

**Ecological consequences of exotic invaders: interactions involving
European earthworms and native plant communities in hardwood
forests**

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Dedicated in loving memory of my father, William A. Hale

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Abstract

During the last few decades, European earthworm species have been invading previously worm free hardwood forest ecosystems in the cold temperate regions of North America. Anecdotal observations suggesting that the abundance and diversity of native plant species and tree seedlings declines following earthworm invasion raised concerns about the potential widespread loss of native forest plant species and the stability of hardwood-forest ecosystems. Incipient earthworm invasions, in the form of visible leading edges, exist in many hardwood forests in northern Minnesota, providing a rare opportunity to document the dynamics of earthworm invasions as they are occurring. Dissertation research objectives were to: (1) Use field studies to describe the rates and patterns of change in earthworm populations across leading edges of invasion and associated changes in the forest floor and upper soil horizons, herbaceous understory vegetation and seedling demography; (2) Use experimental mesocosms to examine the differential effects that the 3 widely distributed European earthworm species have on the forest floor, upper soil horizons and understory vegetation. Visible leading edges advanced up to 30 meters over 4 years and were associated with a succession of earthworm species and increasing total biomass leading to rapid declines in forest floor

thickness, development of a thick A horizon, increased soil bulk density and declines in fine root biomass, nutrient availability, tree seedling density herbaceous plant diversity. Understory plant composition shifted from one of high diversity to a community dominated by *Carex pennsylvanica* and *Arisaema triphyllum* with rare occurrences of other native plant species. In the greenhouse mesocosm experiment individual earthworm species led to increased forest floor mass loss and A horizon thickness, but a 3 species assemblage led to increased nutrient availability and plant mortality with decreases in total fine root and total plant biomass. Individualistic responses among plant species to earthworm treatments also occurred, suggesting that this will be an important factor determining the trajectory of compositional changes in hardwood forests following earthworm invasion.

Table of contents

	Page
Acknowledgements	1
Abstract	2 - 3
Table of contents	4
List of tables and figures	5 - 12
Introduction	13 - 17
Chapter 1: Allometric equations for estimation of ash-free dry mass from length measurements for selected European earthworm species (Lumbricidae) in the western Great Lakes region.	18 - 29
Chapter 2: Exotic European earthworm community composition in northern hardwood forests of Minnesota, U.S.A.	30 - 71
Chapter 3: Effects of European earthworm invasion on soil characteristics in northern hardwood forests of Minnesota.	72 - 111
Chapter 4: Changes in cold-temperate hardwood forest understory plant communities in response to invasion by European earthworms.	112 - 147
Chapter 5: A Mesocosm Experiment: effects of selected European earthworm species (Lumbricidae) on forest floor removal, soil characteristics and native understory plants	148 - 181

List of tables and figures

	Page
Chapter 1	
Table 1. Earthworms used in determining allometric equations.	26
Figure 1. Individual allometric regressions relating measured earthworm length (mm) to ash-free dry mass (grams) for four common European earthworm species or genera.	27
Figure 2. Generalized allometric regression relating measured earthworm length (mm) to ash-free dry biomass (grams) for European earthworms.	28
Figure 3. Frequency distributions of earthworms used to develop the allometric equations.	29
Chapter 2	
Table 1. Grouping of earthworm species used to facilitate analysis.	56
Table 2. Summary Statistics: regressions of earthworm species biomass in relationship to sample point position, site and sample year.	57
Table 3. Indicator values for earthworm species in assemblage groups.	58

List of tables and figures (continued)

	Page
Chapter 2 (continued)	
Figure 1. The locations study sites in the Chippewa National Forest in north central Minnesota. An illustration of the sample grid.	59
Figure 2. Mean and standard error scatter plots of forest floor thickness in relation to sample point position across the sample grid.	60
Figure 3. Pie charts of relative mean earthworm biomass for each species group present in each site.	61
Figure 4. Earthworm species biomass illustrating the site specific patterns in species composition and biomass in relation to the leading edge.	62 - 63
Figure 5. Cluster analysis dendrogram of sample points based on earthworm species biomass.	64
Figure 6. Comparisons of change in forest floor thickness and total earthworm biomass in each of four earthworm species assemblage groups.	65

List of tables and figures (continued)

	Page
Chapter 2 (continued)	
Appendix 1. Mean and standard error scatter sample points of earthworm biomass (ash-free dry biomass) by species in relation to sample point position for each study site in each sample year.	66 - 69
Appendix II. Range and median of earthworm biomass for each study site.	70
Appendix III. Climate data for study sites	71
Chapter 3	
Table 1. Grouping of earthworm species used in analysis.	100
Table 2. Summary Statistics: multiple regression results of soil parameters in relation to site and total earthworm biomass.	101 - 102
Figure 1. The locations study sites in the Chippewa National Forest in north central Minnesota. An illustration of the sample grid.	103

List of tables and figures (continued)

	Page
Chapter 3 (continued)	
Figure 2. Mean and standard error scatter plots of total earthworm biomass in relation to sample point position across the sample grid in each study site.	104
Figure 3. Scatter plots of A horizon bulk density in relation to earthworm biomass in each study site.	105
Figure 4. Scatter plots of O and A horizon thickness in relation to earthworm biomass in each study site.	106
Figure 5. Scatter plots of A and E horizon fine root density in relation to earthworm biomass in all study sites.	107
Figure 6. Paired comparisons of mean N or P availability and total earthworm biomass at opposite ends of the sample grid in each site.	108
Figure 7. Pie charts of mean earthworm biomass for each species group present in each study site.	109

List of tables and figures (continued)

	Page
Chapter 3 (continued)	
Figure 8. Comparisons of total earthworm biomass and soil parameters in relation to earthworm species assemblage groups.	110 - 111
Chapter 4	
Table 1. Correlation coefficients (Pearson r) of herbaceous plant species total cover with ordination axis and the number of plots each species was found (total n = 168).	138 - 139
Table 2. Correlation coefficients (Pearson r) of earthworm species biomass with ordination axis.	140
Table 3. Species diversity of herbaceous plants related to earthworm species assemblage groups, which are described in the text.	141
Figure 1. The locations study sites in the Chippewa National Forest in north central Minnesota. An illustration of the sample grid.	142

List of tables and figures (continued)

	Page
Chapter 4 (continued)	
Figure 2. Scatter plots of herbaceous plant species richness and total percent cover in relation to total earthworm biomass in each study site.	143
Figure 3. Scatter plots of sugar maple seedling density and total cover in relation to total earthworm biomass in all study sites.	144
Figure 4. Sample plot overlays of the herbaceous plant species ordination.	145
Figure 5. Ordination of herbaceous plant species with joint plot overlay of earthworm species biomass vectors for <i>L. rubellus</i> and <i>L.</i> species.	146
Figure 6. Herbaceous plant species-area curves in each earthworm species assemblage group.	147
Chapter 5	
Table 1. Mesocosm experimental design and the number of replicates for each treatment combination. The number of earthworms of each species included is in parenthesis.	170

List of tables and figures (continued)

	Page
Chapter 5 (continued)	
Table 2. Summary Statistics: two-way ANOVA of plant and soil parameters among earthworm treatments and plant species.	171 - 172
Figure 1. Mesocosm design in cross section.	173
Figure 2. Mean forest floor mass loss for each earthworm treatment and plant species.	174
Figure 3. Mean O horizon and A horizon thickness among earthworm treatments across all plant species.	175
Figure 4. Mean O and A horizon percent carbon and nitrogen for the all species earthworm treatment and the no earthworm control.	176
Figure 5. Mean nitrogen and phosphorous availability among earthworms and plant species.	177
Figure 6. Plant mortality among earthworms and plant species.	178

List of tables and figures (continued)

	Page
Chapter 5 (continued)	
Figure 7. Mean mesocosm total coarse root biomass, total fine root biomass, total root biomass, total above ground plant biomass and total plant biomass among earthworms and plant species.	179
Figure 8. Linear regressions of root to shoot biomass among earthworm treatments for each plant species.	180
Figure 9. Mean earthworm numbers and biomass among plant species for each earthworm treatment.	181

Ecological consequences of exotic invaders: interactions involving European earthworms and native plant communities in hardwood forests

Introduction

During the last few decades, European earthworm species have been invading hardwood forest ecosystems in the northern tier of states in the U.S. These hardwood forests developed in the absence of native earthworms and many stands historically had thick forest floor (duff) layers that served as rooting medium for many species of forest herbs and tree seedlings. Anecdotal observations suggest that the abundance and diversity of native plant species and tree seedlings declines steeply following earthworm invasion. Some stands have been observed with only one species of native herb and virtually no tree seedlings remaining. Therefore, concerns had been raised about the widespread loss of native forest plant species and the stability of hardwood-forest ecosystems.

Incipient earthworm invasions in northern Minnesota in the form of visible leading edges were first noted in a few isolated hardwood stands 10-15 years ago. In many stands there exist discrete transition zones where forest floor thickness decreases to zero in as little as 75 meters from areas which have forest floor layers up to 10 cm thick. Preliminary investigations indicated a negative correlation between earthworm abundance and forest floor thickness. The existence of these “leading edges” in northern

Minnesota provided a rare opportunity to document the dynamics of incipient earthworm invasions as they were occurring.

My research objectives were to: (1) Use field studies to describe the rates and patterns of change in earthworm populations across leading edges of invasion and associated changes in the forest floor and upper soil horizons, herbaceous understory vegetation and seedling demography; (2) Use experimental mesocosms to examine the differential effects that the 3 widely distributed European earthworm species have on the forest floor, upper soil horizons and understory vegetation.

As a first approximation, the impacts of invading earthworms are related to their biomass. However, direct measurements of biomass can be significantly affected by the moisture conditions under which the earthworms are collected and their relative gut contents. Ash-free dry mass is the best standardized measure of earthworm biomass but requires the destruction of specimens. Chapter 1 presents 5 allometric equations that allow for estimation of ash-free dry biomass based on length (mm) measurements for European earthworm species (Lumbricidae) commonly seen in the United States.

I identified four sugar maple dominated forests in north central Minnesota on the Chippewa National Forest with active leading edges of earthworm invasion. In each site a sample grid of 45 points (30 x 150 meters) 10 meters apart in three parallel transects with 15 points each was established that spanned the visible leading edge. In Chapter 2, I present the results of 4 years of study describing a succession of earthworm species across the visible leading edge due to different patterns of colonization by different earthworm species. Associated with the succession of earthworm species and increases in

total earthworm biomass, were rapid declines in the forest floor thickness and the advancement of the visible leading edge of up to 30 meters over the 4 year period.

Chapter 3 describes the changes in soil bulk density, soil organic matter, O and A horizon thickness, fine root biomass and nutrient availability associated with the gradient of earthworm biomass and species assemblages represented across the leading edges of invasion. Increasing earthworm biomass was associated with decreased O horizon thickness, increased A horizon thickness and bulk density, shifts in the distribution of soil organic matter in the upper soil horizons, decreased fine root biomass and lower nitrogen and phosphorous availability. Further, different earthworm species assemblages had different affects on soil properties related to their feeding and burrowing habits.

I hypothesized that the ability of a given earthworm species to invade forests with a well developed forest floor and it's ability to remove the forest floor would be more closely linked to its expected level of impact on understory plant communities than biomass alone. In Chapter 4, I present results showing that the abundance and diversity of the herbaceous plant community and the abundance and density of tree seedlings in sugar maple dominated hardwood forests decreased with increasing total earthworm biomass. However, changes in herbaceous plant composition were more strongly affected by the biomass of one species, *Lumbricus rubellus*, than by total earthworm biomass. Where *L. rubellus* was absent there was a diverse community of herbaceous plants including species such as *Caulophyllum thalictroides*, *Uvularia grandiflora*, *Trillium* species, *Osmorhiza claytonii*, *Asarum canadensis* and *Polygonatum pubescens*, which are often used a indicators of rich sugar maple dominated hardwood forests in the Great Lakes region. This was the case even in some instances where there was high biomass of other

earthworm species. Where *L. rubellus* biomass reached its maximum, the herbaceous plant community was dominated by *Carex pennsylvanica* and *Arisaema triphyllum* with rare occurrences of other native plant species.

Chapter 5 presents a greenhouse mesocosm experiment designed to examine the effects of three European earthworm species (*Dendrobaena octaedra*, *Lumbricus rubellus* and *Lumbricus terrestris*) commonly found across North America on forest floor removal, changes in soil characteristics and the mortality and biomass of selected native plant species. Four native understory plant species were included in this study (*Carex pennsylvanica*, *Aralia racemosa*, *Aquilegia canadensis* and *Acer saccharum*) that represent different plant types of particular interest in hardwood forests of the western Great Lakes region. The Randomized Block Design contained five plant treatments (four species and a no plant control) and five earthworm treatments (three individual species, a mixed species treatment and a no earthworm control) with 7 or 14 replicates of each for a total of 280 mesocosms. Mesocosms reproduced worm-free conditions similar to those in the field study sites, using soil and intact forest floor layers collected from similar worm-free sites. Randomly assigned earthworm treatments were added to mesocosms after plants had been established, incubated for 13-18 weeks and then destructively harvested to measure forest floor mass loss, O and A horizon thickness, nutrient content and availability, plant mortality and plant above and below ground biomass. Overall, earthworm treatments led to increases in forest floor mass loss and plant mortality but *Aquilegia* and *Acer* experienced larger increases in plant mortality than did *Carex*, suggesting that *Carex* is less impacted by earthworm invasion. Increases in nutrient availability in response to earthworm treatments did not lead to increases in plant

biomass. Total fine root biomass decreased across all plant species in response to earthworm treatments. However, inverse shifts in the root to shoot ratios occurred among plant species. Individualistic responses among plant species to earthworm invasion will be an important factor determining the trajectory of compositional changes in hardwood forests following earthworm invasion.

Chapter 1

Allometric equations for estimation of ash-free dry mass from length measurements for selected European earthworm species (Lumbricidae) in the western Great Lakes region.

Introduction

In the last decade the invasion of exotic earthworms into previously worm-free glaciated areas of North America has stimulated research into their impacts on native ecosystems in the region (Hendrix and Bohlen, 2002). European earthworms have been invading the North American continent since European settlement (Gates, 1982). In the previously worm-free glaciated regions of North America their appearance in native forest ecosystems is often associated with a cascade of changes in ecosystem function and structure (Bohlen *et al.*, 2004 ; Gundale, 2002; Burtelow *et al.*, 1998; Hendrix 1995). In response to earthworm invasion of a birch-aspen forest, Alban and Berry (1994) documented rapid incorporation of the forest organic horizon, creation of an A horizon and changes in soil carbon and nitrogen. Scheu and Parkinson (1994) demonstrated how earthworm invasion can affect plant growth in an aspen forest. In lodgepole pine forests, McLean and Parkinson (1997) documented decreases in organic matter content, total nitrogen, carbon, basal respiration and metabolic quotient ($q\text{CO}_2$) with increasing earthworm biomass.

To a first approximation, these changes are proportional to earthworm biomass.

Research investigating the effects of the invasion by European earthworms on a wide variety of ecological parameters and ecosystems necessitates an efficient determination of earthworm biomass. Preservation of collected specimens for future reference is often desirable, therefore a method is needed to determine biomass that does not result in the destruction of the specimens. Individual earthworms within a species vary in size and body proportions making the total number of individuals a poor measure of total biomass of a species (McLean and Parkinson, 1997).

Earthworm fresh mass can vary greatly depending on the moisture status of the environment in which they are found (Lee, 1985). Earthworms preserved in formalin commonly lose a significant proportion of their fresh body mass (Lee, 1985). Variability in gut content can account for up to 20 percent of both fresh and dry mass measures of biomass (Lee, 1985; Edwards and Bohlen, 1996). For these reasons, ash-free dry mass measures of biomass which remove gut contents from the dry mass measurement are the most accurate and provide data that is comparable between different sites and under different conditions (Edwards, 1998). Although allometric models relating length to total biomass for selected genera or species of terrestrial Oligochaeta (Lumbricidae) are available (Rogers *et al.*, 1977; Schoener, 1980; Lee, 1985; Collins, 1991), equations relating length to ash-free dry biomass measures of biomass do not exist. This paper presents 5 allometric equations that allow for estimation of ash-free dry biomass based on length measurements for European earthworm species (Lumbricidae) commonly seen in the United States.

Earthworm species assemblages can range widely in a given site. Different earthworms within a species vary in both size and proportion. Therefore, specific equations relating length to ash-free dry mass are needed for each species or group of closely related species (Bohlen, personal communication 2001). The allometric equations presented here are for common earthworm species found in the western Great Lakes region and beyond (Reynolds *et al.*, 2002; Reynolds, 1995) including *Aporrectodea* species, *Octolasion tyrtaeum*, *Dendrobaena octaedra* and *Lumbricus* species.

Methods

Earthworms were collected from four sites on the Chippewa National Forest in north central Minnesota as part of a study investigating the impacts of the invasion by European earthworms on hardwood understory plant communities. The four study sites are similar with respect to overstory composition, soils and stand history. Sugar maple (*Acer saccharum*) is the dominant tree species with yellow birch (*Betula alleghaniensis*), paper birch (*Betula papyrifera*) and basswood (*Tilia Americana*) as secondary species. Soils are deep, well-drained and light colored Eutroboralfs (Warba series) associated with the Guthrie Till Plain (USDA, 1997).

Earthworms used to determine the allometric equations were collected in the fall of 2000 by liquid extraction using a mustard solution (Lawrence and Bowers, 2002; Linden and Wallach, unpublished data). Specimens were preserved in the field with 70% isopropyl alcohol and transferred to 10% formalin at the end of each day. Well-preserved and undamaged specimens were selected for determination of the allometric equations for each species with a range of lengths and sexual maturities (Table 1).

Each earthworm collected was measured to the nearest 1.0 mm. Length was measured on the longest axis of the straightened individual. Individuals were dried for 24-48 hours at 60° C and weighed to the nearest 0.0001g. Individuals were then ashed at 500° C for a minimum of 4 hours. The mass of the remaining ash was measured and subtracted from the dry mass measurement to get the ash-free dry mass of each individual. For small specimens (<40 mm) where the dry mass was <0.009 g, the mean ash-free dry mass was determined from 4-10 specimens of equal length combined into a single sample. Where multiple specimens of the same length were used to determine a mean ash-free dry mass, only one resulting data point was used in the regression analysis.

Regression analysis of natural log-transformed data was used to determine the equations describing the relationship between length and ash-free dry mass for each species or species group (Burton, 1998; Gould, 1965). While a high level of predictive power can often be achieved using non-transformed data ($r^2 \geq 0.90$), the relation of a volumetric measure such as biomass to a linear measure such as length necessitates the use of a power function (Gould, 1965). Earthworm ash-free dry mass (grams) and length (mm) were fit to the natural logarithmic transformation of the standard allometric function, yielding:

$$(1) \quad \ln(\text{ash-free dry mass}) = b \ln(\text{length}) + \ln a$$

Eq (1) not only increases the predictive power (r^2) but also improves the fit of the model at both the small and large extremes of earthworm length.

Results and Discussion

There were no significant differences in either the slope or intercept of the regressions of adults versus juveniles for any species (Glantz, 1992). Therefore, a single regression was calculated for each species, including both juvenile and adult specimens (Fig. 1).

Octolasion tyrtaeum was the only species whose allometric equation of mass vs. length was significantly different from the other species (Fig. 1). This is not surprising since *Octolasion tyrtaeum* generally appears to have a more uniform diameter from head to tail, so biomass would be expected to increase more rapidly with length than in the other species which have a more tapered body shape.

The allometric equations for *Aporrectodea* species, *Lumbricus* species and *Dendrobaena octaedra* individually were not significantly different from each other, indicating that individual equations relating length to ash-free dry mass may not be necessary for these species (Figs. 1, 2). The size range of *Aporrectodea* and *Lumbricus* specimens used to determine the allometric equations overlapped completely, while the size range of *Dendrobaena octaedra* specimens was smaller than those of *Aporrectodea* and *Lumbricus*. The lower r^2 for the regression for *Dendrobaena octaedra* alone is likely due to the small masses and range of masses for that species: even when combining 10 individuals, the dry masses and ash masses were in the range of 0.001 to 0.01 grams making accurate weighing difficult. Inclusion of all three genera (*Aporrectodea*, *Lumbricus* and *Dendrobaena*) in the combined regression yields a generalized allometric equation with high predictive power over a wide range of earthworm sizes.

The maximum lengths of specimens used to determine the allometric equations here are smaller than those reported in taxonomic literature for each species (Reynolds, 1977). However, the range in length used here represented the full range of specimens collected at our study sites over a four year period (Fig. 3). This illustrates the range in variability that is possible within species in different contexts. The regression equations shown here may not provide accurate estimates of biomass for individual specimens larger than those used to develop the regressions. While it is not yet known how different soil types may affect earthworm size and these allometric relationships, ash-free dry weight measures would be expected to be the least variable across different soil types because the gut contents are removed from the measurement.

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Table 1. Earthworms used in determining allometric equations.

Species	# adult	# juvenile	Total #	Length Range (mm)	
				Adult	Juvenile
<i>Octolasion tyrtaeum</i>	20	37	57	15-44	/ 13-39
<i>Aporrectodea</i> species ^a	72	169	241	30-86	/ 10-77
<i>Lumbricus</i> species ^b	23	65	88	30-137	/ 15-125
<i>Dendrobaena octaedra</i>	14	143	157	18-23	/ 10-22

^a included *Aporrectodea rosea*, *Aporrectodea caliginosa* and *Aporrectodea tuberculata*

adults and juvenile *Aporrectodea* where species designation was impossible.

^b included *Lumbricus rubellus* and *Lumbricus terrestris* adults and juvenile *Lumbricus*

where species designation was impossible.

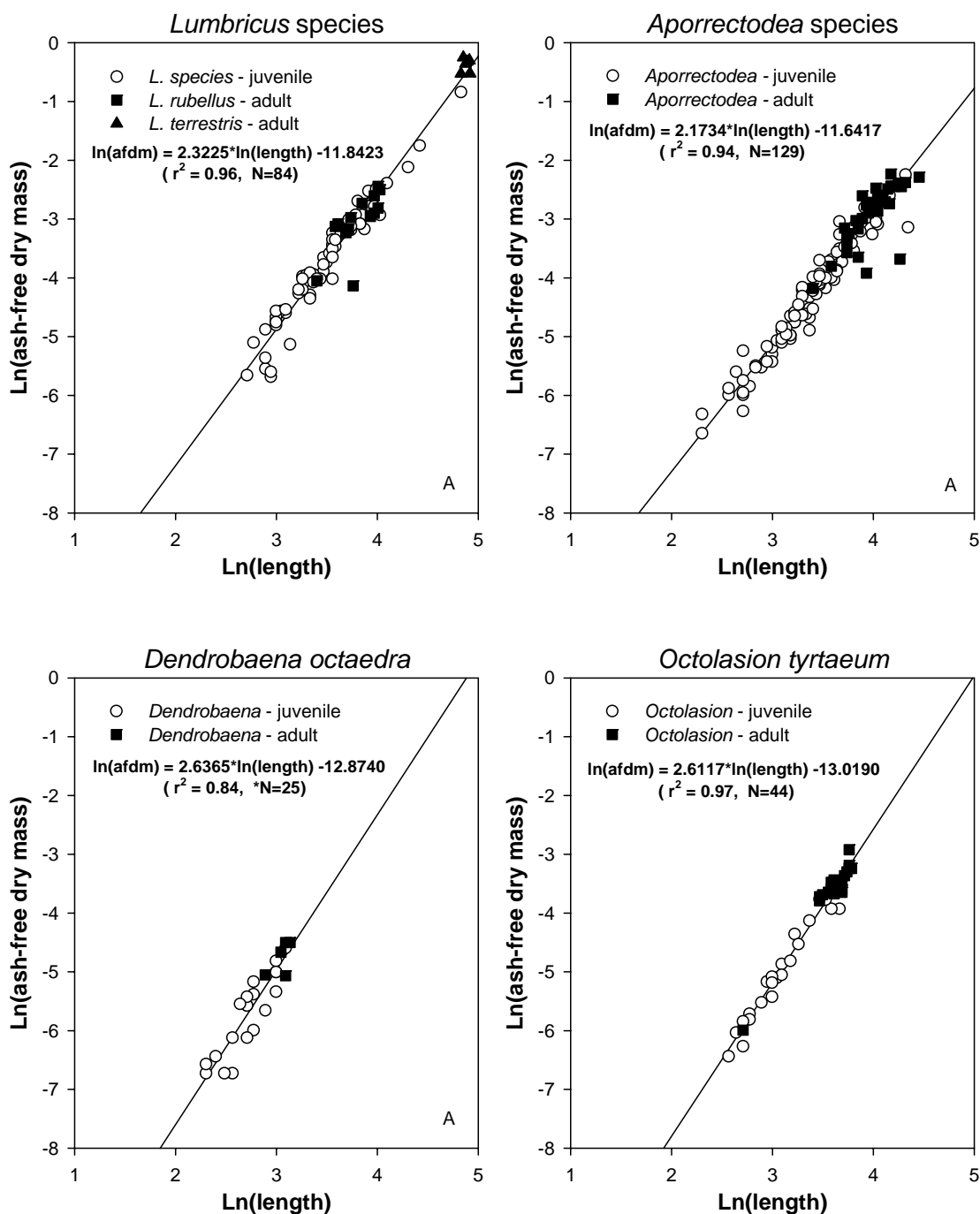


Figure 1. Individual allometric regressions relating measured earthworm length (mm) to ash-free dry mass (grams) for four common European earthworm species or genera. Species groups with the same letter (A) do not have significantly different regressions ($P < 0.01$). * The N value for *Dendrobaena* represents the number of data points rather than the actual number of worms. This was due to the need to determine mean ash-free dry masses for small specimens by combining multiple individuals.

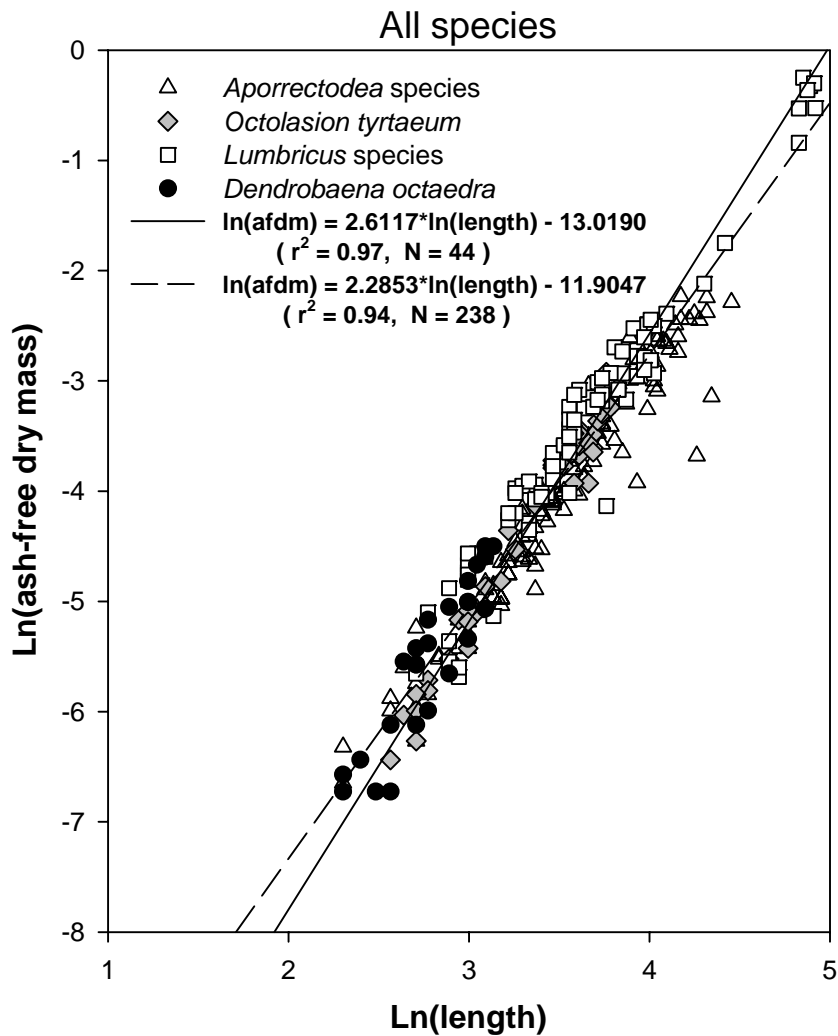


Figure 2. Generalized allometric regression relating measured earthworm length (mm) to ash-free dry biomass (grams) for European earthworms. The dashed line represents the regression for *Aporrectodea* species, *Lumbricus* species and *Dendrobaena octaedra* combined. The solid line represents the regression for *Octolasion tyrtaeum* which was significantly different ($P < 0.01$) than all other species and the generalized regression.

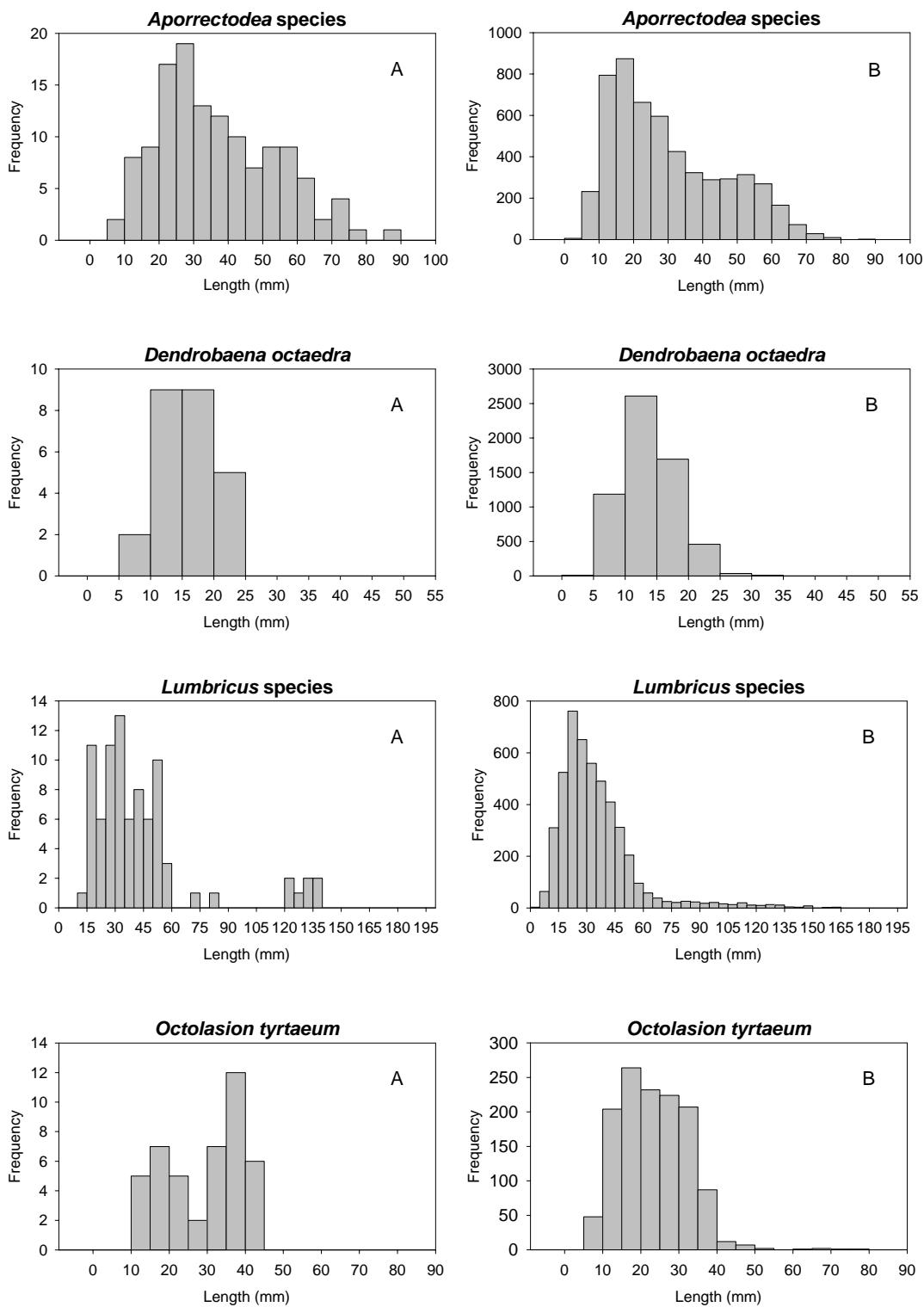


Figure 3. Graphs labeled (A) are the frequency distributions of earthworms used to develop the allometric equations for each species group. Graphs labeled (B) are the frequency distributions of earthworms collected in 1998 - 2001 for each species group.

