Possible Interactions Between Deer Herbivory and Earthworm Invasion Impacts on the Regeneration of Sugar Maple (*Acer saccharum*) in Northern Hardwood Forest.

BIOS 35502: Practicum in Field Biology

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ABSTRACT:

The sugar maple, *Acer saccharum*, has been in decline in forests of the Great Lakes Region for the last five decades. Two possible reasons for the decline are the overabundance of white-tailed deer, *Odocoileus virginianus*, and the introduction of invasive earthworms. We predicted that sugar maple densities and green biomass would be lowest in areas of high deer use frequency and in areas of high worm denstity. We also predicted that there would be a synergistic inhibition between these two stressors. We surveyed sugar maple seedling abundance and performed leaf counts in areas of high and low deer use frequency, and high and low earthworm density. Neither worms nor deer were found to have an effect on sugar maple growth characteristics. There was no evidence of an interaction between the two variables. At least seven species of earthworm were found on UNDERC property, representing three ecological groups of worm. There was a significant positive correlation between earthworm abundance and pH, and epigeic earthworm abundance and pH. The sugar maple is important to the economy, heritage, and ecological integrity of the Great Lakes region. It is essential to understand the factors contributing its decline.

INTRODUCTION:

The sugar maple (*Acer saccharum*) has been in decline in forests of the Great Lakes Region for the last five decades (Storer 2011). Sugar maple regeneration rates are negatively correlated with the presence of white-tailed deer (*Odocoileus virginianus*) (Tilghman 1989). Deer densities in the Upper Great Lakes Region were 2-4 deer/ km^2 pre European settlement. Due to extirpation of predators, forest fragmentation, and favorable management practices, deer densities may exceed 19 deer/km^2 today (MDNR 2010, Alverson et al 1988). A previous BIOS 33502 student project found deer densities at

UNDERC to be between 5.74-6.4 deer/km^2 (Allen and Solchik unpublished data). Deer limit sugar maple recruitment directly by means of herbivory upon seedlings, and indirectly by conferring a competitive advantage to understory species they find less palatable, such as Pennsylvania sedge, *Carex pensylvanica*, and balsam fir, *Abies balsamifera* (Ullrey et al. 1968, Horsley and Marquis 1983, Kraft 2004, Hale et al. 2006, Powers and Nagel 2009). Sugar maples are especially vulnerable as seedlings, when herbivory is most likely to result in mortality (Augustine and Frelich 1998).

Invasive non-native earthworms are another factor that may influence the decline of sugar maples in the Great Lakes region (Bohlen et al. 2004b). The Great Lakes Region has had no native earthworms since the last ice age. Non-native invasive species are widespread in the region, introduced by agriculture, logging, and the dumping of unused fishing bait (Hendrix and Bohlen 2002). Invasive worms affect sugar maple recruitment directly through herbivory on roots of seedlings, and indirectly by altering the mineralization rates and availability of carbon, nitrogen, and phosphorus in forest soils (Alban and Berry 1994, Bohlen et al. 2004a, Bohlen et al. 2004b, Suárez et al. 2004). Earthworms of the Great lakes region may be split into three ecological groups. Epigeic species live in and feed on leaf litter. Endogeic species live in the mineral soil layer and feed on organic matter found there. Anecic species live deep in the mineral soil, but burrow to the soil surface to feed on leaf litter. The three different feeding guilds work synergistically to accelerate litter decomposition and nutrient cycling rates (Hale et al. 2006, Frelich 2006, Bohlen 2004).

Although many papers have been written on the effects of deer herbivory and earthworm invasion upon forests, none have studied these two stressors in synchrony or investigated possible interactions between them (Russel et al. 2001, Bohlen at al. 2004b). This study served to investigate the possible interaction of these effects. Earthworms may lower seedling densities below a stable state threshold, to a point at which populations are highly vulnerable to deer herbivory, producing a synergistic inhibition upon sugar maple regeneration (Augustine 1998).

I studied whether deer and/or worms affect sugar maple growth characteristics in a Great Lakes northern mesic forest.

1) If deer are inhibiting sugar maple regeneration, then leaf counts and seedling abundances should be lower in areas of high deer frequency.

2) If worms are inhibiting sugar maple regeneration, then leaf counts and seedling abundances will be lower in areas of high deer frequency.

3) If there is synergistic inhibition, then growth characteristics will be less at sites with high levels both deer and worms.

