Invasive Earthworm (Oligochaeta: Lumbricidae) Populations in varying Vegetation Types on a Landscape- and Local-scale

By

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ABSTRACT

There have been no landscape-scale studies on earthworm populations in Canada comparing vegetation types; previous studies on edge habitats have been conducted in agricultural systems. I examined the spatial variations of earthworm populations by measuring abundance based on regional municipality, vegetation type, and edge habitat. Earthworms were sampled throughout the season across a gradient of vegetation types including meadow, forest edge, and interior at a local-scale; and at the landscape level with vegetation types including meadow, deciduous forest, pine plantation and mixed forest. Regional effects were more significant than vegetation type likely due to a gradient of soil characteristics in southern Ontario; edges had intermediate earthworm abundance and a higher proportion of epigeic species. My research provides insight into the patterns of earthworm populations in southern Ontario and the possible effects of edge creation through landscape fragmentation. Field sampling of earthworm parasitoid cluster-flies (Calliphoridae: *Pollenia*) using synomones was also discussed.

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GENERAL INTRODUCTION

History and Background on Earthworms

It is generally believed that the last Wisconsonian glaciation eliminated most native earthworm species from Canada about 10,000+ years ago (Callaham et al. 2006, Addison 2009). As a result, the soils, vegetation and ecosystem processes of Canadian forests have developed in the absence of earthworms and their activity. Exotic earthworm species (Oligochaeta: Lumbricidae) were introduced into North America during the late 1800s, presumably through the release of contaminated soil in ship ballasts from Europe (Reynolds 1977, Tiunov et al. 2006). There are currently 19 known species of exotic earthworms in Canada, with 17 present in Ontario (Addison 2009). Some of the most common species found in Canada include: *Lumbricus terrestris* (Linnaeus 1758)*, Aporrectodea tuberculata* (Eisen 1874)*, Eisenia fetida* (Savigny 1826)*, Dendobaena octaedra* (Savigny 1826)*, Dendrodrilus rubidus* (Savigny 1826)*,* and *Allobophora chlorotica* (Savigny 1826) (Addison 2009).

Although they have relatively low diversity, all European earthworm species are capable of colonizing new habitats, spreading rapidly and tolerating a range of environmental conditions, thus making them successful invaders in Canadian forest ecosystems (Bohlen et al. 2004b). Moreover, earthworms are considered to be ‘ecosystem engineers’, able to modify their habitats to suit their needs, thereby contributing to their ability to influence the environment as an invasive species (Eisenhauer et al. 2009, Eisenhauer 2010).

There are two species of native earthworms in Ontario, *Bismastos parvus* (Eisen 1874) and *Sparganophilus eiseni* (Smith 1895), the latter belonging to a separate family (Sparganophilidae) (Reynolds 1977, Addison 2009). Both species are rare and prefer aquatic and limnicolous habitats, respectively (Reynolds 1977), presumably having little competitive interaction with the exotic earthworm species.

Earthworm Dispersal

Humans play an important role in the spread of earthworms to previously earthworm-free habitats through the construction of roads, release of live fishing bait, and movement or dumping of contaminated soil and fill (Bohlen et al. 2004b). For example, *L. terrestris*, better known as the Common Nightcrawler, is widely available to anglers for use as fishing bait and *E. fetida* is the main species used for vermiculture where its high reproductive rate leads to improper disposal into wastelands and forests (Addison 2009). As well, forest management activities may lead to the transport of cocoons through logging equipment or through the planting of tree seedlings (Gundale et al. 2005).

Moreover, it is well studied and understood that areas near roads and fishing sites, second-growth forests, and recently harvested or managed forests have higher earthworm richness and abundance than old-growth forests or interior forests away from human access (Gundale et al. 2005, Hale et al. 2006, Cameron et al. 2007). Although proximity to areas of high traffic and disturbance are generally thought to be related to higher abundance of earthworms, earthworms have been shown to be capable of spreading to areas not previously disturbed such as old growth forests (Tiunov et al. 2006). The range of active, non-facilitated dispersal for these earthworms varies by source and has been estimated to be approximately 5-10 meters per year (Tiunov et al. 2006); however, this may depend on the functional group being studied (Eijsackers 2011).

Functional Groups

Earthworm species can be placed into three main functional groups characterized by their burrowing and feeding behaviour, as well as various other common traits (Bouché 1977, Addison 2009).

Epigeic species, such as *D. octaedra* and *D. rubidus* are found on the soil surface and beneath the leaf litter, feeding on the litter (Jégou et al. 1998, Addison 2009, Eggleton et al. 2009). These species are often small and pigmented, as well as parthenogenic with a relatively high reproductive rate (Addison 2009). Moreover, these species are capable of tolerating low pH and frost (Reynolds 1977). **Endogeic species**, such as *Aporrectodea* and *Octolasion* species, horizontally inhabit the mineral horizons in the soil, feeding directly on soil that is enriched with organic matter (Jégou et al. 1998, Eggleton et al. 2009). They are medium-sized and usually non-pigmented (Addison 2009). **Anecic species**, such as *L. terrestris*, excavate deep, permanent burrows (1-2 m) vertically in the soil. They surface to feed on their preferred fresh leaf litter and to deposit their casts (Hale et al. 2008, Addison 2009). These species disturb the soil horizons by drawing organic matter and leaf litter down into their burrows, and by depositing mineral soil on the surface by casting (Asshoff et al. 2010). They are generally the largest of the three groups, slow at reproducing, and pigmented only on the anterior end (Addison 2009).

Lastly, some species are considered to fall into two different groups. For example, *Lumbricus rubellus* (Hoffmeister 1843) is considered **epi-endogeic** due to its habit of feeding on the surface as well as burrowing horizontally in the mineral soil (Addison 2009). As these functional groups are rudimentary in nature, species may not be perfectly categorized based on the entirety of their characteristics. However, these groups provide a means for determining the variations in these worms on the ecosystem.

Impacts on Flora and Soil Fauna

As well as having impacts on soil characteristics and nutrient cycling, invasive earthworms affect native forest flora and fauna. The mulling of the soil and the digestion of the litter layer has had a notable impact on the forest floor. Generally, these changes cause tree seedling and native herb population declines and patches of visible, bare forest floor (Bohlen et al. 2004b, Hale et al. 2006, Corio et al. 2009). *Aporrectodea* species in particular cause the most significant changes in herbaceous community composition in forests (Corio et al. 2009). Other explanations for changes in vegetation include earthworm ingestion and burying of seeds, and seed exposure due to lack of forest floor (Bohlen et al. 2004b).

Earthworms also affect overstory trees due to exposed roots, nutrient leaching and changes in nutrient distribution within the soil horizons, as well as physical disruption and consumption of the fine tree roots (Bohlen et al. 2004b). Decreased abundance and colonization rates of arbuscular mycorrhizae caused by earthworm feeding and soil disturbance could play a role in the impacts on trees (Bohlen et al. 2004b). Consequently, it is possible that these changes result in 20-30 years of decreased radial growth rates in trees that have been recently invaded by earthworms (Larson et al. 2010). Larson et al. (2010) found that trees in areas invaded by earthworms were more sensitive to drought. This may be due to the decrease in mycorrhizae or earthworm ingestion of fine roots. If current climate trends continue to move towards increased frequency and severity of droughts, it is possible that tree species composition and habitat ranges will shift in part as a result of earthworms (Larson et al. 2010).

Madritch and Lindroth (2009) suggested that other invasive species (e.g. common buckthorn (*Rhamnus cathartica* Linnaeus)) may work with invasive earthworms in the facilitation of each others' invasion, termed 'invasional meltdown'. Depending on the tree species composition of a forest, the leaves of common buckthorn may provide a higher quality litter than native tree species such as red oak (*Quercus rubra* L.) and American beech (*Fagus grandifolia* Ehrhart.)

(higher C:N ratio) (Madritch and Lindroth 2009). Other such invasive species that may benefit from the presence of earthworms include garlic mustard (*Alliaria petiolata* (M.Bieb.) Cavara & Grande), because its small seeds do not require a thick forest floor for germination (Hale et al. 2006).

The mechanical disturbance and homogenization of the soil horizons can influence microarthropods found within the soil (such as mites, collembolans, enchytraieds, and nematodes) resulting in decreased biodiversity and abundance of the soil biota (Bohlen et al. 2004b, Eisenhauer et al. 2007). In particular, Orabatida (mites) tend to avoid soil disturbances caused by earthworms; however earthworm burrowing can provide some advantages to other microarthropods by creating increased living space and escape routes from predators (Gutiérrez-López et al. 2010). Due to the variations in burrowing and feeding habits between the different functional groups, the relationships between earthworms and microarthropods are complex and not well studied (Bohlen et al. 2004b, Gutiérrez-López et al. 2010).

Impacts on Soil and Litter Characteristics

Recent research suggests that the invasion of earthworms into temperate forests may result in significant changes to nutrient dynamics and soil structure (Hale et al. 2005). For example, the feeding habits of *L. terrestris* may increase the immobilization of nutrients, as well as increase nitrification and leaching losses, resulting in decreased nutrient availability (Hale et al. 2005). Earthworms may also shift the soil decomposer community from a fungal-dominated system to a bacterial-dominated system or a fungal system antagonistic to decomposing fungi, thereby changing the rate of nutrient cycling and decomposition (Bohlen et al. 2004b, Jayasinghe and Parkinson 2009). 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 through the creation of casts and stable aggregates (Bohlen et al. 2004b).

Changes caused within the soil may vary depending on the functional group being studied. For example, organic matter distribution, soil structure, bulk density and soil nutrients are not significantly affected by epigeic species (McLean and Parkinson 1997). The earthworms have varying impacts on the 'LFH' layers of the soil, consisting of: 'L' - an organic horizon composed of accumulation of leaves and twigs that can still be easily recognized, 'F' - a layer of partially decomposed organic matter with a sometimes mat-like appearance due to fungal hyphae and decaying litter, and ‘H’ - an organic horizon in which the original organic structures cannot be recognized and has been converted to humic material (Denholm et al. 1993, Soil Classification Working Group 1998). Epigeics mix and consume the ‘FH’ layers, while the litter layer (L) remains intact (McLean and Parkinson 1997, Hale et al. 2005). Endogeic and anecic species mix the upper mineral horizons to a depth of 25-30 cm thereby converting 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). In general however, as species richness increases, the thickness of the litter and duff layer decreases (Gundale et al. 2005).

‘Invasion Succession’

It is often difficult to determine when earthworms were introduced to a particular site and their source composition, as well as the progress or stage of the invasion (Frelich et al. 2006). Recent research has suggested the concept of ‘invasion succession’ where certain species pioneer the invasion before other species may follow. Epigeic and epi-endogeic species, particularly *L. rubellus*, are found on invasion fronts. These species are able to feed on the soil surface but also have faster reproduction rates than the other species. Once this initial disturbance occurs, anecic species such as *L. terrestris* are able to create their vertical burrows, proceeding to till and enrich the mineral soil horizons with organic matter. Finally, endogeic species, which require organic matter in the mineral soil to feed on, can invade; these species are often only observed in the most heavily disturbed sites (Suárez et al. 2006a). Holdsworth et al. (2007a) and Hale et al. (2005) found similar patterns of invasion. However, this is a relatively new concept that warrants further research.

Factors Affecting Abundance

Recent studies on the spatial distribution and invasion patterns of exotic earthworms in North America have suggested that source population species composition, varying vectors of transport (human activities and land use), and soil and litter properties are the main factors affecting presence, species composition and abundance of earthworms (Tiunov et al. 2006). However, once earthworm populations have been established, other factors such as soil texture, acidity, richness of base cations, and moisture play an important role in earthworm populations (Bohlen et al. 2004b, Addison 2009). Snyder et al. (2011) found that climatic conditions, in particular moisture, were a major factor in earthworm population and spatial boundary fluctuation.

Two of the important limiting factors of earthworm invasion are acid and frost tolerance (Addison 2009). Epigeic species such as *L. rubellus, D. octaedra,* and *D. rubidus* are capable of tolerating acidic soils, as well as being able to consume lower quality litters (Reynolds 1977, Addison 2009). In Canada, endogeic species *A. tuberculata*, *Aporrectodea trapezoides* (Dugès 1828), and *Octolasion tyrtaeum* (Savigny 1826) were found in coniferous forests, with *L. terrestris* also capable of tolerating lower pH values (Addison 2009). Moreover, some of these species are capable of altering their habitat by raising the pH; the mechanism for this is not fully understood (Addison 2009). Earthworms may be frost tolerant or overwinter deep in the soil; frost tolerance rankings are: *D. octaedra* > *D. rubidus* > *O. tyrtaeum* > *L. rubellus* > *A. calignosa* ≥ *Aporrectodea rosea* (Savigny 1826) > *L. terrestris* (Tiunov et al. 2006).

Context of the Current Study

There has been limited literature discussing regional variations of earthworms (Holdsworth et al. 2007a); existing literature has also been focused solely on deciduous forests. Patterns of earthworm species and abundance in southern Ontario have not been studied in detail. Thus, it is important to understand factors influencing the invasion patterns and extent of earthworms to be able to find accurate predictors of earthworm invasion. Furthermore, few studies have been conducted on the abundance and species composition of earthworms in habitats other than forests, such as meadows. And of those few that have been conducted, all have focused on earthworms as a beneficial organism in terms of agriculture (Smith et al. 2008) or have taken place in parts of Europe where earthworms are native (Zeithaml et al. 2009). More research is needed to understand the differences in species composition and earthworm dynamics between forest and non-forest habitats; this is especially important as these two habitats are often adjacent to one another and have significant interaction. Such studies may become increasingly important as habitat fragmentation in urban and suburban areas continues to increase, creating more edge habitat.

In addition, the study of natural predators of exotic earthworms, such as *Pollenia* species (Diptera: Calliphoridae) has been largely overlooked. The life cycle and biology of these flies is not well known and has only been examined in laboratory environments (Yahnke and George 1972, Thomson and Davies 1973a). In order to study the biology of these flies and their parasitoid relationship with exotic earthworm species, consistent and effective methods for field sampling must be determined.

My thesis addresses these gaps by investigating the relationship of earthworms to varying site habitats. Chapter 1examines the effects of region and vegetation type on earthworm species and abundance in southern Ontario, including the impact of landscape-scale variations and of soil characteristics.

Chapter 2 examines differences in earthworm species and abundance in between a tolerant hardwood forest, an open meadow, and the edge habitat to determine the effect of seasonal soil temperature and moisture on these relationships.

Chapter 3 discusses the effectiveness of two methods for field sampling *Pollenia* species (Diptera: Calliphoridae), a fly parasitoid of earthworms and the impact on vegetation type on sampling success. The thesis concludes with a short summary that integrates the findings and discusses the implications for Canada's changing forests.

Southern Ontario is composed of numerous regions and vegetation types that have been invaded or are at risk of invasion by exotic earthworms. It is well known that deciduous forests are preferred by earthworms over coniferous forests due to their higher litter quality; however, it is not known how vegetation type affects earthworm species and abundance, especially at a regional scale. As most public forests are assessed and maintained by regional municipalities, studying easily visible qualities such as vegetation type is more practical in predicting earthworm abundance than previous studies focusing on the relationship between earthworms and soil characteristics. Moreover, as urbanization increases fragmentation in the landscape, the effect of edge habitats on earthworm populations should become of increasingly interesting, particularly at boundaries between two different vegetation types of variable litter quality.

My research examined these patterns of earthworm invasion and provides insight into the present and future distribution of earthworms in southern Ontario. It also used regional and vegetation information to predict the nature of earthworm species and abundance, and consequently, identified areas at risk for earthworm invasion. By understanding the importance of these factors, targeted monitoring and education programs may be developed in higher risk areas to slow or prevent the spread of earthworms to earthworm-free areas. Adequate field sampling methods for the *Pollenia* parasitoid may lead to future studies of the life cycle and biology of this species, and subsequently, possible methods for biocontrol.

CHAPTER 1

EXOTIC EARTHWORM ABUNDANCE AND COMPOSITION ON A LANDSCAPE-SCALE: IMPORTANCE OF REGION AND VEGETATION TYPE

Introduction

Previous studies have suggested that earthworm abundance and species compositions are determined by factors such as soil and litter quality, as well as source populations and vectors of transport (Tiunov et al. 2006). Unfortunately in Canada, earthworm studies are limited and often rely on secondary data from other studies (Addison 2009). As well, most studies have focused on deciduous forests or coniferous plantations and few comparisons have been made between various forest types, and fewer still between forests and non-forest vegetation types. There is limited literature on the landscape-scale impacts of earthworm species composition and when addressed, regional variations are determined by comparing different studies (Tiunov et al. 2006). The present study examines the impacts of regional and vegetation community on earthworm abundance and species composition, the role of soil characteristics in this variation, and landscape-scale patterns in earthworm populations in southern Ontario.

There are numerous factors driving the study of earthworm populations with respect to region, particularly the lack of research at the county or district level in North America (Holdsworth et al. 2007a). Moreover, habitat heterogeneity being a scale-dependant concept (Aubert et al. 2003), numerous studies have focused on smaller scales as opposed to the stand level or regional-scale. Gutiérrez-López et al. (2010) found that earthworm abundance was related to space rather than soil parameters, while Bohlen et al. (2004a) found that earthworm patchiness may be related to changes in vegetation type (succession from previous land use), rather than recent invasion. Thus, it is interesting to determine whether variations in earthworm invasion are affected by differences in region and/or vegetation type in southern Ontario and the possible explanatory variables that may drive these relationships.

Addison (2009) states that earthworm invasion may progress more slowly as you move north in the Great Lakes-St. Lawrence forest, due to decreases in litter quality (changes in tree species composition), decreases in pH, and decreases in temperature. Joschko et al. (2006) conducted one of the few regional-scale studies in Germany and searched for a similar pattern in earthworm populations. In a 151-km south-north transect, it was found that earthworm abundance increased northwards, following an increasing gradient of soil clay content and pH. In general, earthworms appeared to be most influenced by the regional gradient in soils (caused by previous glaciations) rather than agricultural management methods or vegetation (Joschko et al. 2006). However, it was noted that the transect method of sampling was inadequate, due to the small sample size per site and the large spacing between sites (3 km). Thus, future studies regarding regional scale trends should consist of replicate sampling per site. Since intensive sampling is costly and labour intensive, it is more practical to sample from specific locations based on slope position or vegetation than frequent, random sampling of grids (Whalen 2004).

Other studies in Europe found that landscape-scale distribution of earthworms appears to be dependent on climatic conditions, habitat suitability (pH and litter quality), and human activity and land use practices (Tiunov et al. 2006). However, landscape-scale studies in North America consist mainly of a few studies conducted in the western Great Lakes Region, a smaller-scale area where climatic conditions are assumed to be of lesser importance (Tiunov et al. 2006, Holdsworth et al. 2007a). Earthworm invasion and patterns were similar between the two studied regions, suggesting that variation was negligible on a landscape-scale (Holdsworth et al. 2007a). This may be due to consistencies in soil characteristics, unlike the aforementioned German study. Also, these studies were conducted on national forests dominated by a single vegetation type of mature sugar maple (*Acer saccharum* Marshall).

The impact of vegetation type on earthworm populations has been relatively more studied than landscape-scale influences. It can be presumed that the effects of earthworm invasion are influenced by varying canopy tree species, as these in turn are related to organic matter quality and nutrient cycling dynamics (Bohlen et al. 2004a). Suárez et al. (2006b) found that forest type was the habitat quality that most predicted earthworm distribution; however, their study was based on the presence or absence of earthworms and not abundance. Smith et al. (2008) found earthworm abundance to be highest in deciduous forests, intermediate in old meadows and low in coniferous forests. Similarly, deciduous forests were also highest in species richness; however, coniferous forests were second highest and old meadows were low. However, many of these comparisons between vegetation types are focused on the agricultural aspect of promoting earthworm populations. Overall, vegetation type was found to be related to earthworm populations; however, the variations in earthworm populations between various vegetation types have not been well studied in Canada.

Although it is difficult to determine what stage of invasion exotic earthworms are in, methods for predicting high and low risk areas for earthworm presence and invasion are needed in southern Ontario. The majority of research has focused on soil characteristics, the earthworms' habitat. However, in management scenarios, predictors that can be easily observed by forest managers would be beneficial. By looking for relationships between earthworms and easily visible and determinable factors (such as vegetation type and municipal region), control and prevention methods, such as educational programs, can be focused in high risk areas.

The goal of my study was to determine patterns in earthworm abundance and species richness as related to four regions and four vegetation types within southern Ontario. Three key objectives were to examine: (1) the effect of region and/or vegetation type on earthworm species richness and abundance, (2) the effect of region and/or vegetation type on soil characteristics and discuss the relationship between soil and earthworms, and (3) landscape scale patterns in earthworm populations. I predicted that landscape-scale impacts would be important, with the more southerly regions having greater earthworm species richness and abundance than the northern regions due to differences in soil characteristics. Within each region, I also expected that earthworm species richness and abundance would be greatest in deciduous forests compared to the other vegetation types.

Methods

Study Area

The landscape-scale study was conducted in four regional municipalities of southern Ontario: Simcoe County, York Region, Wellington County, and Halton Region (starting with the northernmost and moving south). These regions encompass both the Carolinian forest and the Great-Lakes-St. Lawrence forest (NRCan 2012). Predominant tree species of southern Carolinian forest include American beech, maples (*Acer* species.), black walnut (*Juglans nigra* L.), hickories (*Carya* species), and oaks (*Quercus* spp.). The Great Lakes-St. Lawrence forest is characterized by increased coniferous content such as red pine (*Pinus resinosa* Aiton), eastern white pine (*Pinus strobus* L.), and eastern hemlock (*Tsuga canadensis* (L.) Carrière), as well as yellow birch (*Betula alleghaniensis* (Britton)), maples, and oaks.

The soils on the landscape were variable with podzolic soils found to the north, and gleysols, luvisols and brunisols in the more southern areas (Table 1.1). Forest management activities in these regions have been overseen by their respective regional foresters, with the exception of Wellington County, where management was conducted by the City of Guelph and the Grand River Conservation Authority. Access to the forested areas is open to the public for recreational activities including hiking, horseback riding, and cycling. Average annual precipitation and temperature are similar between the four regions (Table 1.1).

Table 1.1 - Soil orders found in plot locations, and precipitation, temperature and altitude by region in southern Ontario from 1982-2012.

Region	**Soil Order**	**Average Annual Precipitation[e] (mm)**	**Average Annual Temperature[e] (°C)**	**Altitude[e] (m)**
Simcoe	Podzols[a]	938	6.6	221
York	Grey brown Podzols[b]	857	7.4	198
Wellington	Podzols and gleysols[c]	905	6.6	396
Halton	Gleysols, luvisols and brunisols[d]	884	6.8	221

[a] (Canada Department of Agriculture 1959)
[b] (Agriculture Canada 1977)
[c] (Canada Department of Agriculture 1962)
[d] (Canada Department of Agriculture 1971)
[e] Data taken from weather stations in Barrie, Buttonville Airport, Fergus and Georgetown, respectively (The Weather Network 2012).

Study Design

The experiment was set up in a generalized block design; each treatment was replicated twice within each block. The four regional municipalities were considered as four blocks (regions): Simcoe County (SC), York Region (YR), Wellington County (CG), and Halton Region (HR). The treatments consisted of four vegetation types, including deciduous forest (DF), mixed forest (MF), pine plantation (PP), and meadow (M) (Figure 1.1). Within each of the regions, there were two replicates of each treatment (with the exception of Halton Region where only one suitable meadow site could be found) for a total of eight sites per region (n=31) (Table 1.2) (Appendix 2). Sites were chosen with the aid of the forest manager in each region; sites of similar age and estimated recreational use were selected to decrease variation. At each site, five plots (10 m x 10 m) were sampled from 6 September to 27 October 2011 for a total of 155 plots. The plots were systematically sampled without preconceived bias at 30-50 m towards the interior of the forest but within 100 m from any road or trail, if possible (no obstructions and ensuring the same vegetation type). This method of sampling was selected due to uncertainty regarding the degree of invasion in each region and to the correlation of earthworm abundance to road proximity (Cameron et al. 2007). Sampling was done at least 50 m away from a forest edge to ensure interior habitat. Each plot was sampled once within this period.