Chapter 2

Exotic European earthworm community composition in northern hardwood forests of Minnesota, U.S.A.

Introduction

European earthworm invasions have been occurring throughout North America since European settlement (Reynolds 1994, James 1995, Gates 1976, Gates 1982). Many ecosystems in Canada and the northern tier of the United States have developed without earthworms (Gates 1977, James 1998). Earthworms can affect ecosystems by altering litter decomposition and nutrient dynamics (Lavelle et al. 1997). Because they are detritivores that eat great quantities of leaf litter (Nielsen and Hole 1964), invasion of European earthworms can greatly alter ecosystem processes.

Invasions of European earthworms in the Great Lakes region have been in progress since European settlement over a century ago, but have become more numerous during the last few decades because of widespread use of European earthworms as bait for the recreational fishing industry (Alban and Berry 1994, Gates 1982). Because forested areas in the Great Lakes region contain many lakes used for fishing (14,000 in Minnesota, 8,000 in Wisconsin, and 11,000 in Michigan), there are many centers of infestation at this time. In Minnesota, it appears that nearly all forests have been invaded in the southern part of the state, where human activity has been most intense for the longest period of time (Hale, personal observation, Reynolds et al. 2002). In contrast,

European earthworms have only recently begun to invade many hardwood forests in northern Minnesota.

Leading edges of incipient earthworm invasions in northern Minnesota were first noted in a few isolated hardwood stands 10 to 15 years ago (Mortensen and Mortensen 1998). In many stands across the leading edge of invasions, the forest floor thickness decreases to zero in as little as 75 meters from areas which have forest floor layers up to 10 cm thick (Hale, personal observation). Preliminary investigations indicated forest floor thickness declines with increasing earthworm abundance. The existence of these “leading edges” in northern Minnesota provided a rare opportunity to document the dynamics of incipient earthworm invasions.

In 1958, Elton concluded that invading species are more likely to establish viable populations in disturbed or otherwise simplified communities and successful invaders of ecosystems were hypothesized to share one or more characteristics that make them better invaders (Ehrlich 1986). However, in the last decade it has become clear that there is no simple checklist of characteristics that make a particular species more or less likely to invade or to have large versus small impacts (Williamson 1996). Rather than looking only at the characteristics of the invader or for some perturbation or degradation in the habitat being invaded, an alternative perspective involves looking at the potential relationships, both biotic and abiotic (Sharov and Liebhold 1998), between an invasive species and an ecosystem and how those relationships may lead to important effects.

Generally, a suite of earthworm species invade forests, each with different habitat and feeding preferences (Smith 1928, Stebbings 1962, Alban and Berry 1994, Bohlen et al. 2004). Three broad ecological groups have been described (Bouché 1977) including

epigeic species that live and feed exclusively in the litter layer, endogeic species which live and feed in the mineral soil horizon and anecic species which burrow deeply but feed primarily on fresh surface litter. The feeding and habitat preferences of these groups suggest that they may invade ecosystems differently resulting in different spatial distributions during initial invasion (James 1998) and different species assemblages once stable reproducing populations have been established (Hendrix et al. 1999). Additionally, each of these groups of earthworms has different potential to remove the forest floor and therefore, is likely to have different impacts on the ecosystem as a whole.

The invasion of North American temperate hardwood forests by European earthworms constitutes the first documented invasion by a group of detritivores (Mooney and Drake 1986, Williamson 1996, Shigesada and Kawasaki 1998) and as such, there are no generalizations that can be made as to the probable outcomes based on current invasion theory. However, the fundamental role that detritivores play in ecosystems and the unique ability of earthworms to rapidly remove surface litter suggests that the impacts could be great. The introduction of powerful detritivores, such as earthworms, where they did not previously exist, results in a shift of ecosystem control from the forest floor community to the invasive detritivore. The ability of earthworms to exert this level of ecosystem control has been demonstrated in other ecosystems (Edwards et al. 1995).

Here I discuss a study conducted between 1998 and 2001, in which I detailed earthworm community compositions associated with leading edges in four northern hardwood forests in the vicinity of Leech Lake on the Chippewa National Forest, Minnesota, U.S.A.. I hypothesized that 1) the pattern of colonization by different earthworm species would result in different spatial patterns across the leading edge and

2) that there would be species specific effects on forest floor removal and the development of leading edges.

I expected the strictly epigeic species *Dendrobaena octaedra* to be the most common species present in advance of the leading edge and exhibit the widest distribution in the sites because it is well adapted to live and feed in thick organic horizons and is reported to be parthenogenetic (Gates 1974). However, *D. octaedra* is expected to have little direct effect on forest floor removal because even at large numbers, this small bodied species has relatively low total biomass and may feed more directly on microorganisms such as fungi (Scheu and Parkinson 1994). *Lumbricus rubellus*, an epi-endogeic species, is also expected to be widely distributed, but to have a large effect on forest floor removal and development of the leading edge because this moderately sized species will have higher total biomass and has a more generalized feeding pattern (Hendrix et al. 1999). Endogeic earthworms, such as *Aporrectodea* species, are expected to be restricted to areas where the forest floor has been largely removed and incorporated into the mineral soil horizon, because they have been shown to feed on soil organic matter as opposed to surface litter (Hendrix et al. 1999). *Lumbricus terrestris*, a very large bodied anecic species, prefers to feed on fresh surface litter (Bouché 1977). While *Lumbricus terrestris* may burrow through a thick organic horizon to access this fresh litter it does not appear to directly feed on the forest floor until all surface litter has been removed (Hale, personal observations). Therefore, *L. terrestris* is not expected to readily invade areas with intact forest floor. However, following removal of the forest floor it is expected to dominate due to its large body size and high total biomass.

Methods

Study sites and the forest floor leading edge

The study was conducted over four years (1998-2001) in 4 mature northern hardwood stands located on the Chippewa National Forest of northern Minnesota (Figure 1). Four study sites were selected that contained visible leading edges of earthworm invasion and had similar overstory composition, soils and stand history. Three sites were identified and initially sampled in 1998 (Blackduck, Ottertail, Section 19). A fourth site (Two Points) was later identified and all four sites were sampled during 1999, 2000 and 2001. Sugar Maple (*Acer saccharum*) is the dominant tree species on the study sites and secondary species include Yellow Birch (*Betula alleghaniensis*), Paper Birch (*Betula papyrifera*) and Basswood (*Tilia americana*). Ironwood (*Ostrya virginiana*) and American Elm (*Ulmus americana*) are common in the sub-canopy and sapling layers. Soils are a deep, well-drained and light colored Eutroboralf (Warba series) associated with the Guthrie Till Plain (USDA 1997) that in the absence of earthworms are characterized by a thick forest floor composed of L, F and H layers. The climate is humid, continental, cold temperate. The median growing season is 134 days with snow cover from late November to early April. Normal mean temperatures in January and July are -15 and 20 degrees C, respectively (Minnesota State Climatology Office 2003).

For the purpose of site selection and sample point placement, a leading edge was defined as an area in the forest where an abrupt transition, 75-100 m wide, from a thick forest floor to thin forest floor took place and large patches of bare mineral soil with obvious evidence of earthworm activity were present. At each site, a 30 x 150 meter

sample grid was established, consisting of 45 sample points 10 meters apart in three parallel transects with 15 points each (Figure 1). Transects were placed perpendicular to the leading edge with sample point 8 located where the forest floor first dropped to zero, the approximate front of the leading edge. Thus, a well-developed forest floor layer existed in front of the leading edge (sample points 10-15) and exposed mineral soil existed behind the leading edge (sample point 1).

Forest floor thickness was measured at all sites in 1999 – 2001, 1 meter from the center of the sample point at a randomly selected cardinal direction. A different cardinal direction was used in each subsequent year. In 1999 and 2000 forest floor thickness was measured in soil cores 6 cm in diameter and 15cm deep. In 1999, soil cores were collected adjacent to all odd numbered points and sample point 8 in each site (N=27 per site). In 2000, soil cores were collected adjacent to all points in each site (N=45 per site). In 2001, forest floor thickness was measured by using a hand trowel to penetrate and expose the forest floor horizon at each point (N=45 per site).

Earthworm sampling

The earthworm populations were censused adjacent to each sample point using a combination of liquid extraction and hand sifting methods (Lawrence and Bowers 2002, Bouché and Gardner 1984). Sampling of earthworm populations was conducted during September and October of each year because at that time soil moisture had recovered from the normal mid-summer dry period and earthworms were active with a relatively high proportion of sexually mature individuals that are easier to identify than juveniles. All earthworms collected were killed in 70% isopropyl alcohol and preserved in 10%

formalin for identification in the laboratory. Schwert (1990) and Reynolds (1977) were used as authorities for earthworm species identification. Regression models yielding ash-free dry (AFD) earthworm biomass from the measured length of preserved specimens were determined for each species (Hale et al. 2004). These allometric relationships were used to estimate earthworm biomass of all preserved specimens for the study.

Liquid extraction samples were conducted in 0.12 m² (35 cm x 35 cm) subplots located 2.0 meters from each sample point center at a randomly selected cardinal direction. A different cardinal direction was used in each subsequent year. The liquid extraction solution used was 40 g of ground yellow mustard to 4 liters of water. In 1998, liquid extraction sampling was conducted in three sites (Blackduck, Ottertail, Section 19) at a subset of sample points including 1, 8, 15 in all transects and also points 5-7 and 9-11 in transect B (N = 15 in each site). In 1999, 2000 and 2001, liquid extractions were conducted at all 45 sample points in all four study sites, except that only roughly half the sample points were censused in the Two Points site in 1999 and 2001.

Earthworm populations in a subset of sample points were censused using hand sifting as a control to ensure that the results from liquid extractions were representative. Hand sifted samples were conducted in 0.12 m² (35 cm x 35 cm) subplots excavated to 30 cm depth. The subplots were located 4.0 meters from sample point center adjacent to points 1, 8 and 15 at a randomly selected cardinal direction. All soil and forest floor material from each pit was sifted to collect all worms present. Hand sampling of earthworm populations was conducted in at Ottertail in 1999 and at all four sites in 2000 and 2001.

For the purposes of analysis, earthworm species with similar habitat and feeding preferences were combined into analytical groups (Table 1). These analytical groups are similar to the ecological groups described by Bouché and Gardner (1984) and Reynolds (1977), with some specific exceptions. *Dendrobaena octaedra* and *Dendrodrilus rubidus* are two very small bodied, pigmented litter dwelling species. *Dendrodrilus rubidus* was a rare species detected in two of the four sites with very low numbers and biomass relative to *Dendrobaena octaedra* so the two were combined into the group “Dendrobaena”. It is impossible to separate juvenile *Lumbricus rubellus* and *Lumbricus terrestris*, these specimens are therefore treated as a separate juvenile group called “Lumbricus juveniles”. *Lumbricus rubellus* (adults) merits a unique category since it is considered an epigeic species by Bouché (1977) but has a much greater ability to consume and mix litter into the upper soil horizon than the other epigeic species (Gundale 2002). *Lumbricus terrestris* (adult), the sole anecic species, also merits a unique analytical group. A suite of endogeic *Aporrectodea* species including *A. caliginosa*, *A. tuberculata*, *A. trapezoides* and *A. rosea* were combined into the group “Aporrectodea”. *Octolasion tyrtaeum* is generally described as an endogeic species, similar to the *Aporrectodea* species, but it was found in only one of the four study sites and in conjunction with a full complement of *Aporrectodea* species. Therefore, *Octolasion tyrtaeum* was treated separately in all analyses.

Many earthworm species enter a state of aestivation under hot and dry soil conditions which can lead to unrepresentative sampling of earthworm populations. Seasonal moisture deficits (July through September) were calculated (SMD = precipitation – evapotranspiration) in each year of the study. Precipitation and

evapotranspiration data were obtained from the State Climatology Office, Minnesota Department of Natural Resources - Division of Waters. Evapotranspiration was estimated by a modification of the Blaney-Criddle procedure (USDA 1967).

Analytical methods

For purposes of statistical analysis, earthworm biomass data were log transformed to conform to normal distributions (McCune and Grace 2002):

$$b_{ij} = \log_{10}(x_{ij} + d) - c$$

Where $c = \text{integer of } (\log_{10}(\min(x)))$, $\min(x) = \text{lowest non-zero } x$, $d = \text{inverse } \log_{10}(c)$ and b_{ij} is the log transformed value.

This transformation technique was appropriate for this data set because it preserves both zero values and differences in magnitude that exist in the data set.

Paired t -tests were used to compare total earthworm biomass values from hand samples to those of the liquid samples for each sample point position across the sample grid, in each site and year where both were collected (SAS Institute Inc. 2001). Because mean earthworm biomass from the hand samples did not differ significantly from the liquid extraction samples, only the earthworm biomass data collected by liquid extraction were used in subsequent statistical analysis.

The non-parametric Mantel test in PC-ORD (McCune and Mefford 1999) was used to determine if the spatial patterns in forest floor thickness and earthworm species biomass across the sample grid were non-random. The Mantel tests the significance of the correlation between two distance matrices by evaluating results from repeated randomizations, where the order of the rows and columns in one of the distance matrices are shuffled (McCune and Grace 2002). If randomizations frequently result in correlations between matrices that are as strong as the correlation with the original data, then we have little confidence that the original correlation differs from zero. In this case, forest floor thickness and earthworm biomass distance matrices (Sorensen distance measure) were compared to a geographic distance matrix representing sample point location in the sample grid (Urban et al. 2002) using 1000 randomized runs. The standardized Mantel statistic (r) is calculated as the usual Pearson correlation coefficient between the two matrices and is used as a measure of the strength of the relationship between the two matrices. The significance of the relationship is calculated by comparing the Mantel Z statistic of the non-randomized data to the distribution of the Z statistic from the shuffled matrix. A p -value is calculated from the number of randomizations that yielded a test statistic equal to or more extreme than the observed value.

A combination of cluster analyses and indicator species analyses in PC-ORD were used to identify earthworm species assemblage groups (McCune and Mefford 1999). Independent analyses were conducted for data from 1999 and 2000. For each year, earthworm sample points from all four study sites were sorted into species assemblage groups by the group average linkage method (a hierarchical, polythetic, agglomerative clustering method) as described by McCune and Grace (2002), using

Sorensen distance measures. Six potential earthworm species assemblage groups were initially identified in the cluster analysis process. Then indicator species analysis (McCune and Mefford 1999) was used to select a subset of groups which had unique species assemblages by identifying which subset of groups had both the lowest average p-values and the most significant indicators (Dufrene and Legendre 1997). Monte Carlo randomization tests (1000 runs) were used to assess the significance of indicator values for each species in each potential subset of earthworm assemblage groups.

A subset of sample points for which data existed in both 1999 and 2000 was used to assess the relative effects of earthworm species assemblages and total biomass on changes in forest floor thickness. Using the 1999 species assemblage group designations, ANOVA and paired T-tests were used to test for differences in the mean value of forest floor thickness and total earthworm biomass in each of the earthworm species assemblage groups from 1999 to 2000.

Results

Forest floor and the leading edge in relation to sample point position.

In 1998, at site establishment, the location of the front of the leading edge was determined by visual inspection and assigned to sample point 8 on the sample grid. In subsequent years, forest floor thickness was measured directly and the location of the front of the leading edge identified as the sample point where forest floor thickness decreased to zero (Figure 2). In the Blackduck, Ottertail and Section 19 sites, from 1999

to 2001 the leading edge advanced by 20 - 30 meters from 1999 to 2001. In the Two Points site, the leading edge did not appear to advance during the study although mean thickness of the forest floor decreased.

In all study sites, thickness of the forest floor increased significantly ($P \leq 0.05$) across the leading edge, from sample point 1 through 15, in each year except Section 19 in year 2000 where measurable forest floor existed in only one sample point position (Figure 2). Overall, forest floor thickness ranged from 0 to 7.0 cm. The pattern of change in relation to sample point position was non-linear, with horizon thickness generally increasing from zero beginning at point 7 or beyond.

Earthworm composition and biomass variation among study sites

Earthworm biomass and composition were unique at each site (Figure 3, Appendix I and II). The Blackduck site had the lowest total earthworm biomass of all sites. The community was composed primarily of two species groups, *Dendrobaena* and *Aporrectodea* species, with a few individuals of *Lumbricus* species found in the first and fourth years of the study only. While total earthworm biomass was lowest in the Blackduck site, *Aporrectodea* biomass was higher in Blackduck than in any other site. The Two Points and Ottertail sites had roughly 50% higher total earthworm biomass than the Blackduck site. The Two Points site was composed primarily of four earthworm species groups while the Ottertail site contained five species groups. Section 19 had the highest total earthworm biomass, more than twice that found in Blackduck, and contained all six earthworm species groups.

Interannual variation in earthworm species biomass

Earthworm biomass varied among years concomitant with interannual variation in seasonal soil moisture conditions (Figure 3, Appendix III). The mean seasonal rainfall (July through September) at the study sites is 25.9 cm, mean seasonal evapotranspiration is 32.7 cm with a mean seasonal moisture deficit (SMD = precipitation – evapotranspiration) of -5.5 cm. For 1998 through 2001, the seasonal moisture deficits were -15.1, 9.2, -2.6 and -17.4 cm, respectively.

When large seasonal moisture deficits occurred in 1998 and 2001, earthworm abundances were correspondingly low (Figure 3). It is unknown to what degree lower sampled abundances in the drier years represent lower actual populations vs. poorer earthworm capture per unit effort. Moreover, sampling intensity was much lower in 1998 than in other years and sampling was done in only 3 of the 4 sites, making analyses across all sites and years unwieldy. For the above reasons, I present results for 1999 and 2000 throughout the rest of this paper. Nonetheless, results for 1998 and 2001 (or for all four years combined) were generally comparable to those in 1999 and 2000, but patterns were muted.

Earthworm biomass in relation to sample point position and the leading edge

The patterns of species biomass in relation to the direction of invasion were generally similar among sites and years (Appendix II). In multiple regression analyses of the 1999 and 2000 data from all sites, earthworm species biomass had a significant relationship ($P \leq 0.0001$) with sample point “position” across the sample grid for all species groups except *L. rubellus* (Table 2). There were also significant interactions of

sample point “position” with “site” or “year” in many instances (Table 2), indicating some temporal and spatial variation in these relationships.

Although the patterns of species specific earthworm biomass varied across the sample grids, they were characterized by a succession of earthworm species relative to the leading edge (Figure 4). For the *Dendrobaena* group, biomass increased across the sample grid from sample points 1 to 15 in three of four sites, reaching its maximum 20 meters or more in advance of the leading edge (Table 2, Figure 4).

For all other groups with a non-random pattern, biomass decreased across the sample grid reaching their maximums at or behind the leading edge of invasion (Table 2, Figure 4). The biomass of the *Aporrectodea* species group decreased significantly across the sample grid in three of the four sites. In these sites, *Aporrectodea* species biomass reached its maximum 20 to 40 meters behind the leading edge and was low in front of the leading edge. The relationship of *L. rubellus* (adults) biomass to sample point position was inconsistent; when present, it was detected up to 40 meters or more in advance of the leading edge (Figure 4). *Lumbricus* juvenile biomass decreased significantly across the sample grid in two of the three sites where detected and was rarely found more than 10 meters in front of the leading edge. *L. terrestris* (adults) biomass decreased significantly across the sample grid in all three sites where it was detected and was rare in front of the leading edge. *Octolasion tyrtaeum* biomass decreased significantly across the sample grid in the one site where it was detected and was present only 30 meters or more behind the leading edge.

Earthworm species assemblages

Independent cluster and indicator species analyses of 1999 and 2000 earthworm species data resulted in four earthworm species assemblage groups. Splitting the dendrogram resulting from the cluster analysis into these four groups produced the minimum average P-value and maximum number of significant indicator species (Figure 5, Table 3). Group 1 contained sample points with *Dendrobaena* only. Group 2 contained sample points identified by the presence of *Lumbricus rubellus* (adults) and *Lumbricus* juveniles but also contained *Dendrobaena* and *Aporrectodea* species. Group 3 contained sample points with *Aporrectodea* species and *Dendrobaena* as the dominant species. Group 4 contained all six species groups but was identified by the presence of *Lumbricus terrestris* (adults) and *Octolasion tyrtaeum*.

Using the 1999 earthworm species assemblage group designations and sample points where forest floor thickness and earthworm data were both collected in 1999 and 2000 (N=82), mean forest floor thickness and total earthworm biomass were compared. Sample points in worm Group 1 (*Dendrobaena* only) had very low worm biomass and high forest floor thickness, which did not change from 1999 to 2000 (Figure 6). At the other extreme, sample points in worm Group 4 (dominated by adult *Lumbricus terrestris* and with all six species group present) had very high worm biomass in both years and negligible forest floor. Forest floor thickness decreased markedly ($P \leq 0.005$) from 1999 to 2000 in sample points with worm groups 2 or 3 (Figure 6), decreasing from 2.8 cm to 0.8 cm in group 2 and from 0.5 cm to 0.04 cm in group 3. Total earthworm biomass (AFDg/m²) also increased significantly ($P \leq 0.01$) from 1999 to 2000 with sample points in group 3 (Figure 6).

Discussion

The leading edge and earthworm population dynamics

My hypothesis that the pattern of colonization by different earthworm species would result in different spatial patterns across the leading edge was supported (Figure 4). The spatial distribution of earthworm species in relation to the leading edge reflects the habitat preferences and feeding behaviors of the different species groups (Bouché 1977, Hendrix et al. 1999). Strictly epigeic, litter dwelling, species such as *Dendrobaena octaedra* and *Dendrodrilus rubidus* are present in large numbers well in advance of the leading edge, where the biomass of this species group reaches its maximum. Behind the leading edge, where the forest floor layer has been largely removed, their biomass declines to low levels. Epi-endogeic species were represented in this study by a single species, *L. rubellus*. It was present in two of the four study sites and was distributed nearly uniformly across the sample grid, both in advance of and behind the leading edge. As a generalist feeder (Hendrix et al. 1999), this species has the ability to persist and thrive whether or not a forest floor is present. In this study, the endogeic species *Aporrectodea* and *Octolasion* were generally found behind the leading edge (i.e., in worm-invaded territory) and were never found alone. Their preference for feeding on soil organic matter (Hendrix et al. 1999, Bernier 1998) suggests that the establishment of endogeic populations occurs as a result of prior alterations of forest floor material by epigeic and epi-endogeic species making this pool of organic material more accessible (Shakir and Dindal 1997). The sole anecic species represented in this study, *L. terrestris*, was rarely present in advance of the leading edge and reached its population maximum

20 or more meters behind the leading edge. While this species can colonize areas with intact forest floor, it prefers to feed on fresh surface litter and begins to degrade intact forest floor only after most of the surface litter has been consumed (Hale, personal observation). The net result of the differences in feeding and habitat preference of different earthworm species is the successive appearance of these species across the leading edge of earthworm invasion in these northern hardwood forests.

The possibility that different rates of colonization by different species or ecological groups could result in spatial segregation of earthworm faunas was proposed by James (1998). While James's reference was to the potential distribution of species based on climatic tolerances as these various species move north, such a pattern describes what I see at the leading edges of invasion, but on a much smaller spatial scale. Rather than a pattern distributed over many miles or even regions, I see this pattern over 150 meters within forest stands.

Development of the leading edge

In sugar maple dominated forests undergoing initial invasion by European earthworms, discrete leading edges characterized by rapid decreases in forest floor thickness develop following rapid increase in earthworm biomass. As hypothesized, different earthworm species had different effects on the development of leading edges characterized by rapid declines in forest floor thickness. The most rapid development and advance in the leading edge occurred with the appearance of *Lumbricus rubellus* and *Lumbricus* species (Figures 4 and 6). In the two sites where both of these species groups were present (Ottetail and Section 19) the leading edge advanced a minimum of 30

meters, an average of 7.5 or more meters per year (Figure 2). The combination of *Aporrectodea* species and *Dendrobaena octaedra* also resulted in the formation a leading edge, however the rate of advance was less than that seen when *L. rubellus* and *L.* species were present. In the Blackduck site, which contained only *Aporrectodea* (endogeic species) and *Dendrobaena* (strongly epigeic species) the leading edge advanced a total of 20 meters in four years. The species group *Dendrobaena* was present in all sites but was not associated with decreases in forest floor thickness (Figure 6). *L. terrestris* developed large populations behind the leading edge but does not seem to be important in the development or advancement of discrete leading edges in these forests. However, it may be responsible for the development of diffuse leading edges in situations where it is present but the other *Lumbricus* species is not. For example, in the Two Points site a discrete leading edge did not advance, but rather, the forest floor was removed in a patchy distribution reflecting the distributional pattern and feeding behavior of *L. terrestris*, the dominant species in that site.

Long vs. short term effects of earthworm invasion

The development of stable reproductive populations of *Aporrectodea* species and *L. terrestris* after initial invasion prevents recovery of the forest floor because their populations can rapidly adjust to consume all annual litter inputs (Cothrel et al. 1997, Nielsen and Hole 1963). These species also tend to dominate earthworm population assemblages after initial invasion (Figure 4, Cuendet 1984, Shakir and Dindal 1997) so *Aporrectodea* species and *L. terrestris* (adults) may have the most profound long-term impacts on forested ecosystems. However, epi-endogeic species (*Lumbricus rubellus*

(adults) and *Lumbricus* juveniles) are associated with the most rapid loss of the forest floor during initial invasion and may also facilitate the establishment of the endogeic and anecic species, therefore the magnitude of their effects may be much greater than initially assumed from observations of late-stage stable populations of earthworms compared to worm-free conditions. Under this scenario, the overall impacts of invasion by European earthworms is the result of rapid conversion of the forest floor to an alternate state by epigeic and epi-endogeic species and the persistent changes resulting from the establishment of large populations of endogeic and anecic species that follow.

Factors determining earthworm species assemblages and relative abundance

In northern hardwood forests of Minnesota, the overall assemblage of earthworm species found in any given microsite is related to the time since initial invasion, the probability that any given species is present in the source population and the ability of a given species to survive and reproduce in northern climates.

Time since invasion and source populations

In areas with only incipient invasions, *Dendrobaena* is often the only species present (Figure 4) since it is capable of rapidly colonizing the intact forest floor. Early stage invasions that contain more than one species generally contain both *Dendrobaena* and *L. rubellus*. While *L. rubellus* is clearly capable of colonizing intact forest floor, it appears to do so at a slower rate than *Dendrobaena*. In sites that have been invaded for long periods of time (multiple decades), earthworm populations often contain a full suite of species including epigeic, epi-endogeic, endogeic and anecic species. However, some

sites contain a more limited set of species simply because all species have not yet gained access to the site. In this study, the Blackduck site is an example of a site that is relatively remote with poor road access. Consequently, only two species of earthworms have reached that site as compared to the Ottertail and Section 19 sites which have direct access from a heavily traveled, paved road and are adjacent to a large fishing resort established in the 1950s. The Two Points site is on a moderately well maintained gravel road with low traffic but is close to a small fishing resort where, subsequent to site selection, I found that the owner has spent a great deal of time and energy establishing night crawler populations (*L. terrestris*) for his clients. As a result this site is dominated by *Lumbricus terrestris* with other opportunistic species, but does not include *L. rubellus*, the other common bait species used in the area.

In addition to the suite of species described in this study, there are other exotic earthworm species that are imported and distributed commercially that have not yet colonized native ecosystems in Minnesota. The red wiggler, *Eisenia fetida*, is sold and transported all across the state for vermi-composting and many people have reproducing populations in back yard compost piles (Hale, personal observation). However, the inability of this species to survive Minnesota winters outside of a compost heap appears to have prevented it from colonizing adjacent natural habitats. The Asian genus of earthworms, *Amyntas*, which is also widely distributed through the vermi-composting trade (Clapperton, personal communication 2000) has not yet been seen in Minnesota ecosystems despite the fact that it is reported in forests of New York and Pennsylvania. It is not yet known if *Amyntas* is unable to survive in Minnesota forests or if it has simply not yet been introduced.

Vegetation and soil types

The dynamics of earthworm invasion are not expected to be uniform in all forest types. The size and species composition of earthworm populations will be dependent upon soil texture, moisture conditions and the palatability and quantity of litter (Lavelle 1997; Ponge and Delhaye 1995). Conifer forests are expected to be largely resistant to invasion by many species of earthworms due to both the propensity of these forests to grow in less productive or drier soils and their low quality, unpalatable litter (Dymond et al. 1997). Mesic oak forests (*Quercus rubra*) in Minnesota occur on similar soils and have similar understory plant communities as compared with sugar maple dominated forests (Hale et al. 1999) and may be expected to support similar earthworm populations. However, dry oak forests (*Quercus macrocarpa*, *Quercus alba*, *Quercus velutina*) on sandy soils would be expected to support much smaller and less diverse earthworm populations suggesting that impacts may be small (Shakir and Dindal 1997).

Climate and soil moisture conditions

Depending on soil moisture conditions, the relative abundance of earthworms in any given site can vary greatly from year to year (Figure 3, Appendix III). In this study, 1998 and 2001 had larger than average seasonal moisture deficits. The relative abundance of *Aporrectodea* species and *L. terrestris* (adults) were more negatively affected by drought conditions than the other species groups (Figure 3), although I cannot be sure whether my sampling reflects changes in abundance or their position in the soil profile. In 2001, *L. terrestris* was not detected in the Ottertail site, illustrating that extreme drought conditions can lead not only to underestimation of the abundance of species but may

result in missing drought sensitive species altogether. When quantitatively assessing earthworm populations, the local climate and soil moisture conditions must be considered to evaluate the accuracy and reliability of the data.

The timing of earthworm sampling should also be directed by typical seasonal moisture patterns for the area. For example, the mean seasonal moisture deficit at my study sites is -5.50 cm. During July and August soil moisture conditions normally decline in these areas and rebound again when fall rains begin in late August. For this reason I conducted earthworm sampling late in September when soil moisture conditions have increased, providing me with the most accurate measure of earthworm populations.

