Report: Les Mehrhoff Botanical Research Fund
Invasive earthworms and plant diversity in Massachusetts’s forests
Timothy J. S. Whitfeld, October 6, 2015

Background: The goal of the proposed research project was to establish a series of survey plots to test the hypothesis that invasive earthworms have a direct, negative impact on native plant diversity. The study site was at Ice Glen Preserve in Stockbridge, Massachusetts where there is a clear demarcation between areas of high and low earthworm abundance. Based on the amount of leaf litter and organic “duff” at the soil surface, I was able to identify these areas during an early spring scouting trip. Earthworms consume the duff layer, so in the spring, last year’s leaf litter lies directly over mineral soil (Figure 1A and 1B).

![Figure 1. (A) area with low earthworm abundance showing last year’s leaf litter, partially decomposed organic “duff”, and mineral soil. (B) area with high earthworm abundance (note earthworm burrows) lacking leaf litter and organic duff.](image)

Methods: In June 2015, with assistance from two Brown University undergraduates (Sophie Duncan and Andrew Pizaturo), we located six survey areas at Ice Glen. Based on the early spring leaf litter and duff layer observations, three of the survey areas were placed where earthworm abundance appeared to be high and three where it appeared to be low. At each site, we established three circular survey plots (each 10 m diameter, 18 plots total) by taking a random compass bearing (between 0° and 360°) from the estimated center of each site and a random distance (between 0 m and 50 m) to locate each plot. Within each survey plot we identified and measured the dbh (diameter at breast height) of all trees and shrubs ≥ 1 cm diameter and estimated percent cover of each species. In a 4 m diameter nested plot located at the center of each 10 m plot we identified all herbaceous species and estimated percent cover for each. We also walked through the 10 m plot and compiled a list of herbaceous species found during a standardized 10 minute search. Finally, we sampled earthworm abundance at two points just outside the 10 m plot, at 0° and 180° from the plot center. At each earthworm sampling point we cleared leaf litter from inside a 35 cm² plot frame and into this area we poured 1 gallon of mustard solution (1 gallon water, 40 g of mustard powder) in three stages over 15 minutes (Figure 2). As earthworms emerged from the soil, they were collected in ethanol for later identification. Finally, we took soil samples from three points just outside each 10 m plot at 0°, 120°, and 240° from the plot center for later analysis of texture and pH. We also recorded slope angle and slope aspect at each plot and took hemispherical canopy photographs, using a fish-eye lens, at the center of each...
plot as a proxy for light intensity (Figure 3). At the end of August, we repeated the herbaceous plant surveys in each plot, also resampled earthworms (this time at 90° and 270° from the center of each plot), and took a second set of hemispherical photographs.

![Figure 2. Earthworm sampling using the mustard extraction method](image)

![Figure 3. Example of a hemispherical canopy photo, used for estimating light levels in each plot](image)

**Preliminary data (n.b., these data are from the June surveys only):** we documented a total of 67 vascular plant species across all survey plots (27 woody species and 40 herbaceous). One of these, *Panax quinquefolius* (American ginseng), we reported to the Massachusetts Natural Heritage and Endangered Species Program since it is listed as rare in the state. We documented each species with a voucher specimen, deposited in the Brown University Herbarium (BRU). In addition, we collected 327 earthworms. Analysis of earthworm abundance suggests a significant difference between the high and low earthworm survey areas identified from the springtime observation of leaf litter and duff layers (Figure 4).

![Figure 4. Average earthworm abundance in survey plots located in areas of putative low and high abundance, based on a springtime visual assessment of leaf litter and organic duff (p < 0.01)](image)
Furthermore, our preliminary analysis of mean herbaceous species density in the low and high earthworm environments, supports the hypothesis that earthworm abundance influences plant diversity (Figure 4A). Overall earthworm abundance was also significantly related to herbaceous species density (Figure 4B).

![Figure 4A](image1)

![Figure 4B](image2)

Figure 4. (A) Herbaceous species density (i.e., species per survey plot) in low and high earthworm sites (p < 0.01). (B) Herbaceous species density versus earthworm abundance ($R^2 = 0.24, p = 0.04$)

**Further work:** after processing is complete, plant and earthworm data from the August census will be incorporated into a comprehensive analysis. We will include earthworm abundance, soil texture, soil pH, light, slope angle, and slope aspect data in an overall statistical model predicting the diversity of vascular plants. In addition, we will generate a phylogeny of all plants in each plot (using the online tool Phylomatic) to estimate the effects of earthworm abundance on plant phylogenetic diversity. All analyses will be included in a manuscript to be submitted for publication in a peer-reviewed journal.