4) If earthworms influence sugar maple growth characteristics by altering soil nutrients, then there will be a significant relationship between worm abundance and N and P levels.

METHODS:

I studied 19 randomly selected plots within sugar maple dominated forests at the University of Notre Dame Environmental Research Center property (UNDERC), located in Gogebic county, MI. The UNDERC property is a northern mesic forest with many homogenous stands of sugar maple (Curtis 1959). Each plot contained three subplots, determined by a randomly selected number of paces along a randomly selected bearing. Each subplot received an identical treatment.

Sugar Maple Density

All sugar maple seedlings (individuals < 25 cm tall) within the 1 m^2 subplot were counted, and leaf count of each individual was taken as a means of inferring green biomass.

Nutrients, Moisture, and pH

Approximately one cup of topsoil was gathered from each of the 1 m^2 subplots. These samples were transported from the field on ice, and stored frozen until they were ready to be analyzed. The samples were then placed in a drying oven at 60° C for 48 hours. Soluble N and P were measured from these samples by means of LaMotte NPK soil kits (LaMotte Company; Chestertown Maryland). Soil pH and moisture at each subplot was measured in the field by means of a Kelway soil tester probe (Kel Instruments Co., Inc; Wyckoff, NJ).

Worm Densities

Worm densities were estimated by hot mustard extraction, as described in Gundale (2002). A .25m^2 barrier was placed in the center of each subplot, and the soil surface confined in this area was cleared of leaf litter. A solution consisting of 40g of mustard seed powder dissolved in 3.8L of water was then poured onto the .25m^2 area. All worms that rose to the surface within five minutes were collected and placed in 95% ethanol. The worms were then identified to ecological group (NRRI 2011). In order to obtain ash free dry mass of each ecological group at each subplot, the worms were dried in an oven at 60° C for 24 hours, and ashed at 500° C for 4 hours (NRRI 2006). A histogram of total worm abundance per site was used to infer a value to serve as a cutoff measurement. The break in the histogram coincided with the median value of the data. Sites with greater than 0.5g dry mass earthworm / m^2 were classified as "high" density, and sites with 0.5g dry mass earthworm / m^2 or less were classified as "low density" (Wironen and Moore 2006).

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Deer Densities

Density of deer was inferred from wolf use patterns as demonstrated by Mech (1977) and Flagel (unpublished data). Areas of high wolf use were assumed to have low deer density, and vice-versa.

Statistical Analysis

Analyses were perform in Systat 13 (Cranes Software). Data was analyzed by means of two-way ANOVAs, with sugar maple growth characteristics as the dependent variable. Deer density and worm density served as factors. High and low worm densities were defined based upon observed clumping in plenary data analysis (Wironen and Moore 2006). The interaction term of these two factors provided insights into possible synergistic inhibition of deer and worms upon sugar maple recruitment.

These analyses rely on the assumption that higher earthworm densities are correlated with higher earthworm damage. To determine if this assumption was supported, soil samples were taken at sampling sites, and soluble nitrogen and phosphorus were qualitatively measured. Correlation analyses were performed to examine the nature of the relationship between earthworm abundance and soil nutrients. I predicted a negative correlation between earthworm densities and soluble N and P present in the soil samples.

RESULTS:

Sugar Maple Abundance

The hypothesis test determined that there were no significant differences between high deer densities (mean = 2.51) and low deer densities (mean = 2.79) ($F_{1,15}$ =.0.069 p = 0.79; Figure 1). There was no difference between high worm densities (mean = 2.93) and low worm densities (mean = 2.37) ($F_{1,15}$ = 0.266 p = 0.61). The interaction between worms and deer was not significant ($F_{1,15}$ = 1.348 p = 0.61).

Sugar Maple Leaf Count

The hypothesis test determined that there were no significant differences between high deer densities (mean = 2.30) and low deer densities (mean = 2.99) ($F_{1,15}$ =.1.377 p = 0.26; Figure 2). There was no difference between high worm densities (mean = 3.01) and low worm densities (mean = 2.28) ($F_{1,15}$ = 1.52 p = 0.23). The interaction between worms and deer was not significant ($F_{1,15}$ = 1.00 p = 0.76).

Worm mass was normalized by means of a log transformation. There were significant positive relationships between total worm mass and pH (p = 0.05; Figure 3) and epigeic worm mass and pH (p=0.01; Figure 4).