In conclusion, I find that declines in the forest floor thickness at leading edges develop in response to rapid increase in earthworm biomass associated with a succession of earthworm species. Different species had different abilities to remove forest floor material resulting in different patterns and rates of loss of the forest floor during initial invasion. The establishment of stable reproducing populations of earthworms behind the leading edge of invasion prevents recovery of the forest floor.

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Table 1. Grouping of earthworm species used to facilitate analysis.

Analytical group	Ecological Group	Species included
Dendrobaena	epigeic	<i>Dendrobaena octaedra</i> , <i>Dendrodrilus rubidus</i>
L. rubellus adults	epi-endogeic	<i>Lumbricus rubellus</i> adults
L. juveniles	epi-endogeic / anecic	<i>Lumbricus</i> juveniles
Aporrectodea	endogeic	<i>A. caliginosa</i> , <i>A. tuberculata</i> , <i>A. trapezoides</i> , <i>A. rosea</i>
Octolasion	endogeic	<i>Octolasion tyrtaeum</i>
L. terrestris adults	anecic	<i>Lumbricus terrestris</i> adults

Table 2. Summary Statistics: regressions of earthworm species biomass in relationship to sample point position, site and sample year.

Analytical groups	Position	Site	Year	Position*Site	Site*Year
Dendrobeana	+	Yes	No	Yes	Yes
Aporrectodea	-	Yes	No	Yes	Yes
L. rubellus adults	NS	Yes	No	Yes	Yes
L. juveniles	-	Yes	No	Yes	No
L. terrestris adults	-	Yes	No	Yes	No
Octolasion	-	Yes	No	Yes	No

Symbols - or + indicate the direction of the relationship of earthworm biomass to increasing sample point number ($P \leq 0.0001$). NS indicates no significant relationship. Yes or No in subsequent columns indicate significant or non-significant main and interaction effects, respectively, at $P \leq 0.05$.

Table 3. Indicator values for earthworm species in final species assemblage groups.

Analytical groups	<u>1999 Groups</u>				<u>2000 Groups</u>			
	1	2	3	4	1	2	3	4
Dendrobeana	32	30	25	7	28	30	27	9
Aporrectodea	0	10	41	40	1	7	40	40
L. rubellus adults	0	11	1	44	0	43	0	34
L. juveniles	0	43	0	56	0	40	2	51
L. terrestris adults	0	1	2	44	0	0	1	64
Octolasion	0	0	0	56	0	0	0	56

Indicator values are significant ($P \leq 0.01$) for all analytical groups in each year.

For 1999, the number of sample points in groups 1, 2, 3 and 4 were 11, 22, 24 and 27, respectively.

For 2000, the number of sample points in groups 1, 2, 3 and 4 were 16, 41, 67 and 51, respectively.

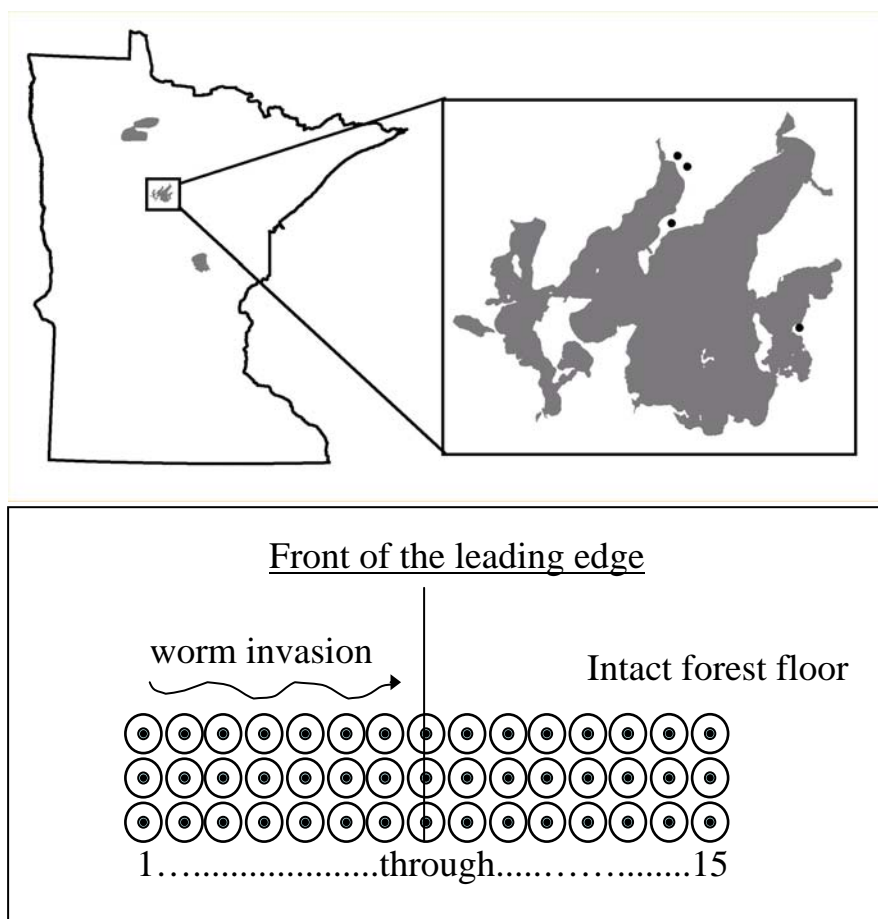


Figure 1. The locations of four study sites in the Chippewa National Forest near Leech Lake in north central Minnesota. An illustration of the sample grid (30 m x 150 m) of 45 points laid perpendicular to the leading edge of earthworm invasion in each study site. Sample points are 10 m apart. Earthworm populations were sampled in a randomly located 35 cm x 35 cm area ($.12 \text{ m}^2$) 2 m from each sample point. Soil cores 6 cm in diameter and 15cm deep were collected at randomly selected locations 1 m from each sample point.

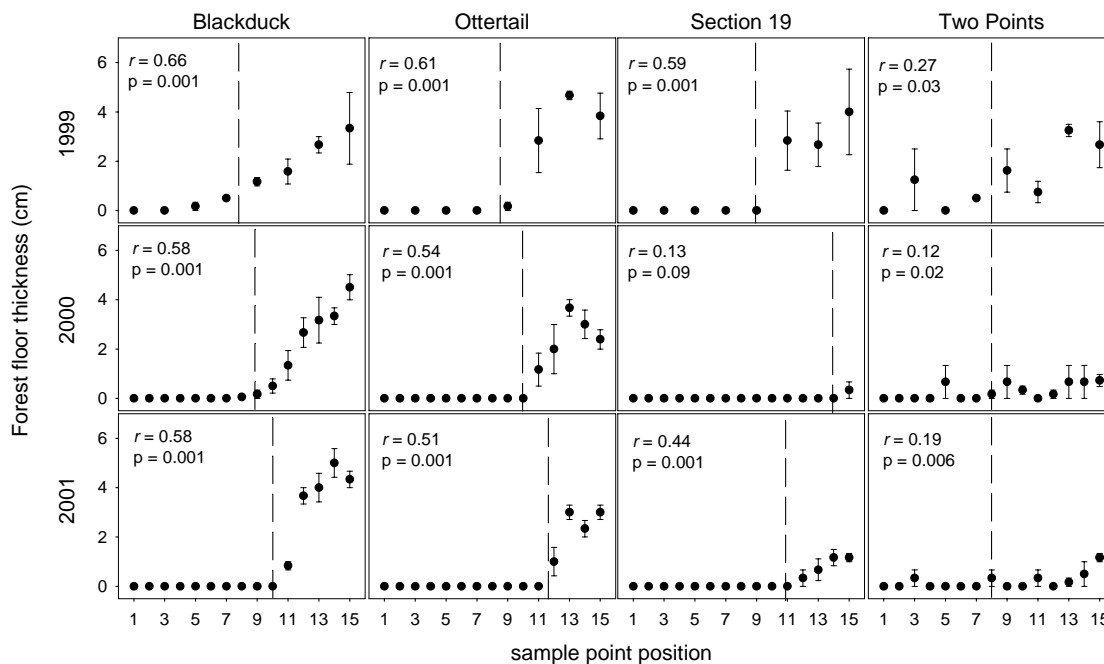


Figure 2. Mean and standard error scatter plots of forest floor thickness (cm) in relation to sample point position across the sample grid in each of four study sites for years 1999 through 2001. Vertical dashed reference lines indicate the position of the leading edge for each site in each year, given that sample point 8 was determined to be the front of the leading edge by visual inspection at establishment of the sample grid. In subsequent years, forest floor thickness was measured directly and the location of the front of the leading edge identified as the sample point where forest floor thickness decreased to zero. The standardized Mantel statistic (r) indicates the strength of the relationship between sample point position and forest floor thickness and the associated p -value indicates the significance of that relationship. In 1999, $N = 24$ per site. In 2000 and 2001, $N = 45$ per site.

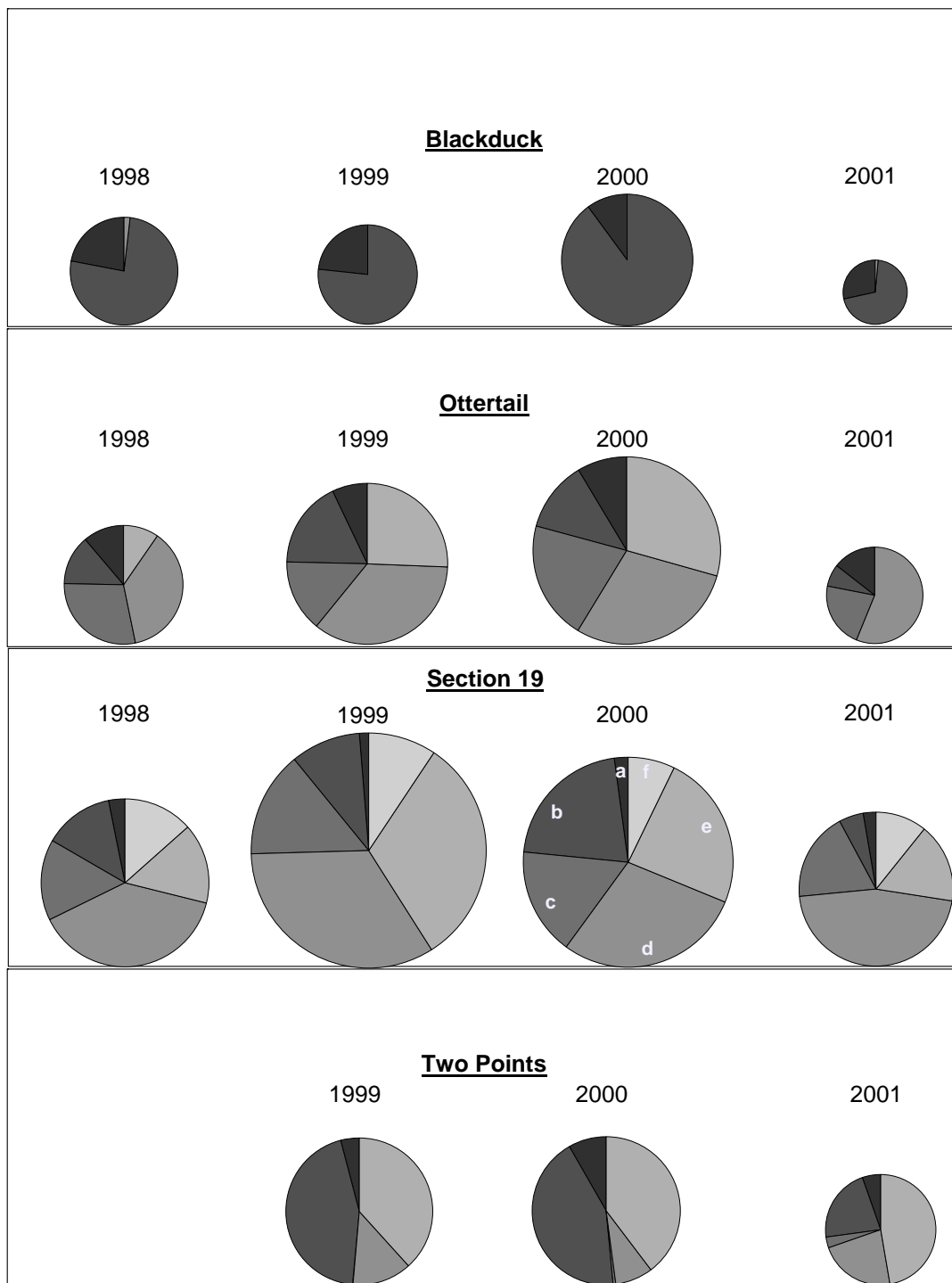


Figure 3. Pie charts of relative mean earthworm biomass for each species group present in each site, where a = *Dendrobaena*, b = *Aporrectodea*, c = *L. rubellus* adult, d = *L.* juveniles, e = *L. terrestris* (adult) and f = *Octolasion*. The size of pie charts is proportional to total earthworm biomass among all sites and years.

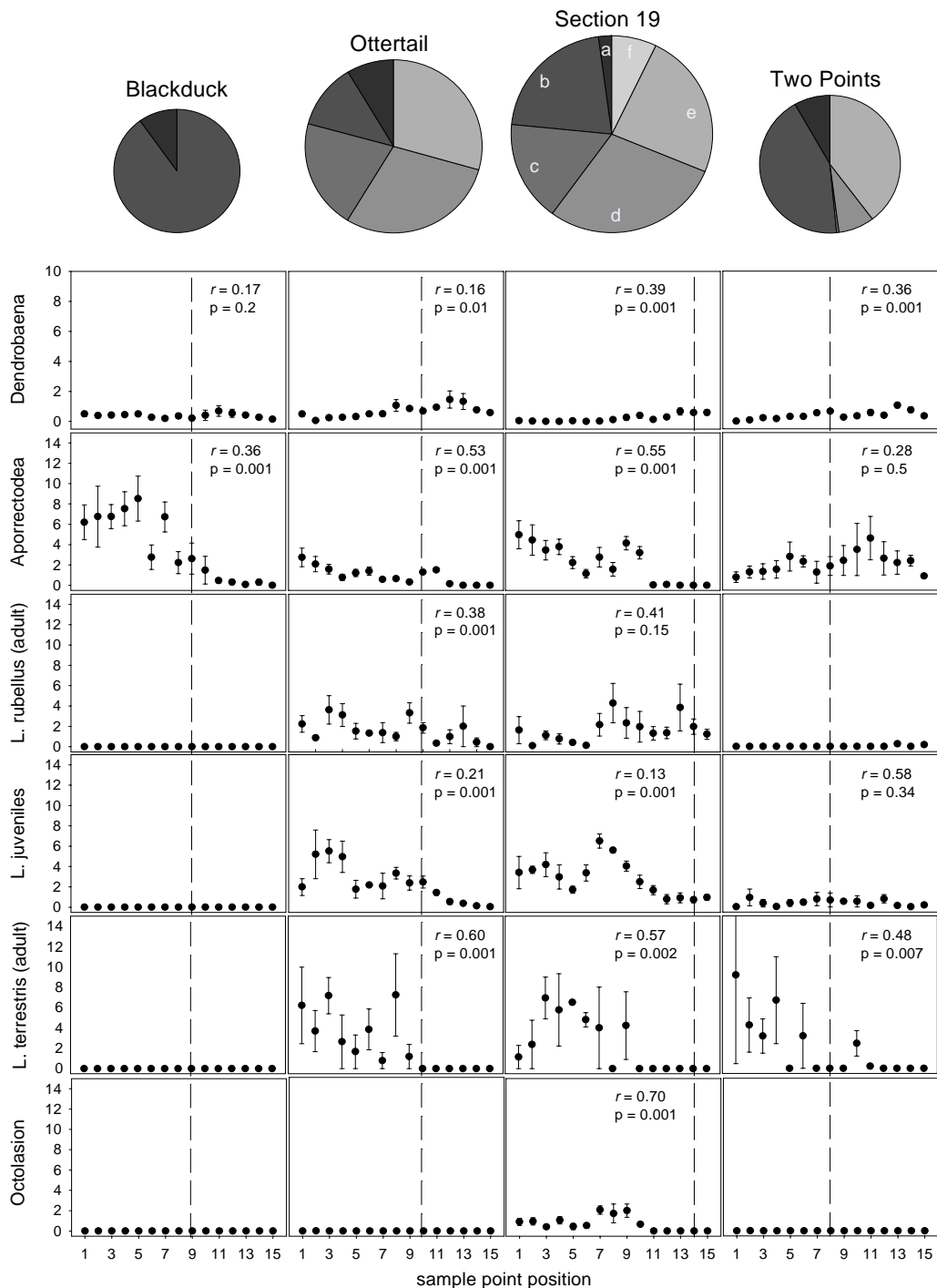


Figure 4. Earthworm species biomass data from year 2000 illustrating the site specific patterns in species composition and biomass in relation to the leading edge. Pie charts of relative mean earthworm biomass for each species group present in each site, where a = *Dendrobaena*, b = *Aporectodea*, c = *L. rubellus* (adult), d = *L. juveniles*, e = *L. terrestris*

(adult) and $f = Octolasion$. The size of the pie chart is proportional to the total earthworm biomass for each site. Mean and standard error scatter plots of earthworm biomass (ash-free dry g/m^2) for each of six species groups in relation to sample point position and the leading edge in each study site. Vertical dashed reference lines indicate the position of the leading edge for each site in year 2000. The standardized Mantel statistic (r) indicates the strength of the relationship between sample point position and earthworm species biomass and the associated p-value indicates the significance of that relationship. $N = 45$ in each site.

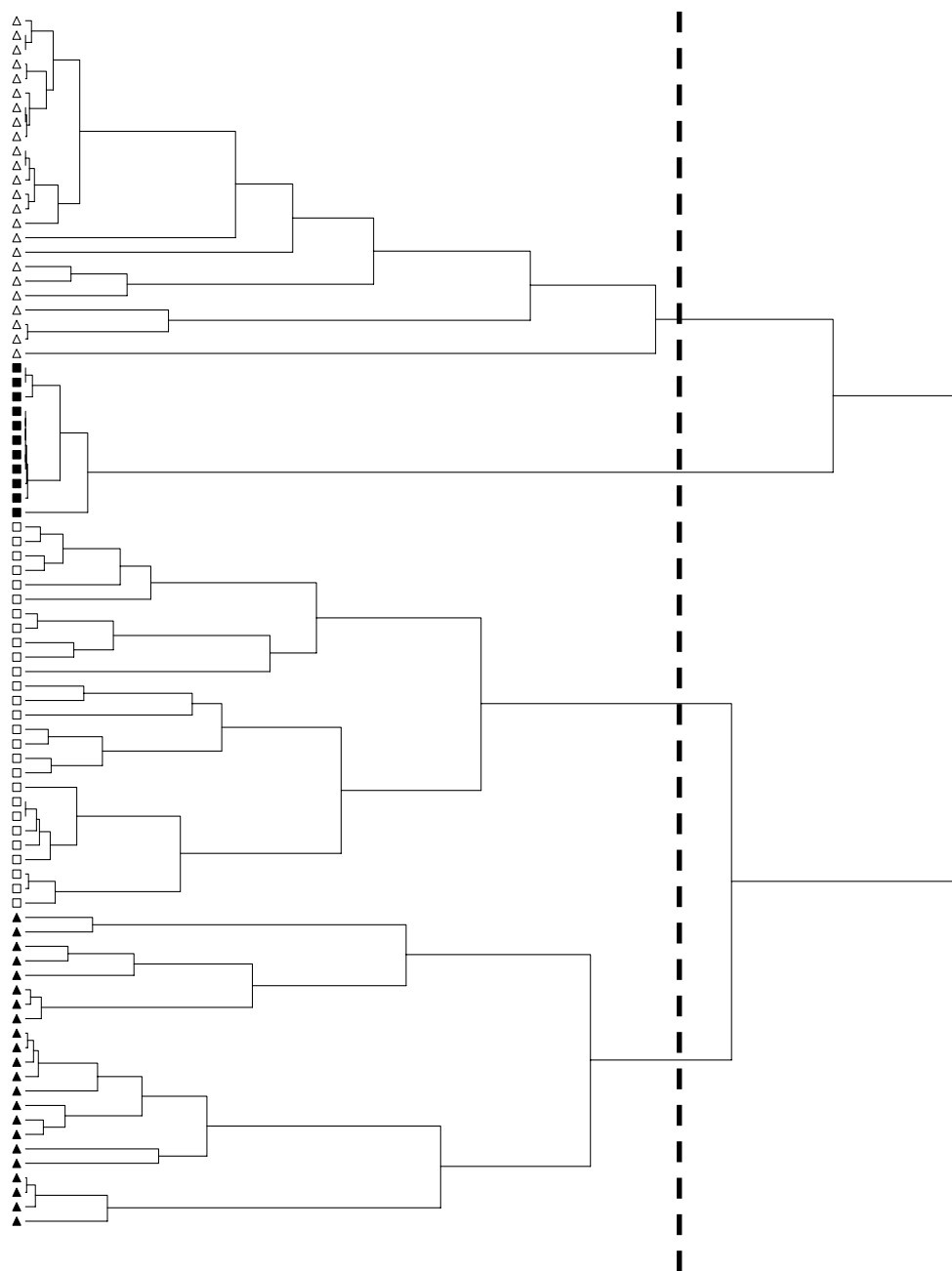


Figure 5. Cluster analysis dendrogram of sample points based on earthworm species biomass in 1999. The vertical dashed line indicates where the dendrogram was cut based on indicator species analysis, identifying four earthworm species assemblage groups, solid squares = sample points with *Dendrobaena octaedra* only; solid triangles = sample points identified by the presence of and *Lumbricus* species (juveniles) and *Lumbricus rubellus*; empty triangles = sample points with *Aporrectodea* species and *Dendrobaena octaedra* as the dominant species; empty squares = sample points identified by the presence of *Lumbricus terrestris* and *Octolasion tyrtaeum*.

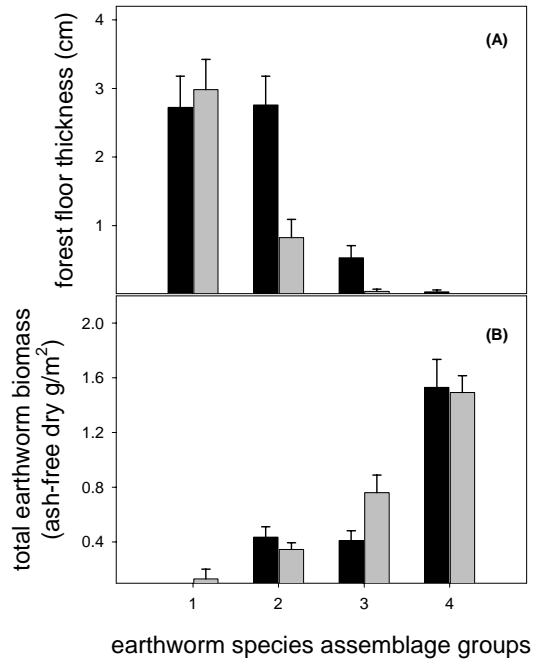
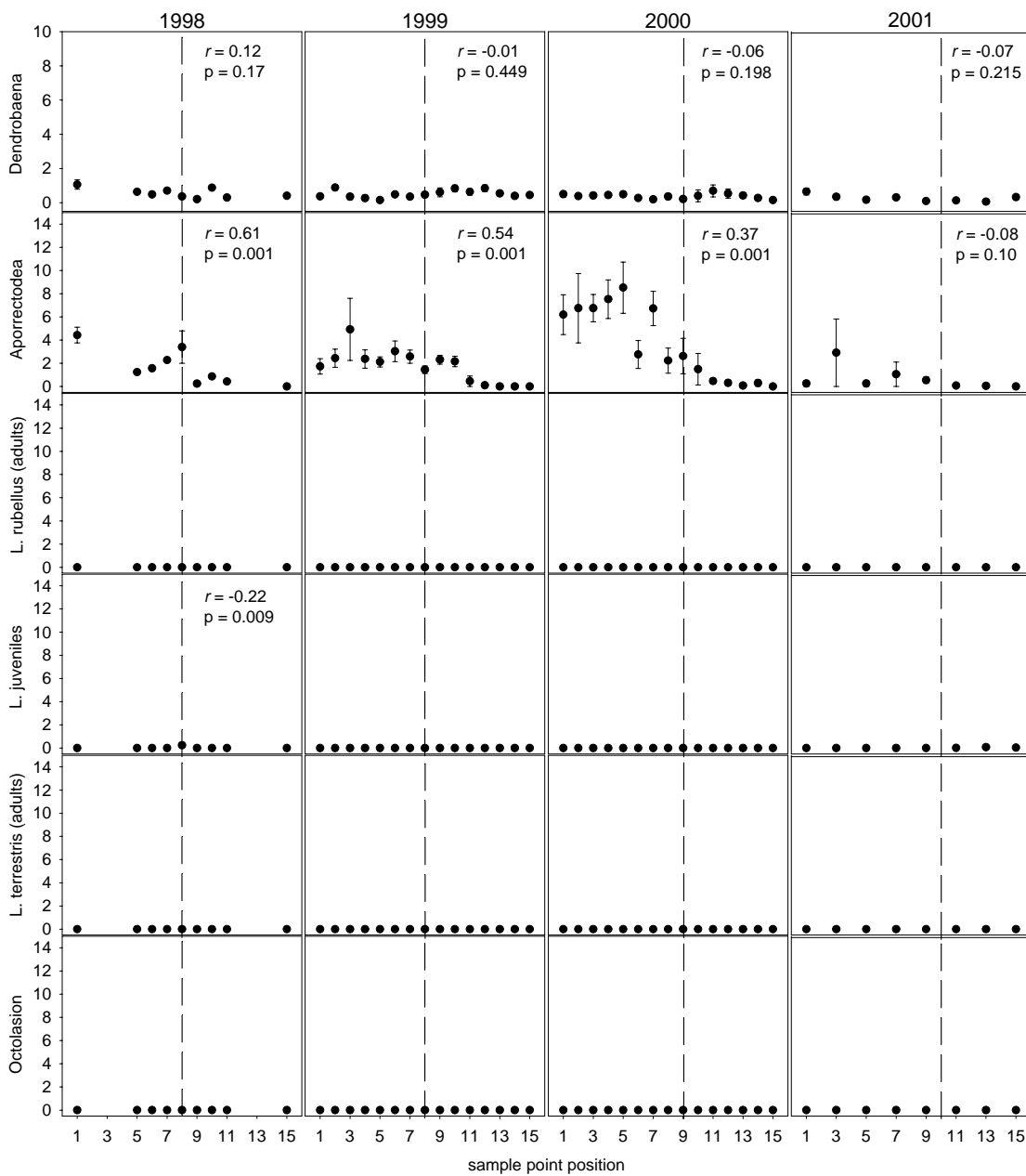


Figure 6. Comparisons of change in forest floor thickness (A) and total earthworm biomass (B) in each of four earthworm species assemblage groups from 1999 (black bars) to 2000 (gray bars). Group 1 contains *Dendrobaena* only (n=10), group 2 is dominated by *Lumbricus* juveniles and *L. rubellus* (adults) (n=22), group 3 is dominated by *Aporrectodea* and *Dendrobaena* (n=24) and group 4 is indicated by the presence of *L. terrestris* (adults) and *Octolasion* (n=27).

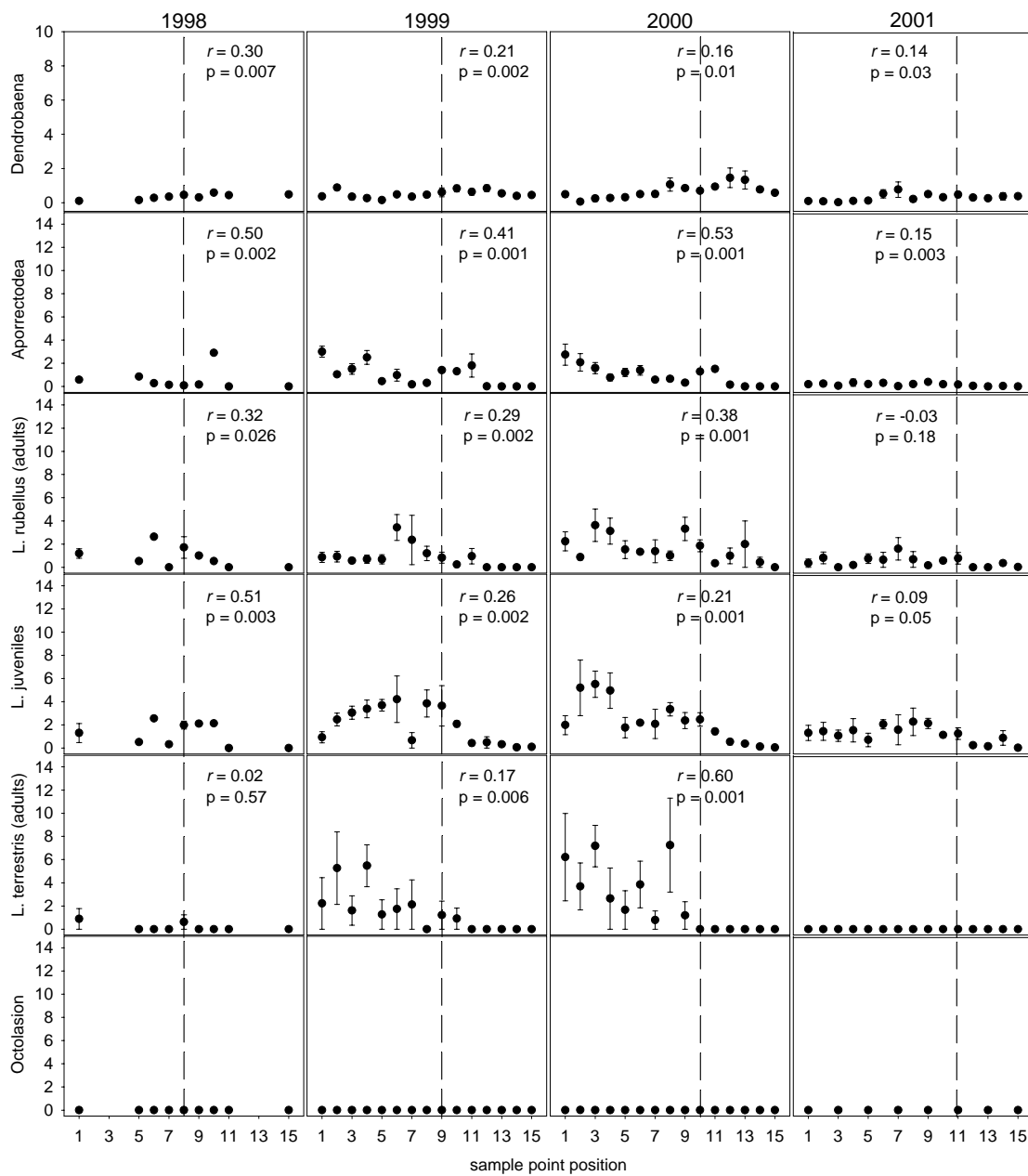
Appendix I.

Mean and standard error scatter sample points of earthworm biomass (ash-free dry biomass) by species in relation to sample point position for each study site in each sample year. The standardized Mantel statistic (r) indicates the strength of the relationship between sample point position and earthworm biomass and the associated p -value indicates the significance of that relationship ($N = 45$ per site).

