that the *Lumbricus* component (adults and immatures) was

380   largely composed of the epi-endogeic species *L. rubellus*, which is also capable of tolerating low

381   pH and functions similarly to the other two epigeic species found. In contrast, the most southern

382   regions of Halton and Wellington both had very low densities of epigeics and were dominated by

383   endogeic species. The epigeic species that we saw in the northern regions, *Dendrobaena*

384   *octaedra* and *D. rubidus*, were not found in the two southerly regions, while *A. rosea* was not

385   found in the northerly regions. The lack of *Octolasion* species and *L. terrestris* in the northern

386   Simcoe Region was somewhat surprising given that it was in close proximity to large bodies of

387   water where frequent introductions of these species would be thought to occur from sport

388   fishing. *Lumbricus terrestris* has a relatively slow rate of spread among the species considered

389   here, so its absence in the north could be explained if the original introduction was in the

390   southern sites. Although these variations in functional group composition and earthworm

391   abundance are consistent with predictions from the theory of invasion succession (Hale et al.

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392   2005), there may also be other factors, especially habitat, soil characteristics, and the relatively

393   low sample size that contribute to the observed patterns and must also be considered.

394   In contrast to our predictions, earthworm biomass and the species assemblage

395   composition did not differ significantly among the four habitat types. Based on soil pH, it was

396   predicted that the Plantation Forests would have the highest proportion of epigeic species,

397   followed by the Mixed Forests and Deciduous Forests. Epigeic species were not expected to be

398   abundant in the Meadow sites because the litter layer there would have been reduced by regular

399   agricultural tilling, leaving them unsuitable for earthworm habitat (Nuutinen et al. 1998).

400   Plantation Forests had five times fewer *Lumbricus* species than endogeic species, which was

401   surprising because endogeic species are not usually found in forest plantations whereas *L.*

402   *rubellus* is an acid-tolerant species frequently collected from them (Ammer et al. 2006). The

403   relatively high pH of the Plantation Forest sites, especially in the more southerly regions (Table

404   5), might account for this unexpected result, while the relatively high bulk density of the

405   Meadow sites might account for the low overall abundance of earthworms found there. Smetak

406   et al. (2007) showed that younger urban habitats had increased bulk density due to compaction

407   than more natural habitats, and that this resulted in lower overall earthworm abundance.

408   The stage of succession in the conversion of Plantation Forests to more natural

409   Deciduous Forests potentially had an influence on the earthworm communities we observed in

410   this study. Two of the conifer plantations in the Wellington Region never thinned since being

411   planted in 1982 (M. Neumann 2012, pers. comm.) had no earthworms present whereas the

412   Plantation Forests in Halton Region that had been thinned to promote deciduous regeneration

413   (Gartner Lee Ltd. et al. 2002) averaged 7.38 earthworms per m^{-2}. Thus, variations in forest

414   management history and practices between the different regions are important in helping to

415   explain local earthworm abundance and assemblages.

416   Based on the general lack of historical information for the timing of earthworm invasion

417   in our study areas, it is difficult to determine definitively the cause of variation in their

418   earthworm communities across the four regions. We do know that the southern sites were settled

419   for agriculture slightly earlier (e.g. late 1700s to early 1800s) than the northern sites (e.g. late

420   1800s to early 1900s). In addition, agricultural activities have tended to be more intensive in the

421   southern than in the northern regions, and past land use and management have likely had a

422   profound influence on any associated soil characteristics. Thus, it is likely that our results

423   identify the presence of a latitudinal gradient in earthworm invasion for this region as soil

424   variables were well within the range for survival and likely had little effect on historical

425   movement and introduction patterns. The spread of earthworms is primarily anthropochorous,

426   and species distributions in forests differ depending on the type of human activity (e.g.,

427   settlement, roads, fishing) and land-use history (e.g., Bohlen et al. 2004; Gundale et al. 2005;

428   Cameron et al. 2007; Cameron and Bayne 2009; Sackett et al. 2012). Human population

429   estimates in the northern region were 92/km^2 whereas those in the southern region averaged

430   550/km^2 (Statistics Canada 2011) supporting the likelihood that human density is an important

431   explanatory variable for differences in the earthworm communities we observed here, and this

432   suggests further investigation is warranted into the effects of human settlement. Our results

433   provide new baseline information on the status, distribution, and relationship of functional

434   earthworm communities with respect to habitat characteristics in south-central Ontario. Armed

435   with this improved understanding, forest managers will be better able to plan for these soil

436   invaders as they continue to advance throughout Canada's forests.

