

**Leaf Litter Mass Relating to Earthworm Community Biomass and  
Soil Characteristics in Hardwood Wisconsin Forests**

BIOS: 569: Practicum in Field Biology

Luritta E. Whiting

Advisor: David Costello

2007

## Abstract

Invasive earthworms have populated many areas of the hardwood forests of Wisconsin, changing soil composition and forest characteristics. Specific changes due to the presence of the invasive species are not clearly understood, and this survey examines the relationship between belowground factors and leaf litter. I found that there is a significant relationship between the increasing biomass of earthworm communities and the pH of the soil. I also measured the leaf litter mass and found a significant relationship between the total litter mass in a subplot with the pH, and with the worm species *Dendrobaena octaedra*. From the survey, an earthworm species composition was constructed, with 37.77% *Aporrectodea*, 29.99% *Dendrobaena octaedra*, and 32.32% *Lumbricus terrestris*, *Lumbricus rubellus*, or *Lumbricus juvenile*.

## Introduction

Earthworm invasions provide rare opportunities to observe changes in soil characteristics due to the density of the population. Research thus far has shown that the presence of invasive earthworms has an effect on percent organic matter, bulk density, topsoil thickness, and O horizon characteristics (Hale et al. 2005a). In 1988, earthworms (phylum Annelida, class Oligochaeta, order Opisthophora) were classified to sixteen different families worldwide. There are six aquatic or semi-aquatic families, and ten terrestrial that are considered the standard earthworm (Hendrix 2002).

The forest floor can be greatly altered by invasive earthworm species. Invading worms can change the understory plant communities, reduce plant regeneration by ingestion and burial of seeds, as well as affect the composition of soil nitrogen and phosphate (Bohlen 2004). Since the last glacial period, native earthworms are not in northern temperate forests, so all of the species found in the region have migrated and

invaded the area (Bohlen 2004). The seventy species of earthworms found in the Northern United States and Canada have been imported from Europe by humans for agriculture, horticulture, waste management, and land bioremediation both on purpose and by accident. The specific effects of earthworm invasion on the forest soil are unknown thus far (Hendrix 2002).

Europeans introduced most exotic earthworms to the United States through human migration and commerce. The popular fishing pastime introduces many nonnative species of earthworms to new habitats. The impact that earthworms have on the forest floor was first studied, described, and cataloged by Gordon E. Gates (Hendrix 2002). Gates' research led him to believe that invasive earthworms are easily spread and have a substantial impact on the global environment. Currently, there are no significant regulations regarding the importation or use of exotic earthworms, with the exception of some regions in Minnesota, though the impact of each nonnative species to an area may be detrimental to the forest habitat (Hendrix 2002).

The linkage between belowground invasive species and aboveground ecological processes is poorly understood and requires further research (Hendrix 2002). Specific changes in soil characteristics have shown a decrease in availability of ammonium, nitrate, and phosphate due to an increase in earthworm biomass. Studies have also observed a significantly lower content of foliar nitrogen and foliar carbon in an inverse relationship with total earthworm biomass measured (Hale et al. 2005a). In 2002, Hendrix showed an increase in compacting surface soil, an unnatural dispersal of weeds, and an increase in erosion according to the total earthworm biomass in the soil. Ecosystems containing invasive earthworms can be compared to ecosystems lacking earthworms for a comparison of soil characteristics (Hale 2005b).

The O horizon of the soil (the top layer) contains leaf litter that can be qualitatively and quantitatively measured for comparisons between earthworm communities and non-earthworm communities. Hale and Pastor (2005a) observed a lower percent organic matter present with an increase in the total earthworm community biomass in both the A and O horizon, while the other soil horizons showed no change. The A horizon shows a soil thickness increase with increasing biomasses of earthworm communities, while the O horizon thickness is usually lacking when a worm community is present (Hale et al. 2005a). The body size and total biomass of an earthworm community can also be measured in order to reflect a relationship between the size of the community and the impact on soil characteristics (Hale et al. 2005b).

