Exotic Earthworms in Minnesota Hardwood Forests:

An investigation of earthworm distribution, understory plant communities, and forest floor dynamics in northern hardwood forests.

Final Report for proposal #100005905

November 26, 2002

Lee E. Frelich
Center for Hardwood Ecology, University of Minnesota

Andrew R. Holdsworth
Conservation Biology Graduate Program, University of Minnesota

Department of Forest Resources
University of Minnesota
1530 Cleveland Ave. N.
Saint Paul, MN 55108

Introduction

For over 12,000 years Minnesota's forests developed without the influence of earthworms. If North American earthworm species ever inhabited Minnesota, they were extirpated during the last glaciation (Figure 1) (James 1990). Without earthworms, forests' fallen leaves accumulated and developed a thick duff layer that provided an excellent rooting zone for herbs and tree seedlings. Currently, over fifteen species of European earthworms inhabit Minnesota (Reynolds et al. 2002). Over the last 150 years European earthworms were likely accidentally and intentionally introduced with the importation of plant material and soils from Europe and the use of worms as fishing bait across the region. Ongoing studies suggest that invasive European earthworms have a notable effect on forest understory plant diversity and composition (Hale, in preparation), nutrient cycling (Groffman et al. 2001), and soil properties (Darwin 1881, Langmaid 1964, Satchell 1967, Wallwork 1983, Tomlin et al. 1995). Since the 1980's forest managers on the Chippewa National Forest have been concerned about the loss of understory plant cover and diversity in areas with high earthworm populations. Exotic earthworms are considered a major factor in the population decline of the state threatened goblin fern *(Botrychium mormo)*.

Figure 1: Southern limit of Wisconsinian Glaciation. Any North American earthworm species living north of this limit were extirpated by glaciation. Native species have been very slow to recolonize this region. (Source: James 1995)

At least seven different European earthworm species inhabit Minnesota's maple-basswood forests. Earthworm species are often divided into three broad ecological groups: epigeic, endogeic, and anecic species.

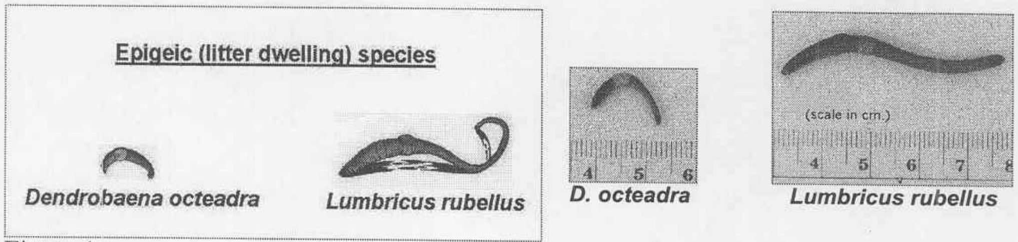


Figure 1a.

Epigeic species live in the leaf litter, above the mineral soil. They are reddish brown in color and small in size, usually less than 3 inches long when mature.

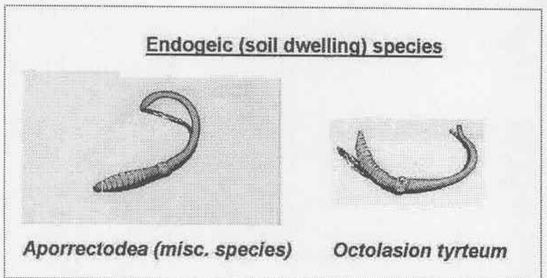


Figure 1b.

Endogeic species live in the upper layers of the soil. They mostly consume highly decayed humic organic matter in the mineral soil. They are easily distinguished from epigeic species by their color: endogeics are a light grey. They are also large in size, usually 3 to 5 inches long when mature.

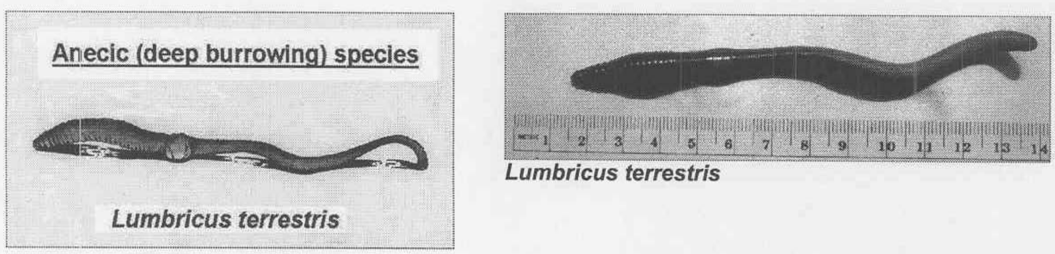


Figure 1c.