DISCUSSION:

Our results suggest that neither deer nor worms have significant effect on either sugar maple seedling abundance or leaf count. However, our results were highly variable, which may have prevented us from being able to detect significance. This is particularly supported by the fact that trends were visible from the analysis. Seedling abundance and leaf count were higher in areas of high worm densities. Earthworms increase the rate of nutrient cycling. By rapidly making the nutrients available, worms could increase growth of sugar maple seedlings, but ultimately deplete the soil of nutrients and substrate necessary for the health of later maple life history stages. Future studies may want to incorporate a greater sample size to support or refute the trends observed in this study.

I discovered UNDERC to be invaded by at least six species of earthworm. All are of European origin, in the family Lumbricidae. Every functional group of earthworms was represented: Anecic (*Lumbricus terrestris*), Epigeic (*Dendrobaena octahedra*, *Lumbricus rubellus*, *Dendrodrilus rubidus*), and Endogeic *(Apporrectodea calignosa, Octolasion spp*.). These three ecological groups work in concert when present at the same location, decreasing surface litter horizons and increasing mineral soil horizons such that they resemble the soils of the Lumbricids native forests (Frelich 2006). Invasions of both epigeic and endogeic worm species result in less understory diversity than invasions of only endogeic species (Hale et al 2006). Earthworm density is known to increase with pH and we see this pattern as well at UNDERC (Leighton and Fahey 2011).

Nutrients in Sugar Maple forests at UNDERC do not vary on a large scale. My methods were not precise enough to detect minute variation. A future study may want to look into a more fine scale assessment.

The sugar maple is important to the economy, heritage, and ecological integrity of the Great Lakes region. It is essential to understand the factors contributing its decline. Further research is necessary to fully understand the relationship between deer herbivory and earthworm invasion upon sugar maple at all stages of its life history.

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FIGURES:

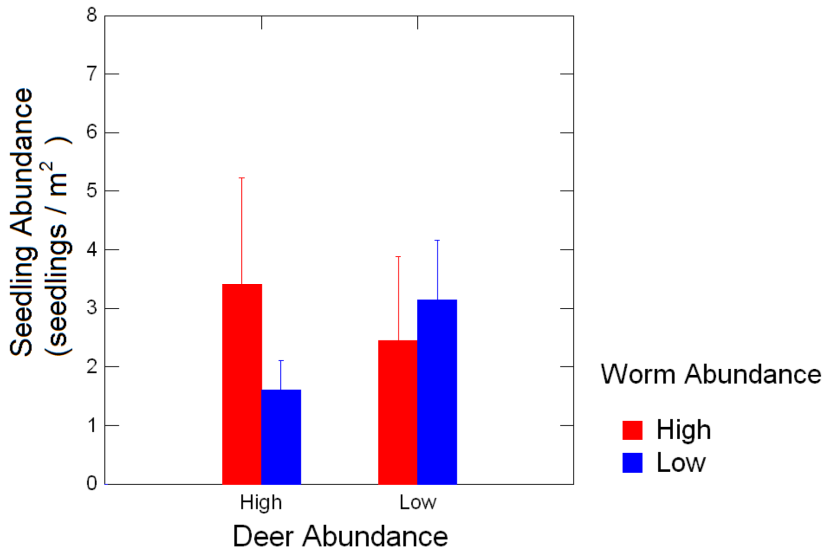

Figure 1. Comparison of *Acer saccharum* seedlings per square meter according to deer abundance. There was no difference between deer abundances ($F_{1,15}$ =.0.069 p = 0.79) or worm abundances ($F_{1,15}$ = 0.266 p = 0.61). There were no significant interactions between deer abundance and worm abundance ($F_{1,15}$ = 1.348 p = 0.61).