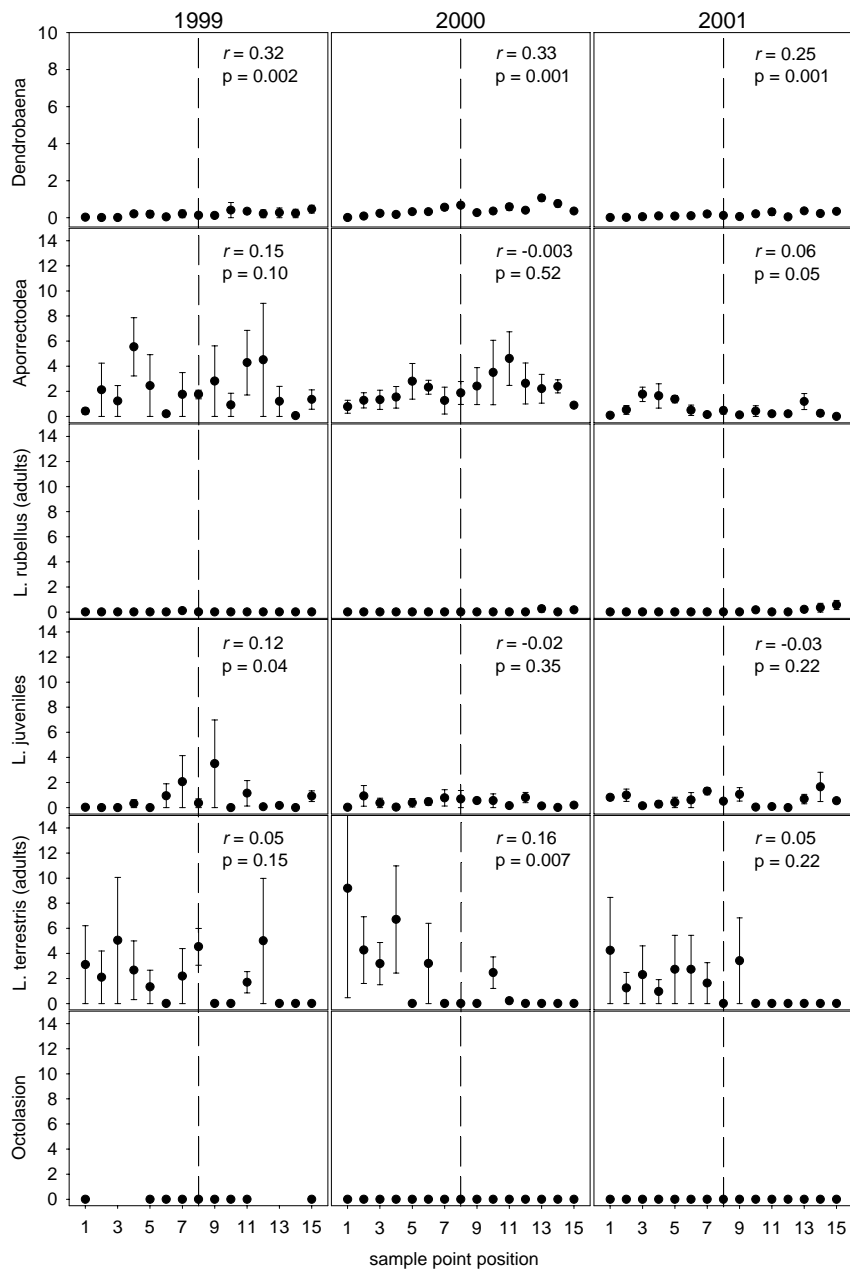
Blackduck



Appendix I. (continued)

Ottertail

Appendix I. (continued)

Two Points

Appendix II.

Range and median (in parenthesis) of total earthworm biomass and species specific biomass (ash-free dry grams/m²) per sample point for each study site (in each site N=15 for 1998, N=45 for 1999 – 2001 except Blackduck 2001 where N=27).

Site Species group	1998	1999	2000	2001
Blackduck total biomass	.18 - 7.16 (2.05)	.27 - 10.15 (1.86)	0 - 13.28 (2.66)	0 - 9.13 (.36)
<i>Dendrobeana</i>	.18 - 1.50 (.51)	.81 - 1.16 (.45)	0 - 1.40 (.32)	0 - .86 (.18)
<i>Aporrectodea</i>	0 - 6.17 (1.56)	0 - 9.48 (1.46)	0 - 12.91 (2.45)	0 - 8.73 (0)
<i>L. rubellus</i> adults	0	0	0	0
<i>L. juveniles</i>	0 - .37 (0)	0	0	0 - .13 (0)
<i>L. terrestris</i> adults	0	0	0	0
<i>Octolasion</i>	0	0	0	0
Ottertail total biomass	.36 - 6.15 (3.30)	.36 - 14.85 (4.73)	.45 - 22.13 (5.77)	0 - 6.74 (1.87)
<i>Dendrobeana</i>	.009 - .64 (.35)	0 - 1.38 (.30)	0 - 2.48 (.63)	0 - 1.67 (.21)
<i>Aporrectodea</i>	0 - 2.90 (.14)	0 - 3.85 (.82)	0 - 4.29 (.64)	0 - .96 (.050)
<i>L. rubellus</i> adults	0 - 3.49 (.53)	0 - 5.39 (.34)	0 - 6.01 (1.20)	0 - 3.34 (0)
<i>L. juveniles</i>	0 - 2.93 (.63)	0 - 7.64 (1.60)	0 - 9.71 (1.91)	0 - 4.15 (.89)
<i>L. terrestris</i> adults	0 - 2.66 (0)	0 - 10.81 (0)	0 - 13.99 (0)	0
<i>Octolasion</i>	0	0	0 - .013 (0)	0
Section 19 total biomass	1.17 - 16.40 (6.57)	.72 - 36.75 (10.49)	1.08 - 23.87 (10.2)	.81 - 14.42 (4.38)
<i>Dendrobeana</i>	0 - .74 (.13)	0 - 1.47 (.05)	0 - 1.00 (.07)	0 - .73 (.04)
<i>Aporrectodea</i>	0 - 2.85 (.48)	0 - 4.92 (1.04)	0 - 7.72 (1.85)	0 - 1.78 (.02)
<i>L. rubellus</i> adults	0 - 4.17 (.53)	0 - 13.62 (1.03)	0 - 8.27 (.78)	0 - 3.56 (.79)
<i>L. juveniles</i>	.54 - 5.42 (1.97)	0 - 15.50 (3.28)	.18 - 7.85 (2.40)	.27 - 10.40 (2.00)
<i>L. terrestris</i> adults	0 - 8.62 (0)	0 - 24.97 (0)	0 - 12.25 (0)	0 - 7.35 (0)
<i>Octolasion</i>	0 - 2.46 (0)	0 - 9.51 (.05)	0 - 3.51 (.43)	0 - 3.82 (.25)
Two Points total biomass	Not sampled	0 - 19.53 (2.66)	0 - 28.44 (3.35)	0 - 13.08 (1.46)
<i>Dendrobeana</i>	Not sampled	0 - .82 (.07)	0 - 1.39 (.35)	0 - .65 (.08)
<i>Aporrectodea</i>	Not sampled	0 - 9.31 (.84)	0 - 8.52 (1.76)	0 - 3.51 (.25)
<i>L. rubellus</i> adults	Not sampled	0 - .20 (0)	0 - .75 (0)	0 - 1.24 (0)
<i>L. juveniles</i>	Not sampled	0 - 6.99 (0)	0 - 2.57 (.02)	0 - 3.96 (.39)
<i>L. terrestris</i> adults	Not sampled	0 - 10.06 (0)	0 - 26.60 (0)	0 - 12.69 (0)
<i>Octolasion</i>	Not sampled	0 - .05 (0)	0	0

Appendix III.

Climate data for study sites from the State Climatology Working Group
(<http://climate.umn.edu>)

Cass County data

Year	Annual	May	<u>Precipitation (cm)</u>			
			June	July	Aug	Sept
1998	73.12	10.59	14.61	10.54	3.61	7.57
1999	80.26	17.34	9.65	16.69	13.26	9.40
2000	72.87	8.10	10.90	7.26	12.37	4.98
2001	68.14	9.63	8.89	5.84	7.26	4.34
30 year mean :	66.04	6.99	8.89	10.80	8.89	6.99

Leech Lake Dam station data

Year	Annual	May	<u>Precipitation (cm)</u>			
			June	July	Aug	Sept
1998	70.26	10.92	19.48	10.03	5.26	5.44
1999	84.28	12.83	13.87	19.15	16.05	7.90
2000	71.12	7.06	11.43	6.66	17.48	6.22
2001	60.71	9.70	8.89	6.76	6.12	4.90
To date mean^a:	64.47	7.70	10.03	9.96	9.04	6.91

Year	Annual	May	<u>Evapotranspiration^b (cm)</u>			
			June	July	Aug	Sept
1998	64.75	9.55	10.01	13.31	13.01	9.45
1999	59.82	7.19	10.80	14.33	12.09	7.54
2000	59.28	8.18	9.60	13.21	12.42	7.34
2001	62.05	8.38	11.07	13.77	13.64	7.75
To date mean^a:	58.32	7.24	10.57	13.08	11.84	7.80

Year	Annual	May	<u>Seasonal moisture deficit^c (cm)</u>			
			June	July	Aug	Sept
1998	11.94	1.37	9.47	-3.28	-7.75	-4.01
1999	24.46	5.64	3.07	4.83	3.96	0.36
2000	11.84	-1.12	1.83	-6.55	5.06	-1.12
2001	-1.35	1.32	-2.18	-7.01	-7.52	-2.85
To date mean^a:	5.39	-0.89	-1.04	-2.26	-2.34	-0.86

^a nearly continuous data since 1898

^b Blaney/Criddle potential evapotranspiration

^c seasonal moisture deficit (SMD) = precipitation – Blaney/Criddle ET

Chapter 3

Effects of European earthworm invasion on soil characteristics in northern hardwood forests of Minnesota, U.S.A.

Introduction

European earthworms have been colonizing the North American continent since European settlement (Steinberg and others 1997, Reynolds 1977, Gates 1982). Although exotic earthworm species are invading much of North America, the large glaciated regions of North America with no native earthworms (James 1998, Gates 1982) may have the greatest potential for large impacts following exotic earthworm invasion. Recent research suggests that earthworm invasions may have widespread negative effects on native understory plant communities (Hale and others 2000, Gundale 2003), soil structure and nutrient dynamics in cold temperate, North American forests (Bohlen and others 2004a, Alban and Berry 1994, Scheu and Parkinson 1994). Yet the nature and extent of earthworm invasions and their impacts on these forest ecosystems are largely unknown across the region (Hendrix and Bohlen 2002).

Earthworms may affect ecosystem structure and function directly by ingesting large amounts of organic matter and mineral soil (Lavelle and others 1998) and by changing soil structure, chemistry and biology (Devliegher and Verstraete 1997). Because different species of earthworms often have different feeding and burrowing

behaviors their effects on soil structure and processes will also vary and the effects of species assemblages are not always simply the sum of their individual effects (Lavelle 1997, Pitkänen and Nuutinen 1997, Tomlin and others 1995, Muys and Lust 1992, Hendriksen 1990, Shaw and Pawluk 1986, Bouché 1977, Neilsen and Hole 1964).

Most research on earthworm effects on ecosystem function has been conducted in agricultural systems, where earthworms have been nearly universally credited with improved soil structure and nutrient cycling dynamics (Parmelee and others 1998, Lavelle and others 1998, Lee 1995). However, effects of earthworms on soil structure, organic matter dynamics and nutrient cycling processes are highly scale dependent, and contradictory results may be observed at different levels of spatial and temporal scales (Chapuis-Lardy and others 1998, Blair and others 1997, Derouard and others 1997, Lavelle 1997, Subler and others 1997, Blanchart and others 1996, Tomlin and others 1995, Barios and others 1987, Scheu 1987, Syers and others 1979, Parle 1963, Hoeksema and Jongerius 1959). Much of the work done on earthworm effects on soil organic matter and nutrient dynamics are done at the cast or microsite level, but the net long term ecosystem effects are largely unknown (Shakir and Dindal 1997, James 1991).

Cold temperate hardwood forests of North America have the potential to support large and diverse exotic earthworm populations (Neilsen and Hole 1964, Reynolds 1972, Ponge and Delhay 1995, Chapter 2). Increasing earthworm biomass and species diversity in previously worm-free hardwood forests have been associated with decreasing forest floor thickness and the development of thick A horizons (Bohlen and others 2004b, McLean and Parkinson 1997, Shakir and Dindal 1997, Coderre and others 1995, Alban

and Berry 1994, Wagner and others 1977, Langmaid 1964, Neilsen and Hole 1963,).

As is the case in agricultural soils, nutrient content and mineralization in forest soils appears to increase in fresh cast material (Spiers and others 1986, Alban and Berry 1994, Steinberg and others 1997, Bohlen and others 2004b). However, increased leaching and immobilization of nitrogen and phosphorous (Scheu and Parkinson 1994, Bohlen and others 2004b, Suárez and others 2004) may ultimately lead to lower availability.

Earthworm effects on plant roots are poorly understood. Earthworms may affect fine roots through direct consumption (Cortez and Bouché 1992, James and Cunningham 1989) or indirectly through alteration of the chemical and physical characteristics of the rhizosphere (Blair and others 1995, Edwards and others 1995). In the one published study that examined root dynamics following earthworm invasion in forest soils, earthworms were associated with decreased fine root biomass and affected the distribution and function of fine roots (Fisk and others 2004).

Studies comparing conditions before and after earthworm introductions, with few exceptions (Yeates 1981, Stockdill 1982, Hoogerkamp and others 1983), were nearly all conducted in areas where worms were chemically or physically removed, as opposed to documenting changes following invasion in areas free of earthworms altogether (Edwards and Lofty 1977, Springett and others 1992, Gilot 1994, Edwards and others 1995, Tomlin and others 1995, Pashanasi and others 1996). Therefore, the value of these results in predicting impacts to previously worm-free hardwood forests is uncertain.

Leading edges of earthworm invasion in forests of northern Minnesota provide an opportunity to document the gradient of changes in soil characteristics associated with

earthworm invasion (Chapter 2). The leading edges also exhibit gradients of earthworm populations that represent the spatial, temporal and species assemblage variability seen across the regional landscape (Holdsworth and others unpublished data). As such they provide the additional opportunity to examine the effects of different earthworm species assemblages within a given stand rather than simply comparing worm-free sites with heavily invaded sites

The objective of this study is to describe the changes in soil properties including bulk density, soil organic matter, horizon thickness, root biomass and nutrient availability, associated with a local and currently changing gradient of earthworm biomass and species assemblages that represent the early stages of the invasion process. My hypotheses are that increasing earthworm biomass will be associated with decreased O horizon thickness, increased A horizon thickness and bulk density, shifts in the distribution of soil organic matter in the upper soil horizons, decreased fine root biomass and lower nitrogen and phosphorous availability. I further hypothesize that species specific affects will be present, such that 1) that the strictly epigeic species of *Dendrobaena octaedra* will have no effect on O horizon thickness, while *L. rubellus* will have a greater effect than that suggested by biomass alone; 2) that species assemblages dominated by either endogeic or anecic species will have similar physical soil characteristics despite differences in total biomass; and 3) the most diverse species assemblage group, with the greatest range of feeding and burrowing behaviors, will have the largest effect on root biomass and nutrient availability.

Methods

Study sites and sampling design

The study was conducted over four years (1998-2001) in 4 mature northern hardwood stands which contain leading edges of earthworm invasion located on the Chippewa National Forest of north-central Minnesota (Chapter 2, Figure 1). The study sites had similar overstory composition, soils and stand history. Sugar maple (*Acer saccharum*) is the dominant tree species making up 50-90% of basal area among stands with Yellow Birch (*Betula alleghaniensis*), Paper Birch (*Betula papyrifera*) and Basswood (*Tilia Americana*) as secondary species. Soils are a silt loam characterized as a deep, well-drained and light colored Eutroboralf (Warba series) associated with the Guthrie Till Plain (USDA 1997). Under worm-free conditions these soils are characterized by a thick forest floor composed of L, F and H layers. The climate is humid, continental, cold temperate, with the median growing season length of 134 days, snow cover from late November to early April, and mean temperatures in January and July of -15 and 20 degrees C, respectively (Minnesota State Climatology Office 2003).

The 30 x 150 meter sample grid consisted of 45 sample points 10 meters apart in three parallel transects with 15 points each (Figure 1). Transects were placed perpendicular to the leading edge with the midpoint, sample point 8, at the approximate front of the leading edge. From sample point 8, the transects extend 75 meters into the zone of well-developed forest floor in front of the leading edge with 7 sample points, 10 meters apart up to sample point 15. Similarly, the transects extend in the opposite

direction behind the leading edge for 7 more sample points, 10 meters apart to sample point 1.

Earthworm sampling

In 1999 and 2000, earthworm populations were censused using mustard liquid extraction in 0.12 m² (35 cm x 35 cm) subplots located 2.0 meters from each sample point at a randomly selected cardinal direction (Chapter 2). For each subplot, total ash-free dry (AFD) earthworm biomass was determined for each species (Chapter 1). For the purposes of analysis, earthworm species with similar habitat and feeding preferences were combined into analytical groups (Table 1) including a separate group for juvenile *Lumbricus* specimens where species identification was impossible

Forest floor and upper soil horizons

In 1999, soil cores 6 cm in diameter and 15cm deep were collected adjacent to all odd numbered sample points and point 8 in each site. In each soil core, the thickness (cm) of the O and A horizons were measured (N=96). Percent organic matter was determined by loss on ignition for each horizon present (N=48 and N = 93 for O and A horizons, respectively).

In 2000, soil cores were collected adjacent to all sample points in each site. In each core, the thickness of the O and A horizons was measured (N=179). In cores from all odd numbered sample points and point 8, bulk density (dry g/cm³) was determined for each horizon present (N=22 and N=105 for O and A horizons, respectively).

In 2001, total fine root density was measured in two soil cores collected adjacent to sample points 1, 8 and 15 in each site. Each core was separated into O, A and upper E horizon and the thickness of each horizon collected was recorded. Cores were returned to the lab and washed to collect all fine roots (≤ 1.0 mm) which were dried at 60 degrees C for 24 hours and then weighed. Mean fine root density (mg/cm^3) was calculated for each sample point by averaging the two replicate samples. Total fine root biomass to 10 cm depth (g/m^2) was calculated for each core.

Nutrient availability

To assess nitrogen and phosphorus availability, six ion exchange resin bags, each containing 5 g of Rexyn I-300 (Fischer Scientific, Fairlawn, New Jersey, USA) mixed-bed, cation-anion exchange resin (Binkley 1984), were buried (8-10 cm deep) in the mineral soil at randomly selected locations 2 meters from sample points 1 and 15 at opposite ends of each transect of the sample grid at each site (N=144). Bags were buried on June 28-29, 2001 and removed September 21, 2001. Ammonium, nitrate and phosphate were extracted from 3 g subsamples of resin with 100 mL of 1 mol/L KCl; the resins were shaken for 15 minutes in 25 mL KCl, decanted, shaken again in another 25 mL aliquot, poured into small Buchner funnels (5.5 cm diameter) equipped with pre-washed #1 Whatman filters, and washed several times with additional KCl before the filtrate was brought to 100 mL volume. The extracts were analyzed by standard methods for NH_4 , NO_3 and PO_4 on a Lachat autoanalyzer (Lachat Instruments, Milwaukee, Wisconsin, USA).

Analytical methods

For all analyses, the 2 year mean (1999 and 2000) of earthworm biomass per sample point was used as the best measure of mean earthworm biomass for each sample point (N = 45 in each site). Additionally, because most soil parameters were collected in different years, the two year mean of earthworm biomass provides consistency across all analyses. Where multiple years of soil parameter data existed (horizon thickness) the results of analyses using the 2 year mean of earthworm biomass were comparable to results using individual year data of earthworm biomass.

For purposes of statistical analyses, earthworm biomass data were log transformed using a technique described in McCune and Grace (2002) which preserves both zero values and differences in magnitude that exist in the data set (Chapter 2). The transformed value (b_{ij}) equals...

$$b_{ij} = \log_{10}(x_{ij} + d) - c$$

Where c = the integer of ($\log_{10}(\min(x))$), $\min(x)$ = the lowest non-zero value of x and d = inverse $\log_{10}(c)$.

For percent organic matter, fine root density, total soil organic matter and nutrient availability $\ln(x+1)$ transformations were used. No transformations were required for horizon thickness or bulk density.

A combination of multiple regression analysis and the non-parametric Mantel test (McCune and Grace 2002, Chapter 2) were used to assess the spatial relationships of

earthworm biomass to sample point position across the sample grid. To test the relationship of total earthworm biomass to changes in nutrient availability and O and A horizon thickness, density and percent organic matter a combination of multiple and simple linear regression analysis were used (Montgomery and Peck 1992, SAS 2001).

The relationships of different earthworm species assemblages to changes in soil parameters were assessed using a combination of cluster analysis and indicator species analysis (McCune and Mefford 1999) to sort sample points across all sites into earthworm species assemblage groups (Chapter 2) and then ANOVA and paired *t*-tests were used to test for differences in the means of soil parameters between assemblage groups (Montgomery and Peck 1992, SAS 2001).

Using mean earthworm species specific biomass, all sample points were sorted into species assemblage groups by the group average linkage method (a hierarchical, polythetic, agglomerative clustering method) as described by McCune and Grace (2002), using Sorensen distance measures. Six potential earthworm species assemblage groups were initially identified in the cluster analysis process. Then indicator species analysis (McCune and Mefford 1999) was used to identify a subset of groups which had unique species assemblages by identifying which subset of groups had both the lowest average *p*-values and the most significant indicator species (Dufrene and Legendre 1997). Monte Carlo randomization tests (1000 runs) were used to assess the significance of indicator values for each species in each potential subset of earthworm assemblage groups.

Results

Total earthworm biomass in relation to sample point position

Across all sites, total earthworm biomass decreased across the leading edge of invasion from sample point 1 through 15 ($R^2 = 0.59$, $P < 0.0001$). However, the spatial pattern of change of total earthworm biomass in relation to sample point position was nonlinear and varied with site. In three of the four sites, total earthworm biomass decreased (Figure 2) and the Mantel test (McCune and Grace 2002) indicated that the spatial structure of earthworm biomass was significantly related to sample point position across the sample grid. The fourth site follows a similar pattern but with greater variability.

Soil properties in relation to total earthworm biomass

Bulk density in relation to total earthworm biomass

Among all sample points and sites, A horizon density generally increased with total earthworm biomass ($P < 0.0001$, Table 2) and ranged between 0.15 and 1.1 g/cm³. However, the pattern of change depended on site (Table 2). Examining each site individually shows strong positive correlations between A horizon density and earthworm biomass in two sites and no relationship in the other two (Figure 3). O horizon density had no relationship with total earthworm biomass or site ($P > 0.50$). E horizon density

varied between sites ($P=0.002$) but had no relationship with total earthworm biomass ($P=0.75$).

Percent organic matter in relation to total earthworm biomass

Across all sample points and sites, A horizon percent organic matter decreased with increasing total earthworm biomass ($P=0.01$, Table 2) and ranged between 11% and 54%. O horizon percent organic matter decreased with increasing total earthworm biomass ($P=0.05$) and ranged between 37% and 83%. However, there are no data points above 11 AFDg/m² of earthworm biomass because the O horizon had been eliminated in those sample points. Percent organic matter in the E horizon did not change in relation to either total earthworm biomass or site.

Horizon thickness in relation to total earthworm biomass

In both 1999 and 2000, A horizon thickness increased with total earthworm biomass ($P<0.0001$) across all sample points and sites (Table 2). Moreover, these relationships varied among sites (Table 2, Figure 4).

Many soil cores lacked an O horizon (48% of cores in 1999 and 75% in 2000). The lack of an O horizon is an important measurable impact of earthworms. However, in regression analyses I would expect a large number of zeros in the data matrix to unduly influence the results and for this reason zeros are often removed (Montgomery and Peck 1992). Therefore, analyses using both full data sets and data sets with zero values removed were conducted and compared (Table 2, Figure 4). In 1999 and 2000, O horizon

thickness decreased with increasing total earthworm biomass across all sample points and sites when using the full data set ($P < 0.0001$, Table 2) with similar trends in 1999 for the data set when all zero values of O horizon thickness were deleted from the analysis ($P = 0.03$). These relationships varied somewhat among sites (Table 2, Figure 4).

Total soil organic matter content in relation to total earthworm biomass

A horizon total soil organic matter content (SOM) ranged from 0.8 to 14.5 Mg/m^2 . The combined effect of changes in bulk density, percent organic matter and thickness for each soil horizon caused A horizon SOM to increase with total earthworm biomass ($P = 0.007$, Table 2) across all sample points and sites. O horizon SOM ranged from 0.0 to 7.6 Mg/m^2 . Across all sample points and sites it decreased in relation to increasing total earthworm biomass ($P = 0.001$), with significant variability among sites (Table 2). However, when all zero data points were deleted, O horizon SOM had no relationship with earthworm biomass. E horizon SOM ranged from 1.1 to 7.2 Mg/m^2 and had no relationship with total earthworm biomass. Combined total soil organic matter to a depth of 12 cm ranged from 5.5 to 16.5 Mg/m^2 and was weakly positively related ($p=0.06$) with total earthworm biomass.

Total fine root density and biomass in relation to total earthworm biomass

Across all sample points and sites, fine root density decreased with increasing total earthworm biomass in the A and E horizon ($P=0.04$, Table 2, Figure 5), but not in the O

horizon. Total fine root biomass to 10 cm depth ranged from 127 to 876 g/m² and showed no relationship with either site or total earthworm biomass.

Nutrient availability in relation to total earthworm biomass

Nutrient availability was compared at the extreme ends of each sample grid (sample points 1 and 15). Ammonium, nitrate and phosphate availabilities were lower in sample points with high total earthworm biomass compared with sample points of low total earthworm biomass (Figure 6).

Soil properties in relation to earthworm species assemblages

Earthworm species specific biomass in relation to sample point position

Earthworm species composition differed between sites. The biomass of individual species of earthworms changed significantly across the leading edge (Figure 2), however, the spatial patterns of change differed among species (Figure 7). *Dendrobaena* was found in all four sites and its biomass increased significantly across the leading edge from bare soil to intact forest floor in three sites (Ottetail, Section 19 and Two Points).

Aporrectodea species was found in all four sites and its biomass decreased significantly across the leading edge in three sites (Blackduck, Ottetail and Section 19). *L. rubellus* adults, *L. terrestris* adults and *L.* (juveniles) were found in two or three sites, and their biomass generally decreased across the leading edge. *Octolasion* was found in only one site (Section 19) and its biomass decreased significantly across the leading edge.

Earthworm species assemblage groups

The species assemblage groups were associated with sites due to differences in overall species composition seen between the study sites (Figure 7). Group 1 contained sample points with *Dendrobaena* only and had a total of 20 sample points from three sites (Blackduck, Ottertail and Two Points). Group 2 contained sample points with only *Lumbricus rubellus* adults, *Lumbricus* juveniles and *Dendrobaena* and had a total of 13 sample points from two sites (Ottertail and Section 19). *Aporrectodea* species and *Dendrobaena* were the dominant species groups in Group 3, which contained a total of 74 sample points from two sites (Blackduck and Two Points). Group 4 was identified by the presence of *Lumbricus terrestris* adults and *Octolasion tyrtaeum* but contained all six species groups. It contained a total of 70 sample points primarily from two sites (Ottertail and Section 19). Total earthworm biomass was lowest in group 1, increased to an intermediate value in groups 2 and 3 and reached its highest in group 4 (Figure 8).

Soil attributes in relation to earthworm species assemblages

A horizon bulk density was lowest in the *L. rubellus* group (group 2) relative to all other groups (Figure 8). A horizon percent organic matter declined modestly from the *Dendrobaena* group (group 2) through the *L. terrestris* group (group 4) (Figure 8). In 1999, the *Dendrobaena* and *L. rubellus* groups had similar thickness of O horizons and A horizons, whereas the *Aporrectodea* group (group 3) and *L. terrestris* group had very thick A horizons and thin O horizons (Figure 8). In 2000, these patterns were similar,

except the *L. rubellus* group had switched to a thin O horizon and thick A horizon comparable to the *Aporrectodea* and *L. terrestris* groups.

Changes in bulk density, percent organic matter and horizon thickness compensated each other and so did not lead to changes in total soil organic matter content to 12 cm depth in relation to earthworm species assemblage groups. However, comparisons with the *L. rubellus* group were impossible due to small sample size. Soil organic material content of the O horizon was higher in the *Dendrobaena* group relative to the *Aporrectodea* and *L. terrestris* groups and A horizon soil organic matter content was higher in the *Aporrectodea* group compared with the *Dendrobaena* and *L. terrestris* groups (Figure 8).

Fine root density and nutrient availability in relation to earthworm assemblages

A horizon fine root density (g/cm^3) was lower in the *L. terrestris* group compared with all other groups (Figure 8). Mean total fine root biomass (g/m^2) to a depth of 10 cm was lowest in the *L. terrestris* group compared with all other groups (Figure 8). Nitrogen and phosphorus availability, in the forms of ammonium nitrate, and phosphate, were lower in the *L. terrestris* group than in all other groups (Figure 8).

Discussion and Conclusions

The gradients of earthworm biomass and species assemblages associated with leading edges of earthworm invasion provide an opportunity to examine changes in soil characteristics and processes related to exotic earthworm invasion without the confounding factors often associated with comparative field studies. Overall, increasing earthworm biomass homogenized the upper soil horizon. The trajectories of change in bulk density, percent organic matter, fine root density and nutrient availability in relation to increasing earthworm biomass are, in part, the result of decreased variability (Figures 3, 4 and 5). Under worm-free conditions, numerous factors may determine conditions at any given point and the system is highly heterogeneous (Webb 1972). After earthworms invade, they come to have a dominant effect on soil structure and function (Edwards and others 1995).

In response to increasing earthworm biomass, there was a net relocation of organic matter from the forest floor down into the upper mineral soil horizon. Total soil organic matter to 12 cm depth increased slightly with increasing earthworm biomass. O horizon thickness declined commensurate with increases in A horizon thickness and bulk density. A horizon percent organic matter by weight decreased with increasing total earthworm biomass as a result of increased bulk density but only minimal increases in total soil organic matter content.

Fine root density declined with increasing total earthworm biomass. Decreased fine root biomass may be the result of persistent disturbance by epi-endogeic and endogeic species in the rhizosphere (Blair and others 1995).

Nutrient availability was consistently lower in areas with high earthworm biomass. In previously worm-free hardwood forests of North America, nutrient availability declines following earthworm invasion (Scheu and Parkinson 1994, Bohlen and others 2004b, Suárez and others 2004). This decline may be caused by increased microbial immobilization of nutrients by the microbial community as fresh litter is continuously incorporated into the mineral soil and by increased leaching losses. In this study, ammonium, nitrate and phosphorous had very similar patterns despite large differences in potential leaching rates, suggesting that increased immobilization may be the primary cause of lowered availability. If increased leaching were the primary effect of earthworms on nutrient availability then nitrate, which is more mobile, would be expected to decrease more than ammonium and phosphate (Jeffrey 1987).

