

**The Influence of Tree Species on the Activity and Impacts of
Invasive Earthworms**

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Introduction

Most talk of invasive organisms refers to the more noticeable, aboveground invaders. However, we must focus on underground species as well, for they have just as much impact as any other organisms.

One of the most important underground organisms is the earthworm. Charles Darwin once said of the earthworm, “It may be doubted whether there are many other animals which have played so important a part in the history of the world, as have these lowly organized creatures” (Darwin, p. 316, 1883). Earthworms are the foundations of many of the world’s ecosystems. But what happens when these crucial organisms are invasive?

Most regions in the Northern United States and Canada have previously never had any native earthworms, and are entirely dominated by invasive species (Bohlen and Groffman, 1999a). About 11,000 to 14,000 years ago, during the Ice Age, glaciers covered most of the Northern U.S., killing off any native earthworms that may have existed (Hale, 2006a). When the glaciers finally receded, forests in many areas in the Northern U.S.A and Canada developed ecosystems completely devoid and independent of earthworms.

Over the past 200 years, however, earthworms have been traveling farther and faster than they ever could have naturally. Invasive earthworms were most likely brought to the earthworm-free regions of North America in the 1800s when European settlers first arrived and dumped their ballast out onto the New World (Hale, 2006a). Since then, a wide variety of human activities have helped spread earthworms throughout the world, from fishing (where unused bait is carelessly dumped out), to the transportation of mulch and soil.

While earthworms move very slowly, their impacts are very clear. Beneficial activities of earthworms (whether invasive or native) include the decomposition of biomass into nutrient-rich castes (fecal matter), the stimulation of microbial activity, increased nutrient cycling, and the

mixing and aggregation of soil and the increase of soil porosity, which improves the flow of water through the soil (Bohlen and Groffman, 1999).

However, invasive earthworms also have many detrimental activities. It is the goal of current scientists to identify the harmful activities of earthworms, study how these activities are changing forest ecosystem and observe whether the harmful activities outweigh the beneficial ones, and to eventually develop plans to combat these changes.

Bohlen and Hendrix (2002) have highlighted many of the possible negative effects of earthworms. Overactive worm populations can reduce the forest floor, leaving only their fecal castes, which increase soil erosion and make it difficult for new tree and plant seedlings to develop. Earthworm activities also control decomposition in many forests, and too much or too little decomposition can upset the balance of the forests. Additionally, earthworm burrowing activities can harmfully alter the structure of the soil. Deep tunnels, especially those made by the European earthworm, *Lumbricus terrestris* increase nutrient leaching. That is, the tunnels make it easier for rainwater to wash nutrients down to lower levels of the soil where they are unavailable for uptake by plants. Lastly, earthworms and their castes alter the soil nutrient content of the soil. When earthworms consume organic material, they recycle nutrients and release them in the form of fecal castes. When earthworms recycle nutrients, they may be altering the soil nutrient content of the soil enough to harm native plants that require specific, constant nutrient levels. Bohlen and Groffman pointed out (1999a, page 144) that “subtle changes in soil biology can lead to major changes in ecosystem function over large scales” (see

Figure 1).

Figure 1: Invaded vs. Uninvaded Forests (Hale, 2006a)



There are three vital nutrients for plants that many scientists study: Nitrogen, Phosphorous, and Potassium. Scientists have studied the impacts of earthworms on Carbon cycling (Bohlen *et al.*, 1997), but I will mainly be focusing on N, P, and K levels.

Nitrogen is required for the synthesis of proteins, protoplasm, and enzymes, and is a main component of chlorophyll (<http://www.ballance.co.nz/thbook.html>). Bohlen *et al.* (1999b) studied the possible influences of earthworms on Nitrogen cycling. When placed in organic matter rich with nitrogen, such as rye and manure, earthworms thrived and rapidly increased nitrogen cycling. It is clear that active earthworm populations will recycle most available nitrogen. However, many forms of nitrogen (especially nitrate) are highly leachable and can be washed to lower levels of the soil, counteracting any seemingly beneficial recycling the earthworms may have performed (Cindy M. Hale, written communication, 2006b).

