The Effect of Invasive Earthworms on Ectomycorrhizal Diversity and Nutrient Cycling
Edna B. Sussman Internship Final Report 2012

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Proposed Study

There are no native earthworms in glaciated portions of North America. In the absence of large detritivores, northern forests have been characterized by a slowly decomposing, thick litter layer and organic horizon. European and Asian earthworm invasions have caused ecosystem-level changes that have begun to be well-characterized in the last decade. Introduced earthworms quickly decimate leaf litter, homogenize the soil profile, and alter soil hydrology by creating macroaggregates and burrows. These ecosystem-level changes can cause cascading effects on the forest community; seedling recruitment, plant invasion, herbaceous plant diversity, arthropod diversity, and tree growth have all been shown to be affected by earthworm invasions.

All North American trees have obligate relationships with mycorrhizal fungi. Trees transfer photosynthate-derived sugars to these fungi through their fine roots, and in exchange mycorrhizae mobilize soil-derived nutrients and water to the plant roots. This intimate mutualism is essential to tree survival. Different mycorrhizae likely occupy different niches in the soil environment, specializing in attaining different forms of essential nutrients (N, P, K, Ca, etc) in different soil conditions. I was interested in examining the effect of invasive earthworms on the diversity of ectomycorrhizal fungi associating with host trees (*Tsuga*, *Betula*, *Quercus*, and *Fagus* spp. especially) in northeastern forests. I hypothesized that soil homogenized by invasive earthworms would result in a lower diversity of ectomycorrhizal fungi, due to a decrease in the number of unique belowground niches.

The purpose of the proposed work was to establish research sites around the northeastern United States in areas that have been partially invaded by earthworms. This research was partially funded by Mianus River Gorge Preserve (Bedford, NY) through their research assistantship program. In addition to Mianus River Gorge, data was collected at Mohonk Preserve (Gardiner, NY), Heiberg Memorial Forest (Tully, NY) and White Mountain National Forest (Warner, NH).

Work Completed

The first half of the summer (May-July) was spent identifying sites and plots appropriate for this study. In addition to the above research sites, I visited other potential sites in the northeast, including Huntington Wildlife Forest (Newcomb, NY), Harvard Forest (Petersham, MA), Hubbard Brook (Lincoln, NH), and Bartlett Experimental Forest (Bartlett, NH). At each of the sites, I walked parallel transects from potential sites of introduction (roads, streams/ponds, agricultural fields) into the forest. The soil surface was visually scanned for evidence of earthworms (by presence/absence of macroaggregates). Of the visited sites, only Mianus, Mohonk, Heiberg, and White Mountains were deemed appropriate as study sites, with clear areas either invaded or uninvaded. At each site, 10 plots were established, with 5 invaded plots and 5 uninvaded plots.

In August, I collected data at my sites. At each of the plots, earthworms were collected using mustard extraction and preserved for later identification. Leaf litter samples were taken. Soil cores were taken and separated by horizon. Half of each soil core was processed for fine-scale nutrient analysis and the other half was preserved for mycorrhizal fungal analysis. In total, I collected 120 soil samples for later analysis of moisture, pH, C:N, total N, and P, Ca, K, Na, and Mg concentrations. I collected 120 soil samples for mycorrhizal root tip analysis, each of which will likely generate anywhere from 1-12 unique morphological types which will be identified using DNA sequencing. I collected 307 worms for later identification using taxonomic keys.