Anecic species burrow 1-2 meters deep into the soil but feed on leaf litter at the surface. Night crawlers are an example of an anecic species. They are reddish brown in color and generally much larger than either of the other two groups, usually 5 to 8 inches long when mature.

We know very little about the distribution of different earthworm species in Minnesota forests and the extent of impacts they may be having on forest plant communities. We also do not know why some forests seem to lose their forest floors, herbs and tree seedlings in response to earthworm invasion, while others do not. There are numerous factors that could influence how much understory plant communities are affected by the invasion of European earthworms. These factors include litter quality and quantity in the stand (directly related to forest composition and productivity), species and biomass of earthworms present, levels of deer herbivory, soil type, time since earthworms invaded, climate, and disturbance history. In a given stand, litter inputs are a major limiting factor for litter consuming earthworm populations. Litter in a more productive forest with greater litter fall rates is more likely to produce "surplus" litter that surpasses the earthworm community's ability to consume all litter each year. If this litter is of lower quality (higher carbon:nitrogen, lignin:nitrogen ratios, etc.), and less preferred by earthworms, then the forest floor is more likely to persist year round. If the major litter consuming earthworm, *L. terrestris* is absent from the stand, the forest floor is even more likely to persist year round.

This report describes two studies of earthworms in Minnesota hardwood forests.

1) A regional survey of the distribution of earthworms in lakeside mature maple-basswood forests and how this distribution relates to understory plant diversity and cover.
2) An experimental study on the effect of leaf litter type and quantity on the rate of forest floor decay in mature maple-basswood stands with different earthworm communities.

Methods

Regional Survey

We conducted our regional survey in the Chippewa National Forest because there is a full range of areas with varying densities of earthworms, forest records are readily available, and an intensive study of exotic earthworm invasion fronts is present there. Using the Chippewa National Forest stand database and GIS system, we chose 20 northern hardwood stands that met the following criteria: classified as "upland-mesic hardwoods", >60 years old, adjacent to lakes ≥10 acres, similar soil types (all on either the Guthrie or Blackduck Till Plains), no timber management in the last ~50 years (based on FS records and inspection of aerial photos). We chose stands with a range of earthworm introduction probabilities.

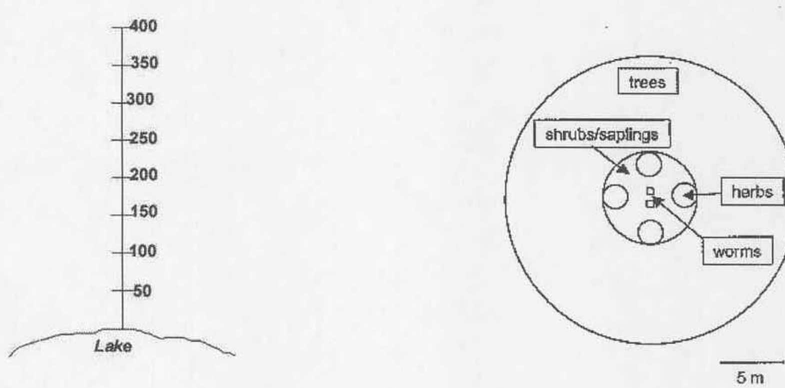
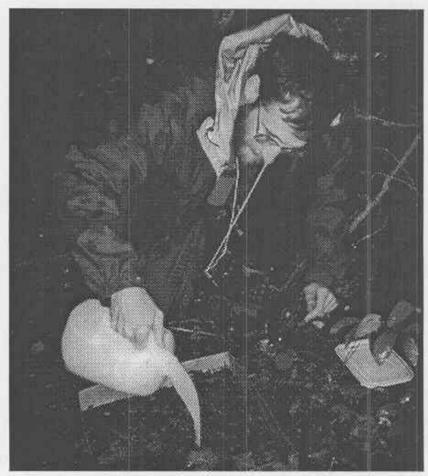


Figure 2: Transect layout (left) and sample plot layout (right) at each of the 8 transect locations.