Invading earthworms in Land O'Lakes, Wisconsin make up the vast biomass majority of terrestrial decomposers, which determine leaf litter abundance and species composition of the soil. A greater earthworm biomass should decrease the total amount of leaf litter present in the area. A greater earthworm biomass should also result in a measurable loss of abundance of native plant species (Bohlen 2004) which are reflected in the leaf composition within a subplot. The impact of earthworm biomass on soil characteristics and leaf litter composition in the hardwood forests of Land O'Lakes, Wisconsin is unknown and requires research.

The purpose of this study is to examine the leaf litter and soil in a deciduous forest in Wisconsin with the presence of an invasive earthworm community. The null hypothesis is that there will be no difference in soil characteristics according to the presence or absence of earthworm community biomass. The main goal is to see if there is a correlating relationship between the soil and leaf litter characteristics and the belowground earthworm communities present. I will also examine the relationships between aboveground and

belowground measurements to examine specific preferences among species of trees and earthworms. I will use models of sugar maple, *Acer saccharum*, yellow birch, *Betula alleghaniensis*, and basswood, *Tilia americana*, to compare abundance of leaf litter within a subplot and combined plot compared with earthworm species of *Dendrobaena octaedra*, *Aporrectodea spp.*, and *Lumbricus spp.*

### **Methods and Materials**

Located in the northern mixed deciduous forests of Land O'Lakes, Wisconsin at the University of Notre Dame Environmental Research Center (UNDERC), East property, located at 46° 13' N, 89° 32' W. My study was conducted in an undisturbed forest area of 7500 acres set aside for research and natural cohabitation. The tree vegetation largely second-growth are primarily sugar maple, aspen-birch, and pine mixes. The plots were selected from this variety of forest types and have an acceptable range of leaf litter diversity for the Northern mesic hardwood forest type.

Earthworms will be extracted from their soil community using ground yellow mustard powder at a concentration of 40 grams per 3.8 Liters of water. One gallon will be used on each subplot of 31 by 31 centimeters. This has been determined an effective method for quickly obtaining a representative sample of the earthworm community present in the sampling site (Gundale 2005). The worms that respond to the mustard technique will be preserved in 70% ethanol and measured in species and length. Using biomass formulas for each species, I will calculate the biomass of each earthworm community from each plot and subplot. Hale et al. (2004) developed specific allometric equations for *Lumbricus spp.*, *Aporrectodea spp.*, and *Dendrobaena octaedra* (Appendix A). I will use this information to analyzing the relationship between total community biomass and characteristics of the forest soil with SYSTAT 12.

Measurements of the soil pH, soil moisture, and soil organic matter content will be recorded in order to observe the relational effects when an earthworm community is present. I will take a sample of soil while on the site, and use a pH probe in lab to obtain a pH value for each subplot. The soil sample will also be weighed, dried, and re-weighed to obtain the total soil moisture. It will then be placed in an ashing oven at 500 degrees Celsius for four hours, and weighed again to calculate the percent organic matter in the soil. Each of these will be changed to a percent by dividing the calculated amounts by the original wet or dry soil sample, respectively.

The study will examine ten carefully selected forest stands with tree community variety and earthworm densities from a survey at UNDERC performed in 2005. The total litter mass and earthworm community composition will be measured for five random subplots within a 50 by 50 m plot. All samples are collected from the five subplots in a 31 by 31 cm sample. The total amount of leaf litter is collected from within the subplot, species counts of the leaves are recorded on site, and the total mass of each leaf species and total leaf litter amount is weighed in lab. The leaf litter mass and specific proportions of present species will aid in the analysis of the relationship between earthworm species and the leaf aboveground community. I will then compare and analyze plot characteristic measurements with all data to find significance.

The relationship between specific aboveground effects of leaf litter on the tree composition in the forest, and the belowground characteristics will also be studied. My team will measure the Diameter Breast Height (DBH) in centimeters of all trees with a DBH greater than or equal to ten cm, located within a 17 m radius from the subplot center. I will look for correlations between the size, species, leaf litter mass, and distance from subplot with the belowground measures.

All of the data will be analyzed using appropriate statistical tests. These will include linear regressions, correlations, and a stepwise multiple linear regression. Excel and SYSTAT 12 will be used in organizing and analyzing the data throughout the process. Data with a p-value of less than or equal to 0.05 will be considered statistically significant.