This information, however, is not definitive because all forests are different. This is an extremely important point for all scientists to take into consideration. Hale and Host (2005, page 1) noted that “little data exist” regarding the distribution of different invasive earthworm species throughout different types of forests, and their impact on soil structure in forests other than those dominated by Sugar Maple. Clearly, it’s important to recognize that what happens in one forest may not happen in another forest. It is therefore imperative that scientists study a variety of different forests composed of different tree species and earthworm species, in order to gain as large a picture as possible of the impacts of earthworm invasion.

Holdsworth (2006) researched forests in Minnesota and found that the type of leaf litter available (that is, the type of trees that were present) directly affected the activity of invasive earthworms. The degree to which the earthworm populations had grown and the effects that earthworm activity had on the forest both correlated to the type of leaf litter that was present.

Hobbie, *et al.* (2006) came to similar conclusions while researching in Southwestern Poland. They studied areas with fourteen different tree species and found that the rate of removal of the forest floor by earthworms was positively related to the type of leaf litter (specifically Ca content of the leaf litter).

Suarez, *et al.* (2006) also sought to study the effects of different invasive earthworms on the decomposition of leaf litter in areas composed of different tree species. The study found that the impacts of earthworms on litter removal and cycling vary greatly as a function of species of earthworms present.

Additionally, Hale and Host (2005) noted that the different feeding preferences of earthworms can lead to varied impacts when they invade. These scientists studied forests dominated by different tree species: sugar maple, beech-maple, and aspen-fir. They concluded that the beech maple forests supported half as much total earthworm biomass as the sugar maple forests, and the aspen-fir forests supported half as much earthworm biomass as the beech maple forests.

The most important paper I came across encompassed every concept discussed in the previous papers and introductory information. Suarez, *et al.* (2003) studied the effects of exotic earthworms on phosphorous cycling in two temperate deciduous forests dominated by different tree species. Phosphorous is a major limiting factor for most plants (<http://www.ballance.co.nz/thbook.html>), and, in forest ecosystems especially, is important seedling germination, young leaf and root growth, and fruit development. Suarez, *et al.* looked at forests in New York both invaded and uninvaded by exotic earthworms. They noticed that areas invaded by earthworms had lower total P in the upper 12cm of the soil than did uninvaded areas. They hypothesized that this P disparity may be attributable to the activities of the earthworms. The earthworms *did* recycle phosphorous, but the phosphorous that they made

available was highly leachable and was easily washed away with water. Additionally, when this phosphorous was leached, it moved to lower levels of the soil and bound with charged soil particles, essentially leaving the ecosystem and becoming unavailable to plants. Phosphorous is a known limiting factor and this leaching could lead to loss of native plant and tree species. However, this is not conclusive; there was a lot of variation between different study sites, and more studies are needed.

This paper and the others mentioned above correlate very closely with my own research. They help to solidify my questions (and possibly my data) regarding the forests near where I live. I live in Westchester, NY, which is encompassed by temperate deciduous forests that are inhabited by earthworms. Just outside my school are two such forests. As I thought about these forests, I began to wonder about the earthworms that were busily tunneling beneath the soil.

In my project, I therefore sought to address many questions regarding these earthworms and the forests. Firstly, are invasive earthworms present in the forests? If so, do the types of deciduous tree species present in the forests influence the species, population numbers, and activity of the invasive earthworms present in the forests? If so, how do these differences in activity affect the soil nutrient content of the forests?

It was hypothesized that, most likely due to the different tree species present, the numbers and species of exotic earthworms, as well as their impacts on soil nutrient content, would vary between the two forests.

Materials and Methods

Transects: First, two forests dominated by trees of different species were located within the vicinity of Horace Greeley High School (my school). The first forest (**Forest 1**), located parallel to the Metro North railroad tracks and the Saw Mill River Parkway, is dominated by a

canopy of Red Oak, Black Oak, and Black Birch. The sub-canopy is composed of small maple seedlings, some small Basswood trees, and small oak species.