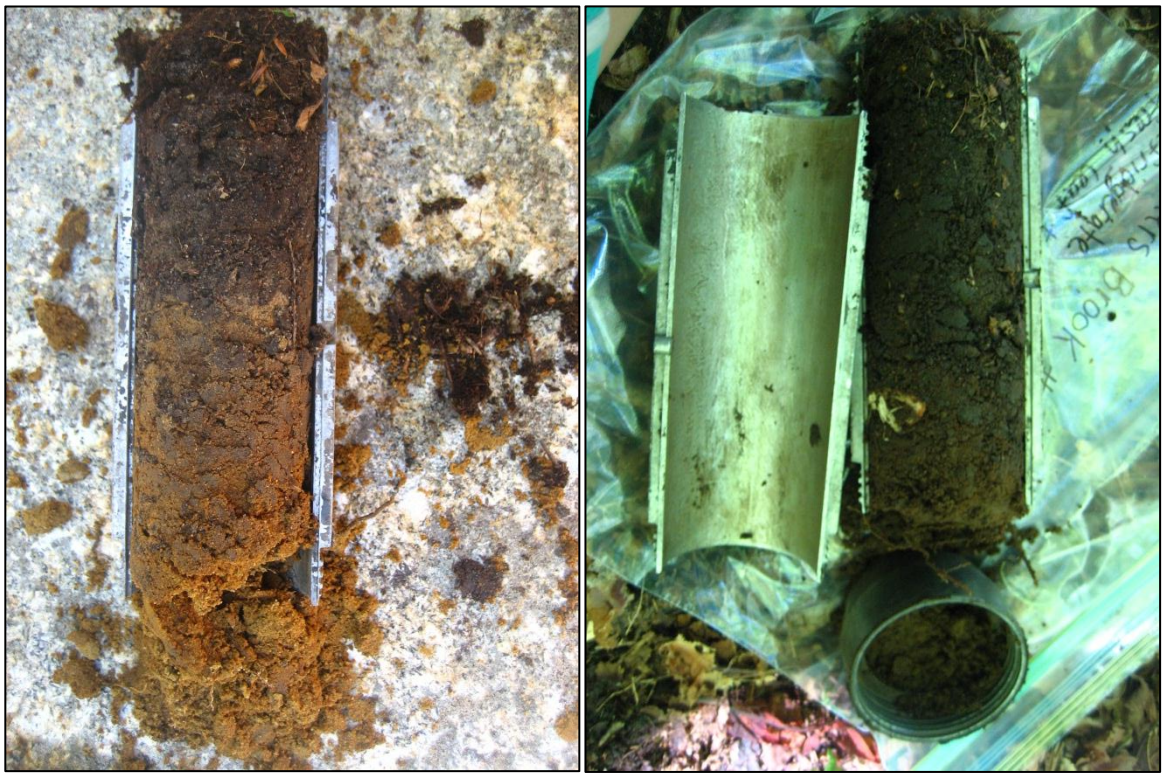
Future Work

Field work continued in September-October, during which I visited each site to resample the plots. In the lab, I weighed the dry mass of leaf litter, in order to assess the effect of earthworms on litter mass. I began my soil analysis, completing calculation of moisture and a have processed a portion of the samples using C:N analysis and cation extraction. Once soil analysis is complete, I will compare soil by horizon between invaded and uninvaded sites, in order to detect any significant differences between soil properties and homogenization. I am in the process of morphotyping the mycorrhizal root-tips collected for DNA analysis. I will begin DNA extraction by January 2013, in order to compare the mycorrhizal diversity and species assemblage by horizon and between invaded and uninvaded plots. I am also in the process of

identifying the earthworms to species (adult) or genus (juveniles). Earthworms collected differ in their ecological role, ranging from surface-dwelling detritivores to geophagous mineral soil-dwellers.

In summer of 2013, I will return to each of the four selected sites to further characterize the effect of earthworms on the aboveground community. I will measure tree species, DBH, and spacing within 10 m of each plot center. I will also complete seedling and herbaceous plant surveys in sampling quadrants.

I would like to thank my advisor Tom Horton, Mianus River Gorge Preserve, and the Edna B. Sussman Foundation for supporting my research project. I will continue to acknowledge the support of the Sussman Foundation in any future publications or presentations.



Soil from an uninvaded site, with clear soil horizons (left) vs. soil from an invaded site, with more homogenous horizons (right)

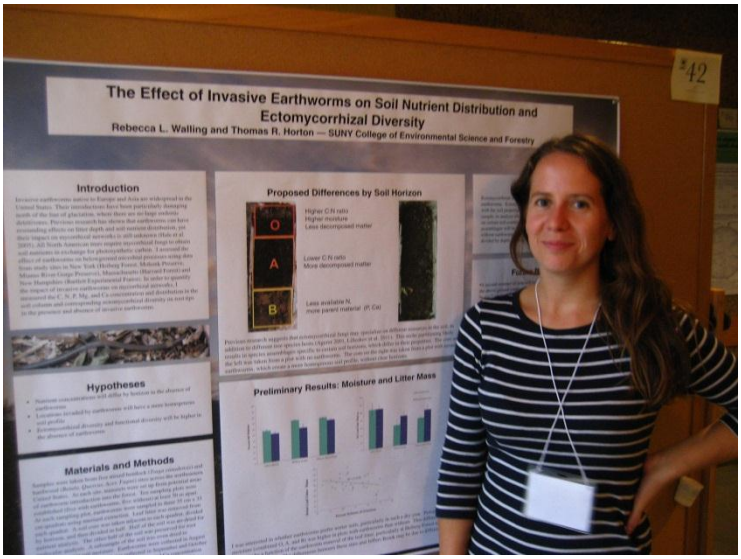
Soil samples being processed for moisture analysis in the lab.

Amynthas spp., an invasive earthworm from Asia, at Mianus River Gorge Preserve

In order to extract earthworms, I poured a slurry of hot mustard powder and water on the soil surface. Worms surfaced within minutes. Here, I am carrying 6 gallons of water to study plots in Harvard Forest, MA.

This *Boletus* spp. is an example of an ectomycorrhizal fungus that may be identified using DNA analysis on a collected root-tip.

Amanita spp. is another ectomycorrhizal fungus that associates with tree roots.



I presented my research in a poster at the University of Massachusetts conference on Plant-Microbe Interactions.