Stands believed to have a high introduction probability were adjacent to a boat ramp and/or a cabin/resort with road access. Stands presumed to have a low introduction probability were as distant from the above developments as possible. Stands with medium introduction probability fell in between these extremes. Before surveying each stand, we mapped transects approximately parallel to the lakeshore using 7.5 min topographic maps and aerial photographs to avoid large canopy gaps. We conducted the field research in August of 2001 and 2002. Each transect consisted of 6-8 nested plots spaced at 50 meter intervals. At each plot (Figure 1) we recorded species and percent cover of all understory vegetation less than 50 cm high in four 1 m radius plots, shrub species and cover and sapling species and density in a 3.5 m radius plot, tree species by 5 cm diameter classes in a 10 m radius plot, and canopy cover. We scored deer browse on all understory vegetation, shrubs, and saplings. In the center of the plot we sampled earthworms by hand sorting all soil in a 0.125 m^2 area to a depth of 30 cm. We also used a liquid mustard extraction (Lawrence and Bowers 2002) over a 0.125 m^2 area. To estimate densities of the anecic earthworm, *Lumbricus terrestris*, we used midden counts in each of the 0.125 m^2 areas. All earthworms were collected for measurement and species identification in the lab. Soil profiles were described to a depth of 30 cm and samples retrieved from each major soil horizon.

Earthworm sampling: hand sorting (left) and liquid mustard extraction (right).

Litter Experiment

In the fall of 2001 we installed a leaf litter experiment at two DNR Scientific and Natural Areas (SNA) with high densities of nightcrawlers *(L. terrestris)* and two sites with low densities of nightcrawlers (Table 1). This suite of sites was chosen because the nightcrawler consumes more leaf litter than any other species in Minnesota forests and may be one of the species affecting Minnesota hardwood forests the most. After raking away the pre-existing litter layer, we placed known quantities of different litter types (Table 1) under 0.5 m^2 rectangular wooden frames with wire mesh tops. For the mixed litter types we placed 1/2 X, 1X and 2X annual litter fall in the frames. In the summer of 2002 we sampled remaining biomass of the experimental litter layers and % of bare ground in June and July. We only report the results of the medium litter quantity (1X annual litter fall) treatments here.

Site 1: High Density of L. terrestris Site: Wood-Rill SNA	***Site 2: Low Density of L. terrestris*** Site: Mary Schmidt Crawford Woods SNA
5 litter types (5 replicates each): *1) Tilia Americana* (American Basswood) *2) Acer saccharum* (Sugar Maple) *3) Quercus rubra* (Red Oak) 4) Even mix (equal portions of *1,2,3)* 5) Native Mix (mix of litter present at site)	5 litter types (5 replicates each): Same
3 litter quantities of Even Mix and Native Mix (5 reps.) 1. low (0.5X annual litterfall) *2.* medium (1X annual litterfall) *3.* high *(2X* annual litterfall)	3 litter quantities of Even Mix and Native Mix (5 reps.) Same
2 Controls 1. Frame with wire mesh (placed over undisturbed forest floor) *2.* Frame without wire mesh	*2* Controls Same
Replicate site: Wolsfeld Woods SNA - 5 reps. of Even Mix, medium quantity only	Replicate Site: Partch Woods SNA - 5 reps. of Even Mix, medium quantity only

Table 1: Litter Experiment Design

Results

Regional Survey

This report focuses on the main elements of interest in this study, the relationships between earthworm introduction probability, earthworm presence, and plant diversity and cover. We studied 155 plots in a total of 20 mature hardwood stands found along the shores of 11 lakes in the Chippewa National Forests. Only 3% (5 of 155) of the plots were completely worm free so we lumped this class with the plots with one ecological group. This class only includes the epigeic earthworm, *Dendrobaena octaedra* (Figure 1a), which is only -3 cm in length and has minor effects on the forest floor. Twenty-seven percent of the plots had only 0 or 1 ecological groups (*D. octaedra* only). Over half (57%) of the plots had two ecological groups of earthworms present. This nearly always includes *Lumbricus rubellus* and one or more endogeic species. Finally, only 16% of the plots had all three ecological groups, generally the above species with the addition of the nightcrawler, *Lumbricus terrestris* (Figures).

Probability of earthworm introduction and the presence of earthworm ecological groups

There was highly significant correlation between the probability of earthworm introduction and the number of earthworm ecological groups present (Chi-square test, N=155, 4 df, $p<0.0001$). Plots with 1 or fewer ecological groups were almost exclusively (98% of plots) found in low introduction probability areas (without cabins, resorts, or boat landings). However, many low probability plots did have 2 and 3 ecological groups

[1] A map of lakes and transect locations is available upon request.