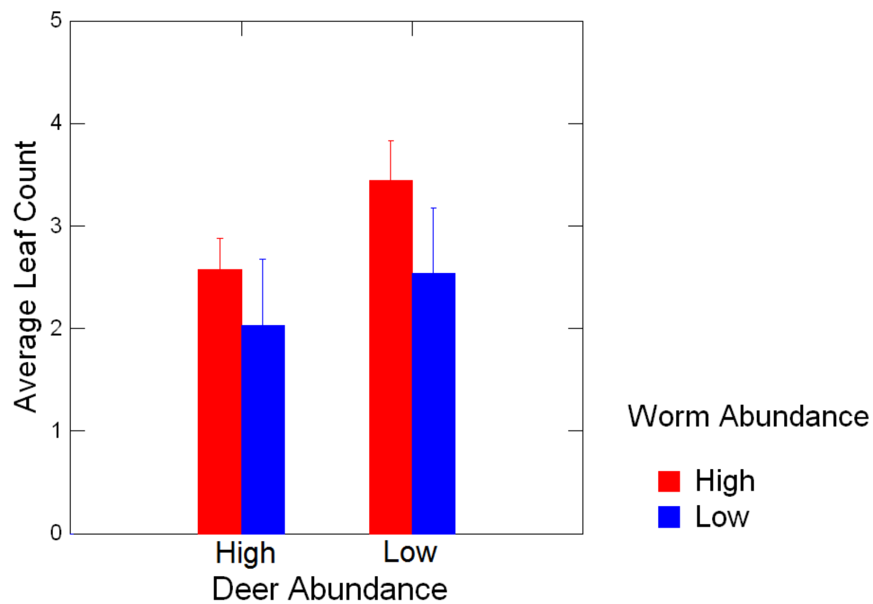

Figure 2. Comparison of *Acer saccharum* leaf count according to deer abundance. There was no difference between deer abundances ($F_{1,15}$ =.1.377 p = 0.26) or worm abundances ($F_{1,15}$ = 1.52 p = 0.23). There were no significant interactions between deer abundance and worm abundance ($F_{1,15}$ = 1.00 p = 0.76).

