The effects of invasive earthworms on maple (*Acer*) seedling germination and growth

BIOS 35502: Practicum in Field Biology

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Abstract

All earthworms in the Upper Great Lakes region are invasive and have been shown to have detrimental effects on area forests. *Acer saccharum* (sugar maple) forests are particularly affected, with both sapling abundance and growth are hindered by earthworms. However, little work has been done on *Acer rubrum* (red maple), which is becoming an increasingly important component of northern forests. *A. rubrum* seeds were grown in the presence of *Lumbricus terrestris, Aporrectodea caliginosa*, or *Lumbricus rubellus* and compared to seedlings grown in the absence of earthworms. A survey of the University of Notre Dame Environmental Research property was also performed to see if the negative correlation between maple abundance, height, and earthworm density reported in past studies elsewhere is seen on property. My results show a significant difference in total biomass of seedlings, with the *Aporrectodea caliginosa* treatment having a higher biomass than both control and *Lumbricus terrestris* treatments. No significant difference was found between the germination rates of different treatments and no significant relationships were found between earthworm densities and the growth or height of maple saplings around property. It is likely the maple saplings on property are more affected by deer over browsing than earthworm effects. This study shows red maples may not be hindered by earthworms as sugar maples are which has future implications as earthworms continue to invade the Upper Great Lakes region.

Introduction

Native earthworms were extirpated from the Great Lakes region during the last ice age. However, non-native earthworm populations currently occupy the majority of the Great Lakes forests mainly through introduction by humans, specifically anglers dumping bait and horticulture practices (Hendrix and Bohlen 2002). These introduced earthworms alter soil characteristics, affecting forest community dynamics that developed since the last ice age in their absence (Bohlen et al. 2004).

Earthworms have been found to have significant effects on soil structure and nutrient cycling in forest ecosystems, mixing soil layers and reducing organic matter (Gundale 2002). Non-native earthworm populations also increase nutrient leaching causing a net loss in N over time (Costello and Lamberti 2008, 2009, Frelich et al. 2006, Hendrix and Bohlen 2002). It has also been shown that earthworms cause a decrease in soil C storage (Bohlen et al. 2004, Hendrix and Bohlen 2002). High earthworm densities significantly decrease leaf litter thickness and mass in areas where they are present, which hinders the regeneration and growth of many plants (Wironen and Moore 2006).

In sugar maple dominated forests, earthworms cause a loss of plant diversity and plant cover as well as a reduction in the abundance of tree seedlings (Frelich et al. 2006). Plant species richness in these forests also decreases as earthworm densities increases (Holdsworth et al. 2007). Notably, *Acer saccharum* seedling density and earthworm density have been shown to correlate negatively (Corio et al. 2009, Holdsworth et al. 2007). When *A. saccharum* is grown in mesocosms, earthworm presence decreases biomass and increases mortality of seedlings (Hale et al. 2007). Though previous studies have focused specifically on *Acer saccharum* (sugar maple) (Hale et al. 2007, Holdsworth et al. 2007, Corio et al. 2009), none to my knowledge have studied any other maple species. *Acer rubrum* (red maple), a generalist maple species, is becoming increasingly more abundant in Great Lakes forests (Abrams 1998). As *A. rubrum* is a tree species that is becoming more abundant in the Upper Great Lakes (Abrams 1998), studying earthworm effects on this species' growth may be able to give an important insight into *A. rubrum's* ability to grow in Great Lakes forests.

Based on results found from other studies stating earthworms decrease sugar maple seedling biomass (Hale et al. 2007), I hypothesized that the presence of earthworms would negatively affect total biomass of *Acer rubrum* seedlings when grown in the presence of earthworms. Also, I predicted that germination rates would be lower where earthworms are present. I also examined the relationship between sapling abundance and earthworm density using an understory plant survey in a Great Lakes forest. This examination helped determine if the negative relationship between sapling abundance and earthworm density found in other areas of the Great Lakes region is maintained in my field site. I hypothesized that I would find the same pattern on property between earthworm densities for both sugar and red maple saplings.

Methods and Materials

This study was conducted at the University of Notre Dame Environmental Research center (UNDERC,) which is located on the border of Vilas County, Wisconsin and Gogebic County, Michigan. The habitat is mostly composed of northern mesic forest (Curtis 1959).