(45 and 7%, respectively). We found that over half of the plots with all 3 ecological groups existed in areas with nearby cabins, resorts, and/or boat landings (Figure 3).

Contingency Table

Probability of Introduction	Count / Total % (# Ecological Groups)	0-1	2	3	
	1	41 26.45	39 25.16	6 3.87	86 55.48
	2	1 0.65	23 14.84	5 3.23	29 18.71
	3	0 0.00	27 17.42	13 8.39	40 25.81
		42 27.10	89 57.42	24 15.48	155

Figure 3: Relationship between probability of earthworm introduction and the # of earthworm ecological groups present at a plot. Only 0-1 ecological groups are most likely to be found at sites with low introduction probability (1) while 3 ecological groups are most likely to be found at sites with high introduction probability (3).

Relationships between earthworm presence and plant diversity and cover

We focused our analysis on the 8 most frequently encountered species (found in at least 56 of 155 plots) that had an average percent cover greater than 1% across all plots. These species are the following: *Acer saccharum, Uvularia grandiflora, Osmorhiza claytoni, Aralia nudicaulis, Carex pennsylvanica, Asarum canadense, Aster macrophyllous and Laportea canadensis.* Anecdotal reports suggest other species that may be especially sensitive to earthworm invasion: *Smilacina racemosa, Aralia racemosa, Caullophyllum Thalictroides* and *Trillium spp. Arisaema triphyllum* is observed to be more abundant in earthworm invaded stands. We evaluated these potential "indicator" species as well.

Scientific Name	Common Name
Acer saccharum	Sugar Maple
Aralia nudicaulis	Wild Sarsparilla
Aralia racemosa	Spikenard
Arisaema triphyllum	Jack-in-the-Pulpit
Asarum canadense	Wild Ginger
Aster macrophyllous	Large-leaved Aster
Carex pennsylvanica	Pennsylvania sedge
Caullophyllum Thalictroides	Blue Cohosh
Laportea canadensis	Wood Nettle
Smilacina racemosa	False Solomon's Seal
Trillium spp.	Trillium species
Uvularia grandiflora	Large-flowered Bellwort

On average the plots with the all 3 ecological groups of earthworms had 14% fewer species than plots with 0-2 ecological groups. However, there was no significant difference (Figure 4). Plots with 3 ecological groups had 18% less total cover of herbs and seedlings relative to plots with 0-2 ecological groups (Figure 5).

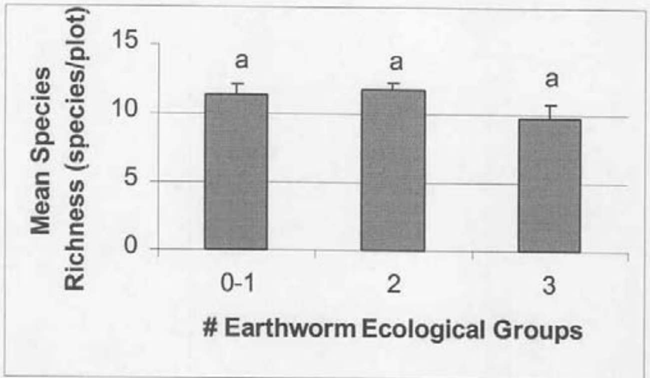


Figure 4: Mean species richness in all plots (N=155) for different numbers of earthworm ecological groups. Data are means + 1 SE. There is no significant difference (Tukey-Kramer HSD at p = 0.05.)