## Results

In five selected plots of sampling in the hardwood forest of Wisconsin, and ten random subplots within each plot, 573 worms were identified by species and length. The DBH and species was recorded for 2332 trees, while the soil characteristics were measured for all 50 subplots. Using SYSTAT 12, I found a trend, although not statistically significant between *Dendrobaena octaedra* and *Lumbricus spp.* (p-value = 0.0605 and  $t = 2.18$ : Figure 4) and *Dendrobaena octaedra* and *Aporrectodea spp.* (p-value = 0.0623 and  $t = 2.16$  Figure 5). A linear regression found that the average pH of each subplot and the worm biomass average of all types related with a p-value of 0.039 and a F-ratio of 6.076 (Figure 1). The total mass of the leaf litter for each plot was related to pH and the biomass of *Dendrobaena octaedra* with respective p-values of 0.0499, and 0.038, with F-ratios of 5.318 and 6.107 (Figures 2 and 3). These leaf litter mass totals are inclusive of all litter, including fern fronds and peat moss.

I did not find significance among different species of leaf litter and earthworms, though tendencies are shown. Using a stepwise regression, I found that as *Aporrectodea spp.* biomass increases, the total measured mass of sugar maple, basswood, and yellow birch decreases. These relationships were not statistically significant, but a stronger relationship with *Aporrectodea spp.* was found than other worm species (Figure 6). The total leaf litter mass showed a stronger preference for *Lumbricus spp.* with a non-significant p-value of 0.105 and F-ratio of 2.726 (Figure 7).

## Discussion

I was unable to find statistical significance between the aboveground factors and the belowground measurements. These included all data recordings of tree species, tree DBH, tree distance from subplot, leaf litter species, leaf litter weight, pH, percent organic matter in the subplot soil, percent moisture, and species of worms or biomasses found within the subplots. The data suggests that the controlling factor between the relationship of above and belowground organisms is the overall pH. With this relationship, the mass of the leaf litter determines the pH of the soil. The extent of a relationship between tree species and number with pH is an interesting one that disperses uncharted impacts throughout the forest floor.

According to the increased leaf litter mass in more neutral pH soil, it is not a stretch to see that there may also be an increased number of trees with a more neutral pH, although we found no such relationship. The large trees (DBH > 10cm) that were included in the study do not change characteristics as rapidly as soil and belowground characteristics are able to change. It is possible that the stable environment challenges ideas about complex relationships between organisms and species of trees and worms. Many of the trees started developing before worms were present, and developing trees and small saplings would give more accurate data on the topic.

A higher litter mass shows a higher preference for *Dendrobaena octaedra*, with a p-value of 0.038 over the other worm species. This is due to the nature of their environment. *Dendrobaena* do not live in the soil like most *Aporrectodea* and *Lumbricus*, but instead, consume and live in the leaf litter. *Aporrectodea* live in and consume the organic matter of the soil, though the correlation is not statistically strong. This is probably because a higher organic content is normally in an environment of higher moisture. Newly

hatched worms have a difficult time surviving moisture fluctuations in the soil (Gerard, 1967). Further investigation in this area could find the most ideal soil and litter conditions for worm species to live in according to moisture, organic matter, pH, and temperature. Gerard found a difference in worm casting activity according to temperature, and calls for further research as well (Gerard, 1967).

In such a way, the pH of the soil has a relationship with the biomass of the worm community present in the soil with a p-value of 0.039. The range of pH values in within the regression relationship was 3.57 to 5.78, which is fairly acidic. As the pH nears 6, the biomass of worms increased significantly, meaning that worms like a slightly more neutral environment, though they function well in relatively acidic soil as well. Founding research in 1921 by Arrhenius discovered that reactivity of the soil is highly influential on earthworm activity. Earthworms prefer neutral and slightly acidic soils for high production (Arrhenius, 1921). With this, there is an indirect connection between the trees of the hardwood forest and the invasive earthworm communities, connected by leaf litter, pH, and earthworm biomass relationships.

The data shows the percent biomass of each earthworm species that we obtained from the soil (Figure 8). 37.77% are *Aporrectodea spp.*, 29.99% are *Dendrobaena octaedra*, and the remaining 32.23% are *Lumbricus spp.* It is important to note that the sampling method did not favor or discriminate against any particular species. I accounted for this by waiting five minutes after a complete sampling infiltration (minimum of twenty minutes) for each subplot to insure large body family like *Lumbricus* an opportunity to be extracted (Gundale, 2005).