The second forest (**Forest 2**), located along Route 117 in Chappaqua, NY, is dominated by a canopy of White Ash with a sub-canopy containing some small maple seedlings, and small Ash species.

Forest 1 (Oak and Birch) is on somewhat of a slope, so two 50m transects were created in the forest to account for the differences in elevation (one transect at the top of the slope, another at the bottom). .5m by .5m cardboard squares were placed every 5m along each transect in order to make the transects visible. Each cardboard square was mapped using GPS to create an aerial map (see **Figure 2**). These two transects in Forest 1 will be known as the **Lower** transect and the **Middle** transect.

In the forest dominated by Ash (Forest 2), the same type of transect was constructed and mapped using GPS. However, this was a much smaller forest and did not have a slope, therefore only one transect was necessary. This transect will be known as **Phil** (named after a fellow student who lives nearby). All factors, such as worms, soil, and fallen biomass, were collected along these transects.

Earthworm Collection Transects



Figure 2: Transects located in forests surrounding Horace Greeley High School in Chappaqua, NY: Transect farthest in the upper left is **Lower**; Transect directly to the right of that is **Middle**; transect all the way on the bottom right is **Phil**.

Earthworms: Random spots along each transect were selected to find earthworms. At each spot, a 2/3 m by 2/3 m square of land was cleared of leaves and biomass. Then, approximately 2 gallons of hot mustard solution (see Hale, 2006a for formula) was poured over the ground and allowed to soak in. After the solution had soaked into the ground for ten to fifteen minutes, all worms that had risen up out of the soil were collected. I dug through the first few inches of soil in order to get any extra worms that hadn't completely risen. This procedure was repeated at each transect.

Then, all worms from each transect were brought back to the lab, where they were anesthetized in rubbing alcohol, and then killed and preserved in formalin in labeled test tubes. Lastly, worms were counted and identified using a key provided by Dr. Cindy M. Hale of the University of Minnesota.

Soil Temperature: Two electronic soil temperature loggers were placed at each transect: one approximately .5m beneath the soil at each transect, and another on the surface of the soil. They are very independent and log the temperature every hour for up nine months.

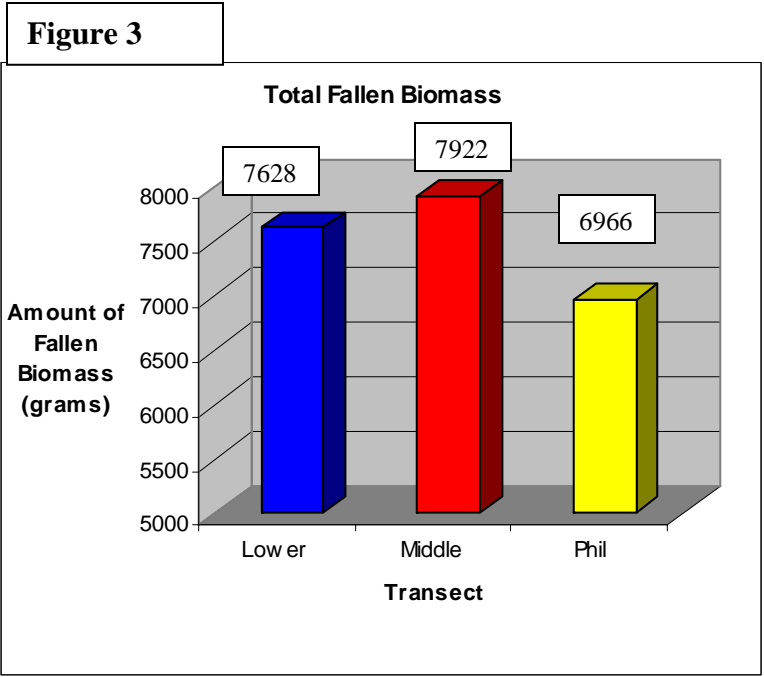
Soil Nutrient Content and pH: Soil cores were collected at random locations along each transect using an approximately 13cm long soil core digger. Multiple cores were collected and mixed together, to get a general idea of the upper 12-13cm of soil at each transect. Soil samples were then dried at 100 degrees Fahrenheit overnight. When completely dry, soil samples were tested for Nitrogen, Phosphorous, Potassium, and pH levels using a HACH-NPK1 soil test kit and its procedures.