Treatment Descriptions

Given that forest can be defined as an area consisting predominantly of tree species and having a canopy cover of greater than 60% (Lee et al. 1998). Four vegetation communities were selected based on the following criteria: (a) deciduous forest as forest dominated by deciduous species such as sugar maple (*A. saccharum*) or ash (*Fraxinus* species) (comprising of >50% of the total tree cover) with a minor component of coniferous species, if any; (b) mixed forest as forest consisting of >25% of total canopy cover of coniferous species such as hemlock and usually having a higher diversity of tree species than deciduous forests; (c) plantation as a forest of originally planted with a monoculture or few species of coniferous species such as white pine, red pine or white spruce (*Picea glauca* (Moench) Voss) usually in rows; and (d) meadow as an area of <25% cover of tree and shrub species and composed of grasses and forbs such as goldenrods (*Solidago* spp.), and should not be actively maintained for agricultural uses (Table 1.2) (Lee et al. 1998).

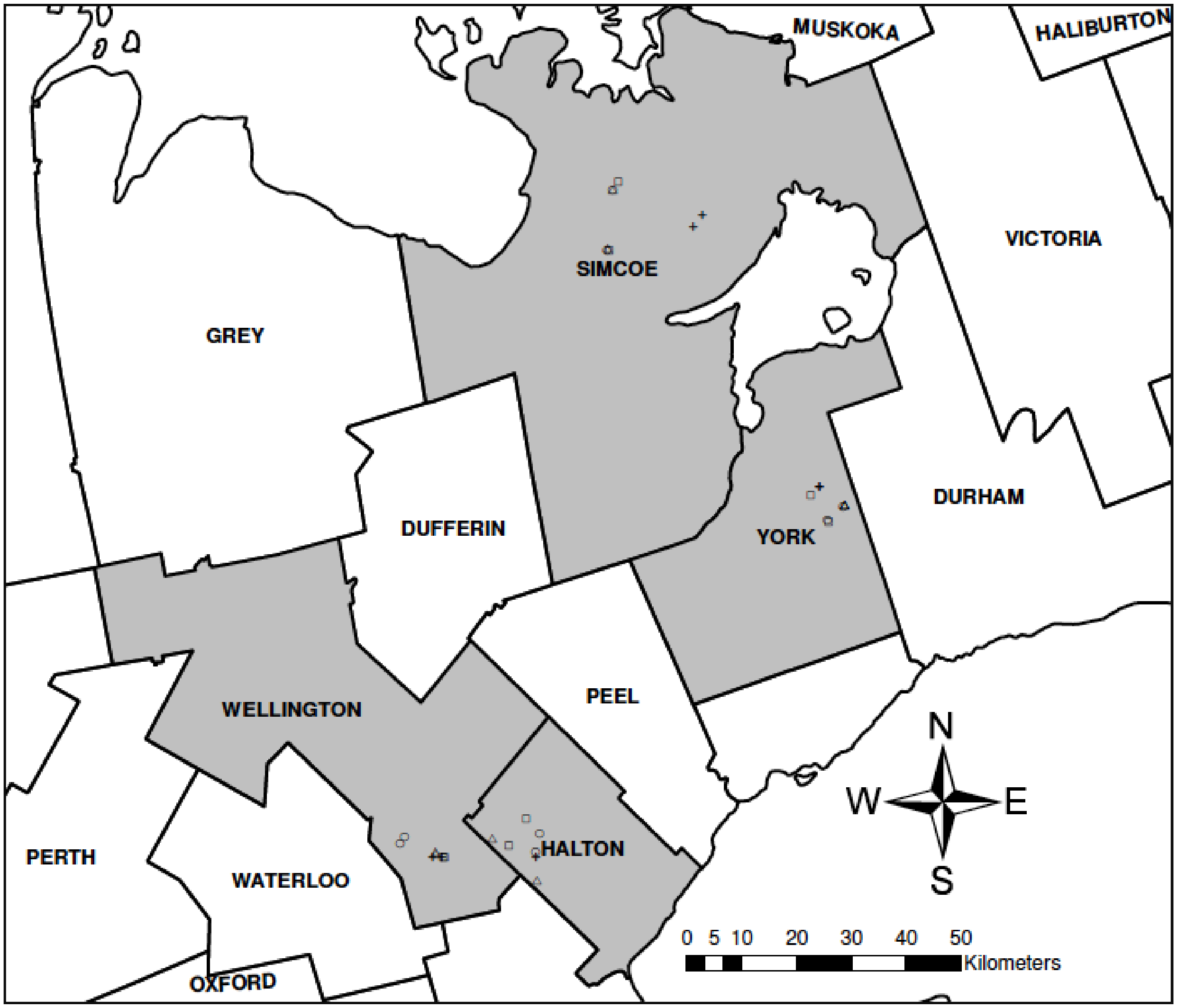


Figure 1.1 - Centroid locations of the 31 study sites in the four regions of Simcoe County, York Region, Wellington County, and Halton Region in southern Ontario (shaded). ○=deciduous forests (DF), △=mixed forests (MF), □=pine plantations (PP), +=meadows (M). Site codes, descriptions and geographic coordinates of the sites are shown in Table 1.2.

Table 1.2 - Study sites in southern Ontario during 2011, representing the four vegetation treatments, and general vegetation type, approximate age, and geographic location.

Treatment ***Site code***	**Dominant species**[a]	**Age (years)**	**Previous land use**	**Centroid location**[b] Northing	 Easting
Deciduous					
SCDFA	Mh (57%), Aw (21%)	99	Natural	44.49425	-79.8148
SCDFB	Mh (91%)	107	Natural	44.59271	-79.7997
YRDFA	Mh (60%), Aw (19%)	80	Natural	44.04603	-79.3227
YRDFB	Mh (55%)	102	Natural	44.06822	-79.2864
CGDFA	Ag (53%), Msi (31%)	>40	Agriculture	43.52492	-80.3005
CGDFB	Ag (48%), Msi (48%)	>40	Agriculture	43.53581	-80.2914
HRDFA	Mh (82%)	65	Pasture	43.54029	-79.9865
HRDFB	Mh (52%)	75	Agriculture	43.51122	-79.9960
Mixed					
SCMFA	He (39%), Mh (29%)	80	Natural	44.49651	-79.8137
SCMFB	Mh (48%), He (36%)	98	Natural	44.59346	-79.8002
YRMFA	He (51%), Mh (19%)	132	Natural	44.07059	-79.2836
YRMFB	He (50%), Mh (18%)	132	Natural	44.07044	-79.2819
CGMFA	He (30%), Ag (28%)	>40	Agriculture	43.51015	-80.2209
CGMFB	Ag (33%), He (17%)	>40	Agriculture	43.51073	-80.2218
HRMFA	Mh (25%), He (14%)	57	Forestry	43.53203	-80.0940
HRMFB	Pw (23%), Bw 19%)	75	Agriculture	43.46323	-79.9934
Plantation					
SCPPA	Pr (67%)	52	Agriculture	44.49373	-79.8096
SCPPB	Pr (86%)	71	Agriculture	44.60543	-79.7865
YRPPA	Pr (81%)	53	Cleared (unknown)[d]	44.08769	-79.3596
YRPPB	Pr (85%)	55	Cleared (unknown)[d]	44.04277	-79.3209
CGPPA	Pw (100%)	30	Agriculture	43.50283	-80.2009
CGPPB	Pw (100%)	30	Agriculture	43.50370	-80.2049
HRPPA	Sw (48%), Pw (28%)	45	Agriculture	43.52029	-80.0558
HRPPB	Sw (48%), Pr (23%)	55	Agriculture	43.56298	-80.0166
Meadow[c]					
SCMA	Solispp, Asclsyri	unknown	Agriculture	44.52879	-79.6164
SCMB	Solispp, Asclsyri	unknown	Agriculture	44.54914	-79.5953
YRMA	Old crop	unknown	Agriculture	44.10129	-79.3388
YRMB	Old crop	unknown	Agriculture	44.10012	-79.3394
CGMA	Solispp, Dauccaro	unknown	Agriculture	43.50385	-80.2071
CGMB	Solispp, Corovari	unknown	Agriculture	43.50360	-80.2276
HRMA	Solispp, Rubuidae	unknown	Agriculture	43.50243	-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 in the meadow is based on dominant 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

Earthworm Sampling and Analysis

Earthworms were sampled using three 25 x 25 cm metal quadrats in each plot, spaced at least 2 m apart. Specimens were extracted using a mustard powder (allyl isothiocyanate) solution at a concentration of approximately 100 mg l^{-1} AITC (Zaborski 2003) (Coja et al. 2008). The 25 x 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. Earthworms emerging within those 10 min were collected and killed in 70% isopropyl alcohol (Hale et al. 2008). Specimens collected from the three quadrats were pooled into one sample per plot. Earthworms were fixed using 10% formalin solution for 24 h as soon as possible and then placed back into 70% isopropyl alcohol for preservation. Isopropyl alcohol was chosen over ethanol due to the latter's tendency to destroy pigmentation.

Preserved earthworms were separated into three age classes based on clitellum development: juveniles (lacking clitellum), adults (clitellate), and pre-/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 *D. octaedra* or *D. rubidus* which could be identified to species as juveniles. *Octolasion* juveniles and adults were grouped for analyses; juveniles could not be confidently identified to species; however, only *O. tyrtaeum* adults were found.

For functional group analyses, earthworms were grouped accordingly: (i) **epigeic species** – *D. octaedra* and *D. rubidus*; (ii) **endogeic species** – *Apporectodea* and *Octolasion* species; and (iii) ***Lumbricus* group** – consisting of the epi-endogeic species, *L. rubellus,* and anecic species, *L. terrestris,* as well as all *Lumbricus* juveniles. Although the two groups included in the *Lumbricus* group have varying functional traits, they could not be satisfactorily separated due to the high proportion of juveniles, and the even distribution of adults from the two species. Species richness (number of species per sample) was estimated by counting the number of unique species per sample. Juveniles of a particular genus that could not be identified as a species were only included in the species count if adult individuals of the same genus were not found in the sample (Smith et al. 2008). This may result in species richness estimates that are slightly lower than true values.

Vegetation Sampling

Tree species composition, size classes and basal area

All five of the forest plots were assessed using a standard 'stand analysis field sheet' (Ontario Ministry of Natural Resources 2004) with the utilization of a BAF2 prism. 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). This method determined tree species composition, size class distribution and basal area. The results of the five plots were pooled into one form per site location.

Herbaceous and tree regeneration list

Absence or presence of understory shrub and herbaceous species was recorded for all plants observed within the 10 x 10 m plots. These figures may be conservative due to the difficulty of identifying some plants past genus (e.g. goldenrods and honeysuckles) when not flowering. Also included in the analysis was the presence and species of tree seedling regeneration. Meadow plots were assessed similarly to the forest plots; however, only forb species were recorded, as graminoid species were difficult to identify. The results of the five plots were pooled into one form per site location.

Stand age, management history, and land use history

To determine the approximate stand age and management history, the forest managers of each region were consulted. A record of previous harvesting practices was maintained by all regions. Previous land use (prior to current state as a forest or meadow) was also attained from records.

Soil Sampling and Analysis

Approximately 10 cm^3 of soil was collected from the top 10cm of each plot to determine soil moisture, texture, organic matter, and pH. A separate sample was taken from the top 10 cm of soil of each plot utilizing a metal ring of fixed dimensions (7.6 cm D x 4.4 cm H) and thus, a fixed volume (199.7 cm^3) to determine bulk density. A wooden block was used to pound the ring into the ground until the top of the ring was just level with the surface of the soil. The ring was carefully removed from the ground with a shovel, ensuring to retain all the soil within the ring. The five soil samples at each site were pooled for analysis in lab.

Gravimetric Soil Moisture Content

Numbered aluminum pie tins were weighed and recorded on a 0.00g scale. Approximately 27g of fresh soil from each sample were added to the tins and the weights of both were recorded. The tins were placed in an oven at 100°C for 24 h. The samples were removed and let cool for 10 min. After cooling, the tin and oven dry soil were weighed and recorded. The following equation was used to determine gravimetric water content:

$$\text{Gravimetric Soil Moisture (u)} = \frac{(\text{Weight of wet soil} + \text{tin}) - (\text{Weight of dry soil} + \text{tin})}{(\text{Weight of dry soil} + \text{tin}) - (\text{Weight of tin})}$$

Soil Organic Matter Content (Loss on Ignition)

Labeled crucibles were weighed and recorded on a 0.0000g scale. Approximately 10g of oven dry soil from each sample were added into the crucibles and the weight recorded. The crucibles were placed inside a 500°C muffle furnace for 4 h to burn off carbon content. A lower temperature was used to avoid burning off calcium carbonate that might have been present in the soil; however, tests for calcareousness using 10% HCl determined that most of the soils were non- to weakly calcareous (Denholm et al. 1993). After four hours, the crucibles were removed and allowed to cool for 15 min before being weighed and recorded. The following equation was used to determine percent soil organic matter:

$$\begin{aligned}&\%\ \text{Soil Organic Matter}\\&= \frac{(\text{Weight of oven dry soil} + \text{crucible}) - (\text{Ash} - \text{free dry weight} + \text{crucible})}{(\text{Weight of oven dry soil} + \text{crucible}) - (\text{Weight of crucible})}\end{aligned}$$

Soil Texture

Due to the organic matter found when sampling from the A horizon, remaining soil from the loss on ignition analysis was used to perform the soil texture analysis. The soil was rubbed in between two fingers to break apart aggregates. Two sets of 40mL beakers were labeled, weighed and recorded (one set for silt and clay fraction and one set for clay fraction) for a total of 50 beakers. One set of aluminum tins were labeled, weighed, and recorded (sand content). Weights were recorded to 4 decimal places.

Ten grams of the soil were added into labeled mason jars with 5mL of 5% Calgon solution (sodium hexametaphosphate) and approximately 200mL of distilled water. Lids were tightly closed onto the jar and jars were shaken using an oscillating shaker for 10 min. The jars were allowed to sit overnight. The soil solution was then transferred to labeled 1000mL graduated cylinders, careful to ensure no particles remained in the jars. Distilled water was used to fill the graduated cylinder to the 500mL mark. The cylinders were marked to indicate 10cm below the top of the water column.. A plunger was used to agitate the soil solution until its contents were evenly dispersed (approximately 10 sec) and let settle for 46 sec. After 46 sec, the tip of a 20mL pipette was inserted to the 10cm mark and 20mL of the soil solution was extracted and placed into the first set of 40mL beakers (silt and clay fraction). This was repeated for all 25 cylinders. The beakers were then placed in a 100°C oven to dry overnight. Beakers were re-weighed when dry using the same scale. The plunger was then used to agitate the soil solution again, as noted previously and allowed to settle for 8 hours. At 8 h, a 20mL-pipette was inserted to the 10cm mark as before and 20mL was extracted and placed into the second 40mL beaker (clay fraction). This was repeated for all 25 cylinders. The beakers were then placed in a 100°C oven to dry overnight. Beakers were re-weighed when dry using the same scale.

The contents of the cylinders were poured through a 63µm-sieve to isolate the sand content. Care was taken to ensure no particles remained in the cylinder. The contents in the sieve were rinsed with distilled water to remove remaining silt, clay and Calgon solution. The sand fraction in the sieve was carefully transferred to the pre-weighed aluminum tins. The tins were placed in a 100°C oven to dry overnight. Tins were re-weighed when dry. Sand was divided into two size classes: 63µm - 0.25mm and >0.25mm. Sand was poured through a 250µm sieve when dry and both size classes were weighed. Due to issues with aggregation of clay and silt particles to the sand particles (which did not dispersed within the soil column), only the results for the proportion of sand were reliable and used in further analysis. This may have been a result from using soil from the loss on ignition soil from the A horizon, rather than mineral soil from the B horizon.

$$\text{Weight of silt + clay or clay} = (\text{Weight of dried sample} - \text{dispersing agent weight}) \times \frac{500\text{mL}}{20\text{mL}}$$

Where: dispersing agent weight is 0.01g
Weight of dried sample is the (weight of soil fraction + beaker) – (weight of beaker)

Soil pH

Ten grams of fresh soil were weighed into labeled 120mL plastic cups. Forty milliliters of distilled water was measured using a graduated cylinder and placed into each cup. The lids were tightly closed on each cup and shaken by hand and then placed into an oscillating shaker for one hour. Cups were removed from the shaker and allowed to settle overnight. An ORION (model 250A) pH reader was used to determine the pH by placing the probe into the water in each the plastic cup. The probe was held just above the settled soil but was not touching it. The reading was recorded after the reader beeped that it had reached equilibrium. The probe was rinsed with distilled water following each reading to remove any residual soil water.

Statistical Analyses

Kruskal-Wallis Rank Sum Test

The Kruskal-Wallis rank sum test was used with region and treatment as the independent variables and earthworm functional group abundances and soil characteristics as the dependent variables. Mann-Whitney U test with Holm correction was used for significant Kruskal-Wallis results ($p \leq 0.05$). The objective of these tests was to determine whether data between regions and treatments were related or independent. Statistical analyses were conducted using the program R (version 2.14.1) (The R Foundation for Statistical Computing 2011).

Moran's I

The Moran's I spatial autocorrelation statistic was computed for earthworm functional group composition using longitude and latitude coordinates of the plot locations (n=155). This test was used to assess if nearby plots were more similar in earthworm abundance. The recommended number of distance classes based on a Sturge's Rule for a sample size of 155 plots is 15 distance classes, respectively (Fortin and Dale 2005). For the plot analysis, 13 distance classes were created at increments of 5 km for the first four distance classes, then at increments of 10 km, ranging from 0-130 km. Due to the geographic distribution of the plots however, distances 30-60 km were grouped into one class due to the small number of pairs in this range (pers. comm. M.J. Fortin 2012). The distance classes and number of pairs in each distance class are shown in Appendix 2. Spatial analyses were conducted with the program PASSaGE 2 (Version 2.0.11.6) (Rosenberg and Anderson 2011).

Results

A total of 806 earthworm individuals from 9 different species were sampled within the two week period (Table 1.3). Neither of the native species, *B. parvus* or *S. eiseni,* were found. Two individuals of unknown and unidentifiable species were not included in the analyses. The highest abundances (number of individual earthworms per m^2) of earthworm species comprised the juveniles of *Lumbricus* and *Aporrectodea* species (57% of total), as well as *Octolasion* species (Table 1.3). Earthworms sampled in Halton Region comprised 58% of the total number of earthworms sampled; deciduous forests in Halton Region 35% of the total throughout the regions. York Region, Wellington County and Simcoe County represented 21%, 16% and 5% of the total, respectively. Throughout the regions, deciduous forests represented 50% of the total abundance of earthworms. Mixed forests represented 32%, plantations 15% and finally meadows were the least abundant, comprising only 3% of the total abundance of earthworms.

Upon comparing the presence of earthworm species between the various regions, Halton Region had the highest species richness while Simcoe County had the lowest richness with 7 and 5 species, respectively (Table 1.3). Endogeic species, *A. rosea*, *A. tuberculata*, and *Octolasion* species, as well as *L. terrestris* were absent from Simcoe County. *A. rosea* and *A. turgida* were absent from York County. Both of the epigeic species were absent in Wellington County and *D. rubidus* was absent from Halton Region. All 9 species were found in the deciduous forests (Table 1.4); however, only two individuals of *A. trapezoides* were found in the deciduous forests of Simcoe County. Neither of the epigeic species nor *A. rosea* and *L. terrestris* were found in meadows. Meadows also had the lowest species richness with only six species.

Overall proportion (percentage of average abundance (per m^2) over the total) of the functional groups varied between the regions; Simcoe County had the greatest proportion of epigeic and *Lumbricus* earthworms due to the small proportion of endogeic worms (Figure 1.2). York Region had the second highest proportion of epigeics (Figure 1.2). Otherwise, excluding Simcoe Region, the *Lumbricus* group appeared to increase from York Region to Halton Region, while endogeic species did not appear to vary greatly between the three regions (Figure 1.2).

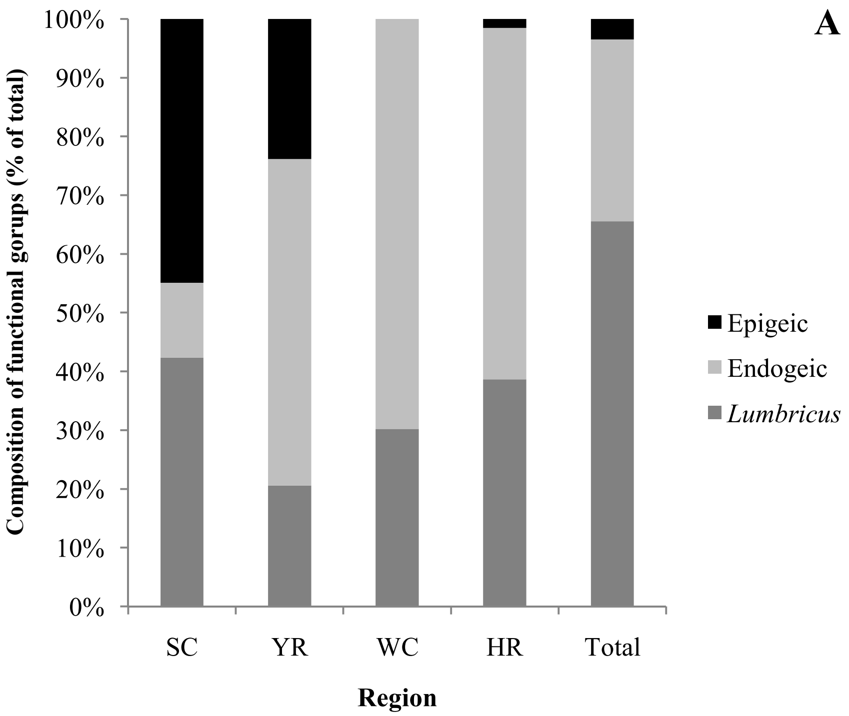
Differences were not as obvious when the functional composition between treatments (vegetation type) was considered. Plantations had the highest proportion of epigeic species, followed by mixed forests and deciduous forests, respectively (Figure 1.2). There were no epigeic species found in meadows. The proportion of the *Lumbricus* group was ranked from highest to lowest: DF > M > MF > PP (Figure 1.2). As before, endogeic species did not vary greatly between treatments. The total functional composition (between all regions and treatments) showed the *Lumbricus* group as dominant (>60%), epigeics as a minimal component (>5%), and endogeic species comprising the remainder of the total.

Table 1.3 - Mean densities of earthworms and range (minimum and maximum values) for earthworm species from the four regions in southern Ontario during 2011, sorted by block (region) (n=155).