Growth Experiment

Acer rubrum grows rapidly in a variety of conditions with germination times of approximately 10-15 days making it ideal for a short term study (Abbot 1974). *Acer rubrum* seedlings were grown from seed in 15.4 cm diameter pots filled with standard potting soil for 42 days. Each pot was watered 25ml and rotated daily to mitigate any local effects due to the placement of grow lights. Grow lights were on for 16 hours a day to mimic summer sunlight amounts in this region (Hale et al. 2007). When more than one plant grew in a single pot, one was randomly selected and removed as soon as it was discovered to prevent the growth of the second plant from significantly affecting the growth of the other.

Each pot contained either an individual species of earthworm or no earthworms for the control. *Lumbricus terrestris* (night crawlers), *Aporrectodea caliginosa* (grey worms), and *Lumbricus rubellus* (red worms) are three common species of earthworms on property (Costello and Lamberti 2008) and therefore were chosen as the three treatments. For each treatment there were ten replicates. Only mature earthworms were used in this study since it is nearly impossible to distinguish *L. rubellus* and *L. terrestris* as juveniles. Earthworms were collected on property after rainfall emergence and extra individuals were purchased from local dealers as needed. All worms were identified to species using Schwert's dichotomous key (1990).

All treatments contained a high density of earthworms (242 g/m^2 fresh mass, 4.4 g/pot fresh mass). High density was defined by the observational findings of Wironen and Moore (2006) of high densities of earthworms being >200 g/m^2. In order to standardize biomass among all treatments, each *Lumbricus terrestris* pot contained a single worm, the *Lumbricus rubellus* pots contained four worms, and the *Aporrectodea caliginosa* pots contained five worms each.

To prevent earthworms from escaping all of the pots were placed in a larger bin (Figure 1) and both the inside edges of the individual pots and the interior of the larger bin containing the pots were coated with Teflon lubricant (D. Cray, unpublished data). After 42 days, the plants were removed from the pots, oven dried at 60°C, and weighed to compare biomass among the various treatments (Edwards and Lofty 1978).

Germination Experiment

To test germination rates between various earthworm treatments, 200 red maple seeds were placed in four pots. Three pots contained a treatment of an earthworm species and one had no earthworms for a control. *Lumbricus terrestris, Aporrectodea caliginosa,* and *Lumbricus rubellus* earthworms were used. Each pot contained an equal biomass of earthworms, consistent with the biomass for the growth treatment. After 19 days, the seeds were dug up and the numbers of germinated seeds were counted (Asshoff et al. 2010).

Earthworm density and sapling abundance survey

I identified 20 areas of UNDERC dominated by maple forest and sampled 3 random locations within each forest. At each location, a 25 cm x 25cm (0.0625 m^2) metal square was place on the ground and the red and sugar maple saplings within the square were counted and their height was measured (Gundale 2002).

As well, earthworm density at these sites was estimated using liquid extraction (Gundale 2002). For each plot, 40 g of yellow mustard powder was mixed with 3.8 L of water. The yellow mustard solution irritates earthworm's epidermis and causes them to move above ground. After the mixture is poured over the area, all earthworms that surfaced within 5 minutes were collected. Earthworms were identified to species then placed in 95% ethanol to preserve them. Since many of the earthworms collected were juvenile and *L. rubellus* and *L. terrestris* are impossible to distinguish as juveniles, I grouped all *Lumbricus sp.* worms together. The collected earthworms were dried in an oven at 60°C and ashed at 500°C to obtain the ash free dry mass (AFDM) (following methodology described in NRRI 2006).

Statistics

The growth biomass data, sapling abundance and height data, and earthworm density data were tested for normality using Shapiro-Wilk's test and data found not to be normal was transformed accordingly. To analyze the results of the growth experiment, a one-way ANOVA was run with the treatments as the independent variable and total biomass as the dependent variable. A Fisher's LSD post–hoc test was then performed to determine which groups differ significantly. A chi-squared test was run to compare the proportion of germinated seeds within the different pots. Four correlations between both total AFDM or *Lumbricus sp.* AFDM at the forest sites and both the abundance and height of red and sugar maples were run to see if earthworms significantly affect the growth and abundance of the maple species on the UNDERC property.