A horizon bulk density increased with total earthworm biomass despite the incorporation of surface litter as a result of earthworm gut mediated processes which modify, compact and mix organic materials with mucus and mineral soil in the formation of earthworm cast material (Devliegher and Verstraete 1995). In this study, A horizon bulk density nearly doubled with increasing earthworm biomass, to values comparable to those reported by Alban and Berry (1994) following earthworm invasion in another Minnesota forest type. This is the opposite of what happens in agricultural ecosystems. In studies of agricultural soils, bulk densities before the addition of earthworms are very

high as a result of compaction by equipment and earthworms reverse the compaction leading to declines in bulk density (Lavelle and others 1998). If earthworm populations dominated by endogeic and anecic species lead to moderate bulk densities, then whether they loosen or compact the soil depends on the starting bulk density before earthworms invade. Although water infiltration rates in arable lands often increase with earthworm activity, the inverse may be the rule in previously worm-free forests with low native bulk densities where earthworm invasion results in increased soil bulk density. The combination of the loss of surface litter and increased soil bulk density is likely to lead to increased surface run off and erosion in earthworm invaded forests (Hale personal observation).

This study demonstrates that different earthworm species assemblages can have different effects on forest soil properties. The feeding and burrowing habits of different earthworm species are important determinants of their impacts on soil structure and function (Shipitalo and others 1988, James and Cunningham 1989, Hendriksen 1990, Scheu and Parkinson 1994). However, increasing species diversity is often associated with increasing earthworm biomass, making it difficult to determine species specific effects under field conditions. The four sites included in this study were on the same landform and soil unit, had the same overstory composition and age structure, had the same land use and disturbance history and were in close proximity so that they experienced the same local climate conditions. Therefore, the site effects seen in this study are attributed to the differences in earthworm populations seen between the sites

and across each leading edge of earthworm invasion (Chapter 2, Bohlen and others 2004a). These population differences are represented by the species assemblage groups.

The *Dendrobaena* group alone did not remove the forest floor or enhance the development of the A horizon, nor did it change other soil parameters. The *L. rubellus* group had a much larger effect on O horizon removal and formation for the A horizon than would be expected from biomass alone. Although, soil bulk density may decrease when the *L. rubellus* group is found in isolation, when found in species assemblages containing endogeic and anecic species soil bulk density increased (Figure 8). The *Aporrectodea* species group also decreased O horizon thickness, but in contrast A horizon thickness, bulk density and total soil organic matter content reached their maxima in this species group, illustrating the importance of endogeic species in the homogenization of the upper soil horizon (Figure 8).

The *L. terrestris* group contained the highest total biomass and the most species, including at least one from each ecological group (epigeic, endogeic and anecic). Therefore, this group represents the net affect of all species present and any synergistic effects that may exist (Shaw and Pawluk 1986). This full species assemblage resulted in the complete elimination of surface litter and the same A horizon thickness and bulk density conditions produced by the endogeic species group (*Aporrectodea*), but total soil organic matter in the A horizon declined compared with the *Aporrectodea* group. Fine root density and biomass and nutrient availability were also lower in this group compared with all other groups.

The four earthworm species assemblage groups in this study represent a typical successional sequence of earthworm assemblages across a leading edge of earthworm invasion (Chapter 2). Incipient invasions often begin with *Dendrobaena octaedra* (group 1) in advance of all other species, followed in succession by the addition of *L. rubellus* (group 2), *Aporrectodea* species (group 3) and finally *L. terrestris* (group 4), with total earthworm biomass generally increasing with diversity.

The *L. terrestris* assemblage group represents a typical stable reproductive earthworm population in stands that have been invaded by European earthworms for multiple decades (Holdsworth and others unpublished data, Hale personal observation) and may therefore represent the long term effects that earthworm invasion may have in hardwood forests of the western Great Lakes region. However, the rapid change in soil characters associated with the appearance of *L. rubellus* illustrates the importance of this species in ecosystem change during initial invasion

The large effect of *L. rubellus* as compared to *Dendrobaena octaedra* also illustrates the need to consider specific feeding and burrowing behavior, and not simply location in the soil profile, when assessing potential impacts. The widely used categories of three ecological groups (epigeic, endogeic and anecic) are too general to adequately describe the diversity in function of different species (Lavelle and others 1998). In particular, *Dendrobaena octaedra* and *L. rubellus* are both categorized as epigeic species but they have very different effects due to the propensity of *L. rubellus* to burrow and cast in the surface mineral soil layer (Edwards and others 1995, Rozen 1982).

In contrast to results of earthworm activity in agricultural ecosystems, changes in fine root dynamics (Fisk and others 2004), declines in nutrient availability, the loss of the forest floor as habitat and seed bed (Baskin and Baskin 1998, Parkinson and McLean 1998) and changes in mycorrhizal fungi composition and colonization rates (Lawrence and others 2003) seen in north temperate hardwood forests represent important changes in ecosystem function that may ultimately lead to changes in forest composition and structure (Gundale 2002, Chapters 4 and 5). The phenomenon of earthworm invasion has been ongoing for many decades but, earthworms have not been recognized as exotic species by forest ecologists in the region (Wagner and others 1977, Coderre and others 1995). In the face of rapid change and multiple pressures on native forest ecosystems, the potential impacts of earthworm invasion must be considered.

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Table 1. Grouping of earthworm species used in analysis.

Analytical group	Ecological Group	Species included
<i>Dendrobaena</i>	epigeic	<i>Dendrobaena octaedra</i> , <i>Dendrodrilus rubidus</i> (rare)
<i>L. rubellus</i>	epi-endogeic	<i>Lumbricus rubellus</i> adults
<i>L. juveniles</i>	epi-endogeic / anecic	<i>Lumbricus juveniles</i>
<i>Aporrectodea</i>	endogeic	<i>A. caliginosa</i> , <i>A. tuberculata</i> , <i>A. trapezoides</i> , <i>A. rosea</i>
<i>Octolasion</i>	endogeic	<i>Octolasion tyrtaeum</i>
<i>L. terrestris</i>	anecic	<i>Lumbricus terrestris</i> adults

Table 2. Summary Statistics: multiple regression results of soil parameters in relation to site and total earthworm biomass.

Soil Parameters	<u>Regression Parameters</u>			
	Full Model ^a	Worm	Site	Site * Worm
Bulk Density				
A horizon	$R^2 = 0.45$	+	Yes	Yes
O horizon	NS	NS	NS	NS
E horizon	$R^2 = 0.18$	NS	Yes	NS
Percent Organic Matter				
A horizon	$R^2 = 0.23$	-	Yes	NS
O horizon	NS	-	NS	NS
E horizon	NS	NS	NS	NS
Horizon Thickness (1999)				
A horizon	$R^2 = 0.53$	+	Yes	Yes
O horizon (all data)	$R^2 = 0.47$	-	Yes	Yes
O horizon (zeros removed)	$R^2 = 0.34$	-	Yes	NS
Horizon Thickness (2000)				
A horizon	$R^2 = 0.53$	+	Yes	Yes
O horizon (all data)	$R^2 = 0.59$	-	Yes	Yes

O horizon (zeros removed)	$R^2 = 0.33$	NS	Yes	NS
Soil Organic Matter Content				
Total to 12 cm depth	$R^2 = 0.42$	+	Yes	NS
A horizon	$R^2 = 0.40$	+	Yes	NS
O horizon (all data)	$R^2 = 0.66$	-	Yes	Yes
O horizon (zeros removed)	NS	NS	NS	NS
E horizon	NS	NS	Yes	NS
Fine Root Density				
A horizon	$R^2 = 0.40$	-	NS	not included ^b
O horizon	NS	NS	NS	NS
E horizon	NS	-	NS	not included ^b

Symbols - or + indicate the direction of the relationship of each soil parameter to increasing total earthworm biomass ($P \leq 0.05$). Yes or NS in subsequent columns indicate significant or non-significant site and site*worm interaction effects, respectively ($P \leq 0.05$).

^a all models $P \leq 0.0001$.

^b Where the interaction term was not significant in the full model it was removed and model run again with only main effects.

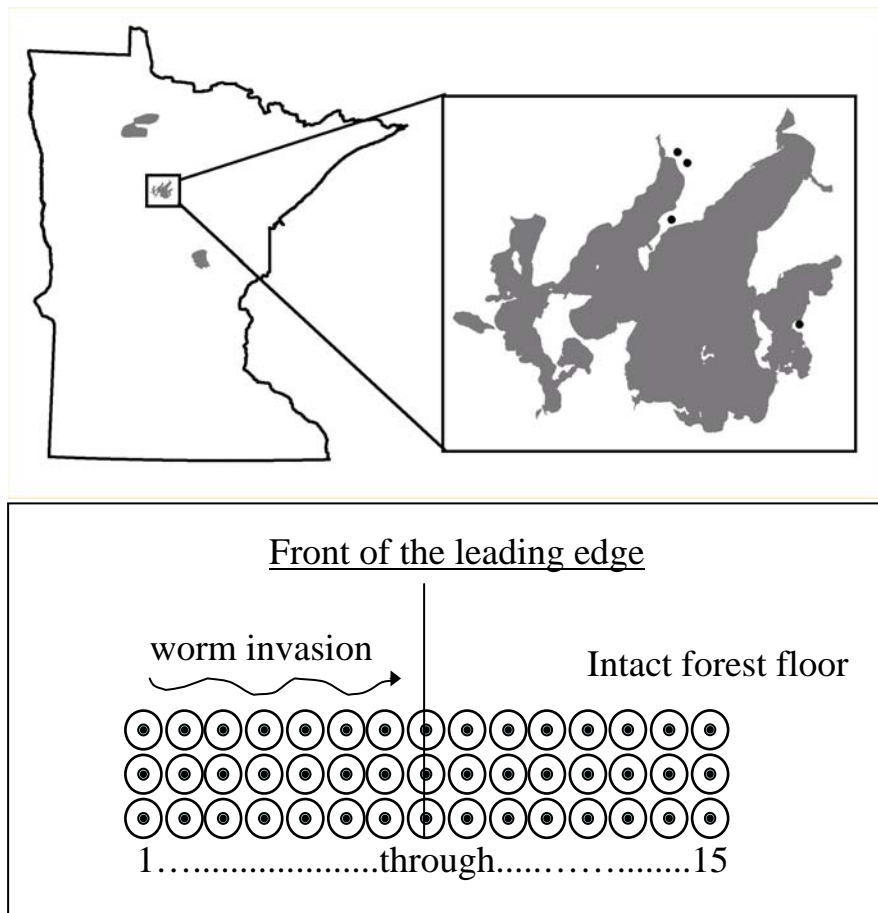


Figure 1. The locations of four study sites in the Chippewa National Forest near Leech Lake in north central Minnesota. An illustration of the sample grid (30 m x 150 m) of 45 points laid perpendicular to the leading edge of earthworm invasion in each study site. Sample points are 10 m apart.

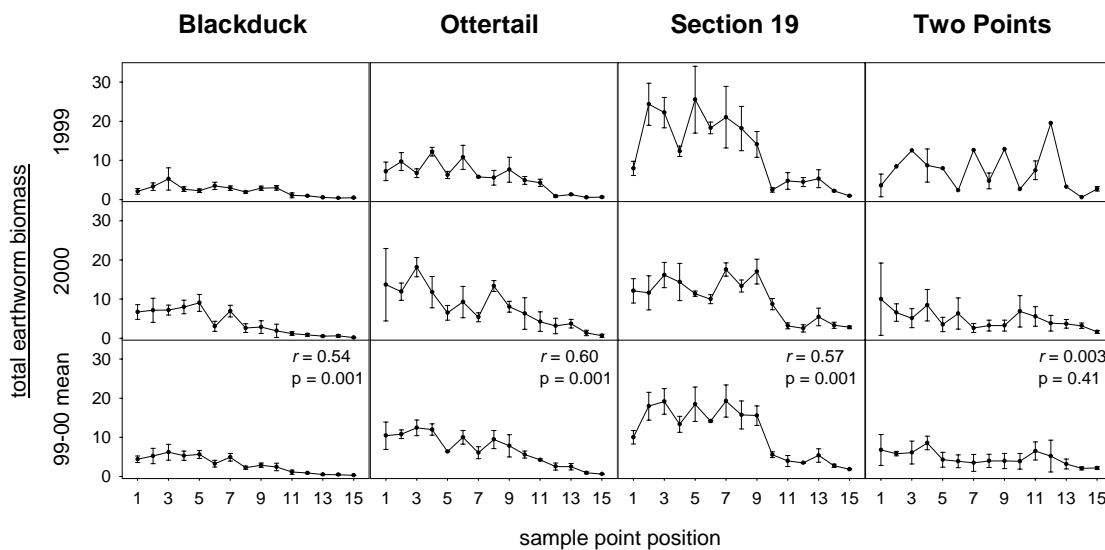


Figure 2. Mean and standard error scatter plots of total earthworm biomass (ash-free dry g/m^2) in 1999, 2000 and the 2 year mean (1999 and 2000) in relation to sample point position across the sample grid in each study site. The standardized Mantel statistic (r) indicates the strength of the relationship between sample point position and earthworm biomass and the associated p -value indicates the significance of that relationship ($N = 45$ per site).

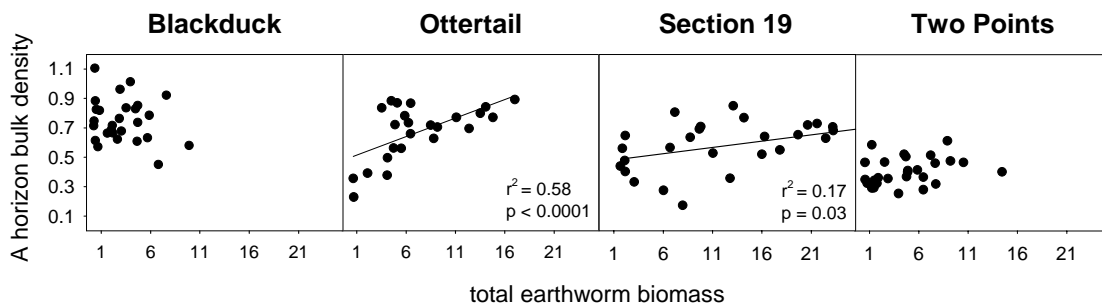


Figure 3. Scatter plots of A horizon bulk density (g/m^3) in relation to mean total earthworm biomass (ash-free dry g/m^2) in each study site. $N = 27$ in each site except Ottertail where $N = 24$.

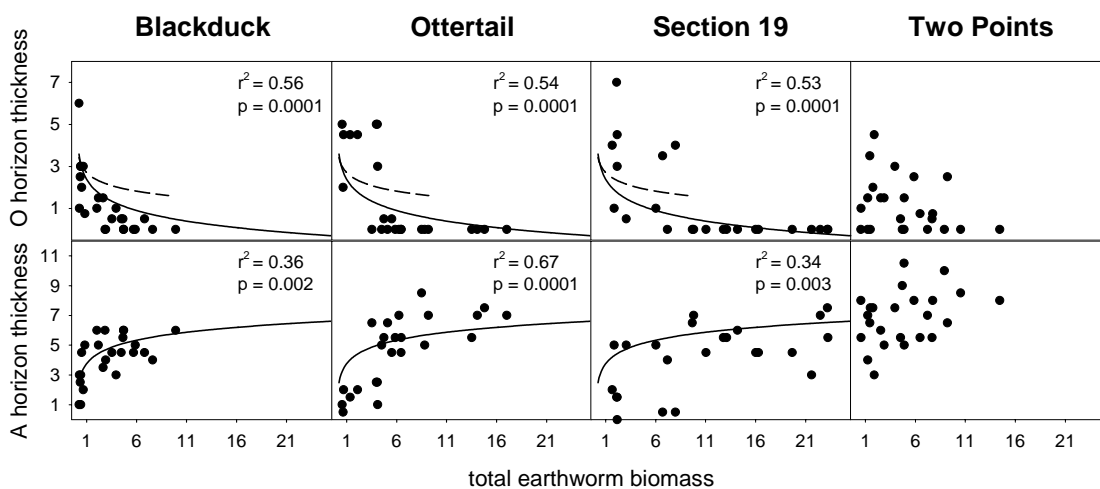


Figure 4. Scatter plots of 1999 O and A horizon thickness (cm) in relation to mean total earthworm biomass (ash-free dry g/m²) in each study site. A = Blackduck, B = Ottertail, C = Section 19, and D = Two Points. Fitted relationships with solid lines include all data points, N = 24 in each site. For comparison, O horizon fitted relationships with all zero values deleted are provided (dashed lines), however, only for the Blackduck site is this relationship significant (r²=0.45, P = 0.004).

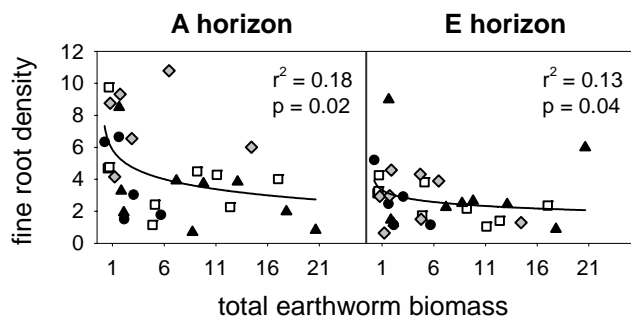


Figure 5. Scatter plots of A and E horizon fine root density (mg/cm^3) in relation to mean total earthworm biomass (ash-free dry g/m^2) in all study sites. Fitted relationships include all data points, $N=29$ and 31 in A and E horizons, respectively; closed circles = Blackduck, open squares = Ottertail, closed triangles = Section 19, gray diamonds = Two Points.

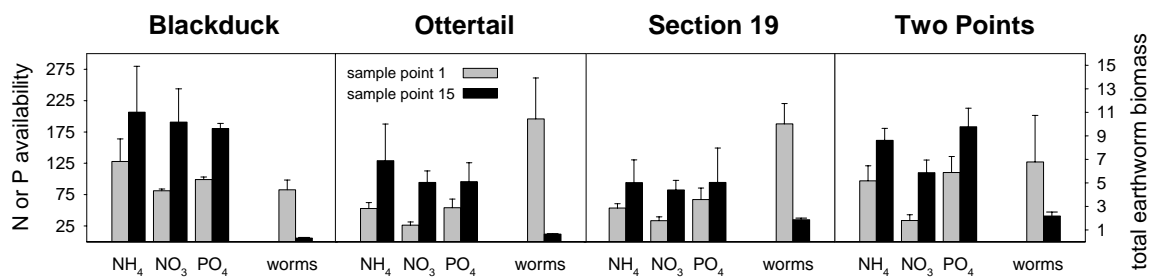


Figure 6. Paired comparisons of mean N or P availability (ug/g resin) and total earthworm biomass (ash-free dry g/m²) at opposite ends of the sample grid in each site, N = 6 in each site. All comparisons are significant at $P \leq 0.02$.

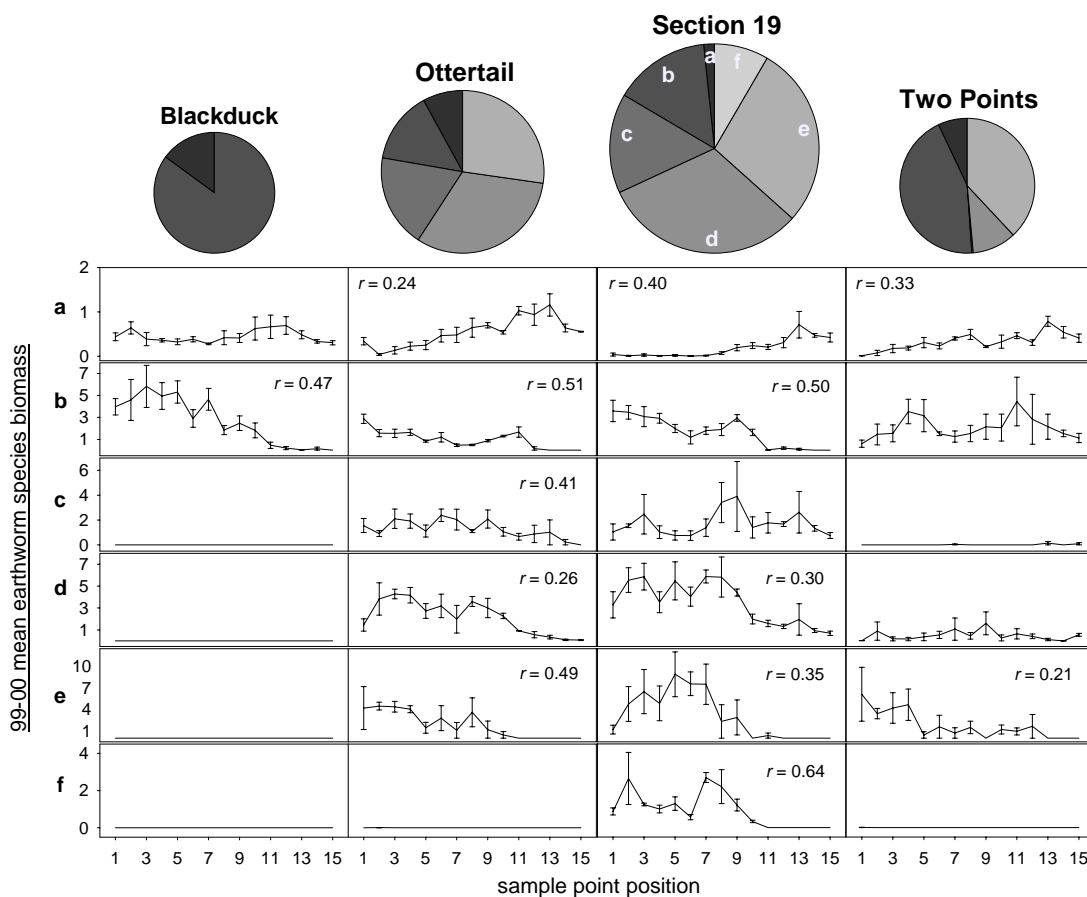


Figure 7. Pie charts of 1999-2000 mean earthworm biomass (ash-free dry g/m^2) for each species group present in each site, where a = *Dendrobaena*, b = *Aporrectodea*, c = *L. rubellus* (adult), d = *L. juveniles*, e = *L. terrestris* (adult) and f = *Octolasion*. The size of the pie chart is proportional to mean total earthworm biomass for each site. Mean and standard error scatter plots of 99-00 mean earthworm biomass (ash-free dry g/m^2) for each species group in relation to sample point position. The standardized Mantel statistic (r), indicates the strength of the relationship between sample point position and earthworm species biomass, when (r) is provided $P \leq 0.01$.

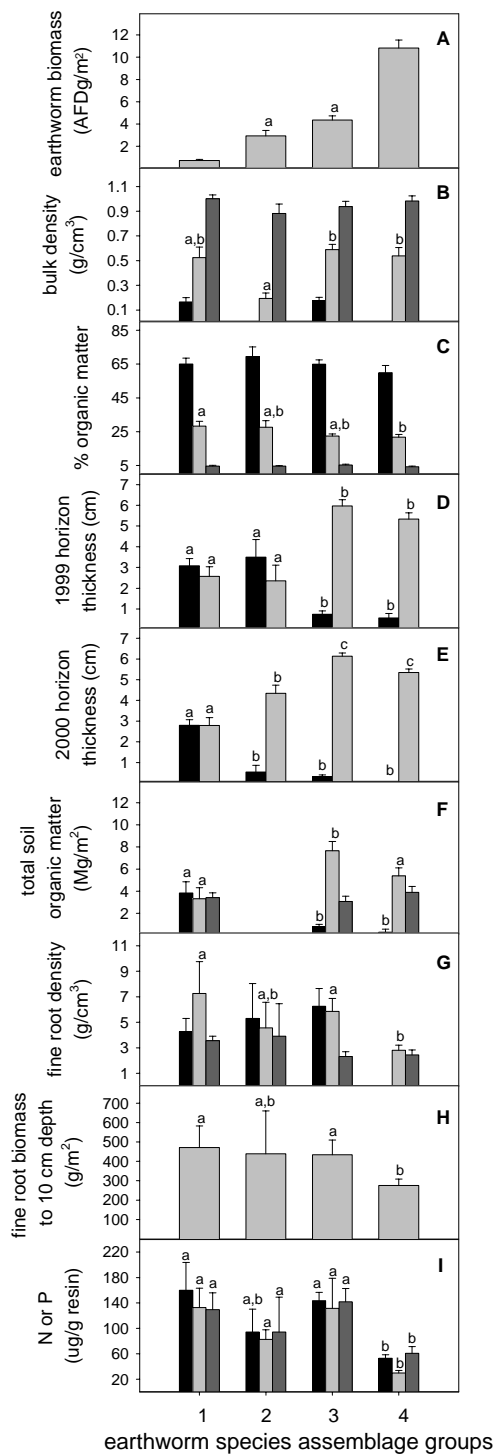


Figure 8. Comparisons of total earthworm biomass (ash-free dry g/m^2) and soil parameters in relation to earthworm species assemblage groups. For graphs B-G, black

bars = O horizon, light gray bars = A horizon and dark gray bars = E horizon. For graph I, black bars = NH_4 , light gray bars = NO_3 and dark gray bars = PO_4 . Where differences existed between assemblage groups, bars with different letters indicate a significant different ($P \leq 0.05$). Group 1 contains sample points with *Dendrobaena octaedra* only (N=20); Group 2 contains sample points with *Lumbricus rubellus* adults, *Lumbricus* juveniles and *Dendrobaena octaedra* (N=13); Group 3 contains sample points dominated by *Aporrectodea* species and *Dendrobaena octaedra* (N=74); Group 4 contains sample points with all species, identified by the presence of *Lumbricus terrestris* and *Octolasion tyrtaeum* (N=70). Sample sizes differed in each group depending on the soil parameter, for groups 1 through 4 respectively: graph A (N= 20, 13, 74 and 70); graph B (N= 11, 7, 42 and 43); graph C (N= 12, 6, 36 and 37); graph D (N= 13, 7, 36 and 38); graph E (N= 20, 13, 74 and 70); graph F (N= 6, 2, 11 and 12); graph G (N= 2, 3, 10 and 12); graph H (N= 3, 3, 7 and 12); graph I (N= 5, 3, 6 and 6).

Chapter 4

Changes in cold-temperate hardwood forest understory plant communities in response to invasion by European earthworms.

Introduction

The effect of individual species on ecosystem function varies greatly (Vitousek 1986, Pastor et al. 1999). Most exotic species have little effect, but a few can alter ecosystem properties, and by changing the rules of existence for all other species, create a new community structure. Vitousek (1990) suggests that invaders can alter ecosystem structure and community composition when they: (1) differ substantially from natives in resource acquisition or utilization; (2) alter the trophic structure; or (3) alter disturbance frequency or intensity.

The hardwood forests of the western Great Lakes region, USA (Minnesota, Wisconsin and Michigan) developed without native earthworms (Gates 1976, James 1995). The introduction of European earthworms (*Lumbricidae*) represents a major change in the detritivore community in these forests. The invasion has been in progress since the major wave of European settlement during the late 1800s, and has accelerated during the last few decades via the widespread use of earthworms as bait for recreational fishing (Gates 1982). It is an example of exotic species that successfully invaded a long time ago and only recently have crossed into the realm of pest, by spreading from human-

dominated habitats to natural ones (Williamson and Fitter 1996). Because the forested part of the region has many lakes used for fishing (33,000 in Minnesota, Wisconsin and Michigan), there are many centers of infestation at this time. Anecdotal reports and personal observations suggest that nearly all forests have been invaded in the southern part of the region, where human activity has been most intense for the longest period of time. In contrast, European earthworms are now invading the hardwood forests of the northern part of the region although many areas remain worm free (Alban and Berry 1994, Mortensen and Mortensen 1998, Chapter 2, Holdsworth et al. in prep).

Without earthworms, northern forests develop a thick forest floor where decomposition controls the rate of recycling and nutrient availability (Bormann and Likens 1979, McClaugherty et al. 1985, Ferrari 1999). Therefore, northern hardwood forests of the Great Lakes region may be particularly predisposed to experiencing large impacts when earthworms invade because earthworms, as detritivores, survive in the forest by eating leaf litter, soil organic matter and associated microorganisms thereby changing the forest floor (Doube and Brown 1998). Earthworms can alter the trophic structure of the forest community by consuming litter much faster than native detritivores, so that the forest floor changes from a thick mat with many years of litter accumulation to a thin covering of litter where nearly all of the annual litter production is consumed within one growing season (Nielsen and Hole 1963, Cothrel et al. 1997, Parkinson and McLean 1998, Chapter 2).

The forest floor serves as a physical substrate and rooting medium for an entire community of native plant species, including tree seedlings and understory plants. Thus,

reducing the amount of detritus also disturbs the forest floor community. After invasion, the trophic structure of hardwood forests may change so that the earthworms alter ecosystem nutrient dynamics, composition, and succession. Forest ecologists have only recently given major attention to the possibility that the community of soil organisms may have a greater level of control over forest dynamics than had been supposed in the past (Ponge et al. 1998, Watkinson 1998). Although invasions of European earthworm species (Lumbricidae) are widespread throughout this region, research documenting these invasions or their impacts on previously earthworm-free native ecosystems has only begun (Alban and Berry 1994, Dymond et al. 1997, McLean and Parkinson 1997, Burtelow et al. 1998, Gundale 2002).

It is unusual for the course of a particular exotic species invasion to be predictable (Williamson and Fitter 1996). However, it may be possible to identify specific relationships that would lead to an invader having large impacts. The forest floor in northern temperate hardwood forests plays a fundamental role in ecosystem organization because it is where most nutrient transformation occurs, it provides habitat for a diverse community of decomposers, fungi, arthropods and small vertebrates and it is the substrate that forms the seedbed for all forest plant species. When the forest floor is removed I would expect a cascade of changes in the ecosystem to follow. Therefore, I hypothesize that earthworm species responsible for the most rapid removal of the forest floor, such as *L. rubellus*, would also have the greatest impact on plant communities during initial invasion (Hendriksen 1990, Bohlen et al. 2004^a, Chapter 2).

Effects of earthworm invasion on plant communities

The most obvious effect of invasion by exotic earthworms in hardwood forest ecosystems is the loss of the forest floor (Nielsen and Hole 1963 and 1964, Alban and Berry 1994, Ponge and Delhaye 1995, McLean and Parkinson 1997, Chapter 2). The magnitude and rate of loss of forest floor mass depends on the biomass, species composition, and activity level of the earthworm populations (Bouché 1977, Springett 1983, Scheu 1987, Cothrel et al. 1997). Earthworms are capable of burying large quantities of organic material (Nielsen and Hole 1963 and 1964, Gallagher and Wollenhaupt 1997) and cause disappearance of the forest floor layer through direct consumption (Pokarzhevskii et al. 1997, Doube et al. 1997), redistribution of organic material within the soil (McColl et al. 1982, Scheu 1993, Devliegher and Verstraete 1997) and increasing decomposition rates (Haimi and Huhta 1990, Devliegher and Verstraete 1997). Epi-endogeic species such as *L. rubellus* lead to the most rapid removal of an established forest floor (Gundale 2002, Chapter 3) and may therefore have disproportionate impacts on plants during initial earthworm invasion.

Invasion by European earthworms in hardwood forests can lead to changes in the understory plant community via many direct and indirect effects (Gundale 2002). Direct mortality of herbaceous plants and small tree seedlings that were rooted in the forest floor layer prior to earthworm invasion occurs when earthworms eat the forest floor out from under them (James and Cunningham 1989, Chapter 5). Plant regeneration may decrease due to direct ingestion and deep burial of seeds by earthworms (Leck 1989) and by loss of the sheltering forest floor layer (Nielsen and Hole 1964, Nixon 1995, Cothrel et al.