	Mean density (individuals per m^2)				
Functional group and species	**Simcoe County (n=40)**	**York Region (n=40)**	**Wellington County (n=40)**	**Halton Region (n=35)**	**Average density per species**
Epigeic					
D. octaedra	0.47 (0-3.75)	0.91 (0-10.00)	0	0.25 (0-3.75)	0.41 (0-10.00)
D. 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 (0-7.5)	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)	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)**	**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 (0-65.00)**	**6.17 (0-65.00)**
Species richness (# of species)	**5**	**6**	**5**	**7**	

Table 1.4 - Mean densities of earthworms and range (minimum and maximum values) for earthworm species from the four vegetation types in southern Ontario during 2011, sorted by treatment (vegetation type) (n=155)

	Mean density (individuals per m^2)			
Functional group and species	**Deciduous forest (n=40)**	**Mixed forest (n=40)**	**Pine plantation (n=40)**	**Meadow (n=35)**
Epigeic				
D. octaedra	0.34 (0-3.75)	0.66 (0-10.00)	0.59 (0-3.75)	0
D. 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)**	**2.5 (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 (0-22.5)**	**0.86 (0-13.75)**
Species richness (# of species)	**9**	**8**	**8**	**4**



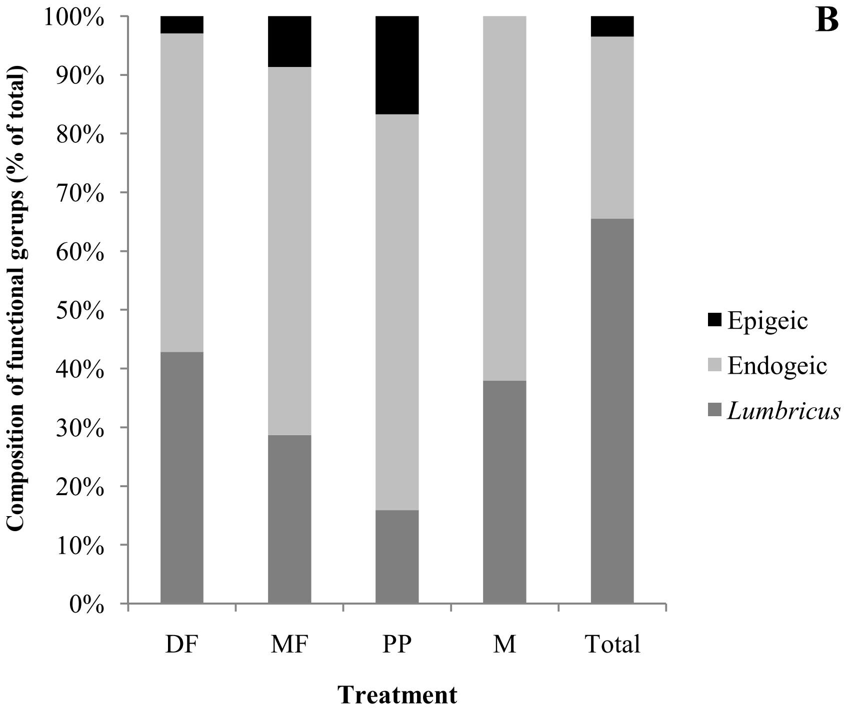


Figure 1.2 - Percentage functional group composition based on region (A) and treatment (B) in southern Ontario during 2011. Functional group composition calculated as a percentage of the number of individuals per functional group divided by the total number of individuals in all groups. Functional group composition appears to vary more when comparing regions than comparing treatments (n=155).

(1) Importance of region and vegetation on earthworm populations

Effect of Region

The effect of region on earthworm abundance was significant for all functional groups and total earthworms ($p=0.002$; $p<0.001$; $p<0.001$; $p<0.001$, respectively). Epigeic species were significantly lower in Wellington County than in Simcoe County and York Region ($p=0.002$; $p<0.001$) (Figure 1.3a).

Endogeic abundance was significantly highest in Halton Region than Wellington County, York Region and Simcoe County ($p=0.001$; $p<0.001$; $p<0.001$, respectively (Figure 1.3b). Endogeic abundance was significantly lowest in Simcoe County than York Region, Wellington County and Halton Region ($p<0.001$).

Similarly with the *Lumbricus* group, Halton Region had the highest abundance than the other regions ($p=0.005$; $p<0.001$; $p<0.001$) (Figure 1.3c). Wellington County had a higher abundance of *Lumbricus* species compared to Simcoe County ($p=0.016$).

Overall, total abundance of earthworms was significantly highest in Halton Region than the other three regions ($p=0.002$; $p<0.001$; $p<0.001$) (Figure 1.3d). Wellington County and York Region also had a higher abundance than Simcoe County ($p=0.025$; $p=0.019$). All functional groups with the exception of epigeic species appeared to show an increasing trend with increasingly southern location (Figure 1.3).

Effect of Treatment (Vegetation Type)

The effect of treatment on earthworm abundance was significant for all functional groups and total worms ($p=0.008$; $p<0.001$; $p<0.001$; $p<0.001$, respectively). Epigeic abundance did not vary significantly between deciduous forests, mixed forests and plantation. However, deciduous forests and plantations did have a significantly higher abundance than meadows ($p=0.028$; $p=0.003$) (Figure 1.4a).

Endogeic species abundance did not differ significantly between deciduous forests, mixed forests and plantations however all three of these treatments were significantly higher than meadows ($p<0.001$; $p<0.001$; $p=0.021$) (Figure 1.4b).

Lumbricus species in deciduous forests were more abundant than in plantations and meadows (p=0.002; p<0.001). Mixed forests showed similar results of having a greater abundance than plantations and meadows (p=0.033; p<0.001). Deciduous forests and mixed forests weren't significantly different in abundance, nor were plantations and meadows (Figure 1.4c).

Overall, deciduous forests had the highest abundance of earthworms over plantations and meadows (p=0.041; p<0.001). Mixed forests and plantations also had a higher abundance of earthworms than meadows (p<0.001; p<0.001). With the exception of epigeic species, there appears to be a trend of highest lowest abundance following the order of: DF > MF > PP > M (Figure 1.4d).

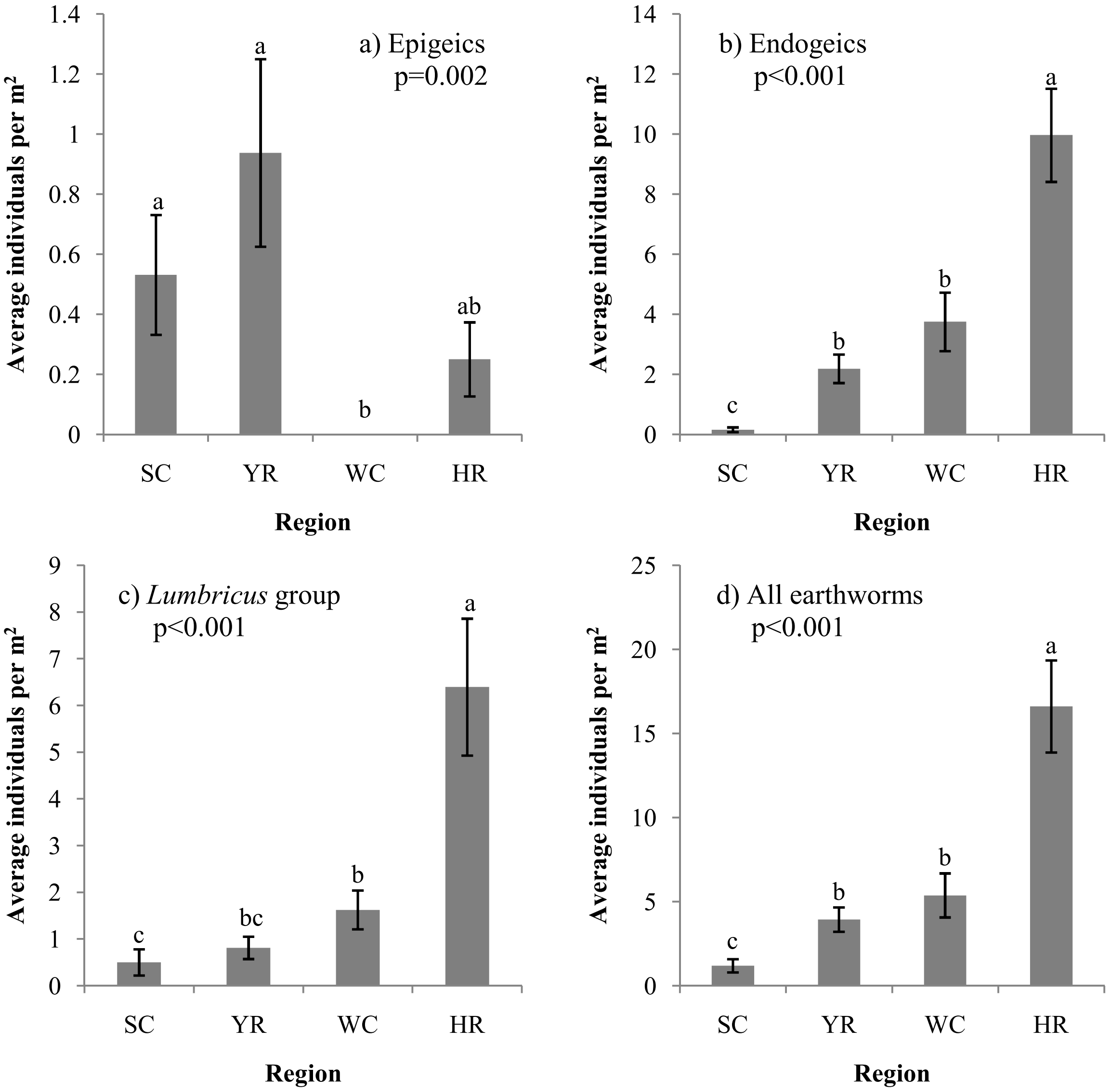


Figure 1.3 - Average abundance (individuals per m^2) (±SE) of (a) epigeic, (b) endogeic, (c) *Lumbricus* and (d) all earthworm species between regions (±SE) in southern Ontario during 2011. SC=Simcoe County, YR=York Region, WC=Wellington County, HR=Halton Region. Non-significant values are marked with the same letter using post-hoc Mann-Whitney U test, significance values derived from a non-parametric Kruskal-Wallis test (n=155).

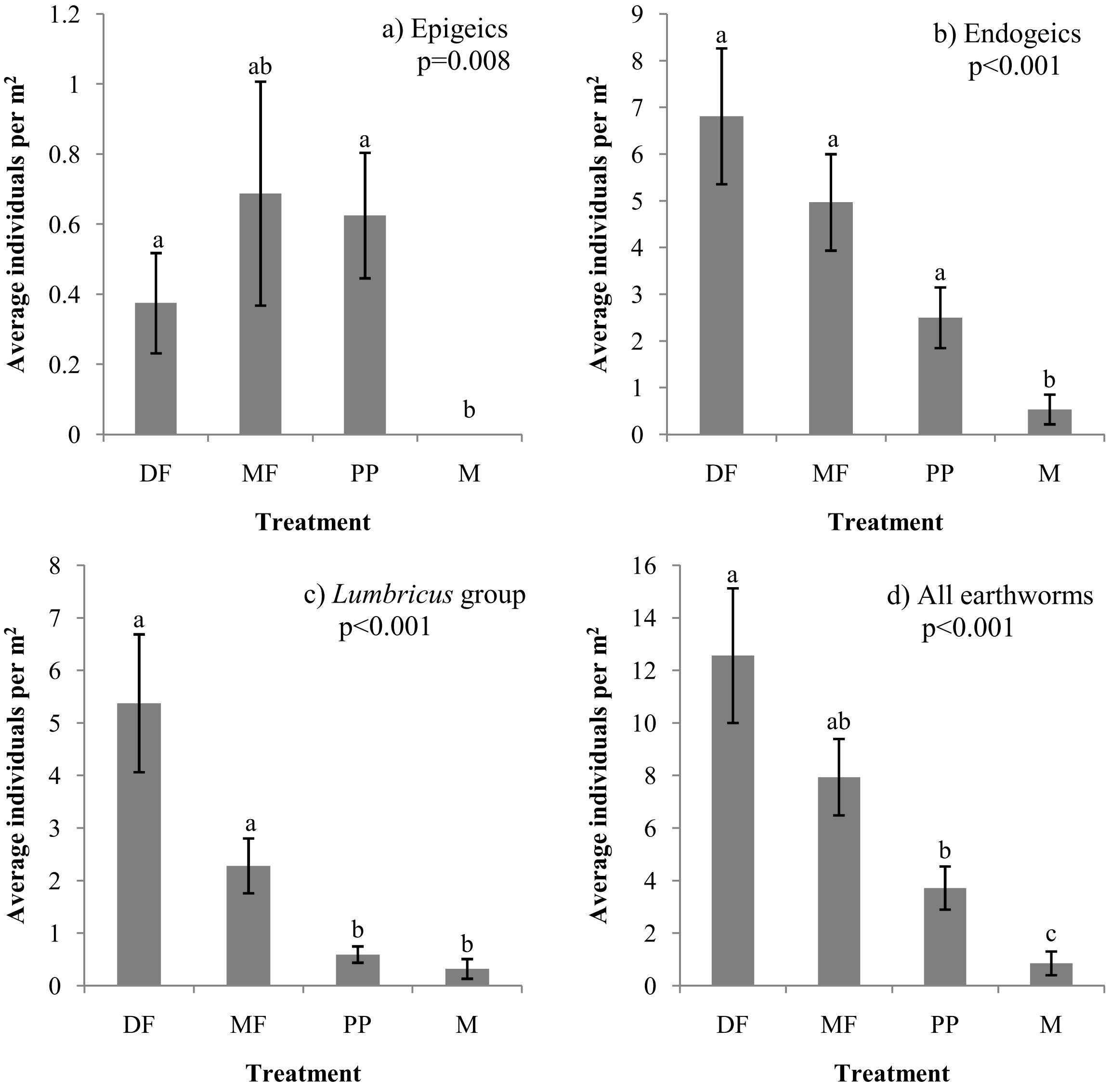


Figure 1.4 - Average abundance (individuals per m^2) (±SE) of (a) epigeic, (b) endogeic, (c) *Lumbricus* and (d) all earthworm species between treatments (±SE) in southern Ontario during 2011. DF=deciduous forest, MF=mixed forest, PP=plantation, M= meadow. Non-significant values are marked with the same letter using post-hoc Mann-Whitney U test, significance values derived from a non-parametric Kruskal-Wallis test (n=155).

(2) Variations in soil characteristics between regions and treatments

Region had an effect on soil characteristics such as pH, gravimetric soil moisture, organic matter, and proportion of two size classes of sand (Table 1.6). Soil pH was significantly lower in Simcoe County than in Wellington County and Halton Region ($p<0.001$; $p=0.002$) with a mean value of 5.70, but not different from York Region (Table 1.7). Soil moisture, however, was significantly lower in Simcoe than York ($p=0.053$), Wellington ($p=0.026$), and Halton ($p=0.009$) while these three regions did not differ (Table 1.7). Soil organic matter was approaching significance and lower in Simcoe than Wellington ($p=0.056$) and Halton ($p=0.053$) but not different than York Region (Table 1.7). Both fine (<250µm) and coarse (>250µm) sand were affected by region (Table 1.6). Fine sand was higher in York Region (68.5%) and Halton Region (43%) than Wellington County ($p=0.014$; $p=0.008$) (Table 1.7). Coarse sand in Simcoe County was significantly higher at 50.1% than York ($p=0.010$), Wellington ($p=0.013$), and Halton ($p=0.009$) which all had less than 15% coarse sand content (Table 1.7).

Treatment had less of an effect on soil characteristics than region. Again, organic matter was affected by treatment ($p=0.024$), as well as bulk density ($p=0.002$), which was not affected by region (Table 1.6). However, while the results were significant in the Kruskal-Wallis test for treatment vs. organic matter, the post-hoc Mann-Whitney U test revealed non-significant results. Bulk density was significantly higher in meadows (0.93 g/m^3) than deciduous forests ($p=0.022$), mixed forests ($p=0.016$), and plantations ($p=0.019$); which all had values under 0.70 g/m^3 (Table 1.7).

Table 1.5 - Mean values of various soil characteristics and respective effect of region and treatment (p-values derived from Kruskal-Wallis test) (n=31) taken from the top 10 cm of the soil in sites from southern Ontario during 2011. Significant values are bolded (alpha=0.05)

Soil characteristics	**Mean (±SE)**	**Region (p-value)**	**Treatment (p-value)**
pH	6.43±0.12	**0.002**	0.077
% Gravimetric soil moisture (gSM)	32.6±2.60	**0.001**	0.428
% Organic matter (%OM)	10.7±0.88	**0.016**	**0.024**[b]
Bulk density (BD) (g/cm^3)	0.69±0.04	0.244	**0.002**
% Fine sand (<250µm)[a]	49.5±3.04	**0.006**	0.744
% Coarse sand (>250µm)	22.5±3.71	**<0.001**	0.343

[a] Percentage sand is the proportion of sand (based on weight) of the total sample

[b] Kruskal-Wallis test was significant but was non-significant in post-hoc Mann-Whitney U test

Table 1.6 - Mean values of soil variables and statistical variation between regions or treatments from the top 10 cm of the soil in sites from southern Ontario during 2011. Rows with the same letter are not significantly different (derived from post-hoc Mann-Whitney U test) (n=31). NS=non-significant Kruskal-Wallis test

	pH	**gSM (%)**	**%OM**	**BD**	**% Fine sand (<250µm)**	**% Coarse sand (>250µm)**
Region						
Simcoe	5.70±0.16 **a**	16.4±2.8 **a**	7.23±1.1 **a**	0.67±0.10 NS	43.9±6.9 **ab**	50.1±6.4 **a**
York	6.41±0.25 **ab**	34.0±4.4 **b**	9.35±1.8 **ab**	0.69±0.09	68.5±5.0 **a**	10.1±5.1 **b**
Wellington	6.80±0.10 **b**	37.1±5.0 **b**	13.9±2.2 **b**	0.76±0.03	42.8±3.0 **b**	14.1±3.6 **b**
Halton	6.89±0.09 **b**	44.3±1.8 **b**	12.6±0.9 **b**	0.69±0.02	43.0±1.9 **a**	14.8±1.1 **b**
Treatment						
Deciduous	6.65±0.16 NS	36.9±5.3 NS	13.4±2.1[1]	0.64±0.04 **b**	41.8±4.8 NS	31.3±8.0 NS
Mixed	6.03±0.24	37.0±5.7	12.5±1.3	0.53±0.07 **b**	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 **b**	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 **a**	51.6±4.9	20.1±6.3

[1] Kruskal-Wallis test was significant but was non-significant in post-hoc Mann-Whitney U test.

(3) Landscape-scale patterns in earthworm abundance

All functional group abundances were significantly related at the first distance class of 0-5 km (Table 1.5; Figure 1.5). Epigeic species were the least autocorrelated (I=0.1322), while endogeic species and the *Lumbricus* group were slightly more correlated at the first distance class (I=0.2594; I=0.2210), respectively. There was a general trend of positive autocorrelation in the first seven distance classes (with the exception of distance class 4 of the *Lumbricus* group), and negative autocorrelation in the last 6 distance classes (Table 1.5).

Upon viewing the correlogram (Figure 1.5), the general trend for endogeics, *Lumbricus* group and all earthworms appeared similar to a slight gradient with unevenly distributed patches (Fortin and Dale 2005). These groups were positively autocorrelated up to a distance of 75 km, at which point became negatively correlated. Epigeic earthworms, followed a different spatial pattern, which was also patchy; however, there did not appear to be a gradient corresponding to the lowest spatial autocorrelation. The distance between epigeic patches appeared to range from 25 to 60 km. For all groups, distance between patches was shorter at smaller distance classes than at the larger distance classes.

Table 1.7 - Moran's I autocorrelation coefficients and associated p-values (based on 999 random permutations) for the three functional groups in the 13 distance classes (range in kilometers). Significant values are bolded (alpha=0.05) (n=155).

Distance class (km)	**Epigeic**		**Endogeic**		***Lumbricus* group**		**All worms**	
	I	p-value	I	p-value	I	p-value	I	p-value
1 (0-5)	**0.1322**	**0.001**	**0.2594**	**0.001**	**0.2210**	**0.001**	**0.2714**	**0.001**
2 (5-10)	-0.0166	0.481	**0.1599**	**0.001**	**0.1155**	**0.001**	**0.1377**	**0.003**
3 (10-15)	-0.0429	0.245	**0.2794**	**0.001**	0.0703	0.075	**0.1642**	**0.003**
4 (15-20)	0.0468	0.086	**0.0916**	**0.010**	**-0.0768**	**0.012**	-0.0020	0.907
5 (20-30)	**0.1167**	**0.044**	-0.1144	0.066	0.0605	0.205	-0.0871	0.126
6 (30-60)	**0.0974**	**0.023**	**0.1922**	**0.003**	**0.0956**	**0.021**	**0.1561**	**0.002**
7 (60-70)	**0.0839**	**0.035**	**0.1207**	**0.009**	**0.0478**	**0.017**	**0.1027**	**0.016**
8 (70-80)	0.0624	0.111	0.0812	0.055	0.0581	0.111	0.0417	0.214
9 (80-90)	**-0.0513**	**0.050**	**-0.2668**	**0.001**	**-0.2543**	**0.001**	**-0.2426**	**0.001**
10 (90-100)	**-0.1225**	**0.002**	-0.0132	0.372	0.0345	0.105	0.0143	0.370
11 (100-110)	**-0.1302**	**0.010**	**-0.2410**	**0.001**	-0.0516	0.186	**-0.1377**	**0.009**
12 (110-120)	-0.0408	0.083	**-0.2938**	**0.001**	**-0.1502**	**0.001**	**-0.2510**	**0.001**
13 (120-130)	0.0051	0.694	**-0.1483**	**0.001**	**-0.0825**	**0.004**	**-0.1144**	**0.004**

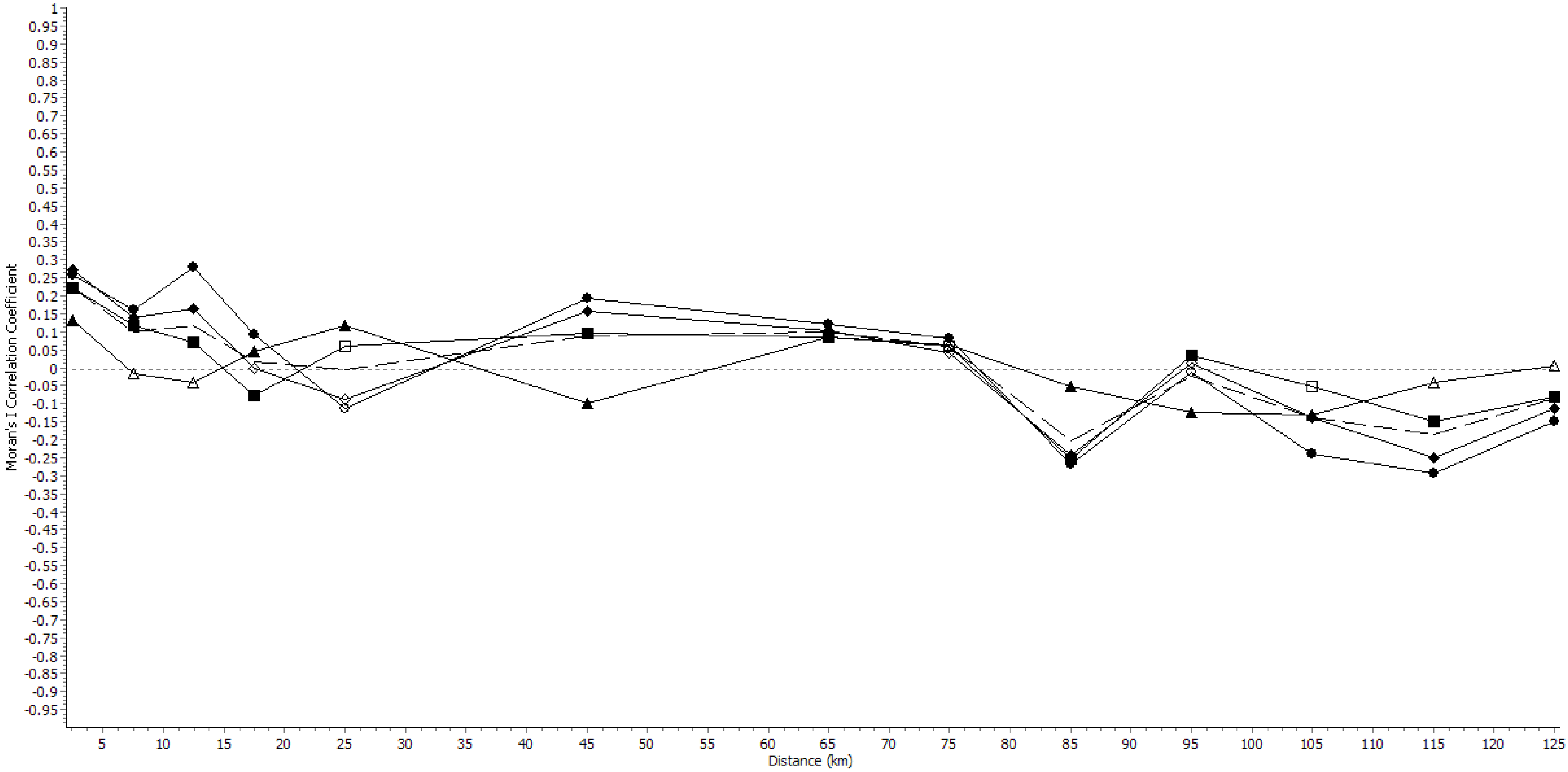


Figure 1.5 - Moran's I correlogram of functional groups of worms (n=155), ranging from a distance of 0 km to 125 km (null hypothesis represented by dotted line). In general, epigeic species (▲), endogeic species (●), *Lumbricus* species (■), as well as all species (♦) positively autocorrelated at smaller distance classes and negatively autocorrelated at further distance classes. Hollow symbols represent non-significant values (alpha=0.05), average values represented by dashed line.