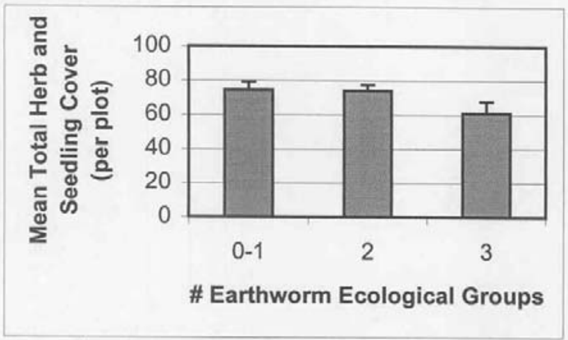
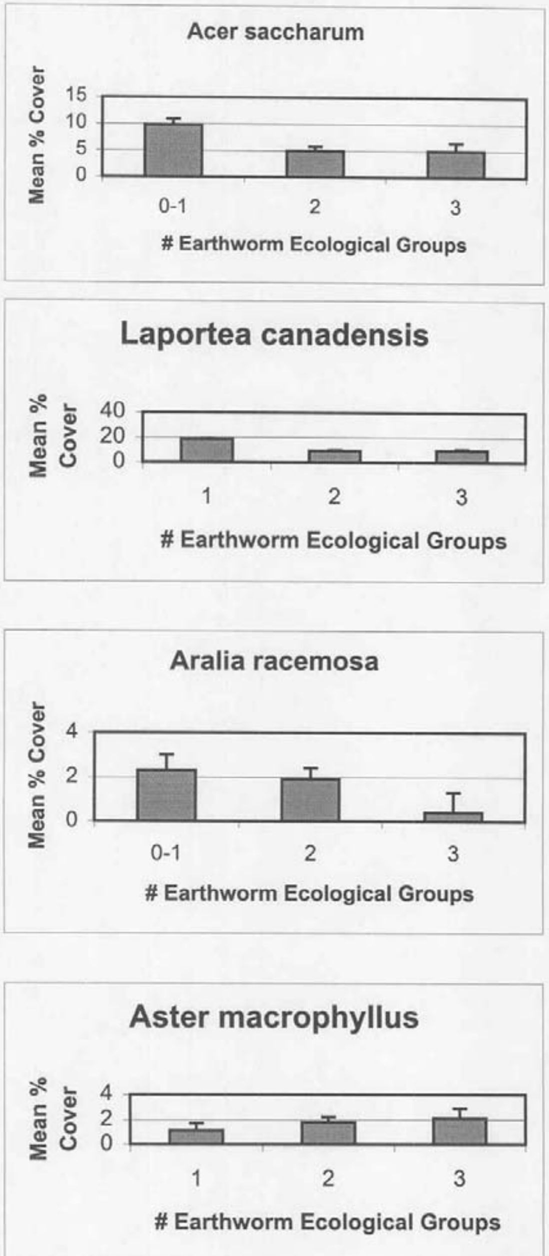
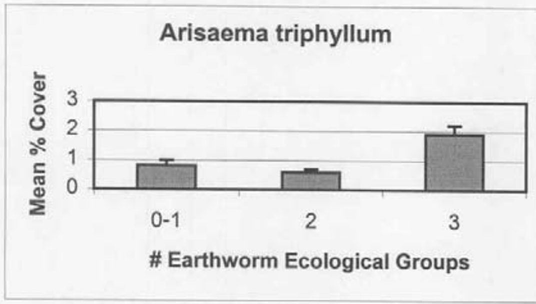
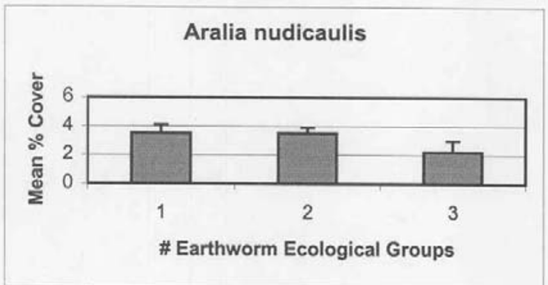


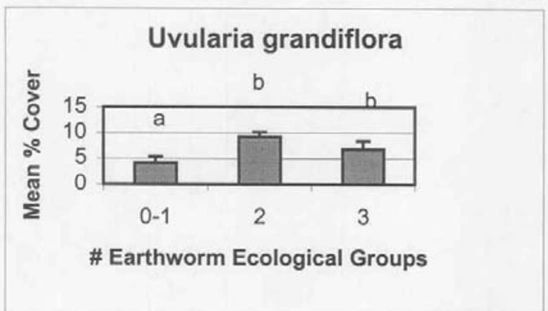
Figure 5: Mean total herb and seedling cover per plot (N=155) for different numbers of earthworm ecological groups. Data are means + 1 SE.

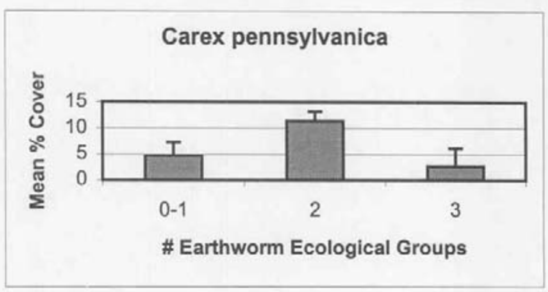
Figure 6: Mean % cover (across all plots, N=155) for selected understory species for different numbers of earthworm ecological groups. Data are means + 1 SE.