% Soil Moisture: Similarly, enough soil cores were gathered from the upper 12-13 cm of soil at each transect and mixed in order to properly represent the transect. The soil from each transect was then weighed, and oven dried at 100 degrees Fahrenheit. When samples were completely dry (usually after 2 days), they were weighed again. The difference between the pre-dried and dried weights equaled the amount of water that had been in the soil.

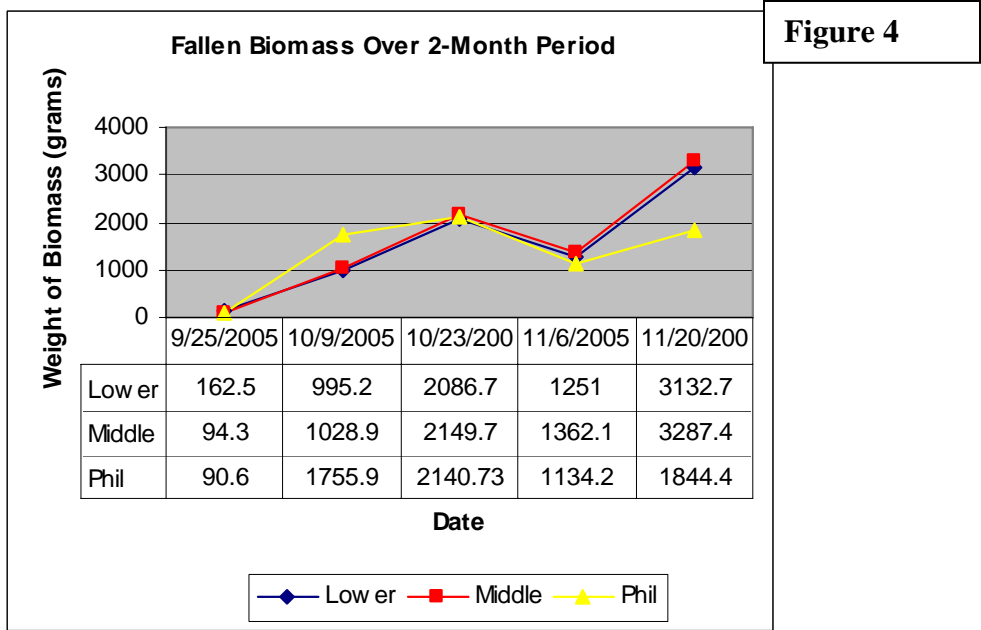
Fallen Biomass: One approximately 3m by 3m enclosure was set up along each transect. Every two weeks, all fallen biomass that had gathered in each enclosure was collected and brought back to the lab. Biomass from each transect was left under the same conditions (in black garbage bags, next to a warm radiator) to dry for several days. When all leaves inside the bags felt dry to the touch, the amount of fallen biomass was then weighed.

Results

Fallen Biomass: Biomass was collected at each transect over a 3-month period when leaves fell the most (9/25/05-11/20/05). Total fallen biomass at each transect and fallen biomass over the 2-month period were calculated and graphed (see **Figures 3 and 4**).



Statistical Analysis using ANOVA (Analysis of Variance between groups) yielded a P= 0.95857.
 -No statistical difference in total fallen biomass between transects.