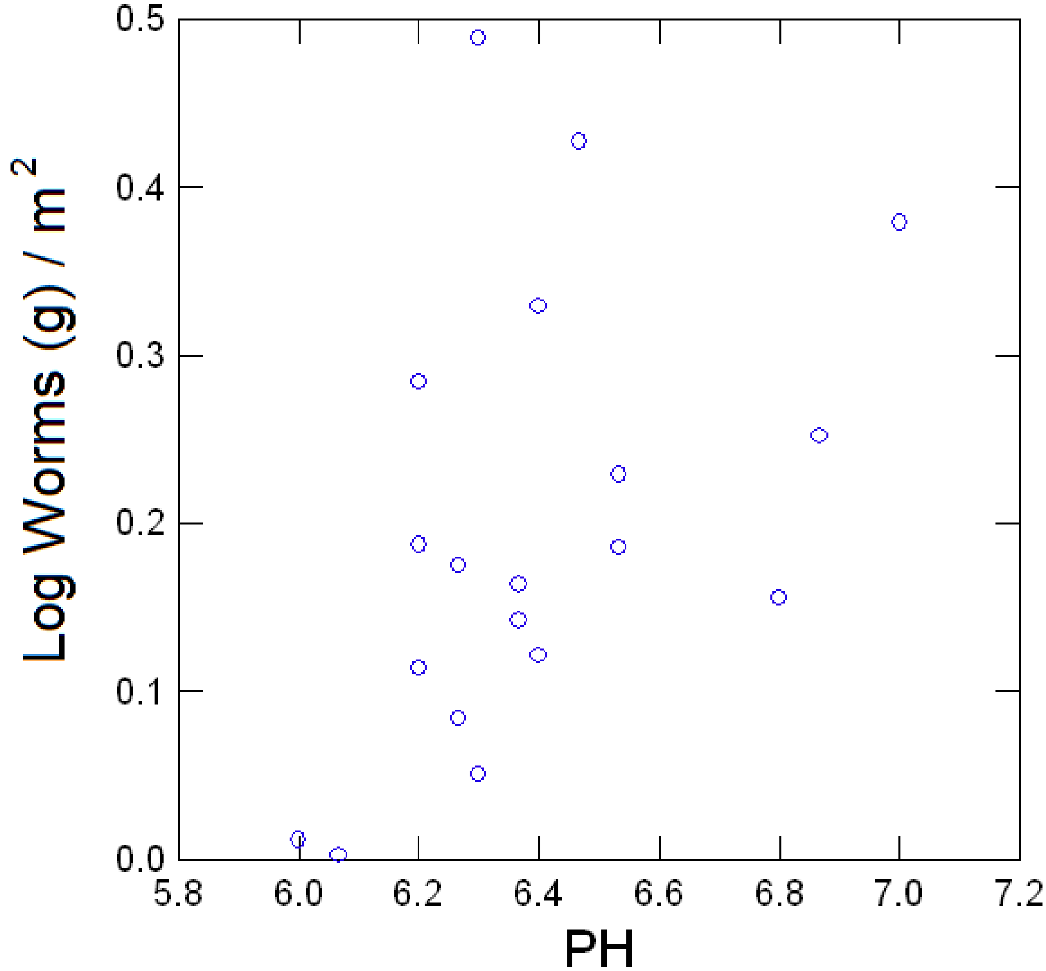

Figure 3. Mass of worms at site (g) in comparison to pH of site. There was a significant positive relationship between worm mass collected in an area and the pH of that site ($p = 0.05$).

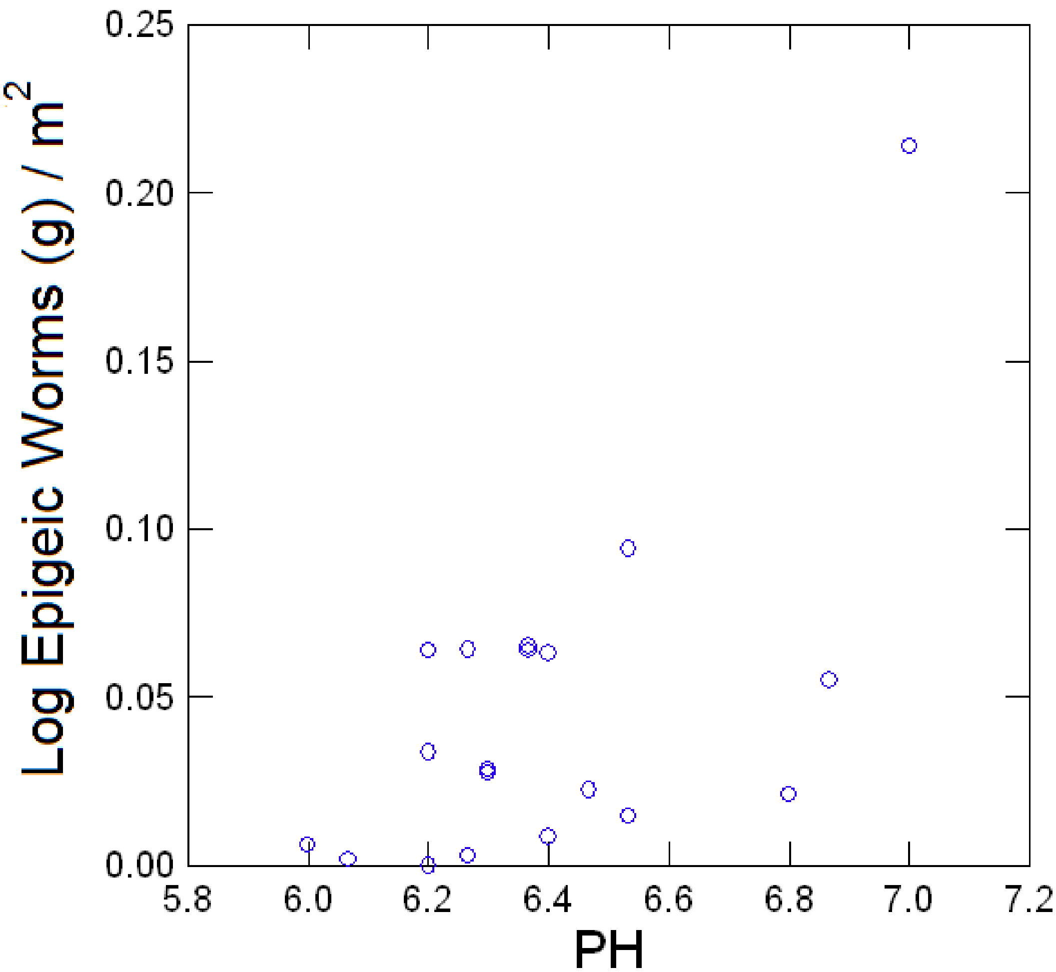

Figure 4. Mass of epigeic worms at site (g) in comparison to pH of site. There was a significant positive relationship between worm mass collected in an area and the pH of that site (p=0.01). Epigeic species found were *Dendrobaena octahedra*, *Lumbricus rubellus*, and *Dendrodrilus rubidus*.