Results

Total biomass between treatments in the growth study was found to be significant ($F_{3,36}$=3.27616, p=0.031973, Figure 2). A Fisher's LSD test revealed significant differences between the *Aporrectodea caliginosa* and *Lumbricus terrestris* treatments (p=0.0338) and the *Aporrectodea caliginosa* and control treatments (p=0.01869). All other groups did not differ significantly.

The germination rates did not significantly differ (p=0.745). Out of the 200 seeds placed in the individual pots, 3 seeds in the control pot, 5 seeds in the *Aporrectodea caliginosa* pot, 6 seeds in the *Lumbricus terrestris pot* and 6 seeds in the *Lumbricus rubellus* pot germinated, respectively.

A correlation run between the transformed data for earthworm density and sapling abundance was not significant (p=0.7297., r^2=0.00679, Figure 3). A correlation looking at total earthworm density and sapling height was also not significant (p=0.519, r^2=0.023, Figure 4). Correlations run between the densities of *Lumbricus sp.* of earthworms and both sapling abundance (p=0.7538, r^2= 0.0056, Figure 5) and sapling height (p=0.4908, r^2=0.0267, Figure 6) were both non-significant as well.

Discussion

My hypothesis that there would be a negative influence from the earthworms compared to the control on the growth of *A. rubrum* was not supported. The differences found were that *Aporrectodea caliginosa* treatments had a higher total biomass compared to both the control and the *Lumbricus terrestris* treatments. *Lumbricus terrestris* also tended to have a lower biomass than the control; however said trend was not significant (Figure 2). These results are interesting because they show that areas possibly dominated by *Aporrectodea caliginosa* may help *A. rubrum* seedling growth compared to areas that are more highly dominated by *Lumbricus terrestris* and compared to those forests that do not have any earthworms. This positive influence found by the presence of *Aporrectodea caliginosa*, as well as the lack of any significant negative influences of the earthworm treatments when compared to the control deviates largely from the drastic negative effects that earthworms are known to have on *A. saccharum* growth and biomass (Hale et al. 2007). *A. rubrum* may be able to coexist well with these earthworms and the data suggest that *A. rubrum* is either not effected or is helped by the presence of earthworms. This observation has implications for forest management and community structure. If earthworms continue to increase in density and their invasion in the area, *A. rubrum* might begin to outcompete *A. saccharum* in forested areas invaded by earthworms.

This study was limited by time, but if plants were grown over many months, even possibly years, long-term effects that earthworms might have could be quantified. Additionally, due to the varying effects seen between *Aporrectodea caliginosa* and *Lumbricus terrestris* it would be advantageous to grow plants within mixed earthworm species treatments to test the combined effects. For instance, if the presence of both *Aporrectodea caliginosa* and Lumbricus *terrestris* would cancel the effects out or if one species would be dominate in their influence.

Since the seedlings in this study were planted in homogenous potting soil, this study focused on the effects of earthworms on topsoil and their mixing effects of various soil layers was not a factor. However, since seedlings are rooted in the very top layer of soil (Hale et al. 2006) and this study was simply looking at the overall effect of biomass change with the presence of earthworms, the results still show an important trend. Future studies should be done in more natural environments to confirm the findings of this study.

The germination experiment yielded no significant results. Germination did not change between the various treatments. This is opposite of other studies done that demonstrated a decrease in germination when earthworms were present (Asshoff et al. 2010). This result again suggests that *A. rubrum* is likely to not be as affected by worm invasions as *A. saccharum*. However, this study was done in a very short period of time and was only able to run for 19 days. Even though the literature germination rate of red maples is 10-15 days (Abbot 1974), the germination observed in the growth pots suggested that normal germination time for the seeds being used was more around 20-25 days. Therefore, if the germination experiment ran for a longer period of time and with more trials, the data might have shown a difference. Also, some mortality was seen in the earthworm pots, specifically within the *Lumbricus terrestris* treatments and these deaths may have affected the results though dead individuals were replaced immediately whenever detected.