The amount of ground covered by vegetation was not measured because the leaf litter mass directly above the worm community was the focus. Future studies should

examine the specific types and numbers of vegetation present according to the earthworm biomass present. The specific relationship between the forest understory and belowground characteristics remain a mystery, even though there may be an important relationship between sapling presence and vegetation abundance with worm biomass and physical soil characteristics. Factors of slope and aspect were also not recorded, though there is a probable relationship between the subplot terrain type and leaf litter amount.

## Figures

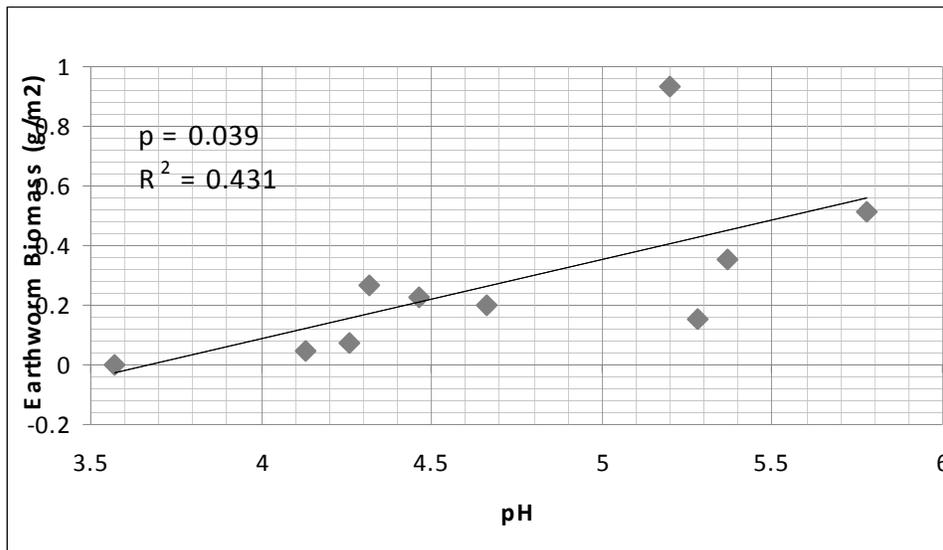


Figure 1: Average earthworm biomass vs. pH average per subplot

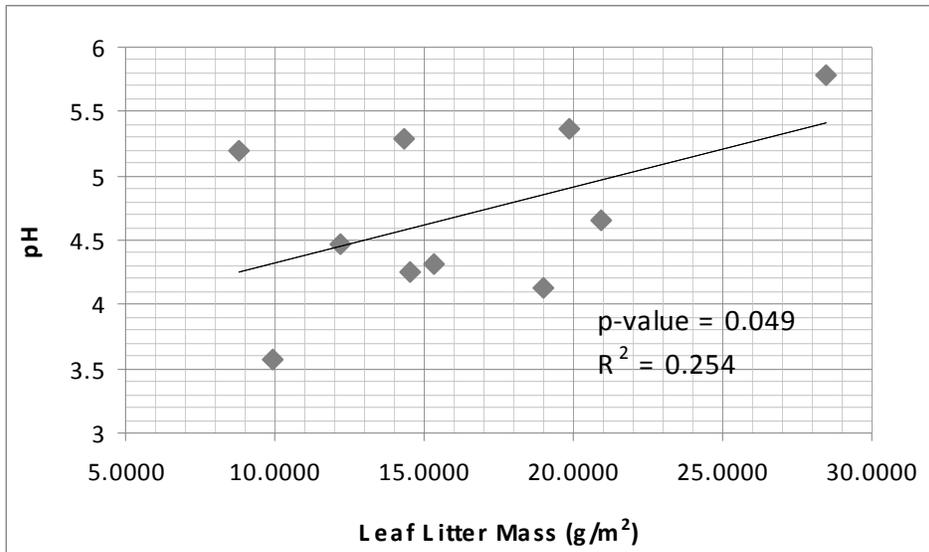


Figure 2: Leaf litter mass in grams vs. pH

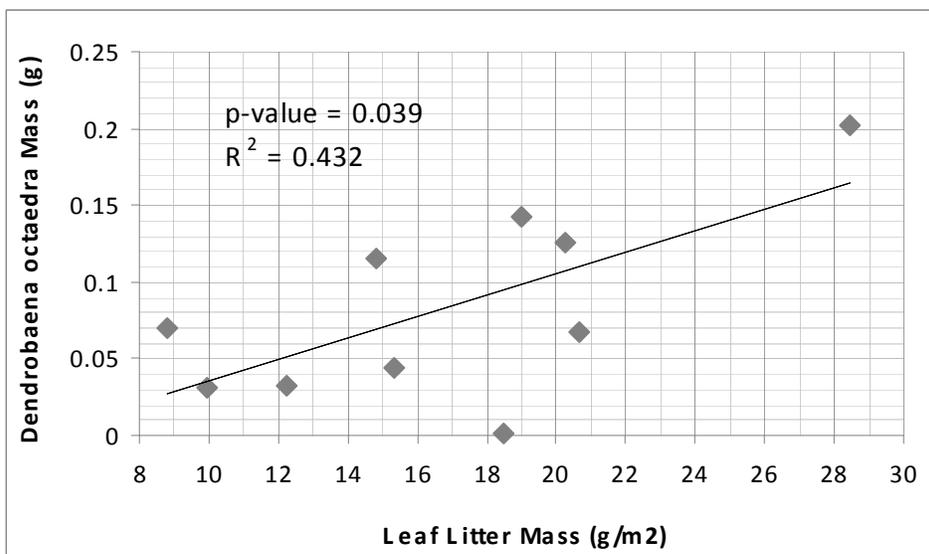