Temperature: Temperature was logged every hour starting on 3/26/06 at 11:00am and ending on 6/28/06 at 1:00 pm. Average temperature (°F) for each month on and under the surface at each transect was calculated and put into tables (see **Tables 1 and 2**)

Table 1:

	Lower Surface	Middle Surface	Phil Surface
March (26-31)	48.23°F	45.08°F	47.15°F
April	50.19°F	49.28°F	49.32°F
May	58.13°F	56.89°F	57.88°F
June (1-28)	65.53°F	64.18°F	64.09°F

Table 2:

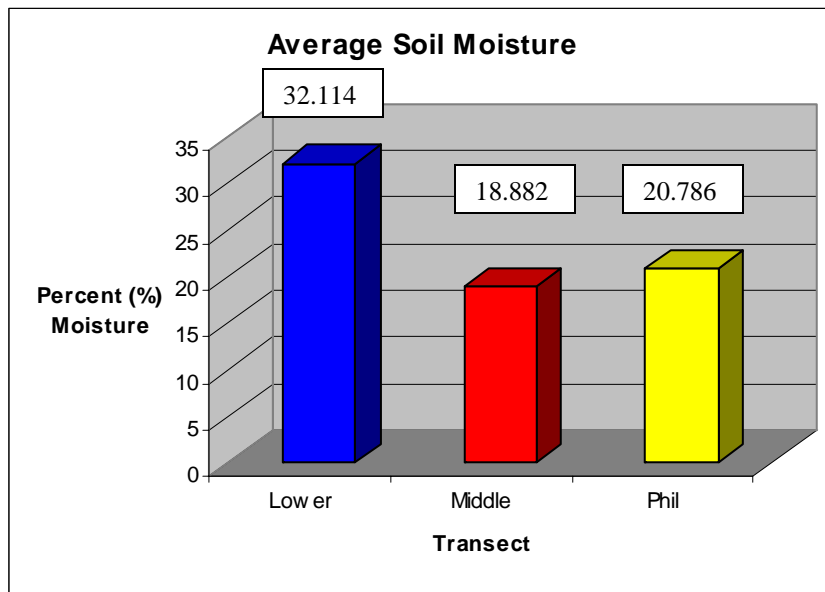
	Lower Under	Middle Under	Phil Under
March (26-31)	41.83°F	41.41°F	40.64°F
April	46.94°F	46.49°F	46.52°F
May	53.22°F	52.92°F	52.58°F
June (1-28)	60.39°F	60.07°F	60.47°F

- ANOVA for the average temperature on the surface at each transect yielded a P=0.95854.
- ANOVA for the average temperature under the soil at each transect yielded a P=0.99543.
- No statistical difference in soil surface temperature or temperature beneath the surface at each transect.

% Soil Moisture: % Soil moisture was calculated once a month over a five month period (from 4/22/06 to 9/10/06). See **table 3** for % soil moisture every month. See **figure 5** for a graphical representation of average soil moisture.

Table 3: % Soil Moisture at Each Transect over a 5-month period.

	Lower	Middle	Phil
4/22/2006	39.03	18.94	24.3
5/7/2006	35.2	17.48	18.76
6/6/2006	32.04	17.53	21.48
7/10/2006	29.5	19.06	18.3
9/10/2006	24.8	21.4	21.09

Figure 5:

-ANOVA for the soil moisture at each transect over the 5-month period yielded a $P=1.40188 \times 10^{-4}$

-Statistical difference in soil moisture at each transect.

Soil Nutrient Content and Soil pH:

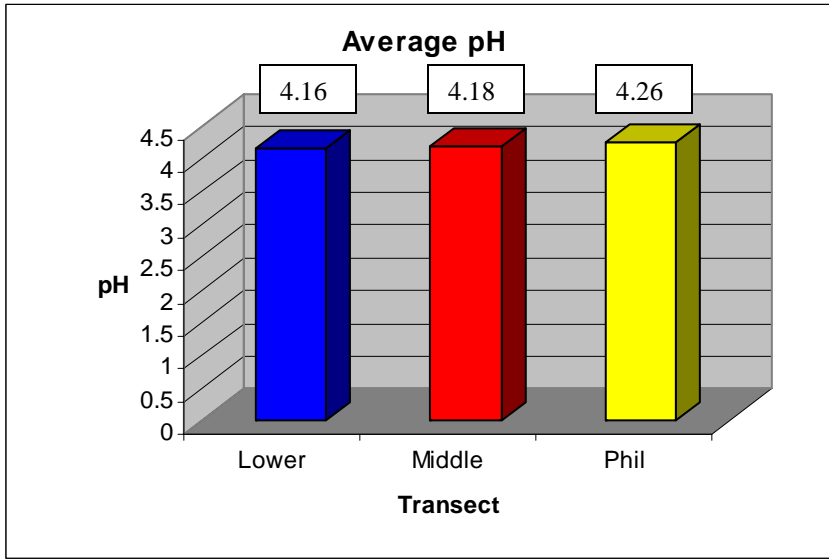
Soil pH and Soil Nutrient content were tested, along with soil moisture, once a month over a 5-month period. Soil pH at each transect can be seen in **figure 6**, below.

Nitrate-Nitrogen content consistently read 0 mg/L and will be disregarded in this study.

Potassium content also had mixed results, and questions were raised as to the accuracy of my tests. Potassium will also be disregarded in this study. These issues will be discussed further in the next section.

Phosphate-Phosphorous content can be seen in **table 4** and **figure 7**, below.

Figure 6



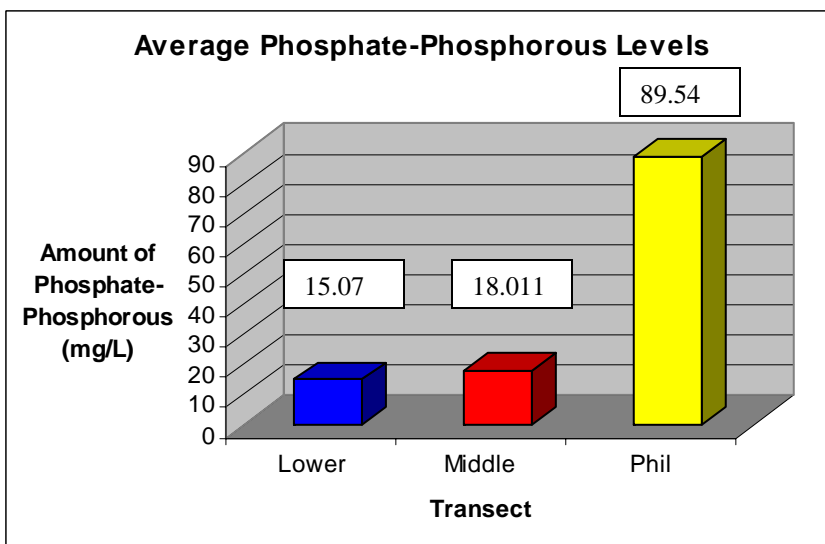
-ANOVA for average pH at each transect yielded a P= 0.26214

-No statistical difference in soil pH between each transect

Table 4: Soil Phosphorous Content at each transect over the 5-month period (mg/L)

5/7/2006	15.4	18.315	83.6
6/6/2006	15.38	17.59	89.1
7/10/2006	14.85	18.4	97.35
9/10/2006	15.4	16.5	86.9

Figure 7



-ANOVA for average Phosphate-Phosphorous yielded a P= 5.41616×10^{-14}

-Large statistical difference in Phosphate-Phosphorous levels between the two forests.

Earthworms:

Earthworms were collected at each transect over a three-month period (May, June, and July 2006). See **tables 5, 6, and 7** for the numbers and species of earthworms that were found.