Discussion

Only 9 of the 17 known species of exotic earthworms in Ontario were found during this study; however, these species were representative of the more common species found in Ontario (Reynolds 1977). One of the more common species, *A. chlorotica*, was not found but this may be due to the fact that this species prefers wetter habitats and these habitats were not part of the current study (Reynolds 1977). Similar to other studies, juvenile earthworms were more abundant (74%) than adults (Suárez et al. 2006a, Sackett et al. 2012). Suárez et al. (2006a) found that the proportion of immature earthworms was approximately 60% in invaded areas and increased towards the invasion front. Whalen (2004) found the range of juveniles to be between 70 to 95% in a study in Quebec, Canada.

Average overall density of all species of earthworms was 6.17 individuals m^{-2}, ranging from 0 to 65 individuals m^{-2} (Table 1.3). These values are relatively low compared to some studies where densities may range as high as 2,621 individuals m^{-2}, but are more commonly around 76-200 individuals m^{-2} (Addison 2009). Values in my study are comparable to those in Michigan where average densities ranged from 4.9 to 17.7 m^{-2} (Gundale et al. 2005, Cameron et al. 2007). The relatively low abundance I observed may be explained by the date of sampling. Eggleton et al. (2009) found a dip in abundance during September and Tomlin et al. (1992) found lowest densities during August to October, depending on the species. Furthermore, it is difficult to compare densities between studies due to differences in methods of sampling and data analyses, as well as variations in species composition of study sites (Cameron et al. 2007). Notably, most past research has focused solely on deciduous forests. Due to the low densities of earthworms in Simcoe County and meadows, these may have decreased the overall average compared to a study only focusing on deciduous forests in Halton Region (28.6 m^{-2}), for example.

(1) Importance of region and vegetation on earthworm populations

Earthworm abundance varied significantly between regions and treatments (Figure 1.3; Figure 1.4). In general, trends in abundance followed: HR>WC>YR>SC for region and DF>MF>PP>M for treatment. Epigeic species, however, did not follow this trend and were less predictable in terms of the effects of region and treatment. The more northerly Simcoe County and York Region had the highest abundances of epigeic species; Cameron et al. (2007) also found that the epigeic, *D.octaedra*, was the most common in northern Canada. Based on preliminary observations, it was expected that Halton Region would have the highest earthworm abundance as this area had visible signs of earthworm invasion, classified by patches of missing litter layer and earthworm middens, casts and burrow entrances (Suárez et al. 2006a).

Low earthworm abundances in the more northerly regions may be an effect of recent earthworm invasion. If the theory of invasion succession were applicable to the current study, newly invaded areas would be expected to have a higher abundance of epigeic species compared to anecic species and endogeic species. This in fact was true for Simcoe County, which had a high proportion of epigeic species and *Lumbricus* species but a small proportion of endogeics. In addition, because *L. terrestris* adults were not found in Simcoe, it is likely that the *Lumbricus* component here was largely composed of the epi-endogeic species *L. rubellus*, which is also capable of tolerating low pH and function similar to the other two epigeic species found. On the contrary, Halton Region and Wellington County had very minimal presence of epigeics and were dominated by endogeic species.

My results suggest that earthworm invasion began earlier and is more advanced in the southerly areas of southern Ontario compared to the northern regions of York and Simcoe possibly due to the differences in functional group compositions. Certain species such as *D. rubidus* were not found in the two southerly regions and *A. rosea* was not found in the more northerly regions. The lack of *Octolasion* species and *L. terrestris* from Simcoe County was surprising, especially for the latter due to the region's proximity to large bodies of water that serve as popular fishing destinations which could result in frequent introductions by sport fishermen. Although these variations in functional group composition and earthworm abundance are conducive to the aforementioned theory of invasion succession, there are other factors that also contribute to these differences, such as soil characteristics, that must be considered.

Treatment had an effect on the composition of earthworm functional groups. It was expected that plantations would have the highest proportion of epigeic species, followed by mixed forests and deciduous forests, based on soil pH. Epigeic species were not present in meadows; this is likely due to the lack of a suitable litter layer habitat characteristic of most meadows and agricultural fields (Nuutinen et al. 1998). It was unexpected that plantations would have a lower proportion of *Lumbricus* species than endogeic species. *L. rubellus* is an acid-tolerant species that is frequently found in plantations; however, endogeic species do not usually inhabit these habitats (Ammer et al. 2006). This may possibly be due to the relatively high pH of plantations found in southern Ontario, especially in the southerly regions (Table 1.7).

The stage of succession and conversion of pine stands was also important as there was a trend of increased earthworm abundance with conversion to deciduous forest; however, these trends were linked to other factors such as palatable litter availability, amount of organic matter and water availability that would also change during conversion and succession (Ammer et al. 2006). The two conifer plantations in Wellington County were planted in 1982 and were never thinned (pers. comm.. M. Neumann 2012); no earthworms were found at either of these sites. In comparison, plantations in Halton Region have been thinned to promote deciduous regeneration (Gartner Lee Ltd. et al. 2002) and earthworm abundance in these plantations averaged 7.38 individuals m^{-2}. Increased deciduous regeneration provides palatable litter for earthworm species. Plantations in Wellington had regeneration of one species whereas Halton had 10 species of deciduous trees regenerating. Thus, variations in forest management between regions may also affect earthworm abundance in the future.

(2) Variations in soil characteristics between regions and treatments

Other factors that may affect earthworm abundance and composition include soil characteristics, as well as litter quality and quantity. The number of palatable litter species, soil pH and temperatures decrease moving north, which may slow the rate at which earthworms can invade and cause changes to forests (Addison 2009). This may be true for Simcoe County, which had the lowest abundance of earthworms paired with the lowest soil pH, moisture, and organic matter. Moreover, York and Simcoe had high amounts of fine and coarse sand respectively, and corresponding low abundances of earthworms. Holdsworth et al. (2007b) found that sandiness was correlated with decreased earthworm abundance. Thus, it was expected that epigeic species would be in higher proportions in the two northerly regions as these species are tolerant of lower pH, and Tomlin et al. (1992) also found the highest abundance of *D. octaedra* in sandy sites.

The effect of treatment on soil characteristics was less significant than region; however, treatment was shown to have an effect on organic matter and bulk density. The low overall abundance of earthworms in meadows may be related to the high bulk density found here compared to the forested habitats. Smetak et al. (2007) found that younger urban systems had increased bulk density due to compaction, resulting in lower earthworm abundance. The lower bulk density in the different forest types may be a result of increased organic matter and fine roots whereas the higher bulk density in meadows may be due to compaction from prior agricultural land use. Subsequently, earthworms will increase bulk density in forests but decrease bulk density in agricultural situations (Hale et al. 2005).

(3) Landscape-scale patterns in earthworm abundance

Generally, regions and treatments that were closer in geographical distance also showed similar earthworm abundances, for example, Simcoe and York or deciduous forest and mixed forests. An analysis of spatial patterns using the Moran's I statistic further confirmed these patterns. Areas closer to each other were more similar in earthworm abundance than areas further away; this was especially true for endogeic species. The distance of positive to negative autocorrelation appears to occur at 75 km, which is approximately the distance that divides the northerly regions from the southerly regions. This could be interpreted as regions further apart being more dissimilar in terms of earthworm populations. Epigeic earthworms again do not follow this pattern as closely although this may be due to their overall low abundance (they are found in small abundances throughout the study area).

Initial patchiness at the shorter distances may be a result of treatment effect; if the nearest neighbour is of a different treatment then spatial autocorrelation of earthworm abundances will be decreased. However, changes in spatial autocorrelation are less substantial at smaller distances than at larger distances. This landscape-scale pattern of earthworm abundance by spatial autocorrelation appears to show that region is more important than treatment in terms of earthworm abundance. It is likely that other factors, such as the aforementioned soil characteristics, are driving this difference between regions such as the study by Joschko et al. (2006) suggesting a gradient of soil conditions was the determining factor.

Conclusions

My results are in line with the original prediction that landscape-scale variation in earthworm populations would be related to differences in soil characteristics, while earthworm populations would vary between vegetation types, with deciduous forests having the highest abundances. These findings are important in that they indicate the status of earthworm populations and species composition in southern Ontario. As well, they provide baseline information on levels of earthworm abundance to enable forest managers to plan for the increasing role of earthworms on forests in the future. Although there are no known effective methods for the control of these species, prevention through monitoring and education will be important to mitigate their expected impacts.

Based on the lack of historical research and knowledge of initial earthworm invasion in the study area, it is difficult to draw firm conclusions as to the cause of variation between regions. The concept of invasion succession is attractive; however, regional variations in soil characteristics cannot be ignored. Therefore, it is difficult to determine whether the low abundances in the northerly regions are due to early stages of invasion or due to unsuitability of habitat (soil characteristics). Moreover, there are many other factors that were not studied in detail here that could have played an important role in these variations, such as age of forest, previous land use, management objectives and practices, and recreational use levels. These are all factors that may vary based on regional jurisdiction and may warrant future research.

For example, previous land use may have important implications on the future impacts of earthworms on forests. The majority of the site locations in the southerly regions were at some point cleared and used for agriculture, along areas in Simcoe and York, which were historically cleared and then planted and converted to conifer plantations (Table 1.2). The deciduous and mixed forests of these more northerly regions may have been unsuitable for agriculture and thus, have never been cleared for agriculture. The soils here would be more representative of a native soil with distinct soil horizons. The impacts of earthworms on the forest floor, such as mulling, are less pronounced in forests that have been previously cultivated since these forests originated on previously tilled soil (Bohlen et al. 2004a). On the contrary, the impact of a suddenly changing soil profile on the natural forests (previously not used for agriculture) will be greater. Migge-Kleian et al. (2006) noted that the mixing of forest floor in coniferous forests by endogeic and anecic species will become of increasing interest in Canada, as these changes may affect the understory and soil communities.

Holdsworth et al. (2007a) predicted the widespread invasion of earthworms into hardwood stands in a national forest of the western Great Lakes area; however, their regional area being studied was composed of 85-89% hardwood forest. In regions such as southern Ontario, the landscape is a mosaic of different vegetation types, interspersed with urban, suburban and rural areas. This fragmentation may have the effect of slowing earthworm invasion as certain less suitable habitats may have a barrier effect on earthworm invasion. This concept will be discussed in more detail in Chapter 2.

CHAPTER 2

EARTHWORM SPECIES AND ABUNDANCE IN AN EDGE BETWEEN FOREST AND MEADOW

Introduction

Urbanization and human activity causes the fragmentation of forest habitats, creating a patchy landscape mosaic (Zeithaml et al. 2009). This often results in stands of forest that are surrounded by agriculture or meadows. These ecotones can represent unique habitats that vary in species when compared to either the forest or the meadow (Zeithaml et al. 2009). This may also be true for earthworm species; however, the few studies conducted in this field have focused on forests in relation to agricultural systems (Whalen 2004, Smith et al. 2008, Zeithaml et al. 2009). Thus, it is important to study the relationship of edges between two natural habitats to determine their role in earthworm invasion. My study examined earthworm abundance and species composition along an edge between deciduous forest and natural meadow to determine whether seasonal patterns in populations, including sexual maturity and proportional size, varied between habitats.

The concept of increased diversity and density of organisms in ecotones compared to adjacent communities, or Leopold's theory, is a relatively old one (1930s) (Leopold 1933, Risser 1995). There are many factors that may affect this phenomenon such as habitat heterogeneity, soil characteristics, species interactions, microtopography and microclimatology, and specific life-history and demographic characteristics of the organisms (Risser 1995). The results from Zeithaml et al. (2009) in Europe support this theory, showing the highest earthworm abundance and richness in areas between forests and agricultural fields. These authors studied earthworm populations along an ecotone transect, starting at 5 m within an oak forest to 100 m from the forest into an agricultural field. The lowest abundance and richness of earthworms was found in the forest, while the highest was at the edge and a distance into the field.

Earthworm diversity and abundance was assessed across a gradient of six systems based on their land-use and disturbance intensity by Smith et al. (2008) with a focus on management techniques in agriculture, including: conventional tillage, no-till, organic agriculture, successional old-field, conifer plantation and old-growth deciduous forest. Contrary to previous work, earthworm abundance and species richness was higher in deciduous forests than in the fields; however, the old field had a greater proportion of endogeic species than the forest. Unfortunately, their study did not investigate the relationship between the edge and two vegetation types.

The successional stage of meadows appears to affect earthworm species composition and abundance. Abundance was found to be lowest in an 11- year-old fallow field but was higher in younger and older successional stages (eventually becoming forest) (Scheu 1992). The decrease in earthworm abundance at mid-successional stage may be due to a decrease in productivity following succession and prior to conversion to forest; anecic species dominance gradually shifted to endogeic dominances during this succession (Scheu 1992). However, field boundaries and edges were not considered to be source areas for earthworm spread into agricultural fields, and highest abundances were found deep into the field; endogeics were higher in the field and epigeics were dependent on edges (Lagerlöf et al. 2002). Based on these studies, it is still unclear as to the expected earthworm species richness and abundance between a forest, meadow and the edge of the two.

Temporally, July seems to have the fewest earthworms compared to other sampling dates during the summer season (Whalen 2004). In contrast, spring and autumn appear to have the highest earthworm abundances seasonally (Scheu 1992). Whalen and Costa (2003) found spatial patches of high earthworm abundance to fluctuate and vary between May and September and considered these variations to be linked to changes in soil temperature and moisture. In laboratory studies, *L. terrestris* biomass and growth appears to be the greatest at 20°C and 30% soil moisture (Berry and Jordan 2001). Soil moisture, as well as soil temperature remains a primary factor in the determination of earthworm abundance on a local-scale (Edwards 2004).

In September, Lagerlöf et al. (2002) found earthworm biomass to be higher within a field than at the field edge. Although the soil, climatic conditions, and source populations may all be similar within a site, earthworms may still occupy different ecological niches, thus resulting in variations in species composition (Smith et al. 2008). Spatially, earthworm populations in forests are considered more stable than in agricultural settings, however, the explanation for this has not been well studied; it is thought to be due to increased heterogeneity of soil and litter in the forest (Whalen 2004). Scheu (1992) found that forests provided a buffering effect on harsh climatic conditions and thus, seasonal changes were less pronounced in his study than others.

Earthworms in different functional groups and having varying life history traits may be compositionally different depending on vegetation types. Endogeic earthworms are capable of aestivating in the soil during hot and dry periods, while anecic earthworms escape these periods by burrowing deep into the soil (Lagerlöf et al. 2002). Both of these groups require greater than one year to complete their life cycle (Tiunov et al. 2006). Epigeic earthworms, however, live on the soil surface and cannot escape these poor conditions and so compensate by using an r-strategy (increased reproduction, parthenogenesis) compared to the k-strategy of the former two functional groups (Lagerlöf et al. 2002, Eijsackers 2011). Epigeic species require less time to complete their life cycle and seem to be tolerant of frost and lower soil pH than endogeic and anecic species (Tiunov et al. 2006, Uvarov et al. 2010). Earthworm populations are generally found to have a high ratio of juveniles to adults (Whalen 2004, Smith et al. 2008) and this high proportion of juveniles appeared to remain fairly consistent across various systems and depths (Smith et al. 2008). Overall, it is generally expected that endogeic earthworms will inhabit the meadows, and that epigeic and anecic earthworms will be found in forested areas.

Based on a review of literature involving the study of edges and non-forested vegetation types, the lack of focus on the relationship between meadows and forests becomes evident. It is hard to ascertain how earthworm abundance and species composition would behave in a forest-meadow study area when most information is available only for agricultural systems. Thus, it is important to understand how fragmentation and urbanization, which both result in an increase of edges from a forestry perspective, affect earthworm species and abundance. Specifically, knowledge of earthworm abundance and composition may provide insight on how these boundaries affect neighbouring earthworm populations. Edge habitats may result in ecotones of increased biodiversity, or they may actually serve as barriers to earthworm invasion.

My study focused on variation in earthworm species and abundance along an edge between forest and meadow to explore the role that soil temperature, moisture, and season might play. The specific objectives were to: (1) examine variation in earthworm species and abundance across a gradient between forest and meadow; (2) determine whether this varied over the season; and (3) determine whether differences in vegetation type and season affected earthworm size and sexual maturity. By using this smaller-scale (vs. the regional-scale study in Chapter 1) approach to investigating earthworm populations, it was also thought that greater insight would be had as to how changes in scale might affect patterns in earthworm populations.

Methods

Study Area

The study was conducted at the University of Toronto's Koffler Scientific Reserve (KSR) located within King Township (Regional Municipality of York) in southern Ontario, Canada (44°02'N, 79°31'W, 300 m elevation). KSR is a 350-ha woodland area located on the Oak Ridges Moraine, a unique glacial feature in the region. The study site varies in elevation and vegetation type, with a variety of other ecological features such as pond, streams, and walking trails. The area selected for this study is comprised of a meadow adjacent to a tolerant hardwood forest. The predominant soil found in the area is a Grey-Brown Podzol (University of Toronto 2010). The average annual temperature is 7.4°C and the annual average precipitation is 857 mm (The Weather Network 2012). Soil temperatures from May to October reached a minimum of 0°C and a maximum of 30.4°C, with the average hottest temperatures in July (Figure 2.1). Volumetric soil moisture content was higher in May, June and October and lower in July, August and September, however monthly averages were relatively stable (Figure 2.1).

Study Design

The study consisted of three treatments: Forest (F), Edge (E), and Meadow (M). There were a total of 18 plots, with each treatment having six replicates (Table 2.2). The forest plots were placed 25 m from the edge into the forest, and the meadow plots were placed 25 m from the edge, with the three plots in a transect (cluster) perpendicular to the edge (Figure 2.2). The clusters were also placed approximately 25 m apart, with the exception of C4 and C5 where there was an obstruction in the way of the meadow plot (Figure 2.2). Similar to the landscape-scale study, the plots were 10 m x 10 m. The diagonals of the square plots were oriented using a compass in a north-south and west-east direction; the corners of the plots were marked with orange flags. Plots were sampled using a repeated measures design throughout the summer starting in 25 May 2011 and ending in 24 October 2011 (Table 2.1). Only one suitable site with three treatments was found and replication was within this site.

Table 2.1 - Sampling period codes with respective sampling dates and plots sampled during 2011 at the Koffler Scientific Reserve near Toronto, Ontario

Sampling period code	Sampling dates	Plots sampled[a]
T1	23-25 May 2011	All
T2	6 June 2011	C1-C3(M, E,F)
T3	20-21 June 2011	All
T4	4 July 2011	C1-C3(M, E,F)
T5	18-19 July 2011	All
T6	8-9 August 2011	All
T7	29 August 2011	C1-C3(M, E,F)
T8	24 September 2011	C1-C3(M, E,F)
T9	24 October 2011	C1-C3(M, E,F)

[a] See Table 2.2 for plot codes and Figure 2.2 for plot locations

Treatment Descriptions

The meadow area was likely used in the past as horse pasture, which has presumably been allowed to naturalize since the property's acquisition in 1995 (17 years). The meadow was dominated by grasses, vetches (*Vicia* species), common milkweed (*Asclepias syriaca* L.), and Queen Anne's lace (*Daucus carota* L.), with no shrub or tree cover. Forest can be defined as an area consisting predominantly of tree species and having a canopy cover of greater than 60% (Lee et al. 1998). At this study site, sugar maple dominated all the plots (>50% of trees), with smaller components of beech, basswood (*Tilia americana* L.), black cherry (*Prunus serotina* Ehrh.), green ash (*Fraxinus pennsylvanica* Marsh.), white ash (*Fraxinus americana* L.), ironwood (*Ostrya virginiana* (Miller) Koch), white birch (*Betula papyrifera* Marsh), red oak and hemlock. The edge of the forest was defined as the outside limit of the tree canopy (or drip line) of the forests; the centre of the edge plots was placed directly underneath.