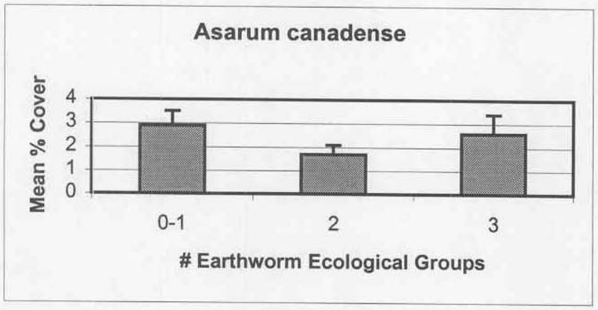


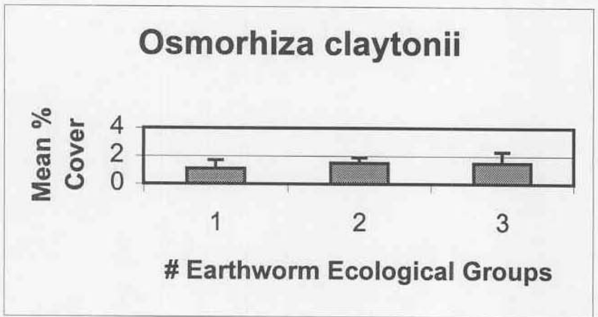

Arisaema triphyllum
Mean % Cover
3
2
1
0
0-1
2
3
Earthworm Ecological Groups


Aralia nudicaulis
Mean % Cover
6
4
2
0
1
2
3
Earthworm Ecological Groups


Uvularia grandiflora
Mean % Cover
15
10
5
0
a
b
b
0-1
2
3
Earthworm Ecological Groups


Carex pennsylvanica
Mean % Cover
15
10
5
0
0-1
2
3
Earthworm Ecological Groups





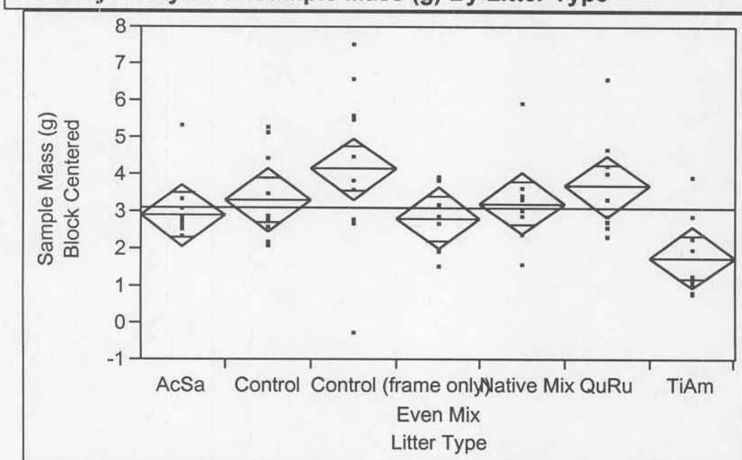
The percent cover of the 8 most common species and the six potential "indicator" species for different numbers of earthworm ecological groups depended greatly on the species. *Acer saccharum, Laportea canadensis, Aralia racemosa, Arisaema tryiphyllum* and *Carex pennsylvanica* showed the most notable differences between plots with different numbers of earthworm ecological groups (Figure 6). *Acer saccharum* and *Laportea canadensis* growing in plots with few earthworms had twice as much cover as in plots with 2 or more ecological groups. *Aralia racemosa* also had more cover in plots with fewer earthworm ecological groups, however the absolute difference is small. In contrast to the previous species, *Arisaema triphyllum* had twice as much cover in plots with 3 ecological groups than plots with 0-2 groups (Figure 6). *Carex pennsylvanica* had over twice as much cover in plots with two ecological groups of earthworms than with 0-1 or 3. There were no notable differences for the other species, *Aster macrophyllus, Aralia nudicaulis, Uvularia grandiflora, Asarum canadense, Osmorhiza claytonii* (Figure 6), and *Trillium spp.*