The relationship demonstrated in other studies (Corio et al. 2009, Holdsworth et al. 2007, Hale et al. 2007) comparing earthworm density and abundance as well as height of maple saplings was not demonstrated on the UNDERC property. The data showed no trends between the height, abundance of maple saplings, and both the overall earthworm density as well as just the density of *Lumbricus sp*. All of these correlations showed no relationship between the two factors. This result is highly contradictory to other studies on this subject. A couple things could explain these results. For one, UNDERC does not have many sites with a "high" density of earthworms, though all sites were occupied. If more plots were sampled with higher earthworm densities, a relationship may have been found. Also, like much of the Upper Great Lakes forest, the UNDERC property has been subject to over browsing by white tailed deer (*Odocoileus virginianus*) for decades, which negatively affects maple sapling/seedling growth (Kraft et al. 2004).

Overall, this study suggests that *Acer rubrum* seedlings are not affected negatively by earthworms and may actually be affected positively by their presence. Other studies doing work with maple seedlings and earthworms have found negative relationships; however all of these studies have focused on a different species, *Acer saccharum*. This study is the only one looking at the effects on *Acer rubrum* and therefore its results open an avenue for future studies and research on why these differences occur and how these findings may affect forest management policies and the future composition of Great Lakes forests.

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Figures

Figure 1. The set up of the *A. rubrum* growth experiment showing how the 10 individual pots were placed within a larger bin to prevent the earthworms from escaping.

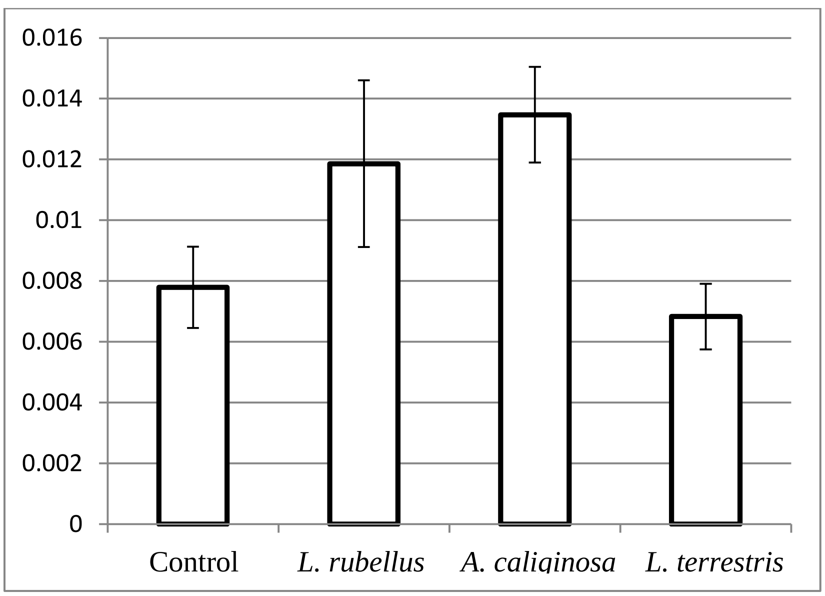

Figure 2. A bar graph showing the averages of total biomass (g) and the standard errors for the *A. rubrum* growth experiment for each earthworm treatment.

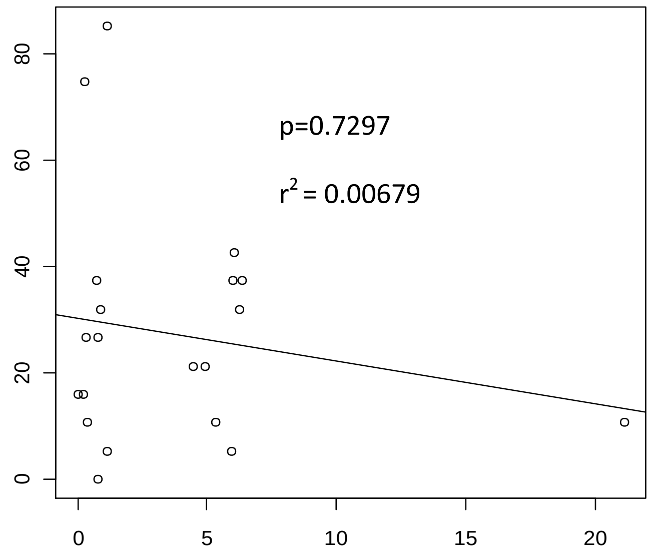

Figure 3. A correlation between abundance of *Acer* saplings (per m^2) on the y axis and total earthworm density (AFDM per m^2) on the x axis.