Figure 3: *Dendrobaena octaedra* mass in grams vs. leaf litter mass in grams

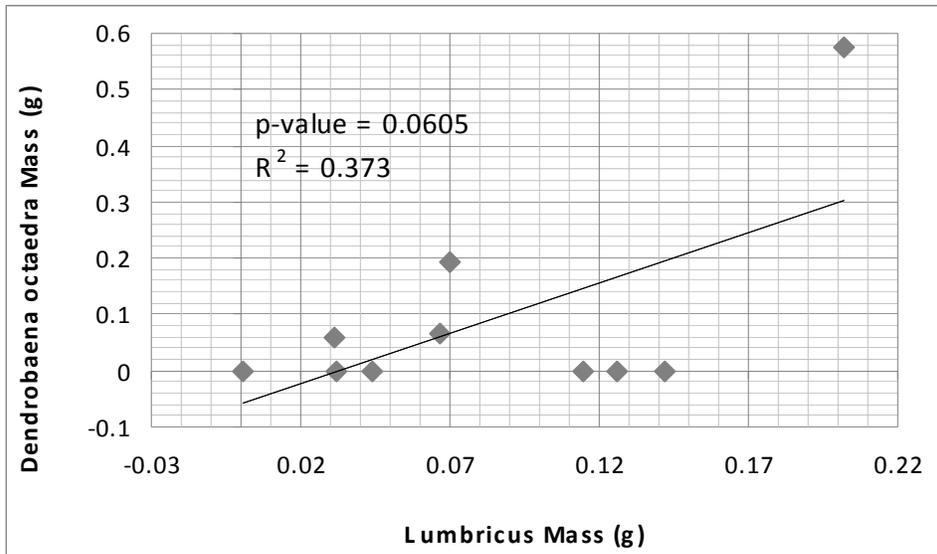


Figure 4: *Dendrobaena octaedra*, and *Lumbricus spp.* biomass correlations

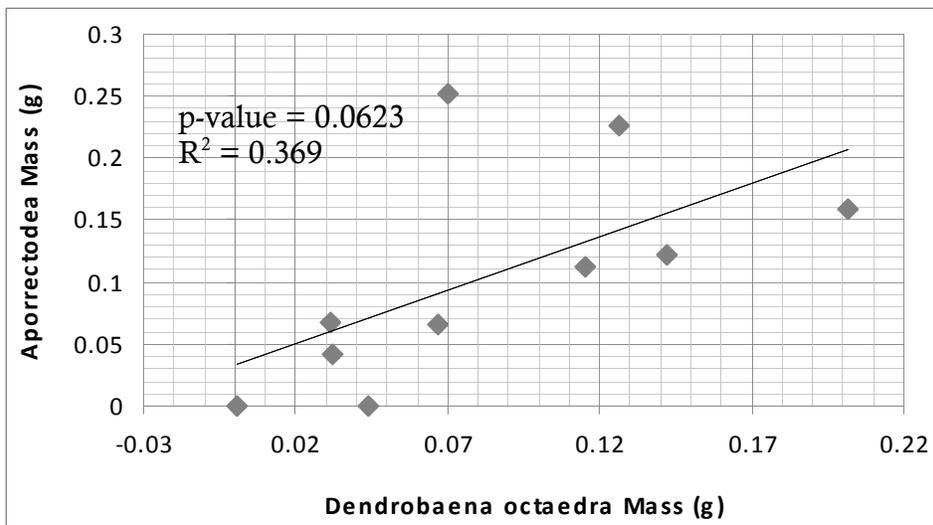


Figure 5: *Dendrobaena octaedra* and *Aporectodea spp.* biomass correlations

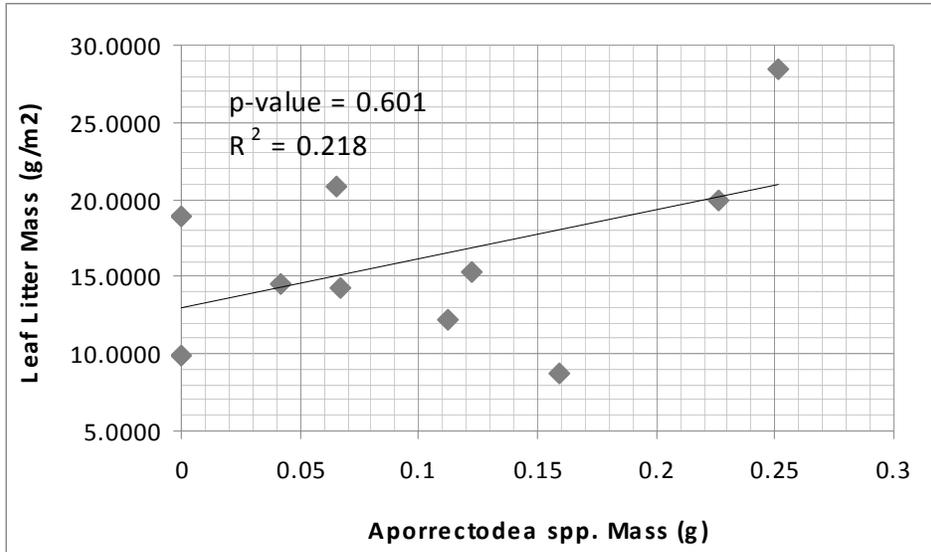


Figure 6: *Aporectodea spp.* and leaf litter mass

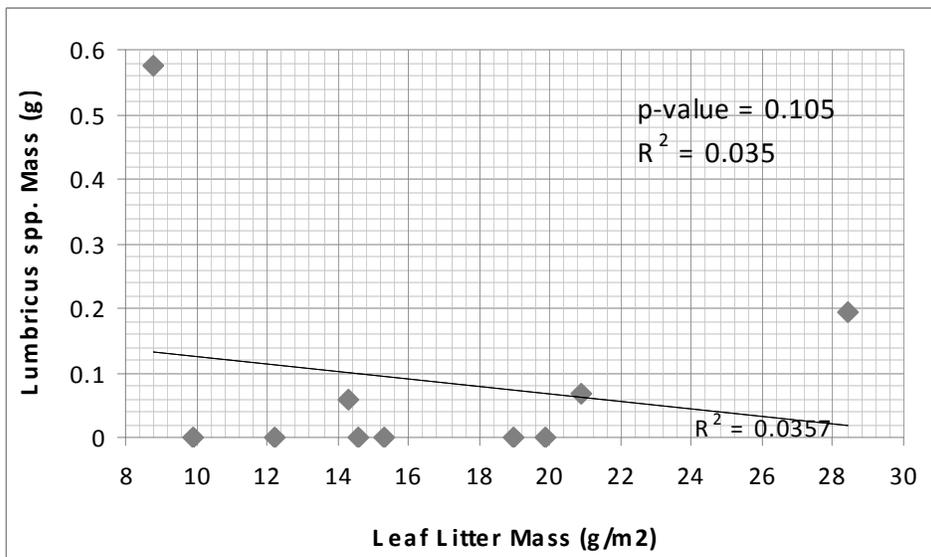


Figure 7: *Lumbricus spp.* and leaf litter mass

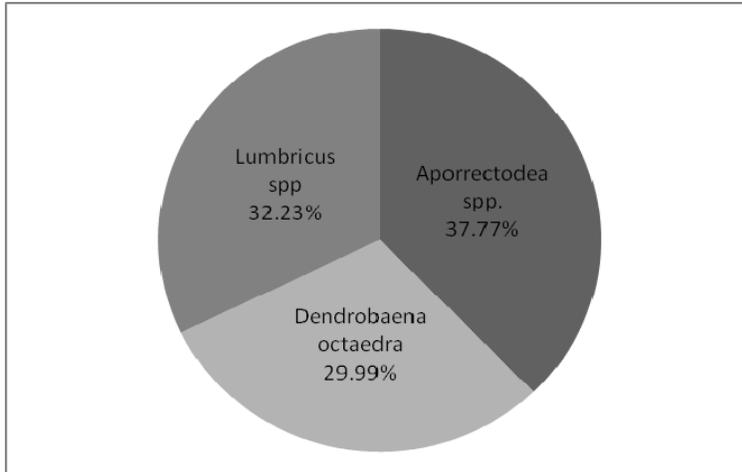


Figure 8: Percent biomass of earthworm species *Aporrectodea* spp., *Dendrobaena octaedra*, and *Lumbricus* spp.

### Acknowledgements

This research was supported by a stipend from the University of Notre Dame Environmental Research Center in Land O'Lakes, Wisconsin and the Costello-Lamberti lab for materials and equipment. Thanks especially to my advisor David Costello for endless support, aid in data collection, and the constant surplus of