Table 5: May

	Lower transect	Middle transect	Phil transect
<i>Dendrobaena octaedra</i>	30 Juveniles 3 Adults	32 Juveniles 4 Adults	18 Juveniles
<i>Lumbricus rubellus</i>	0	0	2 Adults
<i>Lumbricus terrestris</i>	2 Juveniles	1 Adult	0
<i>Aporrectodea rosea</i>	0	0	1 Adult
TOTAL	35	37	21

Table 6: June

	Lower	Middle	Phil
<i>D. octaedra</i>	25 Juveniles 2 Adults	18 Juveniles 3 Adults	5 Juveniles
<i>L. rubellus</i>	0	0	2 Adults
<i>L. terrestris</i>	0	2 Adults	0
<i>A. rosea</i>	0	0	0
TOTAL	27	23	7

Table 7: July

	Lower	Middle	Phil
<i>D. octaedra</i>	17 Juveniles 2 Adults	12 Juveniles 3 Adults	6 Juveniles
<i>L. rubellus</i>	0	0	2 Adults
<i>L. terrestris</i>	2 Adults	1 Adult	0
<i>A. rosea</i>	0	0	1 Adult
TOTAL	21	16	9

Statistical analysis using ANOVA in order to test the differences between the transects with regards to total number of worms in each transect over the 3-month period (regardless of species) yielded $P = 0.15042$.

-No statistical difference in total number of worms at each transect each month.

Discussion:

The fallen biomass data and ANOVA indicate that the amount of fallen biomass present in each forest is not significantly different ($P= 0.95857$). However, the tree species located in each forest (Forest 1, Lower and Middle: Red Oak, Black Oak, Black Birch, Maple, and Basswood; Forest 2, Phil: White Ash, small Ash species, and Maple) were different. This implies that any differences in earthworm species and population numbers that are affected by trees are not affected by the amount of trees and biomass present, but rather the species of trees and leaf litter available to consume.

Additionally, the ANOVA for the temperature indicates that there was virtually no difference in temperature on both the forest floor and underneath the soil in all three transects (Surface, $P=0.95854$ and Under, $P=0.99543$) (see **Tables 1** and **2**). This implies that temperature was most likely not a factor influencing the species and amount of earthworms present in each forest.

ANOVA tests also indicate that soil moisture in the upper 12-13 cm of soil varied slightly between each forest ($P=1.40188 \times 10^{-4}$) (see **Table 3** and **Figure 5**). Middle and Phil transects (different forests) had similar soil moisture levels (18.882% and 20.786% respectively), while the **Lower** transect had moister soil than both (32.114%). This is understandable because the Lower transect is located at the bottom of a slope (where rainwater may drain down) and is located near a pond. However, these differences in moisture are most likely insignificant because both the Lower and Middle transects still had the same species and similar numbers of earthworms present.

Statistical analysis indicated that soil pH in the upper 12-13 cm of soil was similar in each transect ($P= 0.26214$). Soil pH was consistently low (between 4 and 4.5). However, this is

understandable because northern deciduous forests often have low soil pH due to acid rain (Frelich, written communication, 2006).

The data regarding earthworms present in each forest highlighted interesting differences between the two forests. While statistical analysis showed that the total number of earthworms present over the given time period was not significant, the presence of different earthworm species in each forest *is* significant. Both forests and all transects had populations of *D. octaedra*, a small, epigeic (litter/surface-dwelling) species. However, the large anecic (deep-burrowing) species, *L. terrestris* was present in Forest 1 (Lower and Middle transects) and absent in Forest 2 (Phil). Additionally, the epi-endogeic species (upper soil and surface-dwelling), *L. rubellus* and the endogeic (upper soil-dwelling) species, *A. rosea* were both present in Forest 2 (Phil), yet absent in Forest 1.

The fact that most other factors between the two forests were relatively stable, but the species of earthworms present were different, strengthen the argument that the difference in earthworm species may be accountable for any differences in soil nutrient content.

The soil Potassium content and Nitrate-Nitrogen content tests yielded disappointing results. Potassium content varied greatly every time I tested (e.g. Potassium content for the lower transect in April was extremely high, yet in May, there was virtually no potassium), which led me to question the validity of my tests. After consulting many resources and contacting many soil and earthworm scientists, I was unable to come up with an explanation and have decided to disregard soil Potassium in this study.