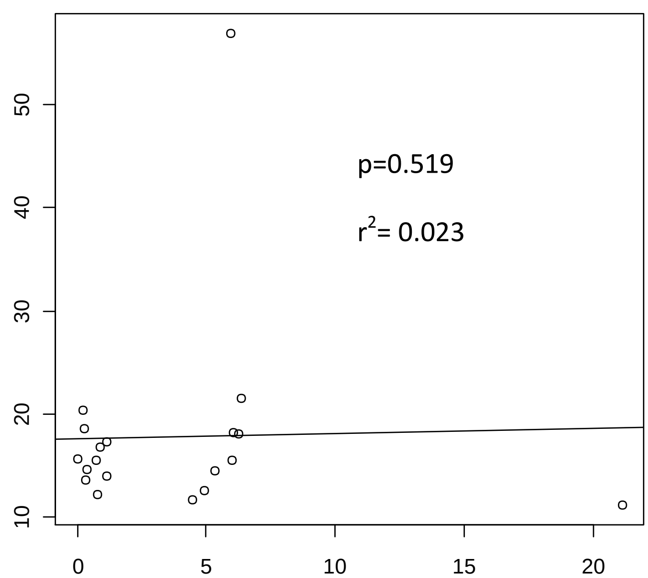

Figure 4. A correlation between the height of *Acer* saplings (cm) on the y axis and total earthworm density (AFDM per m^2) on the x axis.

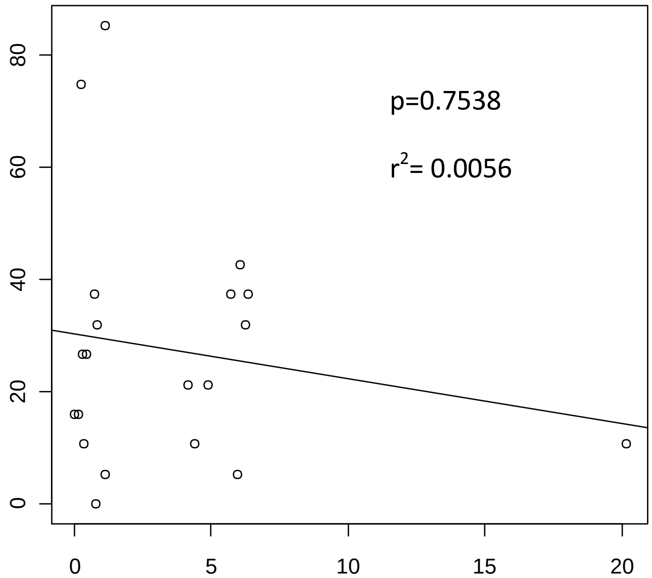

Figure 5. A correlation between the abundance of *Acer* sapling (per m^2) on the y axis and total density of *Lumbricus sp.* (AFDM per m^2) on the x axis.

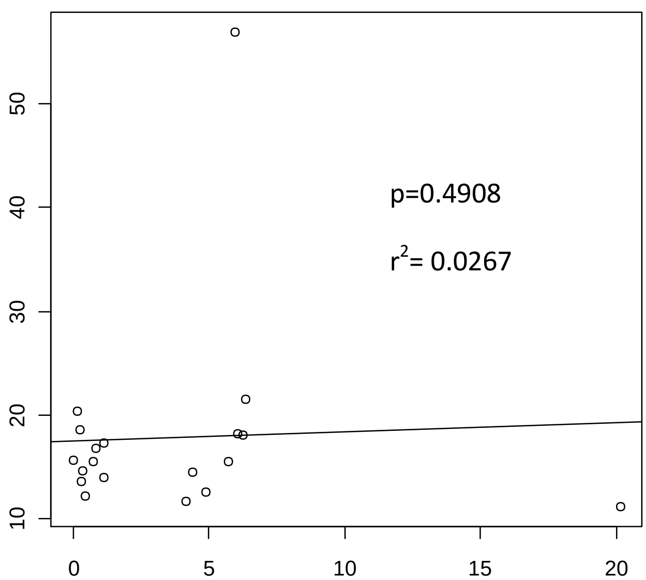

Figure 6. A correlation between the height of *Acer* saplings (cm) on the y axis and the total density of *Lumbricus sp.* (AFDM per m^2) on the x axis.