437

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567   Table 1 –Location, soil order, precipitation, and temperature (1982-2012) of the four study

568   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

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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 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 (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.

[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 2010).

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

[d]unk = 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.

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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					
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 (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	

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583 Table 4.Mean densities and range of earthworm species collected from the four habitat types in

584 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				
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 (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

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589   Table 5. Mean values of soil variables and statistical variation between regions or habitat types

590   based on the top 10 cm of soil from the four sites sampled in southern Ontario. GSM:

591   gravimetric soil moisture, OM: organic matter, BD: bulk density

	pH	GSM (%)	OM (%)	BD (g/cm³)	% 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

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602   **Figure captions**

603

604   Figure 1. Centroid locations of the 31 study sites for the four study regions (shaded in grey) in

605   southern Ontario (shaded).○=deciduous forests (DF), △=mixed forests (MF), □= plantations

606   (PP), +=meadows (M).

607

608   Figure 2. Total earthworm biomass at 15 sites in four different habitat types distributed

609   latitudinally across southern Ontario, Canada. The solid line is the linear regression line and the

610   shaded area is the 95% confidence region (r^2=0.55; p=0.048). Habitat abbreviations are as in

611   Figure 1.

612

613   Figure 3. Ordination (NMDS) plots of earthworm species assemblage composition from 15

614   collections across southern Ontario. Points are individual observations and ellipses enclose 1 SD

615   of the data for samples within the Halton (H), York (Y) and Simcoe (S) Regions. No ellipse is

616   drawn for Wellington (W) Region as only two samples are plotted. Gray arrows indicate the

617   direction of significantly correlated environmental or geographical variables with differences in

618   species composition among sites. Hollow diamonds with species names indicate the relative
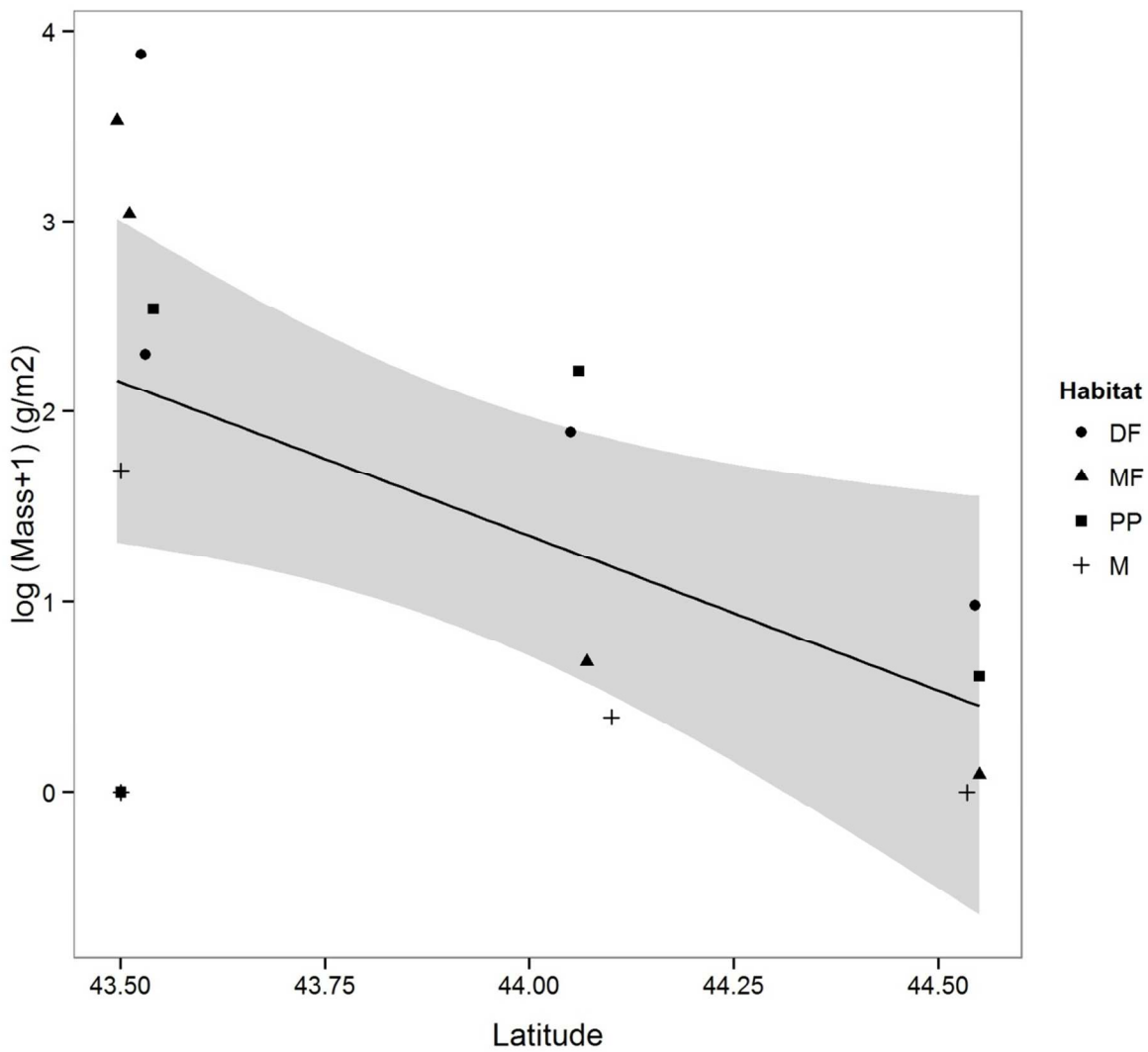
619   distribution of species among sites.