1997) exposing seeds and seedlings to desiccation, and predation by insects, small mammals and other organisms. After initial earthworm invasion, smaller understory plant populations become more vulnerable to the impacts of deer grazing which can lead to local extirpation (Augustine et al. 1998). Changes in soil chemical composition and nutrient dynamics have been documented following invasion in previously earthworm-free soils (Scheu and Parkinson 1994, Blair et al. 1995, Tomlin et al. 1995, Burtelow et al. 1998, Bohlen et al. 2004^a) including increases in N and P loss due to leaching (Suárez et al. 2004) and decreased N and P availability (Bohlen et al. 2004^b, Chapter 3). Finally, changes in soil microbial communities, in particular changes in mycorrhizal fungi associations may lead to declines in native understory plant abundance and diversity (Francis and Read 1994, Groffman et al. 2004).

There are many leading edges of European earthworm invasion across northern Minnesota and the Great Lakes region (Chapter 2, Holdsworth et al. in prep). These leading edges are associated with rapid increases in total earthworm biomass, the successive appearance of up to 8 earthworm species (Chapter 2) and substantial changes in soil physical and chemical properties (Chapter 3). The gradient of earthworm species and associated changes in soil characteristics across the leading edges within forest stands provides an opportunity to assess the relationships of earthworm biomass and species assemblages to changes in the understory plant community while controlling site specific factors such as local soil and climate variability which often confound field based comparative studies.

The objectives of this study were to describe the relationships of earthworm biomass and species composition with the abundance, diversity and composition of the understory plant community across leading edges of European earthworm invasion. I hypothesized that 1) increasing total worm biomass would be associated with decreases in herbaceous plant abundance and diversity and tree seedling density; 2) increasing biomass of the epi-endogeic species *L. rubellus* would be the most strongly related to changes in herbaceous plant composition.

Methods

The study was conducted over four years (1998-2001) in 4 mature northern hardwood stands which contain leading edges of earthworm invasion located on the Chippewa National Forest of northern Minnesota (Figure 1). The four study sites were selected to have similar overstory composition, soils and stand history. Three sites were identified and initially sampled in 1998 (Blackduck, Ottertail, Section 19). A fourth site (Two Points) was subsequently identified and all four sites were sampled in 1999, 2000 and 2001. Sugar maple (*Acer saccharum*) is the dominant tree species with yellow birch (*Betula alleghaniensis*), paper birch (*Betula papyrifera*) and basswood (*Tilia Americana*) as secondary species. Ironwood (*Ostrya virginiana*) and american elm (*Ulmus americana*) are common in the sub-canopy and sapling layers. Soils are a deep, well-drained and light colored Eutroboralf (Warba series) associated with the Guthrie Till

Plain (USDA 1997) and under worm-free conditions are characterized by a thick forest floor composed of L, F and H layers. The climate is humid, continental, cold temperate, with the median growing season length of 134 days, snow cover from late November to early April, and mean temperatures in January and July of -15 and 20 degrees C, respectively (Minnesota State Climatology Office 2003).

During site selection, a leading edge was defined as a discrete area in the forest where a transition from a thick forest floor to a thin forest floor took place within 75-100 meters with large patches of bare mineral soil and obvious evidence of earthworm activity (Chapter 2). In each site, a 30 x 150 meter sample grid was established consisting of 45 sample points 10 meters apart in three parallel transects with 15 points each (Figure 1). Transects were placed perpendicular to the leading edge with sample point 8 on the transect located where at the approximate front of the leading edge. From sample point 8, the transects extend 75 meters into the zone of well-developed forest floor in front of the leading edge and 75 meters in the opposite direction behind the leading edge.

Vegetation sampling.

Each sample point was the center of a set of three nested subplots established for vegetation sampling (Figure 1). In the first and last year for each study site, the species, diameter at breast height (dbh, breast height = 1.4 m) and height of all trees ≥ 10 cm dbh were recorded within a 4.5 meter radius subplot. In the fall of each year, saplings, seedlings and shrubs were sampled in a 1.75 meter radius subplot. All saplings (tree species < 10 cm dbh), seedlings and shrubs were tallied by species and the total percent

cover for each species was recorded. In a 0.75 meter radius subplot, all herbaceous and graminoid species were identified and the number and total percent cover of each recorded.

Earthworm sampling.

In 1999 and 2000, the earthworm populations were censused each fall using mustard liquid extraction in 0.12 m² (35 cm x 35 cm) subplots located 2.0 meters from each sample point at a randomly selected cardinal direction (Chapter 2). For each subplot, total ash-free dry (AFD) earthworm biomass was determined for each species (Chapter 1). For the purposes of statistical analysis, data on earthworm species with similar habitat and feeding preferences were combined into one of six analytical groups including *Dendrobaena octaedra*, *Lumbricus rubellus* (adults), *L. terrestris* (adults), *Aporrectodea* species, *Octolasion tyrtaeum* and a separate group for juvenile *Lumbricus* specimens where species identification was impossible (Chapter 2).

Analytical methods

For all analyses, the 2 year mean (1999 and 2000) of earthworm biomass per sample point was used as the best measure of mean earthworm biomass for each sample point (N = 45 in each site) (Chapter 3). For purposes of statistical analysis, earthworm biomass data were log transformed using a technique described in McCune and Grace (2002) which preserves both zero values and differences in magnitude that exist in the data set (Chapter 2).

Relationships with earthworm biomass

For sapling, seedling, shrub and herbaceous plant total cover and diversity, the two year mean from 1999 and 2000 was used in analyses with the two year mean of earthworm biomass for each sample point. Trees and snag data from 2001 were analyzed in relation to the two year mean of total earthworm biomass. The Shannon-Weaver (H') index of diversity (Shannon and Weaver 1964, Poole 1974) and the Simpson's (D) index of dominance (Magurran 1988) were calculated for the herbaceous plant community. Tree, snag and seedling density along with herbaceous plant richness were log transformed [$x = \ln(x+1)$] to preserve zeros and normalize the data distributions prior to analysis. All percent cover data were converted to ordinal cover classes (Host and Pregitzer 1991, McCune and Grace 2002) which normalized the data distributions and no further transformations were required. A combination of multiple and simple linear regression analyses were used to test the relationship of total earthworm biomass to changes in tree, sapling, shrub and herbaceous plant total cover and diversity measures (Montgomery & Peck 1992, SAS 2001).

The composition of tree, sapling, shrub and herbaceous plant communities in relation to earthworm species specific biomass were assessed using non-metric multidimensional scaling (NMS) ordination with Sorenson's distances (McCune and Grace 2002) in PC-ORD (McCune and Mefford 1999). Ordinations of the herbaceous plant community (2 year mean values of plant percent cover) were conducted with the rarest plant species ($N \leq 3$) and strong outliers (>2.6 s.d.) deleted. Preliminary NMS analysis with 50 runs of real data and 100 runs with randomized data for a Monte Carlo

test of significance were conducted. The optimal number of ordination dimensions was determined based on the lowest stress achievable with the fewest number of dimensions. A series of secondary NMS analyses were conducted with the designated number of ordination dimensions, 50 runs of real data and 100 runs with randomized data. When consistent results after multiple runs from a random starting configuration were found, the secondary analysis with the lowest stress and final instability was used as the starting configuration for the final NMS analysis reported here. Environmental variable overlays were used to assess the relationship of site, sample point position and earthworm species specific biomass to the ordination of the herbaceous plant community.

Relationships with earthworm species assemblages

Richness and species area relationships of the herbaceous plant community were compared among sample points with different earthworm species assemblage groups. Using a combination of cluster analysis and indicator species analysis, four earthworm species assemblage groups were identified (Chapter 3) and each sample point was assigned to one of the four species assemblage groups. Group 1 contained all sample points with the species *Dendrobaena octaedra* and *Dendrodrilus rubidus* only, and had a total of 21 sample points from three sites (Blackduck, Ottertail and Two Points). Group 2 contained sample points with the species *Lumbricus rubellus* (adults), *Lumbricus* (juveniles) and *Dendrobaena octaedra* only, and had a total of 13 sample points from two sites (Ottertail and Section 19). Group 3 contained sample points with *Aporrectodea* species and *Dendrobaena octaedra* as the dominant species with occasional *Lumbricus*

rubellus (adults), *Lumbricus* (juveniles), and had a total of 75 sample points from two sites (Blackduck and Two Points). Group 4 was identified by the presence of *Lumbricus terrestris* (adults) and *Octolasion tyrtaeum* but contained all other species as well, and had a total of 71 sample points primarily from two sites (Ottertail and Section 19).

Richness of the herbaceous plant community was compared among earthworm assemblage groups using Whittaker's (1972) alpha and gamma diversity measures. Alpha diversity is calculated as the average herbaceous species richness per sample point in each earthworm assemblage group. Gamma diversity is the landscape level diversity and is estimated as the total number of herbaceous species across all points in each earthworm assemblage group.

Species area curves of the herbaceous plant community in each earthworm assemblage group were constructed using PC-ORD (McCune and Mefford 1999). In each assemblage group, the sample points were aggregated in every possible combination to produce the average number of herbaceous species and standard deviation for each possible aggregate size (i.e. number of sample points = 1, 2, 3...n). The species area function $S = cA^z$ was fit as...

$$\ln(S) = \ln(c) + z\ln(A)$$

where S is the average number of species found in area A , z is the instantaneous rate by which species richness increases with an incremental increase in area, and c is the expected number of species in a single unit area (Arrhenius 1921). It might be assumed

that any differences in the shape of species curves between assemblage groups are related primarily to differences in earthworm biomass. To test this hypothesis, the z exponent and the c coefficient values from all groups were regressed against the mean earthworm biomass from each group (Pastor et al. 1996).

Results

Plant density, total cover and diversity in relation to total earthworm biomass

Herbaceous plants and maple seedlings in relation to total earthworm biomass

The relationships of herbaceous plant species richness and total cover to increasing total earthworm biomass varied among sites ($P < 0.0001$) (Figure 2). At two sites, herbaceous plant species richness and cover decreased significantly ($P \leq 0.006$) with increasing worm biomass, whereas at a third they increased with worm biomass ($P \leq 0.005$), and there was no relationship at the fourth. The Shannon-Weaver (H') index of diversity and Simpson's (D) index of dominance had the same relationships as species richness to earthworm biomass and sites ($P \leq 0.03$). Sugar maple seedling density and total cover decreased with increasing total earthworm biomass ($P < 0.0001$) in three of four sites (Figure 3).

Trees, snags, saplings and shrubs in relation to total earthworm biomass

Among all sites and sample points, there were no consistent relationships of the density and basal area of trees or snags (>10cm dbh) to total earthworm biomass. The density of trees ranged from 157 to 1258 stems/ha and the density of snags ranged from 0 to 471 stems/ha. The basal area of trees ranged from 0 to 184 m²/ha (mean 32 m²/ha) and the basal area of snags ranged from 0 to 87 m²/ha (mean 6 m²/ha).

Among all sites and sample points, there were no consistent relationships between total cover of large saplings, seedlings or shrubs or the density of dead sapling with total earthworm biomass. The total cover of small saplings (≥ 0.5 m tall to < 2 cm d.b.h.) increased with total earthworm biomass ($P = 0.02$) in only one of the four study sites (Section 19).

Plant composition in relation to total earthworm and species specific biomass

Tree, sapling, seedling and shrub composition

The tree, sapling and seedling populations were strongly dominated by *Acer saccharum* and their species composition did not change in relation to either earthworm species or total biomass. The composition of the shrub community also did not change in relation to either earthworm species or total biomass.

Herbaceous plant community composition

NMS ordination of herbaceous plant species resulted in an optimal 3 dimensional solution (final stress = 19.37656, final instability = 0.00239) with cumulative proportion of variance represented by the 3 dimensional ordination of $r^2 = 0.684$ ($r^2 = 0.330, 0.169$ and 0.184 for axis 1, 2 and 3, respectively). Monte Carlo tests resulted in final stress values well below the range of those of the randomized data set indicating that the ordination is significantly different than that expected by chance.

Axis 1 represents a shift in herbaceous plant community associations related to increasing earthworm biomass, particularly with increasing *L. rubellus* (adults) and *L.* species (juveniles) biomass (Figure 4). Along axis 1 the herbaceous plant community shifts from one of high diversity when *L. rubellus* biomass is low to an herb free or low diversity plant community dominated by *Carex* species and *Arisaema triphyllum* when *L. rubellus* biomass is high (Figure 5). Of the 38 herbaceous plant species included in the ordination, the scores of 32 were negatively correlated with axis 1 relative to the ordination centroid (Table 1). *Carex* species (predominantly *Carex pennsylvanica*) were the most positively correlated plant species with axis 1 ($r = 0.403$). In overlays of earthworm species biomass on the ordination of herbaceous plant species composition *Dendrobaena octaedra* was negatively correlated with axis 1, the *Aporrectodea* species group had no correlation with axis 1 and all other earthworm species groups, as well as total earthworm biomass, were positively correlated with axis 1 (Table 2).

Axis 2 and 3 represent a disturbance gradient in the herbaceous plant community on the Blackduck site because of a blowdown in 1999 that decreased overstory canopy

cover by 30%. This gradient is indicated by high abundance of *Sambucus pubens* and *Galium triflorum* (Figure 5, Table 1). Axis 2 has a moderate correlation with the earthworm group *Aporrectodea* species (Table 2). Axis 3 has no strong correlations with earthworm species biomass (Table 2).

Patterns of plant species abundance were more strongly related to the abundance of *L. rubellus* (adults) and *L.* species (juveniles) than to total earthworm biomass (Figure 4). The relative abundance of *Carex* species increased with the relative abundance of *L. rubellus*. *Polygonatum pubescens* and *Uvularia grandiflora* are absent where *L. rubellus* abundance is the greatest and are highest in abundance in sample points where *L. rubellus* is absent. This pattern is seen with many other plant species as well (Table 1). *Arisaema triphyllum* abundance is the lowest where total earthworm biomass is either high or low, with intermediate abundance elsewhere (Figure 4).

Herbaceous plant richness in relation to earthworm species assemblage groups

Mean herbaceous plant richness per sample point was lower in earthworm assemblages dominated by *L. terrestris*, compared with the other assemblage groups and all sample points combined (Table 3). Total group richness in the *L. terrestris* dominated group was comparable to the group with the small epigeic species *Dendrobaena octaedra* only, which had less than 1/3rd the number of total plots. The *Aporrectodea* dominated assemblage group had comparable number of plots to the *L. terrestris* group but more total plant species.

The species-area curve of the *L. terrestris* group fell below the curves of all other earthworm species assemblage groups (Figure 6). Although, the species curves of the *Aporrectodea*, *Dendrobaena* and *L. rubellus* groups overlapped where sample area was small, near 15 m² the *Dendrobaena* group curve crossed that of *Aporrectodea* on a trajectory of increasing plant species richness compared to all other groups and the *L. rubellus* group begins to fall below the curve of *Aporrectodea*. Regression of the z exponent and the c coefficient values against mean earthworm biomass from each group resulted in no significant correlation, thereby rejecting the hypothesis that changes in z and c among earthworm species assemblage groups are the result of changes in earthworm biomass.

Discussion and Conclusions

The abundance and diversity of the herbaceous plant community and the abundance and density of tree seedlings in sugar maple dominated hardwood forests decreased with increasing total earthworm biomass in three of four sites (Figures 2 and 3). Sample points with earthworm species assemblages containing all species groups detected in this study had the lowest total plant diversity (Table 3) and slower accumulation of plant species with increasing sample area (Figure 6). Although, increasing earthworm species diversity was associated with increasing total earthworm

biomass (Chapter 2), the changes in species-area relationships among different earthworm assemblages were not solely the result of increasing earthworm biomass.

Increasing biomass of *L. rubellus*, which contributes less to total biomass than do *Aporrectodea* species or *L. terrestris*, had the greatest effects on changes in understory plant composition (Tables 1 and 2, Figures 4 and 5). The presence of *L. terrestris* led to the highest total earthworm biomass observed (Chapter 2). However, not all plots containing *L. terrestris* experienced large changes in plant species composition (Figure 5).

Where *L. rubellus* was absent there was a diverse community of herbaceous plants including *Caulophyllum thalictroides*, *Uvularia grandiflora*, *Trillium* species, *Osmorhiza claytonii*, *Asarum canadensis* and *Polygonatum pubescens*, which are often used as indicators of rich sugar maple dominated hardwood forests in our region (Kotar et al. 2002, Aaseng et al. 1993). This was the case even in some instances where there was high biomass of other earthworm species (Figure 5). Where *L. rubellus* biomass reached its maximum, the herbaceous plant community was dominated by *Carex pennsylvanica* and *Arisaema triphyllum* with rare occurrences of other native plant species. In some instances there were no herbaceous plants at all.

Potential mechanisms of earthworm effects on understory plant communities

Plants with high levels of secondary compounds are generally not grazed by herbivores and their rhizosphere may be avoided by earthworms as well. Root herbivory

and changes in the rhizosphere can result in decreases in plant growth and diversity (Brown and Gange 1989, Hobbie 1992, Bever et al. 1997). *Arisaema triphyllum* (Jack in the Pulpit) and *Allium tricoccum* (wild leeks) were both positively associated with increasing earthworm biomass (Table 1, Figure 5). However, both had their lowest abundances in nearly worm-free conditions and where earthworm biomass and diversity were the highest suggesting that an initial fertilization effects may be occurring early on in the earthworm invasion followed by declines in these species after large persistent earthworm populations had become established (Table 1, Figure 5).

Grazing by earthworms can lead to declines in the abundance and changes in the structure of the soil fungal community (Johnson et al. 1992, Scheu and Parkinson 1994, Lawrence et al. 2003). The vast majority of native understory plants in northern temperate sugar maple dominated forests are strongly mycorrhizal (Brundrett and Kendrick 1988, Baskin and Baskin 1998). *Carex pennsylvanica* is one of the only non-mycorrhizal native species in these forests (Brundrett and Kendrick 1988) and was the dominant herbaceous plant species where *L. rubellus* biomass was high (Table 1, Figure 4) suggesting that declines in abundance or colonization rates of mycorrhizal fungi or shifts in fungal community composition may be leading to changes in the understory plant community (Newman and Reddell 1988, Francis and Read 1994, van der Heijden et al. 1998, Brussaard 1999).

Management implications

Invasion of hardwood forests by European earthworms is occurring throughout the range of this ecosystem in North America (James 1995). Concern over the potential widespread loss of native forest plant species and the loss of stability of hardwood-forest ecosystems which could result from such an invasion indicate that management strategies be developed to either prevent further invasions or provide for restoration following invasion (Proulx 2003). Although local control of invasions may be possible in some situations, the magnitude and regional scale of European earthworm invasions suggests that in the next few decades a majority of hardwood forest will be impacted to some degree by earthworms (Scheu and Parkinson 1994, Dymond et al. 1997, Reynolds et al. 2002). Because of their close proximity to fishing lakes and rivers, hardwood forests in Minnesota may be particularly vulnerable to invasion by a number of exotic earthworm species (Wagner et al. 1977, Alban and Berry 1994).

The level of impact in different forest types may vary in relation to site productivity. Therefore, we will need to understand the factors that control or prevent recolonization of native understory species of earthworm-impacted forests across a range of forest cover types. For example, it may be that earthworms alone will not eliminate understory vegetation, but decrease the populations of these species to a level where a shift in the stable state equilibrium under high deer densities causes extirpation (Augustine and Frelich 1997, Augustine et al. 1998). In this situation, control of earthworms would have no affect on recolonization of understory herbs. However, control of deer densities would allow for recolonization from small source populations.

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Table 1. Correlation coefficients (Pearson r) of herbaceous plant species total cover with ordination axis and the number of plots each species was found (total n = 168).

Species	n	Axis 1	Axis 2	Axis 3
<i>Carex</i> species	57	0.403	0.479	0.405
<i>Circaea lutetiana</i>	12	0.136	0.256	-0.222
<i>Oryzopsis</i> species	2	0.128	-0.104	-0.063
<i>Allium tricoccum</i>	3	0.123	0.110	0.041
<i>Arisaema triphyllum</i>	126	0.089	0.141	-0.331
<i>Maianthemum canadense</i>	15	0.030	-0.260	0.184
<i>Viola selkirkii</i>	9	-0.012	0.008	0.012
<i>Aralia nudicaulis</i>	8	-0.013	-0.144	-0.086
<i>Impatiens</i> species	2	-0.047	0.036	0.025
<i>Trillium</i> species	9	-0.054	0.035	0.173
<i>Viola</i> species	8	-0.060	-0.012	0.002
<i>Galium</i> species	4	-0.094	0.049	0.067
<i>Polygonatum commutatum</i>	5	-0.102	-0.259	0.076
<i>Streptopus roseus</i> var. <i>longipes</i>	15	-0.116	-0.247	0.161
<i>Anemone quinquefolia</i>	31	-0.123	-0.148	0.242
<i>Trillium cernuum</i>	5	-0.129	-0.005	0.155
<i>Solidago flexicaulis</i>	6	-0.149	0.049	0.083
<i>Galium aparine</i>	3	-0.153	0.101	-0.057

<i>Matteuccia struthiopteris</i>	13	-0.154	-0.064	0.019
<i>Caulophyllum thalictroides</i>	22	-0.164	0.007	0.093
<i>Equisetum</i> species	20	-0.167	-0.034	0.133
<i>Thalictrum dioicum</i>	4	-0.171	0.004	-0.191
<i>Botrychium virginianum</i>	18	-0.174	-0.024	0.082
<i>Equisetum hyemale</i> var. <i>affine</i>	31	-0.186	-0.032	0.295
<i>Galium triflorum</i>	8	-0.189	0.222	-0.145
<i>Athyrium felix-femina</i>	15	-0.205	-0.141	-0.137
<i>Sanguinaria canadensis</i>	44	-0.211	-0.027	-0.100
<i>Phryma leptostachya</i>	10	-0.237	-0.059	-0.041
<i>Aralia racemosa</i>	14	-0.281	-0.035	0.194
<i>Asarum canadense</i>	15	-0.296	-0.002	-0.004
<i>Sambucus pubens</i>	34	-0.317	0.380	-0.299
<i>Smilacina racemosa</i>	51	-0.381	-0.117	0.304
<i>Viola pubescens</i>	58	-0.385	0.002	0.179
<i>Laportea canadensis</i>	46	-0.418	0.311	0.068
<i>Hydrophyllum virginianum</i>	52	-0.442	0.135	-0.093
<i>Osmorhiza claytonii</i>	47	-0.442	0.119	-0.031
<i>Uvularia grandiflora</i>	82	-0.458	-0.488	0.620
<i>Polygonatum pubescens</i>	58	-0.483	-0.198	0.015

Table 2. Correlation coefficients (Pearson r) of earthworm species biomass with ordination axis.

Earthworm analytical groups	Axis 1	Axis 2	Axis 3
<i>Dendrobaena octaedra</i>	-0.459	-0.143	0.176
<i>Aporrectodea</i> species	0.038	0.219	-0.097
<i>Lumbricus rubellus</i> (adults)	0.694	0.094	-0.025
<i>L.</i> species (juveniles)	0.682	0.037	0.099
<i>L. terrestris</i> (adults)	0.424	0.033	-0.031
<i>Octolasion tyrtaeum</i>	0.493	0.151	-0.250
Total earthworm biomass	0.490	0.194	-0.036

Table 3. Species diversity of herbaceous plants related to earthworm species assemblage groups, which are described in the text.

Earthworm assemblage dominant species	n	* sample point mean richness	+total group richness
group 1 - <i>Dendrobaena octaedra</i>	21	4.45 ^a	36
group 2 - <i>Lumbricus rubellus</i>	13	4.35 ^a	26
group 3 - <i>Aporrectodea</i> species	75	5.60 ^b	44
group 4 - <i>Lumbricus terrestris</i>	71	1.95 ^c	35
All plots combined	180	3.94 ^a	58

*alpha diversity; +gamma diversity

^{a, b, c} means with different letters are significantly different ($P < 0.05$)

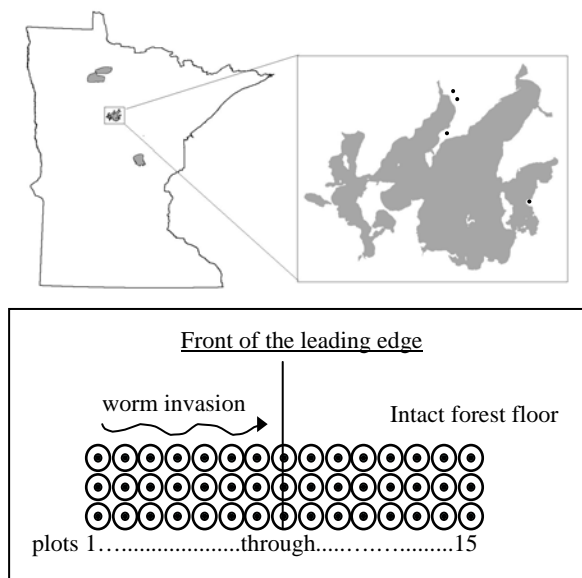


Figure 1. Locations of the four study sites in the Chippewa National Forest near Leech Lake in north central Minnesota. An illustration of the sample grid (30 m x 150 m) of 45 points laid perpendicular to the leading edge of earthworm invasion in each study site. Sample points are 10 m apart.

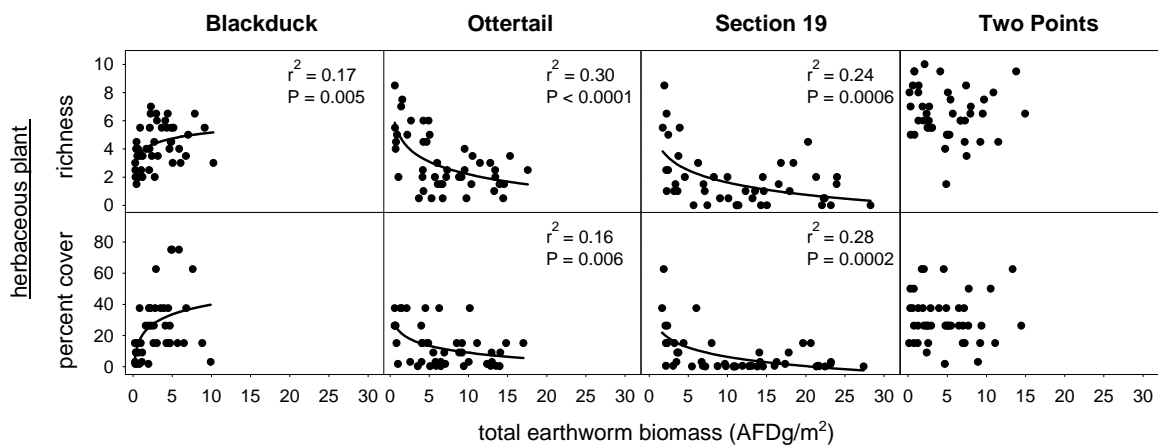


Figure 2. Scatter plots of herbaceous plant species richness and total percent cover in relation to total earthworm biomass in each study site (N = 45 in each site).

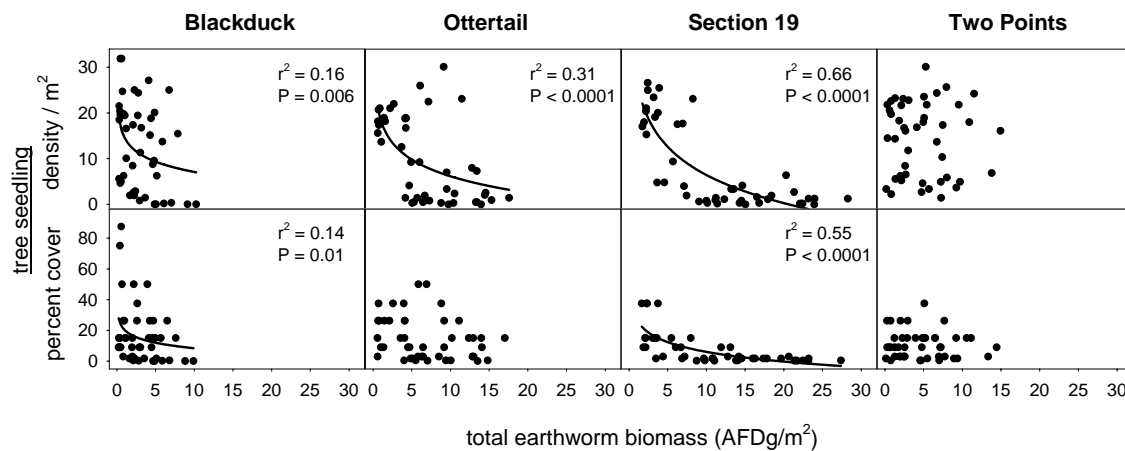


Figure 3. Scatter plots of sugar maple seedling density and total cover in relation to total earthworm biomass in all study sites (N = 45 in each site).

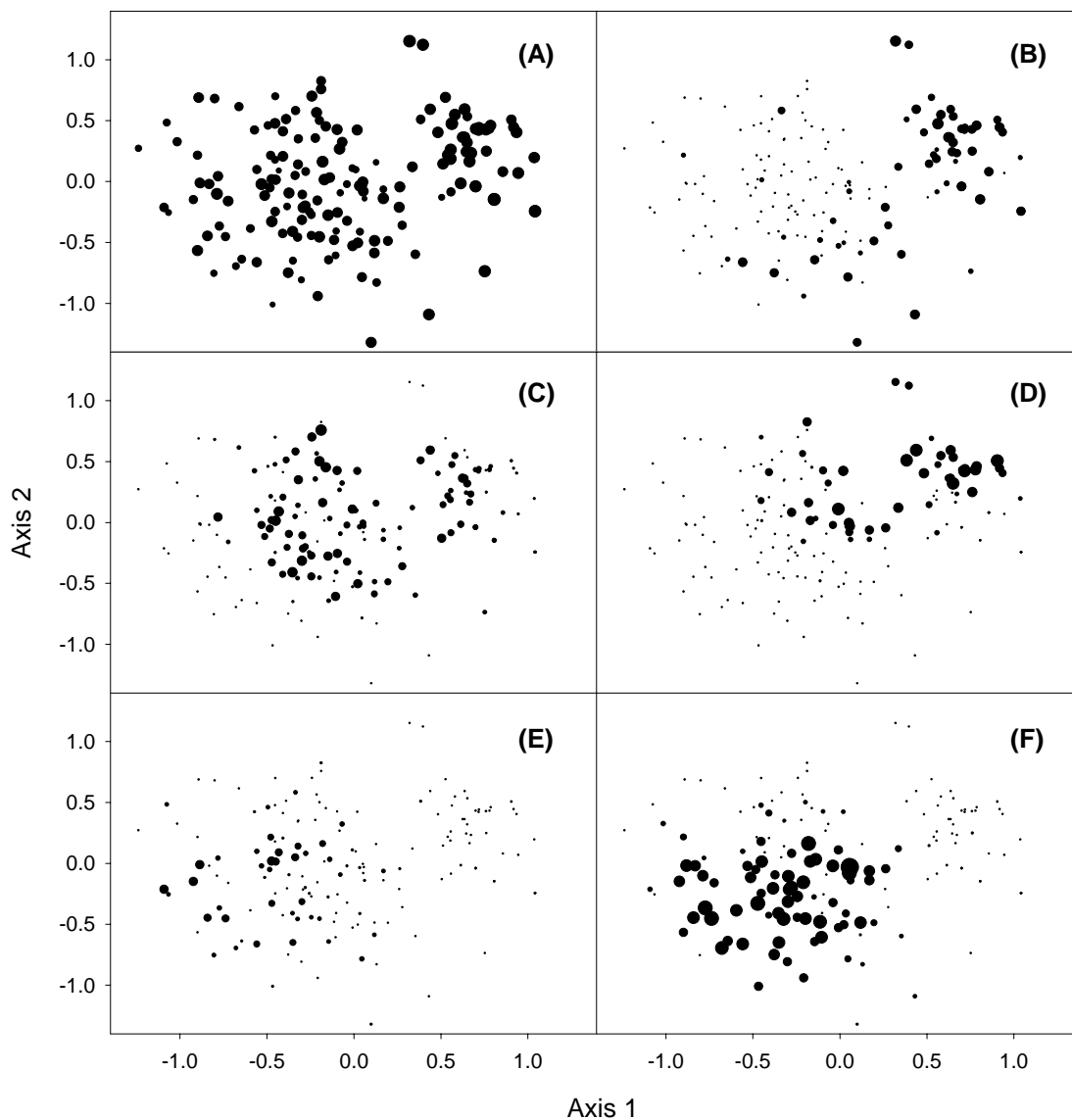


Figure 4. Overlays of sample plots on the herbaceous plant species ordination. Symbol size is proportional to earthworm biomass (A-B) and plant species total cover (C-F) for each sample plot. (A) = total earthworm biomass; (B) = *Lumbricus rubellus* (adults); (C) = *Arisaema triphyllum*; (D) = *Carex* species; (E) = *Polygonatum pubescens*; (F) = *Uvularia grandiflora*.