Table 2.2 - Study plots at the Koffler Scientific Reserve near Toronto, Ontario representing three vegetation treatments, and general vegetation type and geographic location during 2011

Treatment	**Dominant vegetation**[a]	**Plot location**		**Altitude**
Plot code		Northing	Easting	
Forest				
C1F	Mh (50%), He (21%)	44.32990	-79.53431	307
C2F	Mh (70%), Be (20%)	44.33290	-7953478	319
C3F	Mh (56%), He (25%)	44.33400	-79.53520	302
C4F	Mh (100%)	44.33450	-79.53571	313
C5F	Mh (55%), Be (27%)	44.33940	-79.53652	317
C6F	Mh (55%), Be/Or (18%)	44.34210	-79.53696	303
Edge				
C1E	Mh (67%)	44.32770	-79.53465	317
C2E	Mh (43%), Ag (29%)	44.32940	-79.53500	308
C3E	Hb (60%)	44.33100	-79.53551	312
C4E	Mh (80%)	44.33280	-79.53587	306
C5E	Mh (50%), Iw (33%)	44.33820	-79.53669	315
C6E	Aw (75%)	44.33960	-79.53712	311
Meadow[b]				
C1M	Dauccaro, Melialbu	44.32610	-79.53495	308
C2M	Dauccaro, Asclsyri	44.32710	-79.53543	307
C3M	Vicicracc	44.32830	-79.53569	306
C4M	Asclsyri	44.33020	-79.53615	303
C5M	Vicicracc	44.33570	-79.53691	311
C6M	Asclsyri, Cirsarv	44.33680	-79.53732	316

[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] Dominant vegetation in the meadow is based on dominant flowering herbaceous species and does not account for grasses or sedges

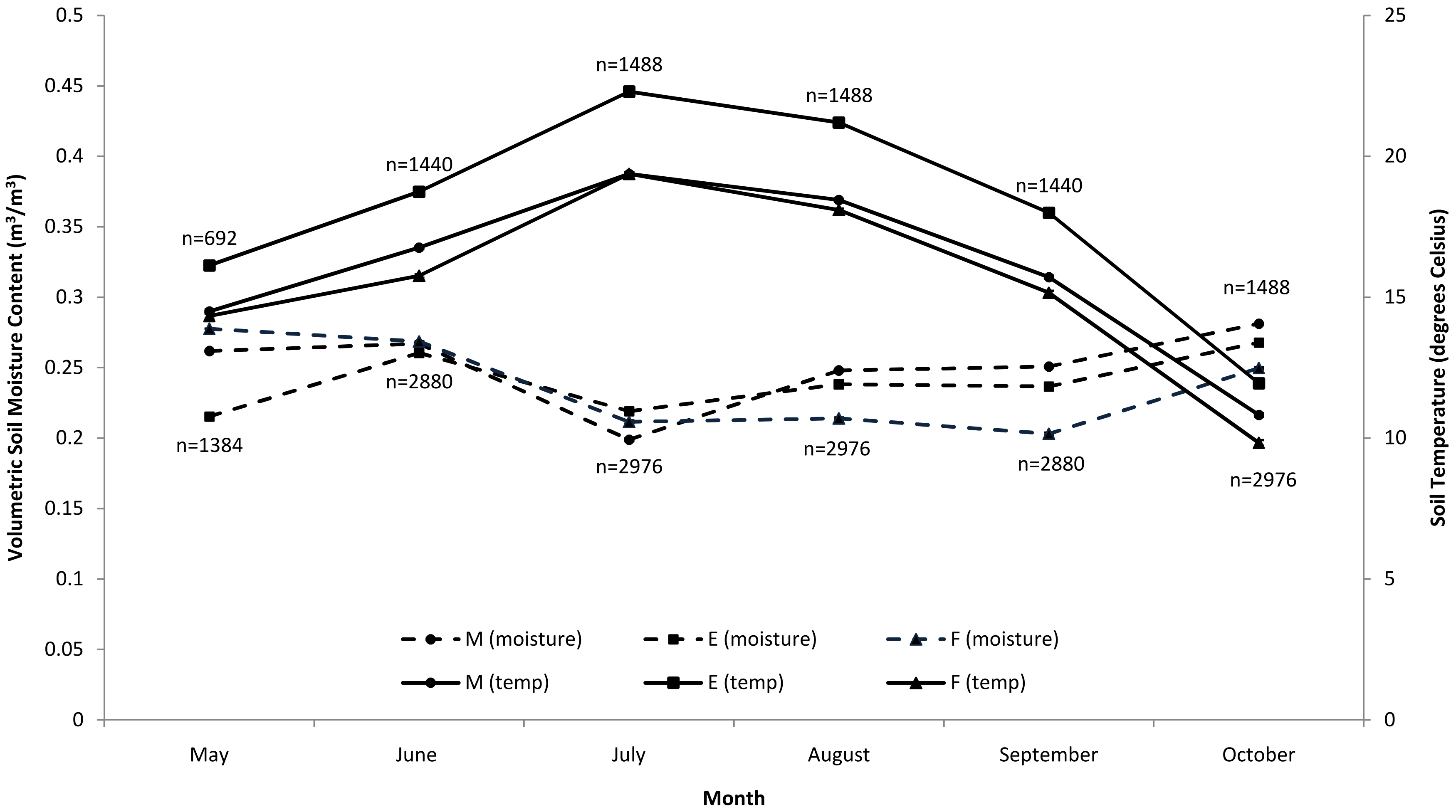


Figure 2.1 - Monthly averages of volumetric soil moisture content and soil temperature from mid-May to end of October (±SE; very minimal due to large sample size) by vegetation type (Sample sizes vary by month and are shown above for temperature and below for moisture; values indicate the common sample size between the three treatments). ●=meadow (M); ■=edge (E); ▲=forest (F). Forest soils were the driest in from August to October. Soil temperatures are highest in the edge plots.

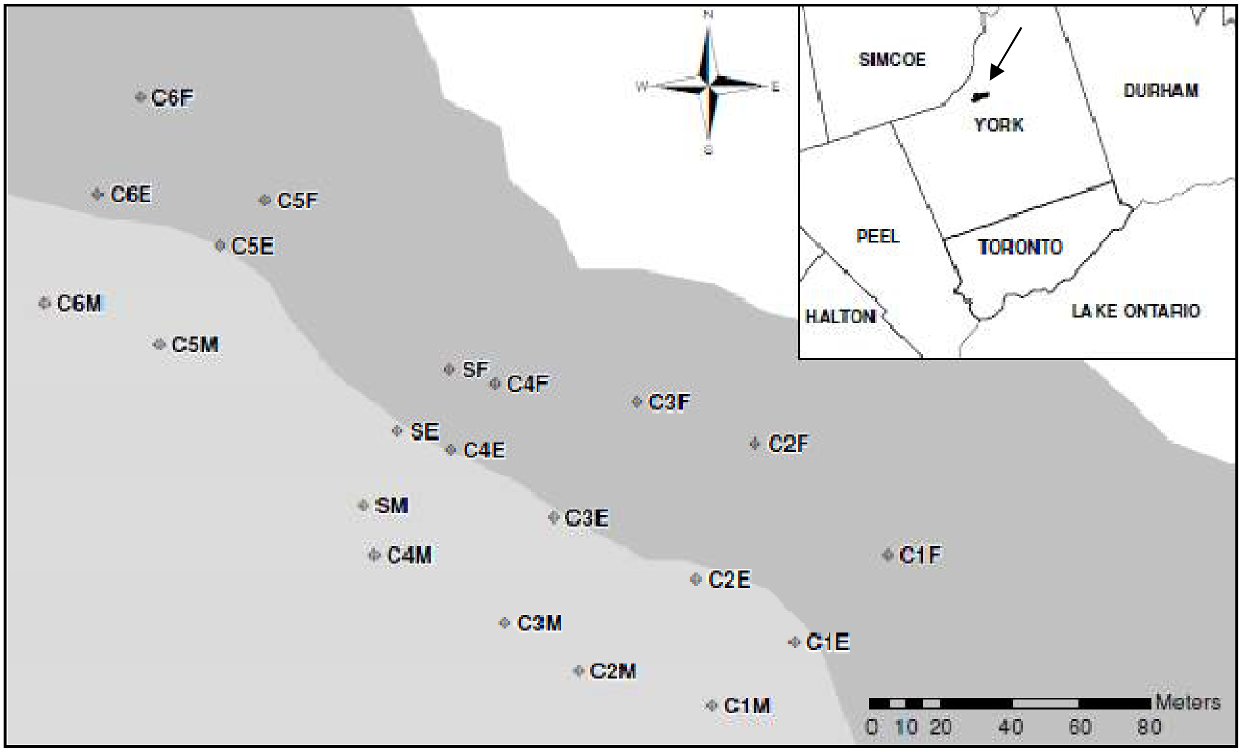


Figure 2.2 - Map showing plot and sensor locations (inset map showing location of Koffler Scientific Reserve, as indicated by arrow, in relation to Toronto in southern Ontario). Forest shown in dark grey, meadow showed in light grey. Location of the soil moisture sensors is also shown (SM, SE, SF). Plot codes, descriptions and geographic coordinates in Table 2.2.

Earthworm Sampling and Analysis

Consistent methods of earthworm sampling were used for all studies. Please refer to ‘Earthworm Sampling’ in the Methods section of Chapter 1 for detailed methodology.

In addition to the aforementioned methods, earthworms were all weighed for biomass. Fixed and preserved worms in alcohol were allowed to settle for a minimum of two days before weighing. Worms were identified and sorted to genus, patted dry with a paper towel and then weighed to the nearest 0.01g (Smith et al. 2008).

Vegetation Sampling

Forest plots were assessed using the standard ‘Stand Analysis’ form (Ontario Ministry of Natural Resources 2004) with the utilization of a BAF2 prism. This method determined tree species composition, size class distribution and basal area. Absence or presence of understory shrub and herbaceous species was recorded for all plants observed within the 10m x 10m plots. Tree species were counted and assessed in the edge plots as the total number within each plot. Meadow plots were assessed similarly to the forest plots, however only flowering forb species were recorded.

Soil Sampling and Analysis

Consistent methods of soil sampling were used for all studies. Please refer to ‘Soil Sampling and Analysis’ in the Methods section of Chapter 1 for detailed methodology.

In addition, a HOBO micro station data logger (H21-002) was utilized in conjunction with 10HS soil moisture smart sensors (S-SMD-M005) to record soil moisture (Onset Computer Corporation 2012). The sensors were placed according to Figure 2.2 with the micro station affixed to a tree by the edge sensor (SE). Sensors were carefully inserted into a slit in the ground so that the sensor was approximately 10 cm below the surface. The slit was carefully pressed closed. Recordings were taken continuously at 15 minute intervals from 17 May 2011 to 3 November 2011.

Six LogTag temperature loggers (TRIX-8) were used to record soil temperature throughout this study (LogTag Recorders Limited 2012). The sensors were placed adjacent to the three moisture sensors and adjacent to the centres of the plots in the C6 cluster (Figure 2.2). The loggers were vertically inserted into a slit in the ground so that the sensor was approximately 10 cm below the surface. The slit was carefully pressed closed. Recordings were taken continuously at hourly intervals from 17 May to 3 November 2011.

Statistical Analyses

Data subsetting

Data were divided into subsets for statistical analyses. The spatial subset consisted of the four sampling periods (T1, 3, 5, 6) where all 18 plots were sampled (n=72). The temporal subset consisted of the five sampling periods (T2,4,7-9) where only nine plots (C1-C3(M,E,F,)) were sampled, as well as the first nine plots (C1-C3(M,E,F,)) from the spatial subset (n=81) (Table 2.1). The complete dataset (All) consisted of all 117 observations. Sampling periods were also divided into subsets of 'Spring' (T1- T3), 'Summer' (T4-T6) and 'Fall' (T7-T9) for analysis of seasonal effects (Table 2.1). These divisions roughly correspond to changes in soil temperature and moisture with summer having increased soil temperature and decreased soil moisture (Figure 2.1). The modified, robust Brown-Forsythe Levene-type test based on absolute deviations from the median for non-parametric data (levene.test {lawstat}) was used to determine whether the three datasets had equal variances. Levene test results were insignificant, proving equal variances; sample sizes, mean earthworm density values and standard errors are shown in Appendix 4. Statistical analyses were conducted using the program R (version 2.14.1) (The R Foundation for Statistical Computing 2011).

Soil Temperature and Moisture

The relationship between soil temperature and moisture, and earthworm abundance was analyzed using the temporal data subset. Due to the periodic method of earthworm sampling in comparison to the continuous sampling of soil moisture and temperature, only data from the five days prior to earthworm sampling were averaged and used. Soil temperature and moisture data started at 6pm five days prior and ended at 6pm the day of sampling (this includes 18 h of data during the actual date of sampling). For example, soil temperature and moisture data used for T1 included 6 pm from 18 May 2011 to 6 pm 23 May 2011. This makes the assumption that only soil temperature and moisture from the five days prior and day of sampling was affecting earthworm abundance. These values were then averaged according to season for analysis. Uvarov et al. (2010) found that populations of *D. octaedra* and *L. rubellus* were affected by extreme daily minimum and maximum values.

Kruskal-Wallis Rank Sum Test

The Kruskal-Wallis rank sum test was used with season as the independent variable and earthworm functional group abundances as the dependent variables. The Mann-Whitney U test with Holm correction was used for significant Kruskal-Wallis results ($p \leq 0.05$). The objective of these tests was to determine whether earthworm data between treatments and seasons were related or independent. This test was employed on both the complete dataset and the temporal dataset. Statistical analyses were conducted using the program R (version 2.14.1) (The R Foundation for Statistical Computing 2011).

Friedman Test

The Friedman test was used as a non-parametric repeated measures analysis for replicated designs. The objective of this test was similar to the Kruskal-Wallis rank sum test; however, was used to take into consideration repeated measures. Sampling period was used as a blocking variable as the same plots were repeatedly sampled, resulting in the lack of independence between samples. This test was used on the complete dataset. Statistical analyses were conducted using the package {agricolae} in the program R (version 2.14.1) (The R Foundation for Statistical Computing 2011).

Results

(1) Variations in earthworm species and abundance in an edge between a forest and a meadow

A total of 905 individuals were sampled during the nine sampling periods (Table 2.1) from all plots; three unknown, unidentifiable individuals were not included in the analyses. Only 8 of the 17 species found in Ontario were found at this location. Neither of the native species was found. The highest abundances of earthworms (number of individuals per m^2) were juveniles of *Lumbricus* and *Aporrectodea* species, as well as *D. octaedra* (24%, 29%, 23%, respectively) (Table 2.3). Endogeic species were the most abundance across treatments and sampling periods (4.72 individuals m^{-2}), almost twice as abundant as epigeic species and the *Lumbricus* group (Table 2.3). Earthworms sampled in the forest plot composed 76.6% of the total number of earthworms sampled, whereas earthworms sampled in the meadow plots only composed 7.4% of the total number of earthworms sampled. All eight species of earthworms could be found in the forest; however *A. tuberculata* was not found in the edge plots and two species (*A. turgida and Octolasion* species) were not found in meadows (Table 2. 3).

Proportional abundance of epigeic species was the greatest in the edge plots (approaching 50%) while in meadow and forest plots epigeic species consisted of less than 20% (Figure 2.3a). On the contrary, endogeic species were the lowest in the edge compared to meadow and forest plots. The relative abundance of the *Lumbricus* group compared to the epigeic and endogeic species differed minimally across all treatments. Functional composition in the edge differed slightly in that epigeic species were dominant compared to endogeic species in the other treatments (Figure 2.3a).

The effect of treatment on earthworm abundance was significant for all functional groups and all worms ($p=0.002$; $p=0.001$; $p<0.001$; $p<0.001$, respectively) with a generally increasing trend from meadows to edge to forest (Figure 2.4). Epigeic species were significantly higher in the forest than in the edge and meadow ($p=0.013$; $p<0.001$). Epigeic species were also higher in the edge than in the meadow ($p=0.003$) with meadows having the lowest overall abundance (Figure 2.4a). Endogeic species were also highest in the forest compared to edge and meadow ($p<0.001$; $p<0.001$). However, abundance of endogeic species was not significantly different between meadow and edge (Figure 2.4b). Abundance of earthworms in the *Lumbricus* group was highest in forests compared to edge and meadow ($p<0.001$; $p<0.001$). *Lumbricus* abundance was also higher in the edge than in the meadow ($p=0.005$) with meadow plots having the lowest abundance (Figure 2.4c). Overall earthworm abundance across functional groups was significantly highest in the forest compared to the edge and meadow ($p<0.001$; $p<0.001$). Abundance in the edge was also higher than in the meadow ($p=0.001$) (Figure 2.4d).

Table 2.3 - Mean densities of earthworms and range (minimum and maximum values per plot) for earthworm species at the Koffler Scientific Reserve near Toronto, Ontario during 2011, sorted by treatment (vegetation type) (n=117)

Functional group and species	**Mean density (individuals per m^2)** **Meadow (n=39)**	**Edge (n=39)**	**Forest (n=39)**	**Total (n=117)**
Epigeic				
D. octaedra	0.26 (0-3.75)	2.21 (0-11.25)	4.20 (0-16.25)	2.22 (0-16.25)
D. rubidus	0.03 (0-1.25)	0.03 (0-1.25)	0.10 (0-1.25)	0.05 (0-1.25)
Total epigeic	**0.29 (0-3.75)**	**2.24 (0-11.25)**	**4.29 (0-16.25)**	**2.28 (0-16.25)**
Endogeic				
Aporrectodea juveniles	1.03 (0-7.50)	0.51 (0-3.75)	6.99 (0-35.00)	2.84 (0-35.00)
A. rosea	0.13 (0-2.50)	0.03 (0-1.25)	0.90 (0-6.25)	0.35 (0-6.25)
A. tuberculata	0.06 (0-2.50)	0	0.61 (0-5.00)	0.22 (0-5.00)
A. turgida	0	0.06 (0-2.50)	0.03 (0-1.25)	0.03 (0-2.50)
Octolasion spp.	0	0.61 (0-5.00)	3.21 (0-13.75)	1.27 (0-13.75)
Total endogeic	**1.22 (0-10.00)**	**1.22 (0-6.25)**	**11.73 (0-40.00)**	**4.72 (0-40.00)**
***Lumbricus* group**				
Lumbricus juveniles	0.54 (0-3.75)	1.02 (0-5.00)	5.45 (0-22.50)	2.34 (0-22.50)
L. rubellus	0.03 (0-1.25)	0.06 (0-1.25)	0.26 (0-5.00)	0.12 (0-5.00)
L. terrestris	0.06 (0-1.25)	0.10 (0-2.50)	0.48 (0-3.75)	0.21 (0-3.75)
Total *Lumbricus* group	**0.64 (0-3.75)**	**1.19 (0-7.50)**	**6.19 (0-23.75)**	**2.67 (0-23.75)**
Average density of all species (m^{-2})	**2.15 (0-11.25)**	**4.65 (0-15.00)**	**22.21 (0-55.00)**	**9.67 (0-55.00)**
Species richness (# of species)	**6**	**7**	**8**	**8**

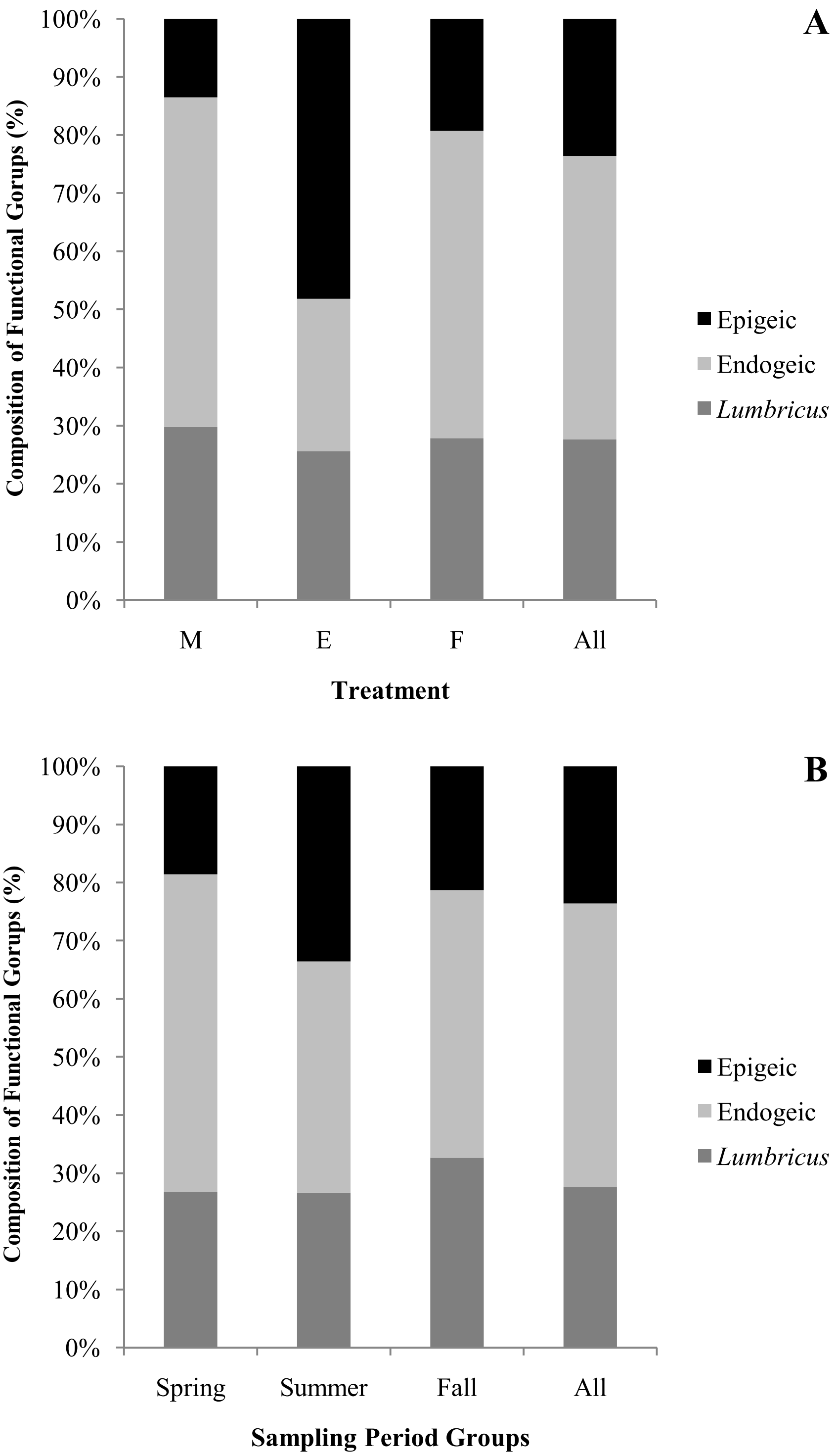


Figure 2.3 - Percentage functional group composition based on treatment (A): meadow (M), edge (E), forest (F) and sampling period (B) at the Koffler Scientific Reserve near Toronto, Ontario during 2011. Proportional functional group composition does not vary greatly, with the exception of epigeic dominance seen in edge plots and increased epigeic composition during the summer.

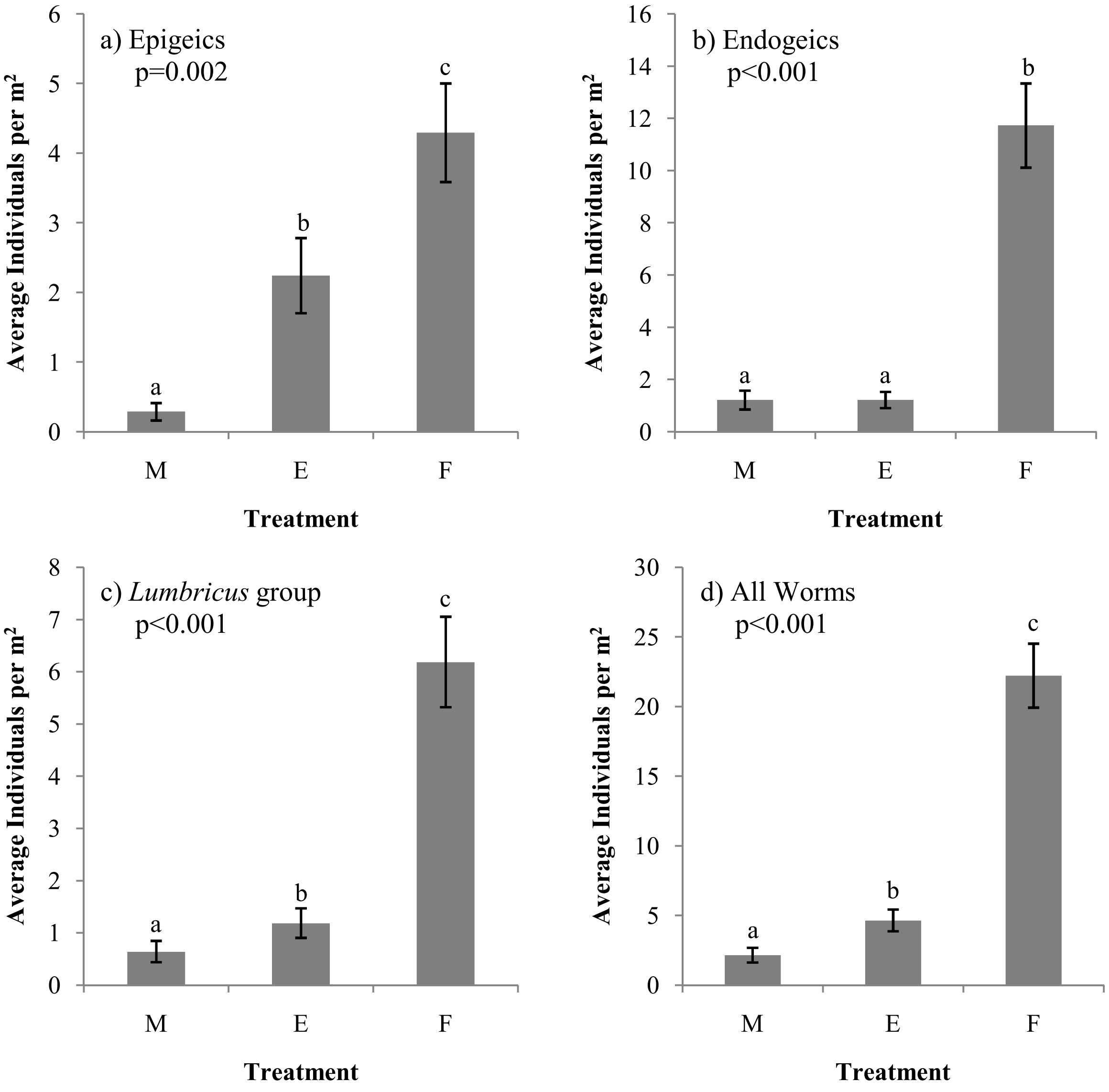


Figure 2.4 - Average abundance (individuals per m^2) (±SE) of (a) epigeic, (b) endogeic, (c) *Lumbricus* group, and (d) all earthworms per m^2 in the three different treatments: meadow (M), edge (E), forest (F) at the Koffler Scientific Reserve near Toronto, Ontario during 2011. Chi-squared p-values are derived from a Friedman test for replicated data, controlling for repeated measures (sampling period); non-significant values are marked with the same letter (n=117; based on full dataset).