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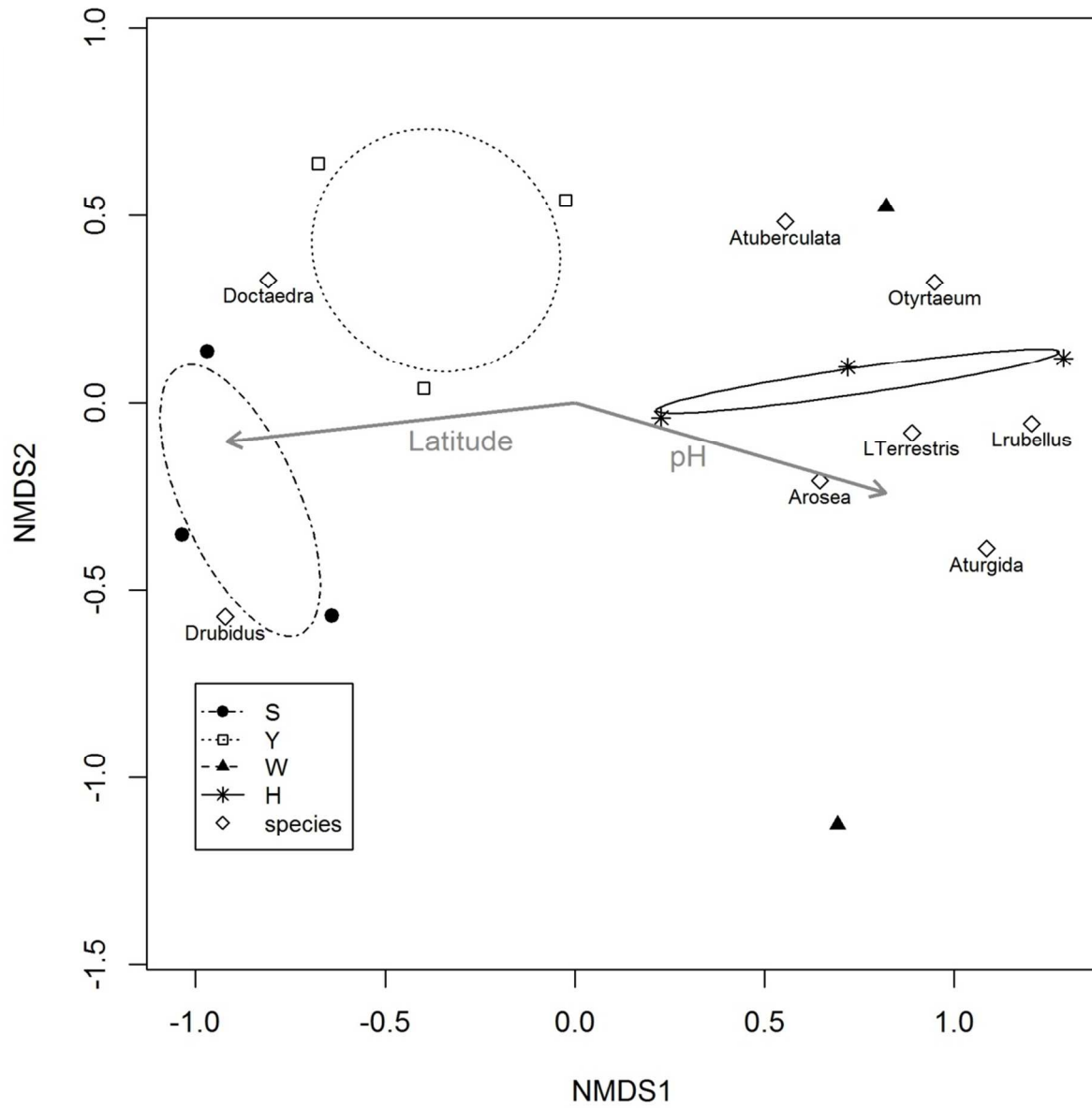