ideas and suggestions. Thanks also to Loretta Brown who is always by my side during work and analysis, as well as Jamin Dreyer, Tim Szevwczyk, and Nathan Payne for their assistance in the field and lab. Thanks for supportive ideas and editing from Dr. Michael Cramer, Luke DeGroot, and Dr. R. Dombkowski.

### Works Cited

- Arrhenius, O. 1921. Influence of Soil Reaction on Earthworms. *Ecology*. 2: 255.
- Bohlen, P., Scheu, S., Hale, C., McLean, M., Migge, S., Groffman, P., Parkinson, D. 2004. Non-native invasive earthworms as agents of change in northern temperate forests. *Front Ecol Environ*. 8: 427-435.

- Gerard, B. 1967. Factors Affecting Earthworms in Pastures. *The Journal of Animal Ecology*. 36: 235-252.
- Gundale, M., Jolly, W., Deluca, T. 2005. Susceptibility of a Northern Hardwood Forest to Exotic Earthworm Invasion. *Conservation Biology*. 19:1075-1083.
- Hale, C., Frelich, L., Reich, P., Pastor, J. 2005. Effects of European earthworm invasion on soil characteristics in Northern hardwood forests of Minnesota, USA. *Ecosystems* 8: 911-927.
- Hale, C., Frelich, L., Reich, P. 2005. Exotic European earthworm invasion dynamics in Northern hardwood forests of Minnesota, USA. *Ecological Applications* 3: 848-60.
- Hale, C. et al. 2004. Allometric Equations for Estimation of Ash-free Dry Mass from Length Measurements for Selected European Earthworm Species (Lumbricidae) in the Western Great Lakes Region. *Am. Midl. Nat.* 151:179-185.
- Hendrix, P., Bohlen, P. 2002. Exotic Earthworm invasions in North America: Ecological and policy implications. *BioScience* 52: 1-11.

**Appendix A:** Equations for length to biomass conversions.

$$Lumbricus \text{ spp. } \ln(\text{afdm}) = 2.3225 * \ln(\text{length}) - 11.8423$$

$$Aporrectidea \text{ spp. } \ln(\text{afdm}) = 2.1734 * \ln(\text{length}) - 11.6417$$

$$Dendrobaena \text{ spp. } \ln(\text{afdm}) = 2.6365 * \ln(\text{length}) - 12.8740$$