(2) Effect of season on earthworm populations and role of soil temperature and moisture

Endogeic and Lumbricus group abundance was highest in the spring; however, epigeic abundance was marginally higher in the summer than in the spring (Table 2.4). Abundance of juveniles of *Lumbricus* and *Aporrectodea* species was also highest in the spring and decreased markedly in the summer and fall. Overall, abundance of all earthworms was also highest in the spring (Table 2.4). All eight species was found in the spring; however, *A. turgida* and *L. rubellus* were not found in the summer, and *D. rubidus* and *L. terrestris* were not found in the fall (Table 2.4).

Functional group composition also did not vary greatly between spring, summer and fall. Epigeic proportions increased slightly during the summer month from approximately 20% in the spring and fall to about 35% in the summer (Figure 2.3b). Proportions of endogeic species were the highest compared to epigeic species and the Lumbricus group. Proportions of the Lumbricus group did not vary greatly between the different sampling period groups. Overall, proportional functional composition was similar between meadow and forest plots, and totals between the three treatments.

The effect of season on earthworm abundance was significant for the *Lumbricus* group (p=0.015) and all worms (p=0.049) with a general trend of increased abundance in the spring and decreasing towards fall (Figure 2.5). The effect of season on endogeic species was almost significant at p=0.057, while the effect of season on epigeic species was not significant (p=0.373) (Figure 2.5). *Lumbricus* species were higher in abundances during the spring than the summer (p=0.037) and fall sampling periods (p=0.037) (Figure 2.5c). Overall earthworm abundance across functional groups was significantly higher in the spring than the fall (p=0.058); however, abundance during the summer sampling periods did not differ from either spring or fall (Figure 2.5d).

Soil variables such as organic matter and bulk density did not vary between treatments. Kruskal-Wallis showed a significant difference of soil pH and percent component of sand by treatment however the post-hoc Mann-Whitney U test with Holm correction did not show a significant difference between treatments (Table 2.5). Soil pH was slightly lower in the forest than in the meadow and intermediate in the edge plots (Table 2.6). The percentage composition of sand was very similar between the meadow and the forest (64.5% and 63.1%, respectively);

however, percent composition of sand was much higher in the edge (80.3%) than both meadow and forest (Table 2.6).

Soil temperature differed significantly between treatments in spring, summer and fall (Table 2.7). In all three seasons, the forest was the coolest, the meadow was intermediate in temperature and the edge was the warmest. The soil temperature on the edge during the summer reached maximum values of 29°C, whereas the meadow and forest both only reached a maximum of 23°C. An average soil temperature at the edge in the summer was high at 21.5°C, whereas averages in the meadow and forest were both under 20°C. Soil temperatures in the meadow, edge and forest all varied significantly between the seasons. This variation was most significant in the meadow (χ^2=864.70; p<0.001) and least significant in the edge plots (χ^2=684.34; p<0.001) (Table 2.7).

Similarly, soil moisture differed significantly between treatments in the spring and fall; however, in the summer, the forest and edge were not significantly different (Table 2.7). The forest was driest in the spring and fall; however, it was the wettest during the summer. On the contrary, the meadow and edge were wettest in the spring and fall and drier during the summer. In the spring, the edge was wetter than the meadow and forest. In the fall, soil moisture in the meadow was higher than the forest and edge. Soil moisture in the meadow, edge and forest all varied significantly between seasons. Unlike soil temperature, this variation was most significant in the edge (χ^2=1134.63; p<0.001 and least significant in the forest (χ^2=375.14; p<0.001) (Table 2.7).

Due to the low sample size for earthworm abundance, significance values may not be entirely reliable. Consequently, there was no significant difference between forests and meadows throughout the seasons even though earthworm abundance was consistently highest in the forests, intermediate in the edge and lowest in the meadows (Table 2.7). There was a similar trend between all the treatments in terms of decreasing total earthworm abundance from spring to fall. Earthworm abundance varied between seasons in the meadow and forest but not in the edge. This effect was strongest in the forest (χ^2=11.28; p=0.004) and weaker in the meadow (χ^2=6.56; p=0.038) (Table 2.7).

Table 2.4 **-** Mean densities of earthworms and range (minimum and maximum values per plot) for earthworm species at the Koffler Scientific Reserve near Toronto, Ontario during 2011, sorted by season (n=117)

	Mean density (individuals per m^2)			
Functional group and species	**Spring (n=45)**	**Summer (n=45)**	**Fall (n=27)**	**Total (n=117)**
Epigeic				
D. octaedra	2.5 (0-12.50)	2.44 (0-16.25)	1.39 (0-3.75)	2.22 (0-16.25)
D. rubidus	0.03 (0-1.25)	0.11 (0-1.25)	0	0.05 (0-1.25)
Total epigeic	**2.53 (0-12.50)**	**2.56 (0-16.25)**	**1.39 (0-7.50)**	**2.28 (0-16.25)**
Endogeic				
Aporrectodea juveniles	4.89 (0-35.00)	1.50 (0-16.25)	1.67 (0-10.00)	2.84 (0-35.00)
A. rosea	0.58 (0-6.25)	0.19 (0-2.50)	0.23 (0-3.75)	0.35 (0-6.25)
A. tuberculata	0.36 (0-5.00)	0.08 (0-3.75)	0.23 (0-2.50)	0.22 (0-5.00)
A. turgida	0.06 (0-2.50)	0	0.05 (0-1.25)	0.03 (0-2.50)
Octolasion spp.	1.56 (13.75)	1.25 (0-11.25)	0.83 (0-5.00)	1.27 (0-13.75)
Total endogeic	**7.44 (0-40.00)**	**3.03 (0-26.25)**	**3.00 (0-16.25)**	**4.72 (0-40.00)**
***Lumbricus* group**				
Lumbricus juveniles	3.03 (0-17.50)	1.94 (0-22.50)	1.85 (0-13.75)	2.34 (0-22.50)
L. rubellus	0.14 (0-3.75)	0	0.28 (0-5.00)	0.12 (0-5.00)
L. terrestris	0.47 (0-3.75)	0.08 (0-2.50)	0	0.21 (0-3.75)
Total *Lumbricus* group	**3.64 (0-18.75)**	**2.03 (0-23.75)**	**2.13 (0-13.75)**	**2.67 (0-23.75)**
Average density of all species (m^{-2})	**13.61 (0-55.00)**	**7.61 (0-50.00)**	**6.53 (0-33.75)**	**9.67 (0-55.00)**
Species richness (# of species)	**8**	**6**	**6**	**8**

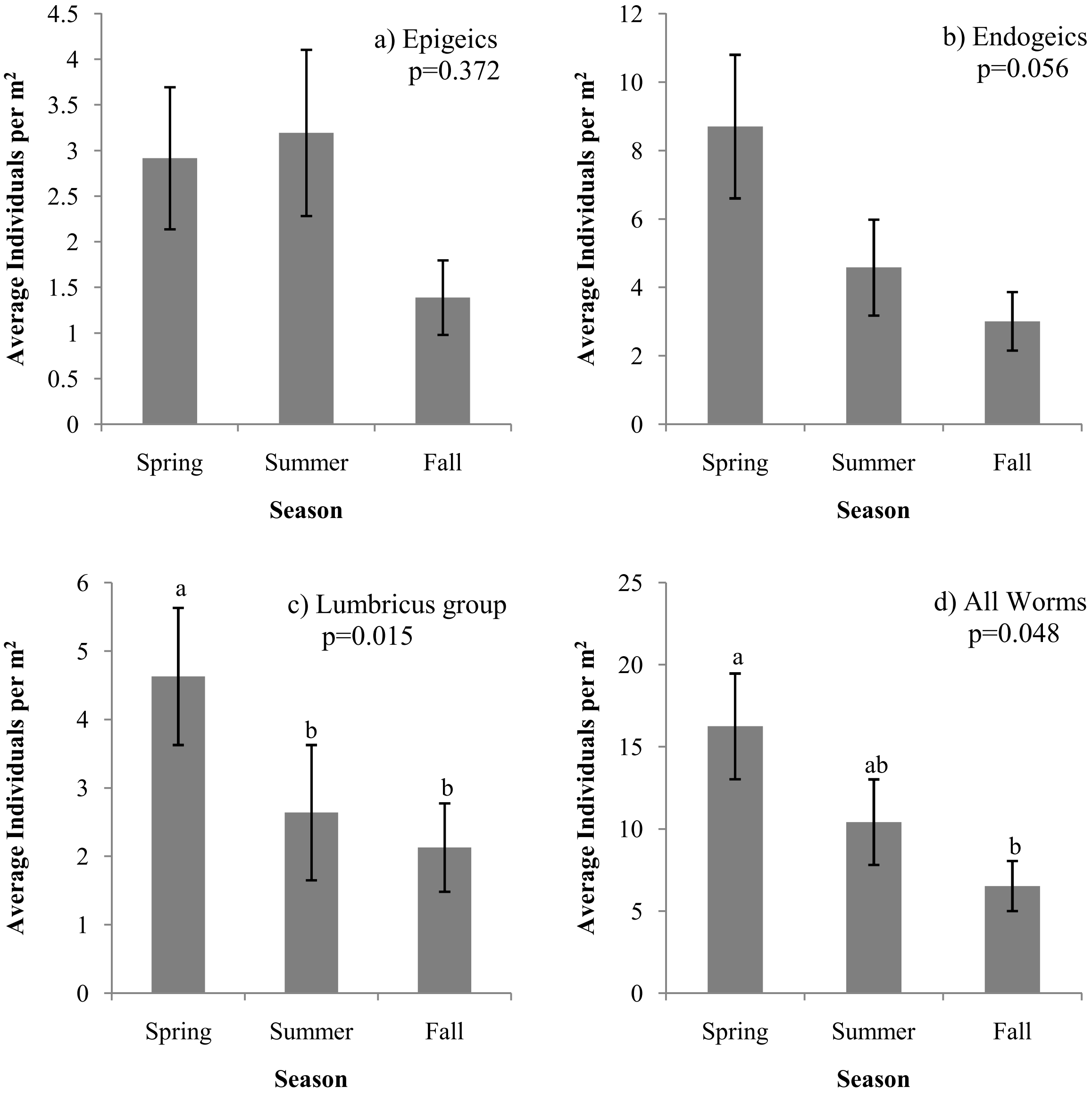


Figure 2.5 - Average abundance (individuals per m^2) (±SE) of (a) epigeic, (b) endogeic, (c) *Lumbricus* group, and (d) all earthworms per m^2 in spring, summer, and fall at the Koffler Scientific Reserve near Toronto, Ontario during 2011. Significance derived from a Kruskal-Wallis test with post-hoc Mann-Whitney U test for significant values; non-significant values are marked with the same letter (n=81; temporal dataset was used to ensure even sample sizes between seasons).

Table 2.5 - Mean values of various soil variables and respective effect of treatment (p-values derived from Kruskal-Wallis test) (df=2) (n=18) at the Koffler Scientific Reserve near Toronto, Ontario. Significant values are bolded (alpha=0.05).

Soil characteristic	**Mean (±SE)**	**Treatment (p-value)**
pH	6.58 (0.14)	**0.038**
Percent Organic Matter (%OM)	6.19 (0.60)	0.300
Bulk Density (BD)	0.77 (0.04)	0.117
Percent Sand[a]	69.3 (3.11)	**0.035**

[a] Percentage sand is the proportion (based on weight) of the total sample

Table 2.6 - Mean values of soil pH and percentage sand (±SE) by treatment at the Koffler Scientific Reserve near Toronto, Ontario. Soil pH and percentage sand composition were significantly different by treatment in the Kruskal-Wallis test (Table 2.5).

Soil characteristic	**pH (±SE)**	**% Sand (±SE)**
Treatment		
Meadow	6.92 (0.16)[a]	64.5 (3.83)[a]
Edge	6.72 (0.24)	80.3 (4.50)
Forest	6.12 (0.21)	63.1 (5.27)

[a] Kruskal-Wallis test was significant but post-hoc Mann-Whitney U test showed non-significant results

Table 2.7 - Variations in soil temperature (with range), soil moisture (±SE), and total earthworm abundance (individuals per m^2 with range), between treatments and seasons at the Koffler Scientific Reserve near Toronto, Ontario during 2011. Earthworm abundance based on temporal dataset (n=81). Between treatment variations were tested using the Friedman Test for repeated measures; non-significant values are marked with the same letter (alpha=0.05). Between season effects were tested using Kruskal-Wallis test; chi-squared values (χ^2) and p-values shown.

Treat-ment	**Spring**			**Summer**			**Fall**			**Between Season Effect**		
	temp. °C	moist. m^3m^{-3}	worms ind. m^{-2}	temp. °C	moist. m^3m^{-3}	worms ind. m^{-2}	temp. °C	moist. m^3m^{-3}	worms ind. m^{-2}	temp.	moist. (χ^2; p-value)	worms
	n=726	n=1443	n=9	n=726	n=1443	n=9	n=726	n=1443	n=9	n=2178	n=4329	n=27
M	15.43 **a**	0.2614 **a**	4.03 **a**	18.60 **a**	0.2129 **a**	1.81 **a**	14.10 **a**	0.2688 **a**	1.53 **a**	**864.70;**	**1043.77;**	**6.56;**
	(12-20)	±0.0002	(0-7.50)	(14-23)	±0.0014	(0-10.0)	(7.7-15)	±0.0007	(0-10.0)	**<0.001**	**<0.001**	**0.037**
E	17.84 **b**	0.2692 **b**	7.50 **b**	21.49 **b**	0.2257 **b**	5.14 **b**	15.67 **b**	0.2540 **b**	4.44 **b**	**684.34;**	**1134.63;**	1.95;
	(13-24)	±0.0003	(0-15.0)	(15-29)	±0.0011	(0-13.75)	(7.4-25)	±0.0008	(0-13.75)	**<0.001**	**<0.001**	0.376
F	14.93 **c**	0.2394 **c**	37.22 **a**	18.30 **c**	0.2324 **b**	24.31 **a**	13.53 **c**	0.2495 **c**	13.61 **a**	**825.30;**	**375.14;**	**11.28;**
	(11-18)	±0.0008	(0-55.0)	(12-23)	±0.0006	(0-50.0)	(6.8-20)	±0.0006	(0-33.75)	**<0.001**	**<0.001**	**0.004**

(3) Changes in proportion of sexually mature individuals and average biomass per individual over time

Due to the low overall abundance of earthworms sampled from meadow plots, no epigeic earthworms were sampled in the fall (Table 2.8). Thus, it is difficult to make inferences based on the significant standard error for some of these values. Nevertheless, it appears that all functional groups in meadow plots had a higher proportion of immature earthworms during the summer and fall compared to the spring even though total earthworm abundance decreased during the summer and fall (Table 2.7). Endogeic biomass per individual was highest in the fall and *Lumbricus* group individual biomass was highest in the summer.

The proportion of immature endogeic and *Lumbricus* earthworms did not vary between seasons (Table 2.8). Proportion of juvenile epigeic species dropped in the summer compared to the spring and fall; however, the average biomass per epigeic individual did not vary between seasons. Although the proportion of immature earthworms did not vary seasonally in the endogeic group, the average mass per individual did in endogeic species. The mass of endogeic earthworms was lowest in the fall compared either the spring or summer (Table 2.8).

In the forest plots, the proportion of epigeic juveniles decreased again in the summer (Table 2.8). Similar to the edge plots, the proportions of immature epigeic and *Lumbricus* earthworms remained high regardless of season. As well, the average biomass per epigeic individual did not vary between seasons, although unlike the edge plots, endogeic species in the forest plots also did not vary in biomass. The average mass per individual of the *Lumbricus* group was greatly decreased in the fall compared either the summer or spring (Table 2.8).

Table 2.8 **-** Percentage of sexual immature individuals (proportion of juveniles and pre/post-clitellates to total number of earthworms) and average weights per individual (biomass divided by earthworms) between treatments for epigeic, endogeic and *Lumbricus* group during spring, summer and fall at Koffler Scientific Reserve near Toronto, Ontario during 2011. Pre/post-clitellates were included as juveniles due to their inability to reproduce at the time of sampling. Only earthworms that were sampled were included in these analyses (zeros were not included in the averages). Statistical analyses could not be performed due to uneven sample sizes.

	Meadow			**Edge**			**Forest**		
	n*	Mean sexually immature (%±SE)	Average mass per individual (g±SE)	n	Mean sexually immature (%±SE)	Average mass per individual (g±SE)	n	Mean sexually immature (%±SE)	Average mass per individual (g±SE)
Epigeic									
Spring	4	25±25	0.045±0.017	8	70±13	0.035±0.006	12	66±10	0.049±0.004
Summer	2	83±17	0.022±0.012	7	41±14	0.044±0.004	10	43±10	0.044±0.004
Fall	0	-	-	4	77±18	0.037±0.009	7	78±9	0.032±0.004
Endogeic									
Spring	7	88±8	0.086±0.025	8	91±9	0.086±0.023	15	82±4	0.147±0.020
Summer	3	100±0	0.070±0.051	4	92±8	0.061±0.026	11	92±4	0.085±0.018
Fall	3	100±0	0.210±0.062	3	100±0	0.028±0.008	8	80±8	0.148±0.031
Lumbricus									
Spring	8	74±14	0.640±0.308	10	92±8	0.351±0.118	15	83±6	1.020±0.265
Summer	1	100	2.004	4	100±0	0.217±0.033	13	93±4	0.628±0.198
Fall	1	100	0.175	4	92±8	0.395±0.197	9	94±6	0.157±0.052
Total									
Spring	10	81±9	0.254±0.074	12	81±7	0.269±0.105	15	81±4	0.297±0.040
Summer	4	97±3	0.554±0.485	9	57±14	0.079±0.023	14	78±4	0.228±0.048
Fall	3	100±0	0.208±0.063	5	86±8	0.159±0.070	9	86±3	0.135±0.032

*n=the number of plots with earthworms sampled; total n=18 (spring and summer), n=9 (fall), based on full dataset

Discussion

Only 8 of the 17 known species of exotic earthworms in Ontario were found during this study. However, these species are representative of the more common species found in this area of North America (Reynolds 1977). One of the more common species, *A. chlorotica*, was not found but this may be due to the fact that this species prefers wetter habitats that were not surveyed here (Reynolds 1977). It was surprising that the abundance of *D. octaedra* was comparable to *Lumbricus* and *Aporrectodea* juveniles; this may have been due to their relatively high abundance in the edge habitats, as well as in the summer months. Likewise, functional group composition did not vary greatly between treatments and seasons; however, epigeic species were dominant in the edge with a relative decrease in endogeic species, and epigeic species increased in proportion during the summer period (Figure 2.3). It was unexpected that earthworm species found in the meadow were not predominantly endogeic as Smith et al. (2008) described; however, this may have been due to differences in species composition of the meadow vegetation and thus, litter quality and quantity. Epigeic species were not expected to be present in the meadows due to the lack of suitable litter layer characteristic of most meadows (Nuutinen et al. 1998).

Average overall density of all species of earthworms was 9.67 individuals m^{-2}, ranging from 0 to 55 individuals m^{-2} (Table 2.3). Similar to Chapter 1, these values were relatively low compared to other studies where densities were much higher (76-200 individuals m^{-2}) (Addison 2009). In particular, earthworm density in meadows was very low (2.15 individuals m^{-2}) and much lower than actively cultivated agricultural fields: hayfield (121 to 220 individuals m^{-2}) (Whalen and Costa 2003) or successional old fields (<300 individuals m^{-2}). Soil in meadows can become very hard and dry resulting in decreased earthworm abundance; agricultural fields, however, are ploughed, which may result in the higher abundance of certain earthworms (Scheu 1992, Lagerlöf et al. 2002). Moreover, the low abundance in the meadow compared to these agricultural fields, may have been related to their age (~17 years old); Scheu (1992) found that successional meadows of intermediate age (11 years fallow), had the lower abundances than both agricultural fields, younger and older fields, and forests. The low abundances in the meadow, as well as the summer and fall may also have decreased the overall average abundance.

(1) Variations in earthworm species and abundance in an edge between a forest and a meadow

Earthworm abundance varied significantly between treatments (Figure 2.4). In general, trends in abundance were significantly greater in the forest, intermediate to low in the edge, and lowest in the meadow. The lower abundance in the edge may have been due to the presence of a trail in the portion of the plot which led to increased soil compaction. It was expected that forests would have the highest earthworm abundance due to increased quantity and quality of litter and organic matter (Scheu 1992). However, the relationship of edges to other habitats has not been well studied. In the Europe, Zeithaml et al. (2009) found that earthworm abundances was lowest in forests, highest in the centre of an agricultural field and intermediate at the forest edge. Also in Europe, Lagerlöf et al. (2002) found that edge habitats have low overall abundance compared to agricultural fields. Both studies reported highest diversity in the edge, possible due to the refuge function for the epigeic and anecic species that are not found in agricultural areas. This relationship may be more important in edges adjacent to agricultural fields where only endogeic species can withstand tilling than in edges between two natural vegetation types. Furthermore, the importance of edge habitat may be less significant if the adjacent habitat is a forest, as in my study where the forest had the highest species richness.

(2) Effect of season on earthworm populations and role of soil temperature and moisture

Season had no effect on epigeic species although approached significance for endogeic species. In the *Lumbricus* group, abundance was higher in the spring than in the summer and fall. Unexpectedly, the fall had the lowest total abundance across all functional groups. Callaham and Hendrix (1997) also found that earthworms were lowest in the late summer and autumn and highest in the late spring and early summer. Similarly, in another study performed in Ontario, earthworm abundance, with the exception of *D. octaedra,* was lowest in August and September (Tomlin et al. 1992). *D. octaedra,* was lowest in May to July; however, this may have been due to site differences (particularly in soil texture), as none of the other species sampled were found on the site with *D. octaedra* (Tomlin et al. 1992).

In the present study, soil pH and percentage composition of sand varied slightly by treatment; pH was highest in the meadow and lowest in the forest, whereas sand was highest in the edge compared to the meadow and forest (Table 2.6). It is unlikely that soil pH was a factor in varying earthworm abundance as the range was minimal and was not acidic enough to cause differences in species composition (Addison 2009). Sandiness, however, may have resulted in the low abundance of earthworms compared to the forest, as earthworms prefer finer textured soils (Holdsworth et al. 2007b). This may have also been related to the higher temperatures and lower soil moisture in the edge.

Soil temperature was affected by treatment and season (Table 2.7); soil temperatures in the edge were consistently warmer compared to the forest and meadow. Surprisingly, forests had the lowest soil moisture, with the exception of the summer season where the forest and edge moisture did not significantly differ. This may be explained by the low chi-squared value showing that between season variations were the least in forest and the highest in the edge; thereby forests have lower average moisture but fluctuations are less extreme. Forests are known to have a buffering effect on harsh climatic conditions (Scheu 1992). Berry and Jordan (2001) found that *L. terrestris* experienced mortality at temperatures of 10°C and temperatures over 20°C and experienced the best growth rates at 25% and 30% soil moisture. Ranges of soil temperatures within forests were closest to this range, whereas maximum values in the edge during summer was as high as 29°C. In terms of forest edges, it is unlikely that earthworms would prefer these over forests as forest edges often have decrease soil moisture, increased temperature and wind (Cameron et al. 2007); consequently, earthworm abundance in the forest were significantly higher than in the edge regardless of season.