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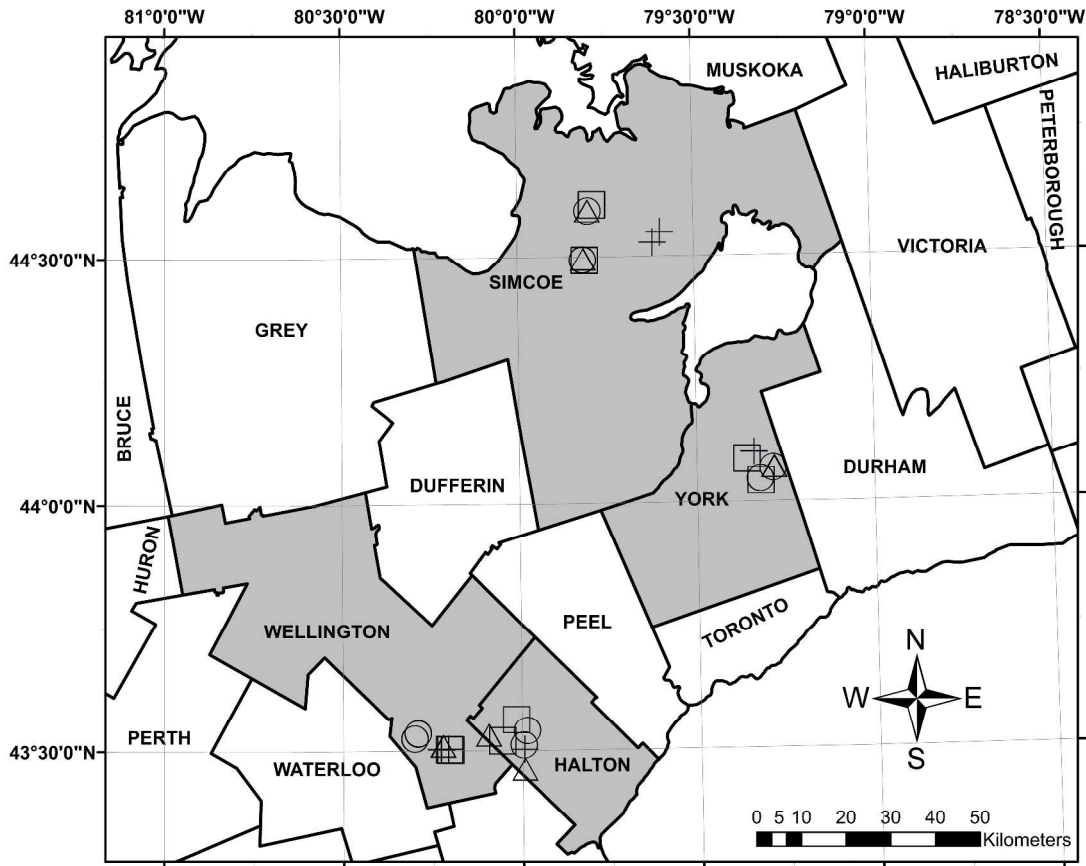
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