Litter Experiment

The local site conditions and litter type significantly affected the amount of leaf litter remaining in June and July 2002 (Figure 8 and 9). Basswood litter (TiAm) decomposed the fastest such that there were negligible amounts left in July at Wood-Rill Scientific and Natural Area. In fact, basswood plots showed over 50% bare ground in June and ~90% bare ground in July versus 0 and 10%, respectively at Mary Schmidt Crawford Woods. Red Oak decomposed the slowest, but was not notably different from the other litter types. Both sugar maple and red oak plots showed approximately 10% bare ground in both June and July at Wood-Rill and approximately 1% bare ground at Mary Schmidt. The even mix of all three litter types did not decompose much faster than the control. However, as time passes, the mixes become more and more oak dominated as the basswood then maple disappears. Results were comparable at the Wolsfeld Woods and Partch Woods SNA sites.

Litter Experiment at Mary Schmidt Crawford Woods SNA. In the fall of 2001 we placed a known quantity of different leaf litter types underneath each 0.5 m^2 frame.

Oneway Analysis of Sample Mass (g) By Litter Type

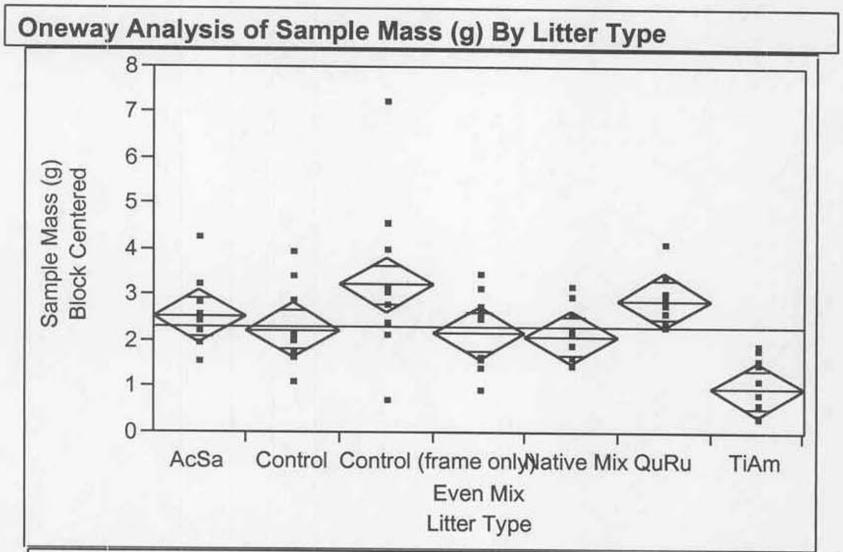


Oneway Anova

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob >
Litter Type	6	33.83579	5.6393	3.2712	0.0074
Site	1	29.21212	29.2121	16.9453	0.0001
Error	62	106.88200	1.7239		
C. Total	69	169.92991			

Figure 8: ANOVA of litter mass in June 2002. Litter types: AcSa (sugar maple), Control (with wire mesh like others), Control (without wire mesh), Even Mix (1/3 maple, oak, basswood), Native Mix (mix found on ground nearby), and TiAm (basswood). All masses were equal at start of experiment in 2001.



Oneway Anova

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob >
Litter Type	6	31.615274	5.26921	5.9890	<.0001
Site	1	3.648006	3.64801	4.1463	0.0460
Error	62	54.548954	0.87982		
C. Total	69	89.812234			

Figure 9: ANOVA of litter mass in July 2002.

The four sites have very different earthworm communities. Wood-Rill and Wolsfeld have *L. rubellus*, endogeic species and a very high density of *L. terrestris* (~32/m^2). Mary Schmidt Crawford Woods and Partch Woods have *L. rubellus*, endogeic species and a very low density of *L. terrestris* (~1/m^2).

Discussion

Our regional survey suggests that there are few areas of completely worm-free mature, northern hardwood forests left on the Chippewa National Forest. In fact we did not find one completely worm-free stand despite surveying stands in some of the most remote areas. This is in contrast to the Chequamegon National Forest where we did find some significant worm-free areas in two designated Wilderness areas. Nearly three-quarters of the Chippewa plots had two earthworm species that affect the forest floor the most, *Lumbricus rubellus* and *L. terrestris*. *L. rubellus* is most associated with the loss of the duff layer and reduced plant diversity during the early stages of earthworm invasion (C. Hale, personal communication). *L. terrestris* is especially associated with bare mineral soil because of the large quantity of leaf litter it pulls into its burrows. In areas of steeper topography, this bare ground can cause notable erosion such as we have observed at Wood-Rill SNA. Still, less than a quarter of the plots had a species (*D. octaedra*) that seems to have negligible effects on the forest floor and understory diversity and cover. Future analysis will explore the likelihood that these forest stands will not be invaded by the earthworm species that affect the forest floor more.