The edge having the highest temperatures did not appear to reflect in the earthworm abundance as this was lowest in the meadows. It is possible that soil temperatures on the edge were not high enough to cause increased mortality or the higher relative soil moisture compensated for this. Also, the edge may have had increased quality litter compared to the meadow as it had more proximity to the forest. Moreover, it was surprising that there was no seasonal difference in earthworm abundance along the edge. This may be explained by the higher proportion of epigeic species and their ability to reproduce at higher rates (Uvarov et al. 2010), leading to a more steady abundance throughout the year. On the contrary, increased variation between seasons in the forest habitats may be due to increased habitat heterogeneity in forests compared to meadows (Whalen and Costa 2003) and may result in patch shifts according to environmental conditions at the time (Whalen 2004). The ability to shift in forest habitats may help sustain earthworm populations during periods of harsh conditions. Overall, it is difficult to determine the detailed relationships between earthworm populations and soil moisture and temperature without analyzing the daily fluctuations, as these have been shown to be important in earthworm growth and reproduction (Uvarov et al. 2010), as well as the spatial dynamics of the variables affecting earthworm abundance.

Moreover, variations in earthworm abundance through the seasons may have been due to factors related to soil temperature and moisture, or methods of sampling. Seasonal changes, such as decrease soil moisture during the summer, causes earthworms to enter quiescent states that are difficult to sample using chemical methods such as formalin (which is similar to AITC extraction) (Whalen 2004). However, Callaham and Hendrix (1997) found that formalin extraction was as effective as hand sorting during June to September, when juvenile earthworms were in lower abundance; AITC extraction, like formalin, is usually less effective than handsorting when sampling for smaller endogeics (Zaborski 2003). Thusly, it is difficult to determine whether the low summer abundances I observed were due to increased earthworm mortality or ineffectiveness of the sampling method used.

As epigeic species did not vary seasonally, it is possible that population fluctuations in endogeic and *Lumbricus* species were due to quiescence and deep burrowing, respectively. In cases such as my study, where forest habitats were sampled, AITC extraction is preferred due its non-destructive nature and ease of use. Moreover, as studies have shown that the majority of earthworms reside in the top 15 cm of the soil (Callaham and Hendrix 1997, Smith et al. 2008), and chemical extraction was efficient at collecting the deeper inhabiting *L. terrestris*, hand digging was not deemed necessary. The disadvantage of variations in sampling methods is that it may be difficult to compare results between studies as different methods may cause variations in earthworm species abundance and composition (Callaham and Hendrix 1997).

(3) Changes in proportion of sexually mature individuals and average biomass per individual through time

Most studies looked at total biomass of earthworms but not changes in size of the earthworm through time (Tomlin et al. 1992, Hale et al. 2005); total biomass often follows similar seasonal patterns as earthworm abundance. Abundance in the meadow was too low to deduce patterns in sexual maturity and average mass per individual; however, there did appear to be a trend of increasing juveniles later in the season. Although the average mass per individual epigeic earthworms did not vary seasonally, the number of mature individuals appeared to be highest during the summer. This result is contrary to the laboratory experiment results from Uvarov et al. (2010) where *D. octaedra* had the highest maturity in the autumn to spring. Endogeic species in the edge and *Lumbricus* group in the forest were smallest in the fall. This may have been due to the high temperatures in the edge during the summer (maximum 29°C) and low temperatures in the forest during the fall (minimum of 6.8°C. Only *D. octaedra* and *L. rubellus* were examined in the aforementioned study; however, this is the only research to date which has been conducted on the effects of temperature and moisture fluctuations on life history of earthworms.

There are many factors that may affect the size and maturity of earthworms aside from environmental conditions, such as soil temperature and moisture. Variations in life strategies between the functional groups may also result in differences in abundance, maturity and growth. For example, epigeic, endogeic and anecic earthworms adapt to extreme environmental conditions in different ways. Epigeic species may combat population declines through their *r*-selected strategies of fast reproduction rates and parthenogenesis (Scheu 1992) whereas endogeic earthworms may become quiescent and likewise, anecic species will burrow deeply until more favourable conditions arise. The latter two have slower reproductive rates and are semivoltine compared to the univoltine, *D. octaedra* (Uvarov et al. 2010). Due to these differences, epigeic species may be able to rebound their populations more quickly and effectively than the other two groups, for example, following winter frost events (Uvarov et al. 2010).

Conclusions

Epigeic species appeared to be able to occupy the edge habitat, regardless of temperature extremes, better than the other two groups. Moreover, this edge habitat had litter qualities of both meadow and forest that appears to be less suitable for endogeic species. The forest-meadow ecotone may be compared to a late successional meadow. In that case, Scheu (1992) found that *D. octaedra* increased with succession and Margerie et al. (2001) found that succession to forest resulted in a decrease of endogeic species and an increase in epigeic and anecic species. As epigeic species may be able to take advantage of this edge habitat, once in the forest, the higher species richness and abundance may lead to increased competition between earthworms (Eriksen-Hamel and Whalen 2007). Epigeic species, such as *D. octaedra*, are less able to compete against other species, such as *L. rubellus,* and therefore, populations decrease (Uvarov et al. 2010). These patterns are also similar to the theory of invasion succession, where epigeic species are initially the most abundant at a site but eventually decrease when other species arrive (Suárez et al. 2006a).

Due to the short timeframe of the present study, trends and patterns on the results should be deemed preliminary. It is difficult to determine the driving factors of the earthworm population fluctuations seen in this study without examining the long-term effects of climate, especially frost and low winter temperatures, as these are known to cause mortality in earthworms (Addison 2009, Uvarov et al. 2010). Moreover, repeated droughty summers may also affect the earthworm population (Eggleton et al. 2009); maximum temperatures and total precipitation for the month of July 2011 were 37.0°C and 50.7 mm compared to 34.7°C and 76.6 mm in July 2010, possibly leading to the decreases in earthworm abundance during this period (Environment Canada 2012). Whalen and Costa (2003) and Nuutinen et al. (1998) found that earthworm patches occurred at 16-21 m to 20-40 m; Whalen (2004) suggested that fixed samplings at intervals of 16-21 m would not account for spatial variations in earthworms. Consequently, the relatively equidistant location of the plots (25 m) in my study may have not accounted for spatial variations, such as patchiness, by missing the areas of higher abundances and thus, possibly leading to low sampling abundances.

Nevertheless, the results of the current study have interesting implications for the importance of edges between two different vegetation types, specifically, on natural, non-facilitated dispersal of earthworms between habitats. Endogeic species were as low along the edge as in the meadow; however, epigeic species were slightly higher. In areas where earthworm-free forests are adjacent to invaded agricultural or meadow areas, edges may provide an important refuge for certain species (epigeic and anecic), which in turn could become a source population of higher species richness for forest invasion. On the contrary, if epigeic and anecic species are not present in an agricultural or meadow setting, edges may slow the natural dispersal of endogeic species. Future studies could examine variations in earthworm rates of dispersal between roads immediately adjacent to forests and roads that are divided from forests by small strips of meadow.

Furthermore, treatment effects were more significant than seasonal variations; however, it was difficult to determine whether low abundances during the summer were due to mortality or sampling error due to quiescence and deep burrowing. It was interesting to note that epigeics were least affected by seasonal changes of all the functional groups, although this could be tied to their differences in life history traits. It was also the only functional group that had proportions of mature individuals that were greater than 50%. Soil temperature and moisture appeared to be important factors in earthworm abundance; however, the relationship between earthworm populations and environmental conditions through time is a complex one that cannot fully be understood in such a short study. If diurnal temperature and moisture fluctuations are important, then monthly and bi-weekly sampling may not be frequent enough to capture relationships between climatic conditions and earthworm populations. Overall, more long-term and detailed studies are needed to determine these relationships and temporal patterns in climate and earthworm abundance, composition and life history.

CHAPTER 3
FIELD SAMPLING OF *POLLENIA* SPECIES (DIPTERA: CALLIPHORIDAE) USING BALL TRAPS AND OLFACTORY FOOD CUES WITH IMPLICATIONS FOR EARTHWORM PARASITISM

Background and Introduction

The European cluster fly, *Pollenia rudis* (Fabricius) has been reported in North America since 1882 (Yahnke and George 1972) and the earliest specimen examined by Jewiss-Gaines et al. (2012) was found in 1904. Thus, it is likely that these flies were introduced concurrently with invasive earthworms. Although these flies have often been viewed as pests themselves due to their habit of overwintering in attics and houses, they are one of the main host-specific parasitoids of earthworms (Yahnke and George 1972).

Thomson and Davies (1973b) completed the most detailed studies on the life cycle of this species to date. They showed that female cluster flies oviposit under specific environmental conditions such as high humidity, soil moisture, and surface vegetation. Eggs are laid in the soil and emergent larvae begin searching randomly for a host. Larvae can be found feeding both on the outside of their host worm or partially inside with their spiracles protruding from the earthworm body cavity (Thomson and Davies 1973b).

Through controlled laboratory experiments, Thomson and Davies (1974) found that the host-parasitoid relationship was greatly affected by the behaviour of the host, and host behaviour was influenced predominantly by soil conditions such as moisture level and bulk density. Average mortality was 73% with a soil temperature of 15°C and increased to 87% with a soil temperature of 20°C; bulk density and soil moisture were also varied between these temperatures. Average mortality increased with decreasing soil moisture and increasing bulk density. Their work demonstrated that encysted worms (worms that became dormant due to low moisture conditions) were unable to lose the infection by the parasitoid, whereas active worms were able to autotomize segments infected by the parasitoid. As worms in low soil moisture and low bulk density are more likely to encyst, these conditions would increase the chance of fly larvae reaching maturity; worms in low soil moisture and high bulk density surfaced and died regardless of infection (Thomson and Davies 1974).

P. rudis has been reported to parasitize the following four species: *A.rosea, A. chlorotica, Apporectodea caliginosa* (Savigny) and *L. terrestris*; however there is some disagreement in the literature (Thomson and Davies 1973a). Moreover, Reynolds (1995) states that *A. caliginosa* is actually a complex of 7 species including *A. tuberculata, A. trapezoides* and *A. turgida*. Laboratory studies suggest that larvae have no preference for a particular host species when feeding on dead earthworm tissue (Thomson and Davies 1973b) so it is likely that different species of earthworms may have varying capabilities of preventing and/or losing parasitoid infections.

In addition, the recent discovery that *P. rudis* may actually be a complex of six species (Rognes 1987) suggests that there may actually be specific host affiliations, and that further study is needed to identify them. The majority of the studies on *P. rudis* were conducted in the 1970s or earlier, whereas *Pollenia* spp. such as *P. pediculata* were not discovered and described in North America until the 1980s by Rognes (Whitworth 2006). It should be stressed that the accurate identification of these species is important to future research and that previous research on the life cycle and biology of these species should not be considered entirely accurate.

Rognes (1987) states that discrepancies between reports on the life cycle and biology of *P. rudis* in North America and Europe are due to the study of different species. Within North America, there are six different species (Whitworth 2006) and it is difficult to determine which species Thomson and Davies (1973a, 1973b, 1974) was actually studying. It is likely that the flies studied by Yahnke and George (1972) were 90% *Pollenia pseudorudis* (Rognes 1985) (syn. *P. pediculata* (Macquart 1834)) and 10% *P. rudis* (Rognes 1987). These two species are the most common in North America (Whitworth 2006). The assumed host of *P. pediculata* and *P. rudis* is *A. rosea*, an endogeic earthworm that can be found in the top 10 cm of the soil (Rognes 1987).

The ecology of *Pollenia* species is not well studied in the field. In the laboratory, captive reared adults mated and laid fertile eggs following 8 days of consistent 27°C temperatures while being fed granulated sucrose and distilled water (Yahnke and George 1972). Thomson and Davies (1973a) placed adult flies in glass jars with moist paper where females oviposited readily. Yahnke and George (1972) found that up to 25% of sampled *A. rosea* were parasitized with *P.*

rudis larvae from mid-May to mid-September near a rural area in Belmont, Ontario. In the northwestern United States, it was concluded that there are four generations per year; in the laboratory, three complete consecutive generations were successfully reared, each taking approximately thirty days (Yahnke and George 1972).

A study conducted on the overwintering status of *P. pediculata* found 119 females and 95 males inside a heated residential house (Greenberg 1998). Of the 16 females dissected, all were nulliparous (never laid eggs) and unmated (no sperm in their spermathecae). It was suggested that their ovaries remained undeveloped until they are ready to mate in the spring (March to April). Approximately 50% of the females showed signs of having recently pupated; however very few specimens of either sex showed signs typical of older flies, such as frayed wings (Greenberg 1998).

Adults of *Pollenia* species feed on nectar from flowering plants (Heath et al. 2004). Souza-Silva et al. (2001) identified flowering plants pollinated mainly by short-tongued dipterous species, such as *Pollenia* species, have light-coloured petals and sepals (white and yellow) with a perceptible aroma. Flowering plants of the Asteraceae family were highly visited; some plants from the Rhamnaceae and Boraginaceae families were also visited (Souza-Silva et al. 2001).

Attempts at field sampling of *Pollenia* species have not been well documented or described in the past. Yahnke and George (1972) mentioned trapping flies using banana as bait during the warmer months or by hand during winter in houses. The type of trap used was not mentioned and the study area was agricultural. Soil conditions appear to be a major factor in the survival of the fly larvae throughout all instars, from egg to adult. Furthermore, it would be expected that adult flies would be found near certain species of flowering plants; however, most flies are collected in agricultural areas and it is likely that these flies are capable of flying distances.

The goal of this study was to evaluate methods for the field sampling of *Pollenia* species with the specific objectives of: (1) determining the effectiveness of traps and the olfactory food cues (synomones) as bait; (2) relating fly presence to vegetation type and/or flowering plant species; and (3) assessing evidence of parasitism through earthworm sampling. As research on invasive earthworms continues, it can be assumed that research on possible methods of biocontrol will also receive interest. To better study the life cycle and ecology of this parasitoid of earthworms, adequate methods of field sampling must be devised.

Methods

Study Area

The study was conducted at the University of Toronto's Koffler Scientific Reserve (KSR) located within King Township (Regional Municipality of York) in southern Ontario, Canada (44°02'N, 79°31'W, 300 m elevation). KSR is a 350-ha woodland area located on the Oak Ridges Moraine, a unique glacial feature in the region. The study site ranges in elevation and vegetation type, with a variety of other ecological features such as pond, streams, and walking trails. Forest vegetation types include cedar, conifer-dominated mixedwood, hemlock-dominated mixwood, locust, mixed-deciduous hedge row, mixedwood, old growth hardwood, red pine and Scots pine plantations, sugar maple, tolerant hardwood, and white pine, as well as other vegetation types including fields, bogs, and barren and scattered areas. The majority of the soil found here is a Grey-Brown Podzol (University of Toronto 2010).

Study Design

The research was conducted concurrently with the study described in Chapter 2 using the same study design. Please refer to 'Study Design' in the Methods section of Chapter 2 for detailed methodology.

Plots were sampled throughout the summer starting in May 2011 and ending in October 2011. The first nine plots (C1-C3) were sampled approximately every two weeks and all of the plots were sampled approximately once a month (Table 2.1).

Vegetation Sampling

Methods of vegetation sampling were consistent with those used in Chapter 2 of this thesis. Please refer to 'Vegetation Sampling' in the Methods section of Chapter 2 for detailed methodology.

In addition, flowering vegetation was recorded throughout the sampling period, in all three vegetation types. Species which were in flower were recorded during each sampling period.

Earthworm Sampling

Consistent methods of earthworm sampling were used for all studies. Please refer to 'Earthworm Sampling' in the Methods section of Chapter 1 for detailed methodology.

In addition, earthworms were also inspected during the identification process for any evidence of parasitism or injury, including any wound-like nodes, cysts, bumps, and so on. Photographs were taken of these worms and recorded.

Soil Sampling

Consistent methods of soil sampling were used for all studies. Please refer to 'Soil Sampling' in the Methods section of Chapter 1 for detailed methodology.

Traps

Trap Description

Two types of traps were implemented in this study: Yellow ball traps (modified McPhail traps) and yellow pan traps. Both of these traps were affixed to a wooden stake so that the bases of the traps were approximately 70 cm above the surface of the ground.

Ball traps (AR934A) are similar to the traditional McPhail trap; however, they are capable of holding twice the amount of liquid attractants (ISCA Technologies 2012). Due to this increase in volume, the liquid held inside the ball trap can be maintained for three times longer than the McPhail trap. Metal wire was used to hang the ball traps from a metal bracket that was affixed to the wooden stake.

Small, plastic yellow bowls (17.78 cm diameter) were used to make pan traps. These were affixed to the wood stake using metal strapping that had been bent into a circular shape and bolted to the wooden stake. The bowls were then filled with a weak solution of dish detergent and water to disrupt the surface to prevent escape by the promotion of drowning.

Olfactory Cues (Synomones)

Pheromone attractants were avoided in order to capture both males and females of the species. Synomones, which are compounds beneficial for both the receiver and sender, are preferred since *Pollenia* adults feed and pollinate flowering plants (Budaa et al. 2009). An example of such a compound is methyl salicylate (MeSa), which is of plant origin and is widespread in many flowering plants (Budaa et al. 2009). The only other known attractant for this species is methyl eugenol (Budaa et al. 2009). It was found that MeSa-baited sticky traps caught significantly more flies than the controls (Budaa et al. 2009); however, this experiment was performed in a greenhouse where post-humous predation by birds was unlikely. Yahnke and George (1972) also used banana as bait to trap flies.

Sampling

The wooden stakes were securely inserted into the ground at the centre of each plot with brackets facing a consistent, but arbitrary, direction (east) on 10 May 2011. A cotton ball was dipped into 99% MeSa (O3695-500) (Thermo Fisher Scientific Inc. 2011) and then placed in the receptacle of the ball trap. 70% isopropyl alcohol was poured into the basin to kill and preserve trapped insects. A two-week trial period was initiated prior to the commencement of concurrent earthworm sampling (as described in Chapter 2). To test alternative baits, two additional traps were set up just outside the sample area on 21 June 2011; one containing bananas and one containing live worms in soil.

Due to the perceived ineffectiveness of the MeSa bait compared to the banana bait following the examination of the exploratory, banana-baited test trap, all traps were converted to bananas on 4 July 2011. Traps were removed from stakes and thoroughly cleaned prior to the change. Sliced, fresh bananas were placed in mesh packets and placed in the basins. Traps were inspected during earthworm sampling periods (Table 2.1) for *Pollenia* species (readily identifiable with a hand lens by their conspicuous golden hairs), which were then collected in a vial and labeled for identification in the lab. Any other remaining species were discarded. Addition of bait and alcohol was performed as necessary.

Pan traps were tested as an alternative method during the ball trap sampling period. The pan traps were filled halfway with water and a drop of dish detergent was added. Pan traps were tested on 11 July 2011 for a total of 24 hours. Due to the difficulty in continuous sampling using this method and the predominance of hymenopteran species sampled, pan trapping was discontinued after this sampling period.

Fly Identification and Preservation

The collected flies were identified in the lab using the keys in Whitworth (2006) and a dissecting microscope. Flies were then preserved in 70% isopropyl alcohol for possible dissection as soon as possible. However, following preservation in alcohol, a contaminant producing a white cotton-like substance presented itself within the vials, consuming the flies and partially dissolving them. Specimens had to be discarded.

Statistical Analyses

Due to the small number of *Pollenia* species collected, statistical analyses could not be performed. As a result, it is important to note that most of the results are observational in nature.

Results and Discussion

(1) Effectiveness of traps and olfactory food bait

No *Pollenia* species were collected until early to late Fall (29 August 2011 to 24 October 11); a total of only 12 specimens were found in the banana-baited traps (Table 3.1). According to Rognes (1987), the first and largest peak in flies occurs in March and sampling for this study did not commence until mid-May. The majority of the flies collected were determined to be *P. pediculata* and the remaining to be *P. rudis*, with a total of ten and two specimens, respectively (Table 3.1). These results follow previous studies noting these two species as the most commonly found in North America (Rognes 1987, Whitworth 2006). However, Jewiss-Gaines et al. (2012) states that collections of *P. pediculata* in Ontario peaked in the summer months, but that they could be found year-round.

Table 3.1 - Number of *P. pediculata and P. rudis* individuals collected during trap inspection dates at the Koffler Scientific Reserve near Toronto, Ontario during 2011; trap inspection dates where no *Pollenia* species were collected are not listed. Flies were only caught in meadow plots.

Trap inspection date	***P. pediculata***	***P. rudis***
29 August 2011	2	0
24 September 2011	2	1
24 October 2011	6	1

All specimens were caught in the meadow plots and although it is not clear what type of vegetation previous studies involved, some of them were focused in primarily agricultural fields (Yahnke and George 1972, Greenberg 1998), which are generally more similar to meadows than forests. Wind may play a role in the dispersal of the olfactory cues, which would also explain the increased catch rate with meadows compared to forests, where the trees may act as wind barriers.

Bycatch in ball traps was high for both MeSa and bananas throughout all sampling dates; however, overall catch rates for all species were obviously higher with banana bait (pers. observ. Choi 2011). However, once the bananas had started to decay, saprophagus species such as carrion beetles (Family: Silphidae) started to appear. Overall catch rates for all species were lowest in the forest, intermediate in the edge and highest in the meadows (pers. observ. A. Choi 2011). Pan traps appeared to predominantly attract species of the order Hymenoptera and thus were discontinued after a single sampling attempt.

Ball traps were effective at holding liquids such as the alcohol and MeSa, as well as solids such as the banana bait. Basins were large and capable of holding a large number of specimens, particularly when sampling for longer periods of time such as in this study. In forest plots, however, the tree canopy resulted in many of the ball traps being filled with water and possibly decreasing the effectiveness of the olfactory cues. The receptacles were a feature that would allow separation of bait from the basin; however, there were multiple occasions where flies were found inside the receptacle compartment still alive and escaped during trap inspection. This was an issue as trap rates for *Pollenia* species were low.

Pan traps were ineffective at sampling the target species. Moreover, olfactory food cues could not be used with these traps. In the meadow plots, some of the liquid in the pan traps would be lost during periods of high winds, thus resulting in the loss of some of the collected specimens. Overall, the ability of being able to sample over long periods and to use different baits gave the ball traps an advantage over the pan traps, which could only be used for short periods (1 to 2 days).

MeSa was less effective than bananas as an attractant for dipterans. An advantage of this substance was that it could be bought in a purified, concentrated form, and could be used consistently between experiments. The oily consistency of this chemical allowed it to remain effective throughout the sampling period without evaporation. Bananas attracted an increased amount of dipterans; however, bycatch was also increased, particularly once the bananas started to decay. Future studies should use liquid banana-scented synomone using the same method as the MeSa.

(2) Relating presence of *Pollenia* species to vegetation type and/or flowering species

A total of 21 flowering herbaceous species were identified in forest plots, 13 species in edge plots, and 5 in meadow plots (Appendix 4). Species richness was highest in the forest and lowest in the meadow. Although species richness was higher in the forest plots, some of these species did not have light-coloured petals and/or shallow corollas that would be adapted for pollination by dipterous species. Moreover, the majority of the forest herbaceous species bloomed in the spring (May to June) (Appendix 4). Edge and meadow species included species with lighter-coloured petals and shallow corollas. Species richness was lower; however, almost all of the flowers bloomed later in the year (June to August) (Appendix 4). Certain species, such as Queen Anne's Lace (*Daucus carota* L.), were dominant and abundant in meadow plots, which was also where *Pollenia* species were sampled. It is important to note that species counts in meadows are conservative as rare species were more easily missed due to the long grass. On the contrary, species counts could be conducted more thoroughly in forest plots; however, plant cover was much less in forest plots than in meadows and it was difficult to determine an obviously dominant species.