I came to similar conclusions with Nitrate-Nitrogen content. I calibrated the nitrogen tests perfectly, and knew that there was nothing wrong with my techniques this time. However, I still got readouts that said that there was consistently 0 mg/L nitrate-nitrogen in the soil at each transect in both forests. However, this was explainable. Nitrate is the most easily leached

mineral form of nitrogen (Hale, written communication, 2006b). That is, rainwater easily washes nitrogen away from the upper layers of the soil (where I was testing). Ammonium (which I wasn't testing) is far less leachable than nitrate. Also, available nitrogen is rapidly absorbed by plants and is also held in bacteria in the soil and may not have been testable using my soil test kit. Although there was plenty of information that helped account for the nitrate-nitrogen data, it would be irresponsible for me to come to any conclusions about the nitrate levels in the forests at this point in time, before further testing can validate the results.

However, the nitrogen and potassium data problems are dwarfed by the substantial implications brought forth by the Phosphate-phosphorous data. The Phil transect had substantially greater amounts of Phosphate in the upper 12-13cm of the soil than did the Lower and Middle Transects ($P= 5.41616 \times 10^{-14}$, indicates the very large difference). Specifically, the Lower transect had an average of 15.07 mg/L of phosphate, the Middle transect, had an average of 18.011 mg/L and Phil had a drastically different average of 89.54 mg/L (see **Figure 7**). Additionally, the phosphate levels at each transect were relatively consistent over the 5-month period from April to September (**Table 4**), indicating that it is unlikely that these results occurred by chance.

These different phosphorous levels are quite reminiscent of the study performed by Suarez, *et al.* (2003). They noticed that in certain invaded forests, there was far less available phosphorous than in uninvaded forests. The activity of certain earthworm species created castes with high levels of highly leachable phosphorous. When it rained, this phosphorous was washed down to lower levels of the soil where it became unavailable to plants. By looking at their paper, it is clear that the same process may be occurring in the two forests that I studied.

Conclusions:

This study aimed to look at a variety of factors within forest ecosystems in order to narrow down possible causes of existing soil nutrient levels. Most factors between the two forests were statistically similar (earthworm numbers, amount of fallen biomass, pH, and temperature). There were differences in soil moisture between the Lower transect and the others (possibly caused by the slope in Forest 1). However, the soil moisture didn't seem to have an impact on the activity of earthworms or their subsequent impacts on soil nutrient content, as both transects in Forest 1 had the same species of earthworms with statistically similar numbers of earthworms.

This elimination of different factors that could have impacted soil nutrient content has led to a possible conclusion. The two forests in which I studied were dominated by different tree species and, in turn, had different species of exotic earthworms present. Additionally, Forest 1 had much lower levels of Phosphate-Phosphorous than Forest 2. It is therefore plausible that differences in tree species have caused different earthworm species to inhabit the two forests, and these different earthworms have therefore had different effects on the Phosphate-Phosphorous levels in the two forests. This implies that tree species impacts the type of earthworms present in forests, as well as their activity and impacts on the soil nutrient content of the forests. This could be a significant piece of the puzzle in understanding the dynamics and implications of earthworm invasions.

However, these results are not entirely conclusive, and more in-depth studies are needed to confirm the results because of the many sources of error (time constraints, lack of professional equipment, and, most importantly, sample size) that could have skewed the data. Also, while I narrowed down a number of different factors, I still cannot wholly attribute any differences between forests to the different tree and earthworm species present in the forests (there are

always other possible causes out there). However, past work by scientists has led me to believe that my possible conclusions are very likely.

If anything, this study opens up the opportunity for many future studies. How are these soil nutrient content changes affecting native plants and animals? How does soil nutrient content differ in deeper levels of the soil (deeper than the upper 12-13 cm)? How do Nitrogen and Potassium levels differ between the two forests that I studied? Many more studies are certainly needed to explore how exotic earthworms respond to different environmental conditions, from tree species to soil moisture.

I'm optimistic and hopeful that I and other scientists are on our way to results that will allow us to properly address the implications of earthworm invasion on the slowly unraveling futures of our northern temperate forests.

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