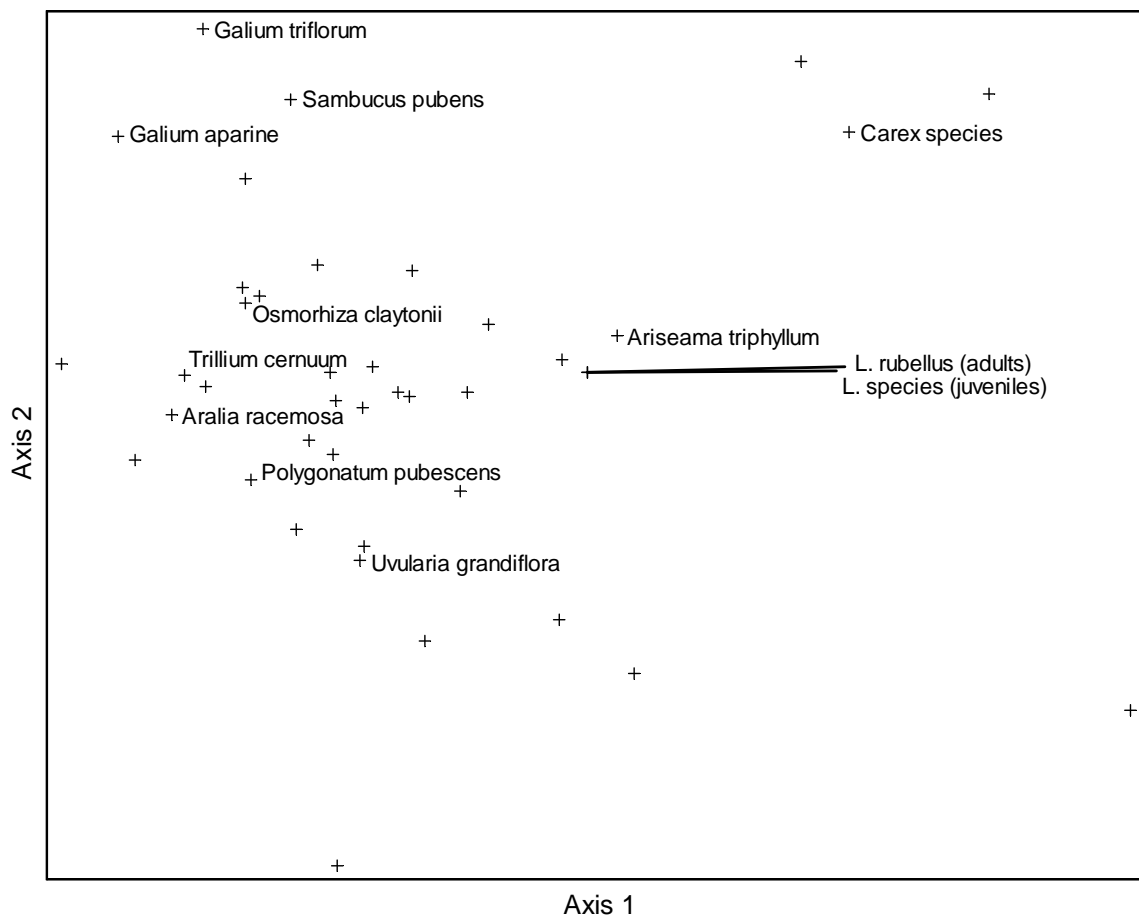


Figure 5. Ordination of herbaceous plant species with joint plot overlay of earthworm species biomass vectors for *L. rubellus* and *L. species* ($r^2 > 0.450$). Selected plant species names listed to the right of the species symbol (+).

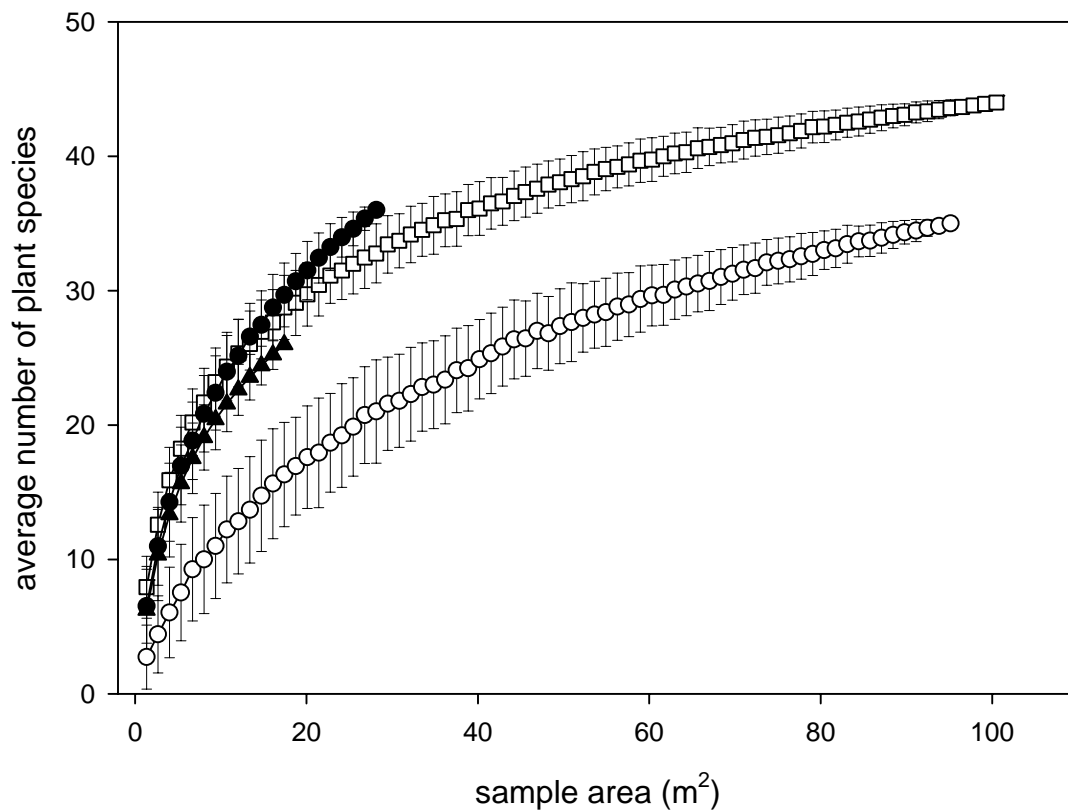


Figure 6. Species-area curves: average herbaceous plant richness (s.d. error bars) with increasing sample area in each earthworm species assemblage group. solid circles = sample points with *Dendrobaena octaedra* only (N=21); solid triangles = sample points with *Lumbricus rubellus* (adults), *Lumbricus* (juveniles) and *Dendrobaena octaedra* (N=13); empty squares = sample points dominated by *Aporrectodea* species and *Dendrobaena octaedra* (N=75); empty circles = plots with all earthworm species, identified by the presence of *Lumbricus terrestris* and *Octolasion tyrtaeum* (N=71).

Chapter 5

A Mesocosm Experiment: effects of selected European earthworm species (*Lumbricidae*) on forest floor removal, soil characteristics and native understory plants

Introduction

Across the cold temperate regions of North America, European earthworm invasions into previously worm-free forests have apparently led to changes in ecosystem structure and function (Chapters 3 and 4, Bohlen et al. 2004^a, McLean and Parkinson 1997). Following exotic earthworm invasions into hardwood forests of the Great Lakes region, the previously thick forest floors have been lost (Chapter 2, Alban and Berry 1994, Nielsen and Hole 1963), the thickness and bulk density of the A horizons have increased (Chapter 3, Nielsen and Hole 1964), nutrient availability has declined (Chapter 3, Bohlen et al. 2004^b, Suárez et al. 2004) and populations of many native understory plant species have declined (Chapter 4, Gundale 2002).

With few exceptions, many earthworm species invade concurrently (Chapter 2, Bohlen et al. 2004^a, Dymond et al. 1997, Alban and Berry 1994), each with specific burrowing and feeding behaviors that can lead to different impacts (Lavelle 1997, Shaw and Pawluk 1986). Because of this, it is difficult to determine the importance of any

individual earthworm species on the changes associated with earthworm invasion in the field (Chapters 3 and 4, Bohlen et al. 2004^a). Additionally, native understory plant species may respond individually to different earthworm species (Brussaard 1999, Bever et al. 1997, James and Seastedt 1986). An understanding of earthworm species specific effects will be important to predict trajectories of native forest ecosystems following European earthworm invasions.

I designed a greenhouse mesocosm experiment to examine the effects of three common European earthworm species (*Dendrobaena octaedra*, *Lumbricus rubellus* and *Lumbricus terrestris*) (Chapter 2, Reynolds 2002, Reynolds 1995), on forest floor removal, changes in soil characteristics and the mortality and biomass of selected native plant species. I focused on three earthworm species that differ in their feeding strategies. *D. octaedra* is a small (~2-4 cm) strictly litter dwelling species that feeds primarily on microorganisms associated with decaying surface litter (McLean and Parkinson 2000, Scheu and Parkinson 1994). *L. rubellus* is a medium sized (~3-7 cm) surface dwelling species that feeds on surface litter but also burrows and casts in the upper mineral soil layer (Hendriksen 1990). *L. terrestris* is a large (~8-15 cm) soil dwelling species that feeds on fresh surface litter but forms deep, persistent vertical burrows and deposits large amounts of cast material in the burrows and at the surface (Pitkänen and Nuutinen 1997). Because of different feeding and burrowing behaviors, each species is expected to have different effects on forest floor removal, development of an A horizon, plant mortality and plant biomass (Hendrix et al. 1999, Bouché 1977). In particular, I hypothesized that *D. octaedra* would have no effect on forest floor removal, soil characteristics and plant

mortality and biomass because it has been shown to have little effect in previous field studies (Chapters 3 and 4). *L. rubellus* was expected to have the largest effects on forest floor removal and plant parameters relative to *D. octaedra* and *L. terrestris* because of its ability to rapidly consume surface litter (Gundale 2002, Shipitalo et al. 1988) and its propensity for feeding in the rhizosphere of plants (Pierce 1978). Although *L. terrestris* can consume large amounts of surface litter (Nielsen and Hole 1964, Cothrel et al. 1997) its burrowing habits are less disruptive to the upper soil horizon than *L. rubellus* and I therefore expected it to have intermediate effects as compared to the other species. Cumulative impacts when all three earthworm species are present were expected to be greatest, largely due to the combined effects of multiple feeding and burrowing behaviors (Lavelle et al. 1998).

Four native understory plant species were included in this study that represent different plant types of particular interest in hardwood forests of the western Great Lakes region (Chapter 4). Earthworms lead to declines in fine root biomass in the upper soil horizon (Chapter 3, Fisk et al. 2004), cause declines in abundance and changes in composition of the soil fungal community (Scheu and Parkinson 1994) and result in decreases in mycorrhizal colonization rates (Lawrence et al. 2003). Therefore, plant species that differ in root architecture and mycorrhizal status may be affected differently by earthworm invasion. *Carex pennsylvanica*, a non-mycorrhizal native sedge species, became the dominant native plant species following earthworm invasion in the field sites (Chapter 4). I hypothesized that *C. pennsylvanica* would have low mortality and increased biomass in response to earthworm additions. *Aralia racemosa* is a large,

perennial herbaceous species that was mostly eliminated following earthworm invasion in field sites and *Acer saccharum* (sugar maple), the dominant tree seedling in the field sites, also declined in abundance following earthworm invasion. *Aralia* and *Acer* are strongly mycorrhizal species with much thicker fibrous root systems than *Carex*. Therefore, I hypothesized that mortality of *Aralia* and *Acer* would increase and biomass would decrease in response to earthworm additions. *Aquilegia canadensis* is a mid-sized perennial herbaceous species that is common in hardwood forests of the Great Lakes region (Hale et al. 1999) but was not present in my earthworm invasion field study sites (Chapter 2). However, based on similarity in growth form and habitat as compared to *Aralia*, I hypothesized that *Aquilegia* would have similar responses to earthworm additions.

Plants affect localized soil conditions that may in turn affect certain earthworm species (Petersen et al. 2001, Brussaard 1999, Hobbie 1992). *L. rubellus* survival was expected to be lower in mesocosms with no plants because of the lack of its preferred rhizosphere habitat. In contrast, because *D. octaedra* is a strictly litter dwelling species and *L. terrestris* is less of a generalist feeder, their abundance was expected to be unaffected by plant species.

Methods

Experimental design and mesocosm assembly

A randomized block design containing five plant species and five earthworm treatments was used (Table 1). *Carex*, *Aquilegia* and *Acer* had 14 replicates in each earthworm treatment while *Aralia* and the no plant control had 7 replicates in each earthworm treatment because of space limitations on the number of mesocosms (total n = 280). For each earthworm and plant treatment, the total number and biomass of earthworms and plants were standardized across all replicates.

Worm-free conditions similar to the earthworm invasion field sites (Chapter 2) were created in each mesocosm (30 x 38 cm plastic buckets) (Figure 1). Discs (30 cm diameter x 5-7cm thick) of intact forest floor layers were collected randomly from a 1 hectare area within a worm-free, sugar maple dominated hardwood forest in Tettegouche State Park in northeastern Minnesota. Each forest floor layer was wrapped in thin plastic sheeting to keep it intact during transport and then frozen at 0° C for 14 days. Worm-free mineral soil of the same silt-loam soil found at the earthworm invasion field sites (Chapter 2) was also collected. The surface vegetation and top ~10 cm of soil were peeled back with a bobcat and then the next ~50 cm of soil collected with a backhoe. A total of ~25 m³ of soil was collected. The soil was spread on tarps, sun-dried for 48 hours and then sifted and mechanically mixed until homogeneous.

The mesocosm containers were assembled with a 5 cm diameter PVC ventilation tube in the center (Figure 1). To discourage earthworms from preferentially burrowing

along the soil-plastic interface the inside surfaces were sprayed with polyurethane and coated in coarse sand. Each mesocosm was filled with ~22 cm of mineral soil and the outer edge (~1.5 cm) of soil compacted, to further discourage earthworm burrowing along the soil-mesocosm interface. The surface of the mineral soil was raked to create a rough surface and a layer of intact forest floor (~5-7 cm) was placed on top (Figure 1). Mesocosms were watered to field capacity, weighed to the nearest 0.25 Kg and randomly placed in the greenhouse.

Plants used in the study were either collected from field sites or germinated and started in a greenhouse. In the first week of June, *Acer saccharum* seedlings were collected from Tettegouche State Park in NE Minnesota and *Carex pennsylvanica* seedlings were collected from Magney-Snively Municipal Park in Duluth Minnesota. *Aralia racemosa* and *Aquilegia canadensis* seedlings were established spring of 2001 from seed in a greenhouse by a regional native plant nursery (Agrecol). *Aralia* seeds were collected in fall 2000 from the earthworm invasion field study sites (Chapter 4) and *Aquilegia* seeds from central Wisconsin were purchased from Agrecol.

Mesocosms were randomly assigned to one of 5 plant treatments (Table 1) and planting occurred June 15-21, 2001. In each mesocosm, six seedlings (~7-15 cm) of the assigned plant species were planted, evenly spaced, by piercing the forest floor layer to the top of the mineral soil with a hand trowel and planting the roots in each furrow. The forest floor was similarly pierced in the no plant control treatment. Plants were allowed to grow for 8 weeks before earthworms were added. During plant establishment, each

mesocosm was weighed and watered to field capacity on a weekly basis. Plants that died in the first three weeks were replaced.

Earthworm treatments were randomly assigned to each plant species (Table 1) and added to the mesocosms August 17-20, 2001. *L. rubellus* (leaf worms) and *L. terrestris* (night crawlers) were purchased at local fishing bait shops. Separation of these two species was determined by size using sexually mature individuals. *D. octaedra* were collected from a field site previously determined to contain only that species and identification of 83 individuals collected confirmed that only *D. octaedra* was present. A mixture of juvenile and adult *D. octaedra* individuals were used. For each species, the number of individuals included in each treatment reflected the average densities of each in field sites (Chapter 2, Table 1). The fresh biomass of earthworms added to each mesocosm was recorded. Mesocosms were incubated for 13-18 weeks, depending on plant species. To minimize earthworm mortality due to high soil temperatures during the experiment, water baths were constructed in the greenhouse that allowed for circulation of cool water around all mesocosms.

Throughout the experiment, greenhouse air temperature was maintained between 15 and 35 degrees C and supplemental lighting was used to maintain a total of 16 hours of daylight. Soil temperatures were maintained between 13 - 21 degrees C by circulating cool water in water baths as needed. Mesocosms were monitored daily and watered as needed to maintain near field capacity moisture conditions. Weekly observations were recorded including visual indications of earthworm activity, forest floor condition and the general health of each plant. Any foliage shed was collected and labeled for each plant.

All mesocosms were rotated in the greenhouse on a weekly basis to minimize the effects of any localized conditions within the greenhouse.

Sample collection and data calculations

Initial fresh biomass of the forest floor layer, individual plants and total earthworms added to each mesocosm were recorded. Initial forest floor dry biomass values for each mesocosm were estimated based on the average fresh to dry weight conversion of 10 samples oven dried for 48 hours at 60° C.

After 13 weeks of exposure to earthworm treatments, final destructive harvesting of mesocosms commenced. Because final harvesting of the mesocosms would take over a month, all mesocosms containing each plant species were harvested together, over approximately 7 days, to minimize the time variation within each plant species. In each mesocosm, O and A horizon thickness were measured at three evenly spaced locations. For each mesocosm assigned to the all species earthworm treatment and the no earthworm control, three 4 gram subsamples of O and A horizon material were collected. The three subsamples within each horizon were combined as a composite sample and total percent nitrogen and carbon were determined for each mesocosm using a LECO CHN-800 elemental analyzer. All remaining forest floor material was collected and doused in a mustard solution (Chapter 2) to extract any earthworms. The forest floor material was then oven dried at 60° C for 48 hours and final dry mass measured. All surviving plants were collected individually and final fresh biomass of above ground plant parts, fine roots (≤ 1.0 mm) and course roots were recorded. All plant parts were

oven dried for 48 hours at 60° C and then final dry biomass recorded. Finally, the soil from each mesocosm was sifted to collect remaining earthworms. All earthworms collected from the forest floor material and mineral soil were killed in 70% isopropyl alcohol and preserved in 10% formalin. Ash-free dry (AFD) biomass of earthworms collected was estimated using previously determined allometric regression equations (Chapter 1).

Individual plant above ground biomass (grams dry weight) was the sum of all foliage collected during the course of the experiment and the final above ground biomass collected. Mean individual plant above ground biomass, fine root, course root, total root and total plant biomass were calculated for each mesocosm. The sum of each plant biomass parameter for all surviving plants in each mesocosm yielded mesocosm total plant biomass values for each parameter.

Nitrogen and phosphorus availability were measured using ion exchange resin bags, containing 5 g of Rexyn I-300 (Fischer Scientific, Fairlawn, New Jersey, USA) mixed-bed, cation-anion exchange resin (Binkley 1984). One resin bag was buried (~15 cm deep) in each mesocosm during assembly June 4-7, 2001 and removed during final destructive harvesting November 16 – December 20, 2001. Resin bags were rinsed with de-ionized water and stored in ziplock bags in a refrigerator until processing.

Ammonium, nitrate and phosphate were extracted from 3 g subsamples of resin with 100 mL of 1mol/L KCl; the resins were shaken for 15 minutes in 25 mL KCl, decanted, shaken again in another 25 mL aliquot, poured into small Buchner funnels (5.5 cm diameter) equipped with pre-washed #1 Whatman filters, and washed several times with

additional KCl before the filtrate was brought to 100 mL volume. The extracts were analyzed by standard methods for $\text{NH}_4\text{-H}$, $\text{NO}_3\text{-H}$ and $\text{PO}_4\text{-P}$ on a Lachat autoanalyzer (Lachat Instruments, Milwaukee, Wisconsin, USA).

Analytical methods

Percent plant mortality was square root transformed to meet assumptions of normality. Earthworm number and biomass, nutrient availability and all plant biomass parameters were log transformed [$\text{Ln}(x + 1)$]. No transformations were necessary for forest floor mass loss and A and O horizon thickness and nutrient content.

A combination of two-way Analysis of Variance with least squared mean comparisons and one-way paired comparisons were used to test for the effects of earthworm and plant treatments (SAS 2001). Linear regression analysis was used to test for differences in root to shoot biomass relationships among earthworm treatments for each plant species (Reich 2002).

Results

The mean incubation time during which mesocosms were exposed to earthworm treatments was 13, 16, 17, 17 and 18 weeks for *Acer saccharum*, no plant control, *Aralia racemosa*, *Aquilegia canadensis* and *Carex pennsylvanica*, respectively. Mean incubation time for the no plant control was not significantly different than any plant species. There

were no significant differences in mean incubation time among earthworm treatments for any plant species.

Forest floor mass loss and changes in O and A horizon thickness

Mass loss of forest floor (gram dry weight) was significantly greater when all earthworm species and *L. terrestris* or *L. rubellus* were present compared to *D. octaedra* and no worm control, in three plant species ($P < 0.0001$, Table 2, Figure 2). Each earthworm species and all earthworm species combined significantly reduced O horizon thickness and increased A horizon thickness compared with the no earthworm control, with increasing magnitudes of impact in the order: *D. octaedra*, *L. rubellus*, *L. terrestris* and all earthworm species combined, respectively ($P < 0.0001$, Table 2, Figure 3).

Nutrient content and availability

Percent carbon and nitrogen were measured only in mesocosms with all earthworm species and no earthworms. Percent carbon and nitrogen were higher when there were no earthworms than when all earthworm species were present for all plant species ($P \leq 0.04$, Table 2, Figure 4), except for nitrogen in the O horizon.

Nitrogen (NH_4 and NO_3) and phosphorous (PO_4) availability were significantly higher when all earthworm species were present compared with mesocosms with only one species or no earthworms. *L. rubellus* was the only single earthworm species that elevated nutrient availability above the no earthworm control and only in NO_3 ($P < 0.0001$, Table 2, Figure 5). Nitrogen availability was dominated by nitrate (NO_3) and *Carex* had

the highest nitrogen availability among plant species while *Acer* had the highest phosphorous availability (PO_4) ($P < 0.0001$, Table 2, Figure 5).

Plant mortality and biomass

Plant mortality was highest when all earthworm species were present compared with *L. rubellus*, *D. octaedra* or the no earthworm control, and intermediate when *L. terrestris* was present ($P = 0.0009$, Table 2, Figure 6). Among plant species, plant mortality was highest for *Carex pennsylvanica* followed by *Aralia racemosa*, *Aquilegia canadensis* and *Acer saccharum*, respectively ($P < 0.0001$, Table 2, Figure 6).

Mean individual plant total biomass did not change with the addition of earthworms compared to the no earthworm control (Table 2). However, mesocosm total plant biomass, above ground plant biomass and fine root, course root and total root biomass (grams dry weight) decreased significantly when all earthworm species were present compared with no earthworms and single earthworm species lead to intermediate biomass values ($P \leq 0.10$, Table 2, Figure 7). Among plant species, *Aralia* had the highest mesocosm biomass values followed by *Aquilegia*, *Carex* and *Acer*, respectively ($P < 0.0001$, Table 2, Figure 7).

Root to shoot biomass relationships

Plant root to shoot biomass relationships were affected by the addition of earthworms in two of the four plant species ($R^2 = 0.88$, $P < 0.0001$, Figure 8). The regression slopes of root to shoot biomass were not affected by earthworms in any plant

species. However, for *Aralia*, the regression intercept was significantly lower when no earthworms were present than when all earthworm species or *L. rubellus* were present. For *Aquilegia*, the regression intercepts were significantly higher when no earthworms or *D. octaedra* were present than when all earthworm species or *L. terrestris* were present. For *Carex pennsylvanica* and *Acer saccharum*, root to shoot biomass relationships were not affected by earthworms.

Earthworm survival

L. rubellus survival was higher in the presence of *Acer* compared to no plants present and intermediate when the other plant species were present ($P < 0.0001$, Table 2, Figure 9). Earthworm survival when all species were combined was higher in the presence of *Acer* and *Aquilegia* compared to no plants present and intermediate when *Carex* and *Aralia* were present. The presence of plants did not affect survival of *D. octaedra* or *L. terrestris*.

Discussion and Conclusions

Earthworm effects on forest floor removal, soil characteristic and plant species

Although *D. octaedra* significantly decreased O horizon thickness and increased A horizon thickness (Figure 3), it did not significantly affect any other property. While *D. octaedra* may have minimal short-term effects on forest soil structure, it does not appear

to affect plant mortality or growth (Chapters 3 and 4, Gundale 2002). However, changes in microbial communities caused by *Dendrobaena* may have important long-term effects in forests (McLean and Parkinson 2000, Scheu and Parkinson 1994).

In contrast with my hypothesis that *L. rubellus* would have the greatest effects of all earthworm species it significantly increased forest floor mass loss (Figure 2), decreased O horizon thickness and increased A horizon thickness (Figure 3), but did not significantly affect any other property. Given the magnitude of forest floor mass removed by *L. rubellus* as compared with the other earthworm species, greater impacts to plant species might have been expected. In field studies where *L. rubellus* was the most strongly related to changes in soil characteristics and plant species composition (Chapters 3 and 4), *Dendrobaena* was always present as well. There may be some interactive effects under field conditions when both species are present that is missing in this study.

As hypothesized, the impacts of *L. terrestris* were relatively moderate, especially given its nearly 3-fold higher biomass compared to *L. rubellus* and *D. octaedra*. *L. terrestris* significantly increased forest floor mass loss (Figure 2), decreased O horizon thickness and increased A horizon thickness (Figure 3). *L. terrestris* also increased the root to shoot ratio of *Aquilegia canadensis* (Figures 8), but did not affect any other property.

All properties were the most affected when all earthworm species were present, despite the fact that the total biomass of earthworms was not always greater compared with the single species (Figure 9). O horizon thickness declined and A horizon thickness increased significantly more than when any single species was present (Figures 2 and 3).

Total carbon and nitrogen content declined (Figures 4). Nutrient availability and plant mortality were higher than when single earthworm species were present (Figures 5 and 6). Mesocosm total plant, total fine root and total root biomass were significantly lower when all earthworm species were present than when no earthworms were present (Figure 7). Root to shoot ratios of *Aralia* and *Aquilegia* were significantly different when all earthworm species were present compared with no earthworms (Figure 8). For many properties, the presence of all earthworm species had larger effects than *L. terrestris* alone even when total earthworm biomass did not increase (Figure 9). The magnitude of responses when all earthworm species were present compared with single species suggests that synergistic effects exist when multiple species with differing feeding behaviors are present.

Plant species responses to earthworms

In field studies documenting changes in native plant communities following European earthworm invasion, increased plant mortality is inferred from the disappearance or decline of populations of many native understory plant species (Chapter 4, Gundale 2002, Kourtev et al. 1999). This study confirms that mortality of mature native understory plants increases as a result of earthworm invasion into previously worm-free conditions.

Although *Carex* had the highest mortality overall, the proportional increase in mortality when a mix of earthworm species were present was greater in *Aralia*, *Aquilegia* and *Acer* (Figure 6), suggesting that *Carex* is less affected by earthworm

invasion. This conclusion is supported by the relative success of *Carex* following earthworm invasions in field sites as compared to most other native understory plant species (Chapter 4). Although the increase in *Aralia* mortality was not significant, the true potential of plant mortality for this species under field conditions may not be fully represented by the mesocosm results. Except in the presence of *D. octaedra*, the root crowns of most *Aralia* plants were completely exposed when any other earthworm species was present and plants appeared dead (personal observation), but under the protective conditions of the greenhouse, re-establishment of the root system occurred and rapid plant growth followed. It is easy to see how the same plants may not have survived under less protected field conditions.

Increased plant mortality following earthworm invasion can result in lower productivity even if individual plant biomass is unaffected or even increased by earthworm invasion. Decreased plant biomass in response to earthworms in this study largely reflected increased mortality because mean individual plant biomass was not affected by earthworms. Other pot experiments have yielded contradictory results of earthworm effects on plant productivity (James and Seastedt 1986, Alpei et al. 1996, Blair et al. 1995) and there are few studies of the effects of earthworm introductions on plant production under field conditions. However, while germination and seedling survival of native North American hardwood tree species were lower in areas with large earthworm populations, those of an exotic shrub species increased (Kostel-Hughes 1995). Increases in native plant species mortality may be an important cause of changes in plant

populations associated with earthworm invasion, even if growth of individual plants increases following an invasion.

Mesocosm total fine root biomass declined in the presence of each earthworm species (Figure 7). However, the root to shoot ratios remained unchanged for *Carex* and *Acer*, increased for *Aralia* and decreased for *Aquilegia* in the presence of all earthworm species and *L. rubellus* or *L. terrestris* (Figure 8). Total fine root biomass sometimes increases following earthworm introductions in agricultural systems (Hendrix 1998, Syers and Springett 1984), however, fine root biomass has also declined following earthworm introductions to systems of native plant species (Bohlen et al. 2004^a, Alpehi et al. 1996). James and Seastedt (1986) found that the root to shoot ratio in a native grass species (*Andropogon gerardii*) increased following earthworm introductions. Differential responses among plant species may lead to different levels of success following earthworm invasions of native ecosystems and ultimately to changes in community structure and composition.