The height of the traps did not reflect the height of the vegetation between treatments. The height of the traps in the meadow plots was similar to the height of the vegetation in the meadows plots; however, the height of the traps in the forests plots was much higher than the majority of the flowering vegetation found in this vegetation type. This may also explain the lack of *Pollenia* species caught in the forest plots.

(3) Evidence and signs of earthworm injury and/or parasitism

Twenty-five earthworm individuals were found to have bodily wounds throughout the study period representing less than 3% of the total number of earthworms sampled. *Lumbricus* species made up 36% of the wounded individuals, followed by *Octolasion* species with 32%, *D. octaedra* with 20% and *Aporrectodea* species with 12%. Six individuals were sampled 23 May 2011, three were sampled 6 June 2011, four were sampled 20 June 2011, four were sampled 4 July 2011, one was sampled 18 July 2011, three were sampled 8 August 2011, two were sampled 29 August 2011 and two were sampled 24 October 2011. No earthworms with signs of parasitism were sampled 24 September 2011.

The majority of the individuals were found in the forest plots than either the meadows or edges; however, this was due to higher abundances of total earthworms in forest plots compared to meadow plots. The actual proportion of wounded individuals to total individuals did not vary greatly between treatments (ranging from 2-3%).

Wounds were classified into four different types based on their general appearance. Type I included round, cyst-like protrusions which were dark in colour (Figure 3.1). Type II included smaller, dark protrusions emerging usually in multiples along the dorsal line, with a characteristic elliptical shape (Figure 3.2). Interestingly, Type II was only found on individuals of *Lumbricus* species. Type III included balloon-like cysts which were light in colour and empty, or sites where a possible cyst of this nature had burst (Figure 3.3). Type IV included any wounds or signs that could not be characterized into the former three groups.

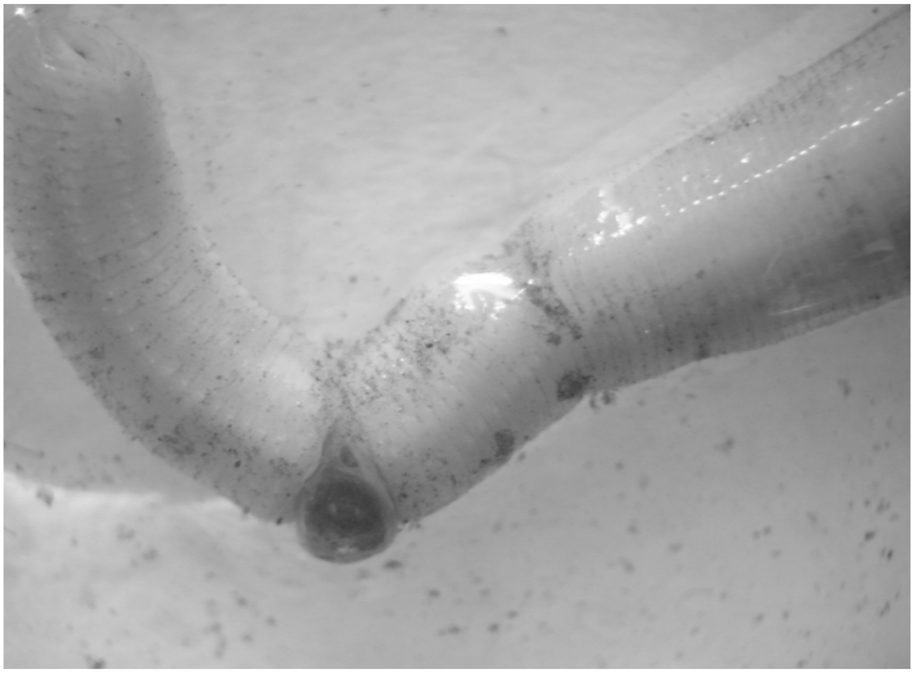


Figure 3.1 - Typical representation of a Type I wound found dorsally on posterior end of an *O. tyrtaeum* adult; note round appearance and dark colour.

Figure 3.2 - Typical representation of a Type II wound found dorsally on a *Lumbricus* juvenile; note the characteristic dark colour and parallel orientation to the dorsal line. This type is usually found in multiples along the dorsal side and was only found on individuals of the *Lumbricus* species.

Figure 3.3 - Typical representation of a Type III wound that has collapsed or burst found dorsally on a *D. octaedra* juvenile. Most of the wounds of this type were already collapsed or burst when examined.

Type I was the most common wound found (32%) and Type II was the least common (16%). Some individuals had wounds that could be placed into more than one category. The majority of the wounds were found dorsally and on the posterior end of the earthworm. Only one earthworm had a wound on its anterior end (Type IV). The low number of anterior wounds could suggest that any injury caused to the anterior end of an earthworm caused increased mortality and thus these worms were not representatively sampled. Thomson and Davies (1973b) found that earthworms parasitized on their posterior ends were capable of autotomizing the infection, reducing mortality.

Pollenia larvae could not be found in any of these wounds and it is hard to infer the cause of these wounds. Unfortunately, past studies where earthworms were parasitized in lab conditions were conducted before high resolution cameras were available to document the appearance of a infected earthworm. Photos from these older studies are blurry and difficult to assess (Yahnke and George 1972, Thomson and Davies 1973a). Moreover, it is possible that the mustard extraction technique may cause irritation to the *Pollenia* larvae as well, causing them to detach before the earthworm reaches the surface. More research is necessary to determine the exact cause of the wounds found in this study.

Conclusions

Overall, attempted field sampling of *Pollenia* species using various traps and synomones did not prove to be successful. A variety of possible factors could have contributed to the lack of flies caught, including low overall density of *Pollenia* species in the field. Although these flies are frequently seen in high abundances when overwintering in buildings, they become greatly dispersed during the warmer months. This is further emphasized in the agricultural areas where these flies are seen as a pest, and where there are only a few residential houses found within a large area.

Due to the gaps in knowledge of the biology and ecology of this fly, as well as the previous issues of misidentification, further studies should focus on determining the life cycle and respective earthworm hosts of these flies in North America. Based on the results of this study, it appears unlikely that there is more than one generation a year, as deemed possible in laboratory rearing in previous studies (Yahnke and George 1972). Moreover, previous taxonomical difficulties have been resolved and visual, photographic keys such as the one developed by Jewiss-Gaines et al. (2012) are helpful and should be utilized in the future, especially by those without strong taxonomic backgrounds. Even so, a future collaboration between an ecologist or biologist and a taxonomist could be of value. It would be difficult to proceed with further attempts to sample flies in the field or study earthworm parasitism without first knowing these important details in life cycle or biology.

CONCLUSIONS AND MANAGEMENT IMPLICATIONS

In the landscape-scale study, earthworm abundances were highest in the most southern Halton Region, as well as in deciduous forests. Functional group composition, particularly epigeic species, varied between regions and slightly between treatments. Epigeic species also followed different trends in terms of earthworm abundance, however, the other groups generally followed the pattern of highest to lowest abundance: Halton Region>Wellington County>York Region> Simcoe County and deciduous forest>mixed forest >plantation>meadow. Patterns of earthworm abundance across the study area show that nearby plots are more similar than plots that are further away from each other. In particular, one could make the interpretation that Simcoe and York are more similar and Halton and Wellington are more similar but the more northerly regions are very different from the more southerly regions. Overall, based on the evidence, region appears to have a stronger effect on earthworm abundance than vegetation type.

It was difficult to determine whether the variations observed in the present study were due to the new concept of invasion succession (Suárez et al. 2006a), or habitat unsuitability based on various other factors such as soil characteristics. As soil characteristics also impact earthworm abundance, relationships between these and region and/or treatment were also assessed. Region had an impact on soil pH, soil moisture, organic matter, and two size classes of sand. Treatment had an impact on organic matter and bulk density. These variations help to explain why region and treatment have an effect on earthworm abundance. As region had a higher impact on earthworm populations than vegetation, factors leading to this trend should be researched in the future. Possible factors that could be related to regional jurisdiction are age of forests, previous land uses, management objectives and practices, and recreational use levels. My research provides insight as to how earthworm populations may vary within a given study area and what factors drive these variations. Future research should focus on identifying factors other than soil characteristics that also may have an impact on the abundance and composition of earthworms, as well as the aforementioned factors tied to municipal region.

In the local-scale study, earthworm abundances were highest in the forest and lowest in the meadow. These results follow the trends that were found in the landscape-scale study. The earthworm abundances in the edge were low to intermediate with intermediate species richness;

these results do not support European studies where edge habitats had the highest diversity (Lagerlöf et al. 2002, Zeithaml et al. 2009). This may be due to the fact that my study was conducted between two natural habitats, whereas previous studies have been conducted between forests and active agricultural fields.

The effects of season were unexpected and the relationship to soil temperature and moisture were complex. It was difficult to fully understand the processes in play due to the short timeframe and lack of multiple locations for this study. However, it is important to note that at the local-scale, climatic variables may play a more important role than the soil characteristics described in the landscape-scale study. Future research should study variations in soil moisture and temperature in more detail, as well as sample for earthworms more frequently. Moreover, it was interesting that patterns of sexual maturity and average biomass per individual earthworm were different in epigeic species than in endogeic and *Lumbricus* species. Similarly in the landscape-scale study, patterns in epigeic species abundance varied from the other two groups. This may be due to the different life strategy of epigeic earthworms; they are univoltine with faster reproductive rates than the other groups (Scheu 1992, Uvarov et al. 2010).

The findings of the landscape and local-scale study have important implications for predicting the spread and invasion of earthworms in southern Ontario. Southern Ontario represents a mosaic of different vegetation types, interspersed with urban areas. As demonstrated in Chapter 1, certain locations and vegetation types have more abundant earthworm populations than others. In Chapter 2, it was shown that earthworm populations in an edge do vary from its adjacent vegetation types, forest and meadow. My results provide insight as to the locations where earthworms are most likely to be found and how edges and fragmentation may affect populations. In the future, certain activities such as continued monitoring, invasive species control, and education should be undertaken by the forest managers of each region.

In terms of continued monitoring, changes in herbaceous species composition should be noted. Certain species, such as *Maianthemum canadense*, are associated with low earthworm densities (Corio et al. 2009), while *Carex pensylvanica* and *Arisaema triphyllum* are associated with high earthworm densities (Hale et al. 2006, 2006, Holdsworth et al. 2007b). Particular species can also be used as indicators to earthworm invasion. *Aralia nudicaulis* and *Streptopus lanceolatus* were found to be indicators of light earthworm invasion while *Maianthemum racemosum* was found in heavily invaded areas (Holdsworth et al. 2007b). Although these species can provide insight on the status of forests, herbaceous species composition does vary between forest type and location and should not be wholly depended on as indicators of earthworm invasion.

The continued control of invasive plants may be important in controlling the spread and abundance of earthworm species. Removal of buckthorn and honeysuckle resulted in significant decreases of earthworm abundance in oak-dominated forests (Madritch and Lindroth 2009). This reduction will be more predominant in forests of low quality litter because invasive species provide a source for palatable litter that would otherwise be unavailable and facilitate growth of earthworm populations. Such an effect would be particularly applicable to the plantations in my study where higher quality litter is largely unavailable.

Education plays an important role in the further spread of invasive earthworms. In a survey of the public, perceptions of earthworms varied greatly, ranging from considering earthworms as pests needing control to earthworms being useful components of ecosystems (Seidl and Klepeis 2011). Ninety percent of the survey respondents from Webb, New York, were familiar with the concept of invasive species; however, only 17% of them recognized earthworms as an invasive species. As the majority of earthworm spread is facilitated by humans, increased education is important to prevent activities such as the dumping of live fishing bait into the forest. Resources for the public, such as the Great Lakes Worm Watch Program developed by the University of Minnesota, provide interactive and easily accessible methods for earthworm education (Callaham et al. 2006, Natural Resources Research Institute 2011).

Finally, it is difficult to determine the role of *Pollenia* species in controlling earthworm populations without understanding the life cycle and host species of these flies. Laboratory experiments have shown that mortality rates of infected earthworms to be as high as 87% (Thomson and Davies 1974). Future research must be conducted to confirm the role of *Pollenia* species in earthworm population fluctuations so that management may include providing overwintering shelters for these flies, for example.

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APPENDICES

Appendix 1 - List of tree and herbaceous species found with respective acronyms

	Common Name	Scientific Name	Acronym
Trees	Balsam Fir	*Abies balsamea* Linnaeus	Bf
	Basswood	*Tilia americana* L.	Bd
	Beech	*Fagus gradifolia* Ehrhart	Be
	Bitternut Hickory	*Carya cordiformis* (Wangenheim) Koch	Hb
	Black Ash	*Fraxinus nigra* Marshall	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
	Ironwood	*Ostrya virginiana* (Miller) Koch	Iw
	Large-toothed Aspen	*Populus grandidentata* Michaux	Alt
	Red Maple	*Acer rubrum* L.	Mr
	Red Oak	*Quercus rubra* L.	Or
	Red Pine	*Pinus resinosa* Aiton	Pr
	Silver Maple	*Acer saccharinum* L.	Msi
	Sugar Maple	*Acer saccharum* Marsh.	Mh
	Trembling Aspen	*Populus tremuloides* Mich.	At
	White Ash	*Fraxinus americana* L.	Aw
	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
	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 Oisier Dog wood	*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

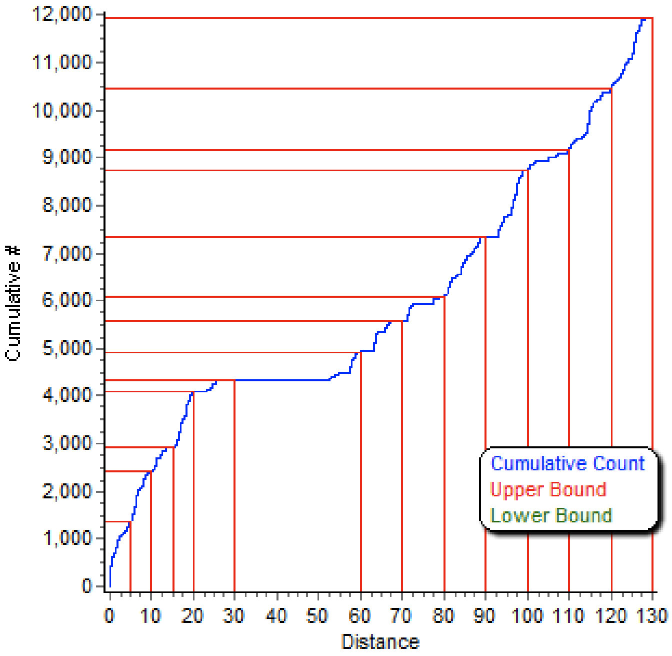
Appendix 2 - Summary of site locations including region, tract name, vegetation type, site code, general location and date sampled

Date	Region	Tract	Vegetation Type	Code	General Location
06/09/2011	**Simcoe**	Coughlin	Plantation	SCPPA	Horseshoe Valley Road W and Highway 27
07/09/2011		Coughlin	Deciduous	SCDFA	Horseshoe Valley Road W and Highway 27
08/09/2011		Coughlin	Mixed	SCMFA	Horseshoe Valley Road W and Highway 27
12/09/2011		Orr Lake	Plantation	SCPPB	South Orr Lake Road and Penatanguishene Road
13/09/2011		Orr Lake	Deciduous	SCDFB	South Orr Lake Road and Penatanguishene Road
14/09/2011		Orr Lake	Mixed	SCMFB	South Orr Lake Road and Penatanguishene Road
15/09/2011		Sandford	Meadow	SCMA	Old Barrie Road West and Line 5 North
16/09/2011		Hutchison	Meadow	SCMB	Old Barrie Road West and Line 7 North
20/09/2011	**Wellington**	Mitchell Woods	Deciduous	CGDFA	Willow Road and Elmira Road North
21/09/2011		Westwood	Deciduous	CGDFB	Willow Road and Westwood Road
22/09/2011		Hanlon Creek (GRCA)	Plantation	CGPPA	Clair Road West and Clairfields Drive W
23/09/2011		Hanlon Creek (GRCA)	Plantation	CGPPB	Clair Road West and Clairfields Drive W
27/09/2011		Hanlon Creek (GRCA)	Meadow	CGMA	Clair Road West and Clairfields Drive W
28/09/2011		Hanlon Creek (GRCA)	Meadow	CGMB	Kortright Road and Hanlon Parkway
29/09/2011		Hanlon Creek (GRCA)	Mixed	CGMFA	Kortright Road and Edinburgh Road
30/09/2011		Hanlon Creek (GRCA)	Mixed	CGMFB	Kortright Road and Edinburgh Road
03/10/2011	**York**	Bendor and Graves	Plantation	YRPPA	Davis Drive and McCowan Road
04/10/2011		Bendor and Graves	Meadow	YRMA	Davis Drive and McCowan Road
04/10/2011		Bendor and Graves	Meadow	YRMB	Davis Drive and McCowan Road
05/10/2011		Hall	Plantation	YRPPB	St. John's Sideroad and McCowan Road
06/10/2011		Hall	Deciduous	YRDFA	St. John's Sideroad and McCowan Road
11/10/2011		Hollidge	Mixed	YRMFA	St. John's Side Road and Highway 48
12/10/2011		Hollidge	Deciduous	YRDFB	St. John's Side Road and Highway 48
13/10/2011		Hollidge	Mixed	YRMFB	St. John's Side Road and Highway 48

17/10/2011	**Halton**	Elliot	Plantation	HRPPA	15 Sideroad and Second Line (Milton)
18/10/2011		Britton (Hilton Falls)	Deciduous	HRDFA	15 Sideroad and Sixth Line (Milton)
19/10/2011		Mahon (Hilton Falls)	Deciduous	HRDFB	10 Sideroad and Guelph Line (Milton)
21/10/2011		Finney	Plantation	HRPPB	15 Sideroad and Sixth Line (Milton)
25/10/2011		Currie (hydroline)	Meadow	HRMA	10 Sideroad and Guelph Line (Milton)
26/10/2011		Conley	Mixed	HRMFA	20 Sideroad and First Line (Milton)
27/10/2011		Snyder	Mixed	HRMFB	Campbellville Road and Twiss Road

Appendix 3 - Distance class ranges, number of pairs in a table and figure

Distance Class	Upper Limit	# of Pairs
1	5	1384
2	10	1051
3	15	500
4	20	1150
5	30	250
6	60	575
7	70	650
8	80	525
9	90	1250
10	100	1400
11	110	425
12	120	1282
13	130	1493



Appendix 4 - The three datasets and their respective sample sizes, mean earthworm densities (individuals m^{-2}), and standard errors for the various treatments and seasons. Modified robust Brown-Forsythe Levene-type test were insignificant showing equal variances between datasets.

Treatment	Dataset	n	Epigeic	±SE	Endogeic	±SE	*Lumbricus*	±SE	Total	±SE
Meadow	All	39	0.28	0.13	1.22	0.36	0.64	0.20	2.15	0.52
	Temporal	27	0.37	0.17	1.44	0.47	0.65	0.22	2.45	0.60
	Spatial	24	0.42	0.19	1.09	0.39	0.78	0.29	2.29	0.70
Edge	All	39	2.24	0.54	1.22	0.31	1.19	0.28	4.65	0.78
	Temporal	27	2.69	0.74	1.48	0.38	1.53	0.37	5.69	1.00
	Spatial	24	1.98	0.64	1.09	0.40	0.94	0.35	4.01	0.91
Forest	All	39	4.30	0.71	11.73	1.61	6.19	0.87	22.21	2.30
	Temporal	27	4.44	0.88	13.38	1.94	7.22	1.17	25.05	2.91
	Spatial	24	4.17	0.84	11.67	2.21	5.68	0.88	21.51	2.80
Spring	All	45	2.53	0.55	7.44	1.49	3.64	0.67	13.61	2.24
	Temporal	27	2.92	0.78	8.70	2.10	4.63	1.00	16.25	3.22
	Spatial	-	-	-	-	-	-	-	-	-
Summer	All	45	2.56	0.62	3.03	0.90	2.03	0.63	7.61	1.71
	Temporal	27	3.19	0.91	4.58	1.40	2.64	0.99	10.42	2.611
	Spatial	-	-	-	-	-	-	-	-	-
Fall	All	27	1.39	0.41	3.01	0.86	2.13	0.65	6.53	1.53
	Temporal	27	1.39	0.41	3.00	0.86	2.13	0.65	6.53	1.53
	Spatial	-	-	-	-	-	-	-	-	-
Total	All	117	2.28	0.33	4.72	0.72	2.67	0.39	9.67	1.17
	Temporal	81	2.50	0.43	5.43	0.92	3.13	0.52	11.07	1.52
	Spatial	72	2.19	0.40	4.62	0.96	2.47	0.42	9.27	1.43

Appendix 5 - Herbaceous species flowering periods and petal colour by treatment from May to August (each column represents a week). x=observed flowering; o=seed/fruit observed

Herbaceous Species																		
Common Name	**Scientific Name**	**Colour**	**May**				**June**				**July**				**August**			
Red/White Baneberry	*Actaea rubra/pachypoda*	white			x	x		o	o		o		o			o		o
Garlic Mustard	*Alliaria petiolata*	white							o		o		o					
Wild Ginger	*Asarum canadense*	red																
Blue Cohosh	*Caulophyllum thalictroides*	yellow							o		o		o			o		
Enchanter's Nightshade	*Circaea lutetiana*	white									x		x/o			o		o
Trout Lily	*Erythronium americanum*	yellow		x														
Fragrant Bedstraw	*Galium triflorum*	white		x	x	x			x		x							
Herb Roberts	*Geranium robertianum*	pink						x	x		x		x			x		x
Blunt Lobed Hepatica	*Hepatica acutiloba*	blue																
Virginia Waterleaf	*Hydrophyllum virginianum*	purple				x		x	x		x		o					
Canada Mayflower	*Maianthemum canadense*	white						x										
False Solomon's Seal	*Maianthemum racemosum*	white		x	x	x		x	o		o		o			o		
Hairy Solomon's Seal	*Polygonatum pubescens*	white		x	x	x		x	o		o		o					
Canada Bloodroot	*Sanguinaria canadensis*	white																
Zig Zag Goldenrod	*Solidago flexicaulus*	yellow																
Dandelion	*Taraxacum officinale*	yellow			x													
Tall Meadow-Rue	*Thalictrum pubescens*	white							o									
Red Trillium	*Trillium erectum*	red		x	x	x			o		o		o			o		
White Trillium	*Trillium grandiflorum*	white		x	x	x			o		o		o					
Painted Trillium	*Trillium undulatum*	pink		x	x	x			o		o		o					
Downy Yellow Violet	*Violette pubescente*	yellow																

Edge Species

Common Name	**Scientific Name**	**Colour**	**May**				**June**				**July**				**August**			
Unidentified Aster	*Hieracium spp.*	yellow						x	x		x		x					
St. John's Wort	*Hypericum perforatum*	yellow									x		x					
Black Medick	*Medicago lupulina*	yellow						x	x		x		x			x		
Alfalfa	*Medicago sativa*	purple											x					
White Sweet Clover	*Melilotus albus*	white						x	x		x		x			x		
Evening Primrose	*Oenothera biennis*	yellow						x	x									
Broad-Leaved Plantain	*Plantago major*	white									x		x					
Yellow Goat's Beard	*Tragopogon dubius*	yellow							x		x							
White Clover	*Trifolium repens*	white							x		x		x					
Common Mullein	*Verbascum thapsus*	yellow											x			x		

**A. syriaca, D. carota, V. cracca* were also found in edge plots

Meadow Species

Common Name	**Scientific Name**	**Colour**	**May**				**June**				**July**				**August**			
Common Milkweed	*Asclepias syriaca*	red									x		x			o		
Canada Thistle	*Cirsium arvense*	purple									x		x			x		
Queen Anne's Lace	*Daucus carota*	white									x		x			x		x
Goldenrod spp.	*Solidago spp.*	yellow									x		x			x		x
Cow Vetch	*Vicia cracca*	purple							x		x		x			x		