Slightly lower plant species richness and understory cover were associated with plots with all three earthworm ecological groups present. This reduction is smaller than anecdotal reports have suggested. If understory richness and cover did decrease during initial earthworm invasion in these stands, our data may suggest that enough time has passed that understory plant diversity and cover recovered. This would especially be possible if there were abundant propagule sources and relatively low deer populations. We did not observe notable differences in deer browse levels in the stands we surveyed.

There are differences in the plant composition between areas with different numbers of earthworm ecological groups. The 50% decline in sugar maple seedlings from plots with 0-1 groups to 2 or 3 groups is notable. How much this will affect forest regeneration is still unknown and should be investigated further. In small remnants of the Big Woods, such as Wood-Rill SNA and Taylor's Woods (Hennepin County) where L. *rubellus* and *L. terrestris* densities are very high, sugar maple seedling densities are so low (Frelich and Holdsworth, personal observations) that maple recruitment could be especially limited. Besides sugar maple, this study suggests that *Aralia racemosa,* and *Aralia nudicaula's* may be sensitive to high earthworm densities. The study also supported anecdotal evidence that *Arisaema triphyllum* cover increases in areas with high earthworm densities. However, the patterns for other potential "indicator species" like *Uvularia grandiflora, Asarum canadense,* and *Osmorhiza claytonii* were not as clear in this study. Our ongoing research will further explore individual species responses to exotic earthworms.

The litter experiment showed the dramatic differences between the decomposition rate of basswood and male and oak leaf litter and the notable differences between the sites with high and low L. *terrestris* densities. Nightcrawlers seem to prefer basswood leaves to oak and maple. After leaf fall when all litter types are available, basswood leaves are clustered around the openings of nightcrawler burrows and preferentially pulled down into the burrows (A. Holdsworth, personal observation). This suggests that the **forest floor of forest** patches with high densities of basswood trees could be more heavily impacted by nightcrawler invasion than patches that have a minor basswood component. Future work will examine the regional survey data for this pattern.

Other future analyses will explore the relationships between biomass of specific earthworm species and plant diversity and composition. For instance, C. Hale (personal communication) has found the biomass of *L. rubellus* to be especially associated with plant community changes. We will also explore relationships between tree and shrub data, deer browse index, and canopy cover.

Literature Cited

Darwin, C.R. 1881. The formation of vegetable mould through the action of worms with observation on their habits. Murray, London, UK.

Groffman, P.M., P.J. Bohlen, and M.C. Fisk. et al. 2001. Invasion of north temperate forest soils by exotic earthworms. Instititute of Ecosystem Studies, Millbrook, NY. http://www.ecostudies.org/research/reports/grofrep2.html.

James, S.W. 1990. Oligoghaeta: Megascoledidae and other earthworms from southern and midwester North America. Pp. 370-386. In: Dindal, D.L. (ed.) Soil *Biology Guide.* John Wiley and Sons, New York.

James, S.W. 1995. Systematics, biogeography, and ecology of nearctic earthworms from eastern, central, southern, and southwestern United States. Pp. 29-51. In: Hendrix, P.F. (ed.) Earthworm Ecology and Biogeography in North America. Lewis Publishers, Boca Raton.

Langmaid, K. K. 1964. Some effects of earthworm invasion in virgin podzols. *Canadian Journal of Soil Science* 44: 34-37.

Lawrence, A.P. and Bowers, M.A. 2002. A test of the'hot' mustard extraction method of sampling earthworms. Soil Biology and Biochemistry 34(4): 549-552.

Satchell, J.E. 1967. Lumbricidae. In A. Burges and F. Raw (eds.), Soil *Biology, pp.* 259-322. London: Academic Press.

Tomlin, A.D., M.J. Shipitalo, W.M. Edwards and R. Prom 1995. Earthworms and their influence on soil structure and infiltration. In P.F. Hendrix (ed.), *Earthworm Ecology and Biogeography in North America, pp.* 159-184. Boca Raton, Fl., Lewis Publishers.

Wallwork, J.A. 1983. *Earthworm Biology.* The Institute of Biology's Studies in Biology, No. 161. London, Edwards Arnold.