Despite increases in nutrient availability with earthworm introductions in this study, total plant biomass declined. It is widely assumed that increases in nutrient availability resulting from earthworm activity would increase plant productivity (Edwards et al. 1995, Lee 1985). However, virtually all research related to plant productivity response to earthworm introductions has been conducted with agricultural plants and these results may not apply to native plant species. Further, the observed increase in nutrient availability may be short term, immediately following removal of the forest floor (Lavelle et al. 1992). Although, nitrogen and phosphorous availability

increased with the introduction of earthworms in this study, percent soil carbon and nitrogen declined due to rapid mineralization of the forest floor material. This is consistent with other studies conducted primarily in agricultural systems (Subler 1997, Parkin 1994, Marinissen and deRuiter 1993, Scheu 1987) and may not capture the long term decline in nutrient availability seen in field studies following earthworm invasions of forests in the Great Lakes region (Chapter 3, Bohlen et al. 2004^b).

Effects of plant species on earthworms

Plant species affected survival of *L. rubellus* (Figure 9) and thus levels of impacts related to this species. *L. rubellus* survival was the lowest when plants were not present and subsequently the rate of forest floor mass loss was much lower than when any other plant species was present (Figure 2). *L. rubellus* has been reported to preferentially feed on soil organisms associated with the plant rhizosphere (Pierce 1978) and the lack of such habitat without plants is a possible contributor to decreased survival of this species as compared to *D. octaedra* and *L. terrestris*.

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Table 1. Mesocosm experimental design and the number of replicates for each treatment combination. The number of earthworms of each species included is in parenthesis.

Plant species ^a	<u>Earthworm treatments</u>				All species
	no worm control	<i>L. terrestris</i> (3)	<i>L. rubellus</i> (15)	<i>D. octaedra</i> (40)	
no plant control	7	7	7	7	7
<i>Aralia racemosa</i>	7	7	7	7	7
<i>Acer saccharum</i>	14	14	14	14	14
<i>Aquilegia canadensis</i>	14	14	14	14	14
<i>Carex pennsylvanica</i>	14	14	14	14	14

^a six individual plants in each mesocosm

Table 2. Summary Statistics: two-way ANOVA of plant and soil parameters among earthworm treatments and plant species.

Plant / Soil Parameters	<u>ANOVA Parameters</u>			
	Full Model ^a	earthworm treatment	plant species	plant * worm
Forest floor mass loss	$R^2 = 0.27$	***	**	**
O horizon thickness	$R^2 = 0.49$	***	**	ns
A horizon thickness	$R^2 = 0.46$	***	ns	ns
% C – O and A horizons	$R^2 \geq 0.26$	**	ns	ns
% N – A horizon	$R^2 = 0.45$	***	*	ns
N availability (NH ₄)	$R^2 = 0.26$	***	***	ns
N availability (NO ₃)	$R^2 = 0.36$	***	***	ns
P availability (PO ₄)	$R^2 = 0.19$	**	***	ns
% Plant mortality	$R^2 = 0.63$	***	***	ns
Mean plant total biomass ^b	$R^2 = 0.78$	ns	***	ns
Mesocosm total plant biomass ^c	$R^2 = 0.78$	*	***	ns
Mesocosm above plant biomass ^c	$R^2 = 0.77$	*	***	ns
Mesocosm total root biomass ^c	$R^2 = 0.77$	**	***	ns
Mesocosm coarse root biomass ^c	$R^2 = 0.78$	*	***	ns

Mesocosm fine root biomass ^c	$R^2 = 0.50$	**	***	ns
Final earthworm numbers	$R^2 = 0.54$	***	***	**
Final earthworm biomass	$R^2 = 0.78$	***	***	**

In each column, “ns” indicates non-significant main or interaction effects; symbols *, ** and *** indicate significant effects where $P \leq 0.10$, $P \leq 0.05$ and $P \leq 0.001$, respectively.

^a all models $P \leq 0.001$

^b mean of all surviving plants for each mesocosm

^c sum of all surviving plants for each mesocosm

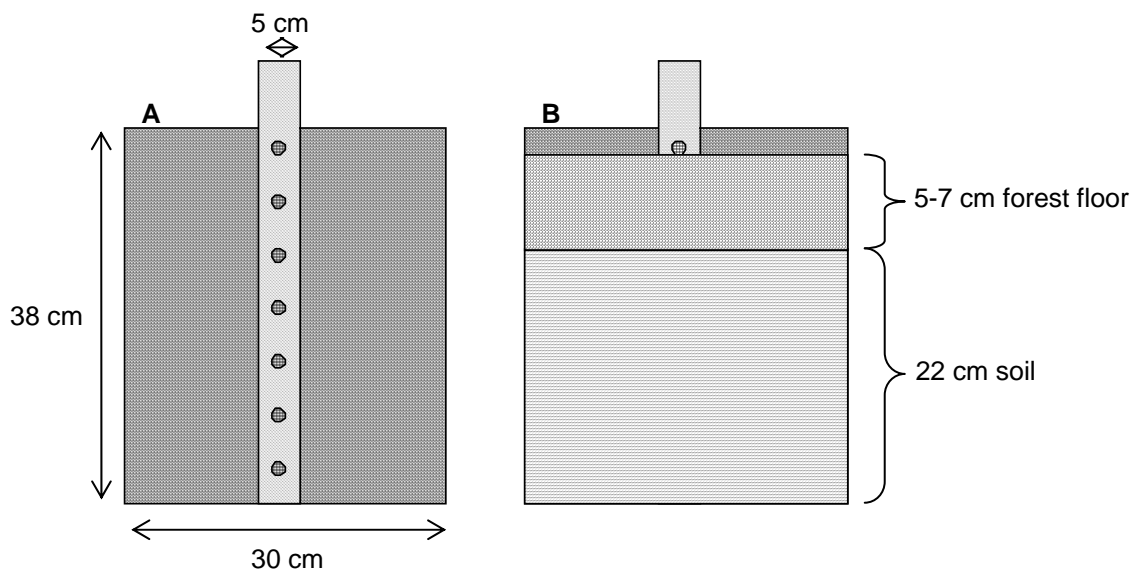


Figure 1. Mesocosm design in cross section. (A) The mesocosm containers were plastic buckets with a PVC ventilation tube in the center. The ventilation tube had 7 – 1 cm holes, one every 5 cm along its length and each hole was covered with fine fiberglass screen. The inside of the bucket and outside of the ventilation tube were sprayed with polyurethane and coated in coarse sand (dark and light stippling). (B) Mesocosms were filled with homogenized, worm-free soil (diagonal hatch marks) and a intact layer of worm-free forest floor (thatching).

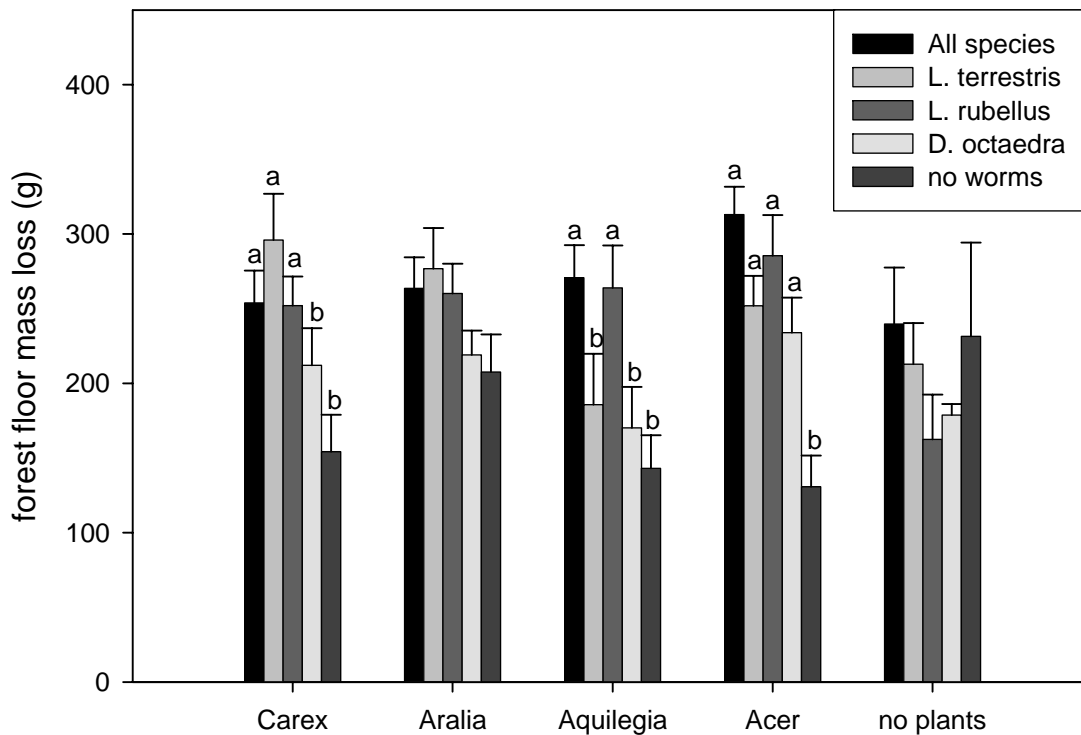


Figure 2. Mean forest floor mass loss (grams dry weight) for each earthworm treatment and plant species. In individual paired comparisons among earthworm treatments within each plant species, significant differences are indicated by different letters ($P \leq 0.05$).

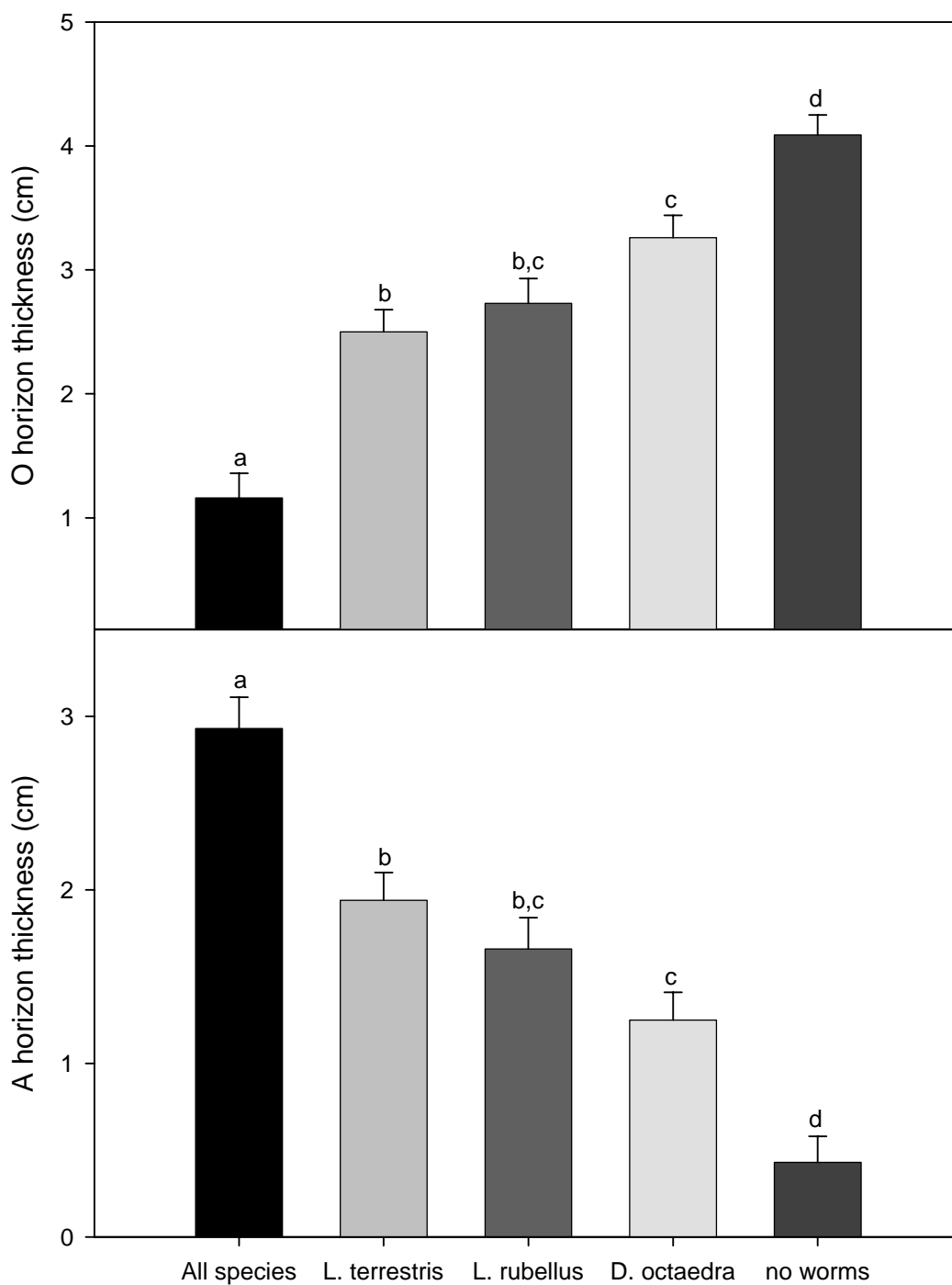


Figure 3. Mean O horizon and A horizon thickness (cm) among earthworm treatments across all plant species. In least squared mean paired comparisons, significant differences are indicated by different letters ($P \leq 0.05$).

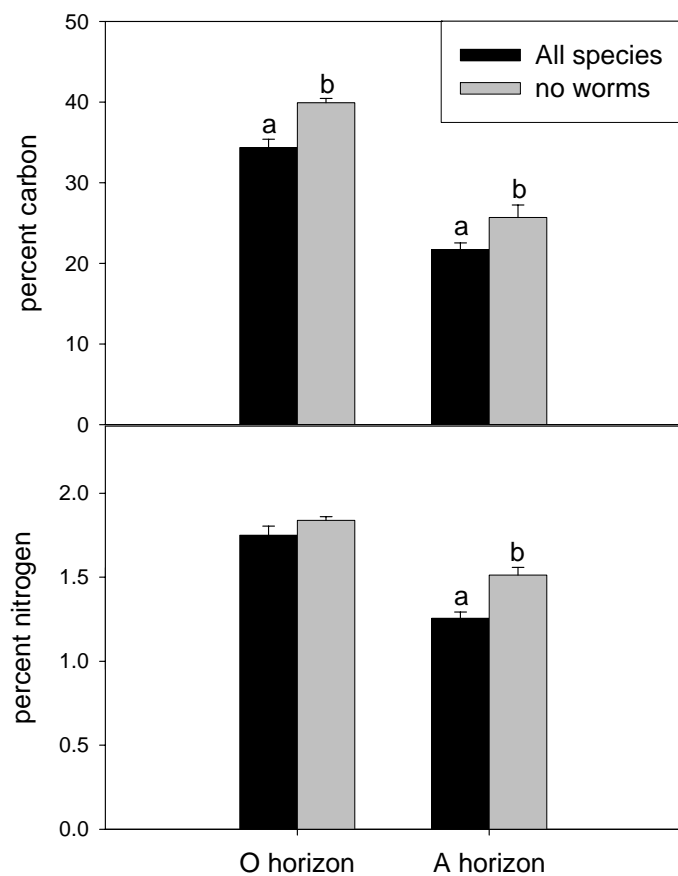


Figure 4. Mean O and A horizon percent carbon and nitrogen for the all species earthworm treatment (black bars) and the no earthworm control (grey bars) across all plant species. In least squared mean paired comparisons, significant differences are indicated different letters ($P \leq 0.05$).

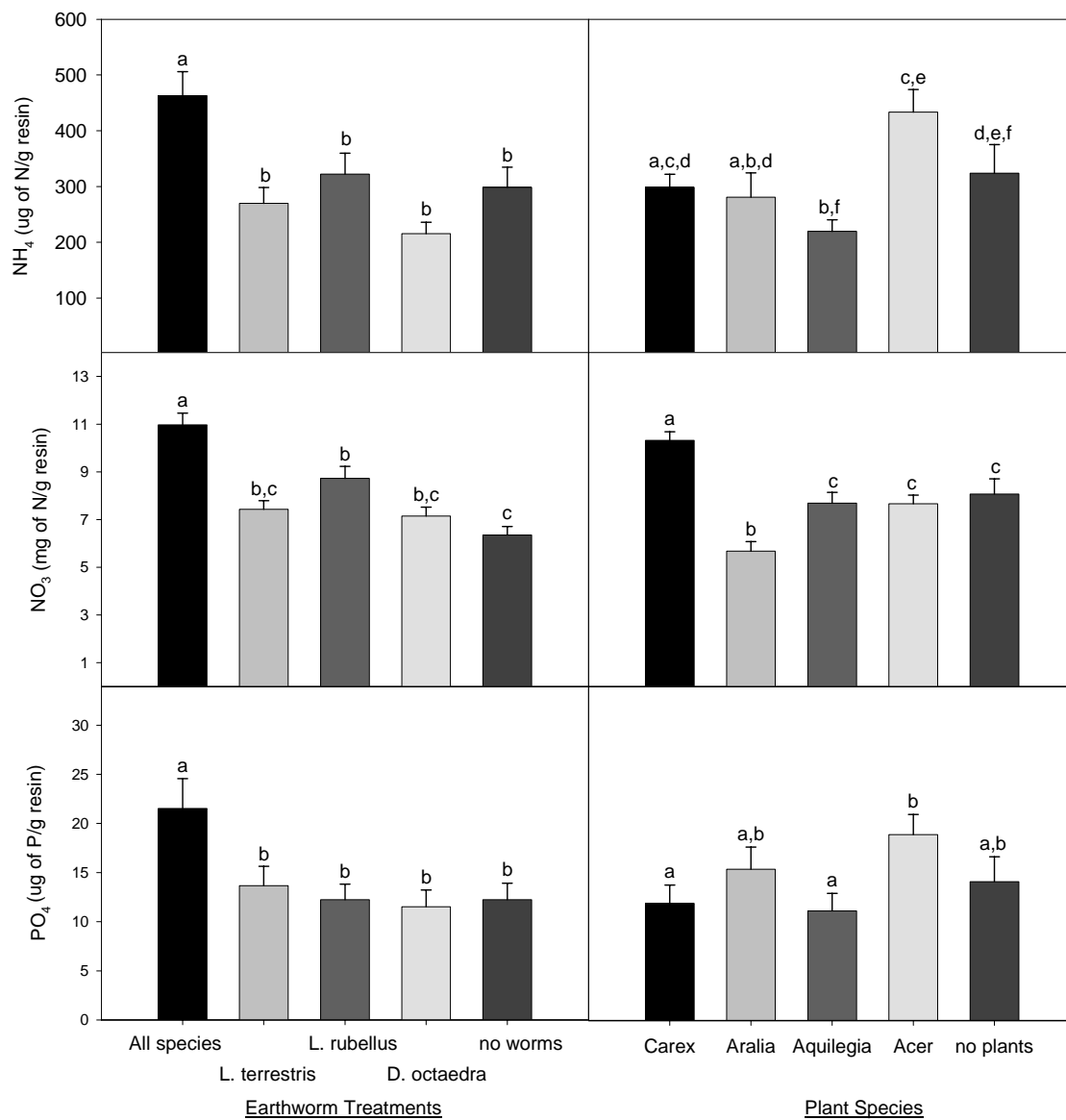


Figure 5. Mean nitrogen and phosphorous availability among earthworms and plant species. Least squared mean paired comparisons are of log transformed data and significant differences among earthworms or plant species are indicated by different letters ($P \leq 0.05$).

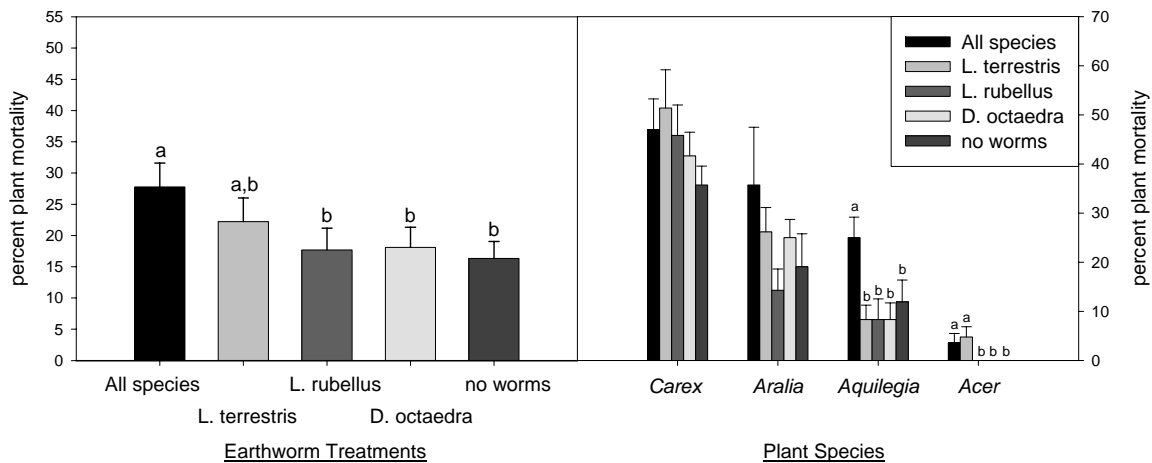


Figure 6. Mean percent plant mortality among earthworms and plant species. Least squared mean paired comparisons are of square root transformed data and significant differences among earthworms are indicated by different letters ($P \leq 0.05$).

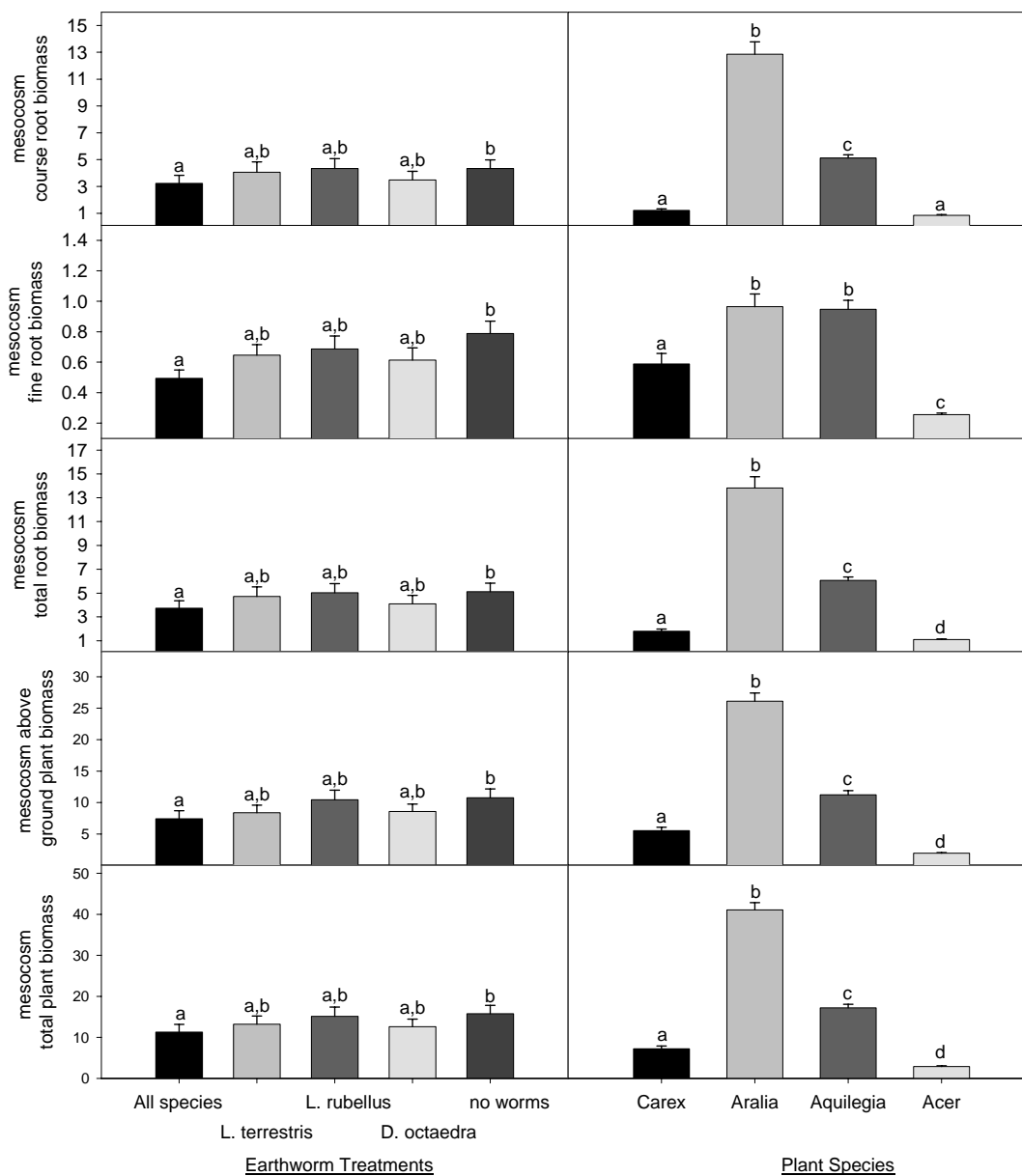


Figure 7. Mean mesocosm total course root biomass, total fine root biomass, total root biomass, total above ground plant biomass and total plant biomass (grams dry weight) among earthworms and plant species. Least squared mean paired comparisons are of log transformed data and significant differences among earthworms or plant species are indicated by different letters ($P \leq 0.05$).

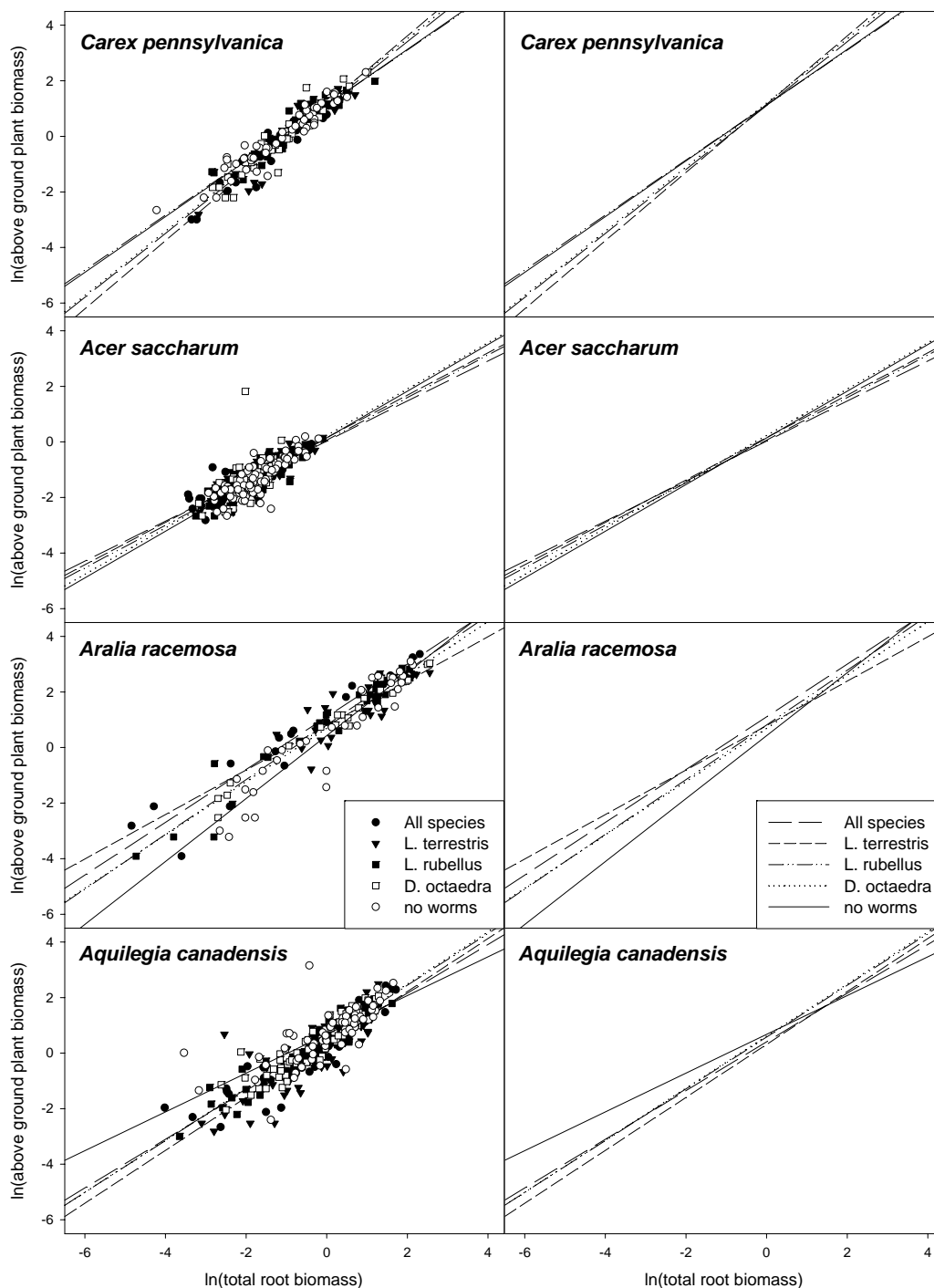


Figure 8. Linear regressions of root to shoot biomass among earthworm treatments for each plant species. Graphs on the left show both data points and regression lines. The graphs on the right show only the regression lines so differences in the regression intercepts are visible [$\ln(\text{total root biomass})$ equals zero].

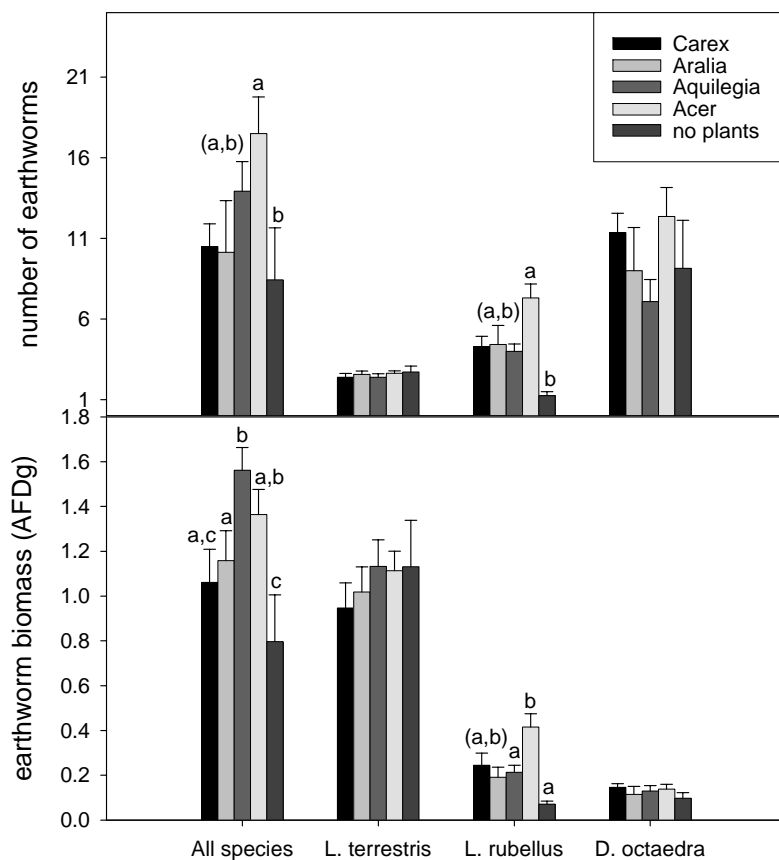


Figure 9. Mean earthworm numbers and ash-free dry biomass (g) among plant species for each earthworm treatment. In individual paired comparisons among plant species within earthworm treatments, significant differences are indicated by different letters ($P \leq 0.05$). Letters in parenthesis correspond to all bars below them.