

The Effect of Non-native Earthworms on Northern Michigan Soils

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Abstract

Earthworms are a non-native species in northern Michigan and their impact on soil structure and ecosystem dynamics is still poorly understood. Alterations in the proportions of nitrogen and carbon in the soil affect primary production, which in turn has consequences for higher trophic levels. Our research investigated the effect of non-native earthworms on carbon and nitrogen in soils of northern Michigan by conducting one manipulative experiment in two soil types and one observational experiment. Our results showed that the total nitrogen and carbon in anecic earthworm burrows was positively correlated with the total values in non-burrows. Also, the earthworms significantly decreased carbon levels in sandy outwash plain soil rather than nutrient rich moraine soil and had negligible effects on nitrogen in both soils. Non-native earthworms catalyze the release of carbon into the atmosphere in outwash plains, therefore, negatively affecting the forest ecosystems. Concurrent with leading research, non-native earthworms stand to be invasive species.

1. Introduction

Over 12,000 years ago, when the Laurentide ice sheet retreated from the Great Lakes region, it left behind glacial features still present today. Some features visible in northern Michigan are moraines and outwash plains. A moraine is a ridge that is composed of accumulated, unstratified material dropped at the leading edge of a glacier. An outwash plain is a broad, flat, alluvial deposit in front of the moraine (Encyclopædia Britannica 2011). The soil of an outwash plain has a high proportion of

sand which causes nutrients and water to drain quickly, whereas moraine soil has the ability to hold nutrients more effectively and can become available for plants. These two major soil types in northern lower Michigan were left without any native species of earthworms after the last glacial retreat (Tiunov *et al.* 2006).

Ecological systems rely on complex and highly evolved webs of interactions to function, and non-native species can have unexpected effects on these natural processes. Small changes in the lowest trophic levels can become magnified and affect all other species in the community. Above ground, these effects have been well studied. For instance, the infamous zebra mussel greatly impacts aquatic food webs in the Great Lakes through bioaccumulation. This concentrates chemical contaminants in species above zebra mussels in the food web, such as fish and waterfowl, which results in decreased abundance and fitness for these species (Wu *et al.* 2010). However, below ground research on the effect of non-native species is sorely lacking and lends only generalizations.

Non-native earthworms from Europe and Asia have been introduced in the past 300 years to the Great Lakes region. Native earthworm populations from the south are also expanding their range back towards the Great Lakes, but this is a very slow process (Bohlen *et al.* 2004). Currently, non-native populations are often closely associated with centers of human activity because of their unintentional introduction through construction, shipping, logging, horticulture, and fishing (Bohlen *et al.* 2004, Tiunov *et al.* 2006). Although their recent invasion has caused changes to forest floor characteristics, such as a reduction in leaf litter, faster decomposition, denser soil, and thinner organic soil layers, it is yet to be researched how these new areas will be affected in the long term (Bohlen and Groffman *et al.* 2004, Frelich 2006).

Our first experiment investigates the impact of earthworms on particular soils in northern lower Michigan. More specifically, we tested earthworms' effect on carbon and nitrogen levels in soil from a moraine and an outwash plain. Previous research shows that earthworms significantly increase the rate of nutrient cycling and decomposition (Bohlen *et al.* 2004). Research by Tiunov (2006) has shown sandy soils to have the lowest earthworm biomass and species richness, though the range of habitats suitable for earthworms varies between species. Other research by Laossi (2010) shows that

earthworms will have a greater positive effect on plant growth in sandy soils than in clayey soils because they are initially nutrient deficient. This led us to predict that earthworms will increase the amount of carbon and nitrogen in the outwash plain soil by a greater proportion than in the moraine soil.

A potential consequence of non-native earthworms further populating sandy outwash plains is that they may significantly increase the nutrients in that soil. In other words, they may improve soil quality and plant productivity (Laossi *et al.* 2010). This makes the environment inviting to new plant species previously not adapted to that environment which changes the plant community. Additionally, continued earthworm presence can significantly change other species' habitats by altering the availability of key resources, and out competing native species. We will measure the magnitude of influence non-native earthworms have, if any, on the different Michigan soils.

2. Materials and Methods

A. Study Organisms

We designed two experiments that took into account three ecological groups of worms. Epigeic species are small earthworms that feed on the fungi, bacteria, and organic material in the surface litter or top layer of mineral soil. Endogeic species live in the mineral layers of the soil and feed on organic material and microorganisms there. Larger anecic species affect both the forest floor and the mineral soil horizon by feeding on surface litter. They pull the litter down into vertical burrows, incorporating those nutrients into deep soil layers, and returning mineral soil materials to the surface through their castings (Eisenhauer 2010). Due to the differing niches of these three groups, we studied their effects on the nutrients in the soil with two separate experiments. The first experiment focused on the combined effects of epigeic and endogeic species on soil carbon and nitrogen levels by isolating the top eight inches of the forest floor in a controlled environment with both moraine and outwash samples. The second experiment focused exclusively on the effect of anecic species on carbon and nitrogen levels in the soil by analyzing core samples from their natural vertical burrows compared to non-burrow samples.

B. Question One: Do Earthworms have a Different Effect on Carbon and Nitrogen Levels in Moraine versus Outwash Plain Soils?

To determine whether non-native species of earthworms influence the carbon and nitrogen levels in the soil there, we designed a manipulative experiment to test the difference in soil before and after earthworm activity. Our first experiment involved ten 5.5 gallon aquariums with five containing moraine soil and five containing outwash plain soil. For each type of soil, three tanks had eight and a half grams each of local earthworms and two were controls without earthworms. Each tank had a representative sample of the leaf litter from our test sites. To maintain a natural environment, we strove to preserve the different soil layers from each site. For example, the moraine soil had a thicker layer of black organic material, whereas the outwash plain soil had a thinner layer of organic material above sandier layers. The tanks were watered twice to prevent the earthworms from drying out. Their sides were also covered to prevent light from penetrating. Carbon and nitrogen were measured from each of the tanks after 12 days and compared to levels before the addition of earthworms in the tanks.

C. Question Two: Will Anecic Earthworms Increase the Amount of Nitrogen and Carbon in the Soil?

Our second experiment was observational and involved taking soil core samples from the burrows of an anecic earthworm population at Colonial Point in Pellston, MI. Anecic earthworm burrows were abundant throughout the sampling site and were recognizable by their distinctive leaf mounds surrounding the opening. Their vertical burrows allowed us to easily take soil core samples. We took ten burrow core samples with corresponding non-burrow samples which were three inches away. In order to get representative samples, the pairs were separated by approximately ten feet.

3. Results

Both experiments tested the soil for total carbon and nitrogen, yielding results in percentages. Our data was normally distributed, the replicates were independent, and equal variances were assumed.

For the first experiment we had to isolate the average effect of earthworms on soil carbon and nitrogen. Therefore, the fluctuations in the control tanks were averaged and subtracted from the total effect of earthworms in the moraine and outwash tanks. This eliminates fluctuations onset by the experimental environment. On average, the percent of carbon decreased by 0.002 (± 0.165) percent in moraine soil and by 0.512 (± 0.129) in outwash soil. There was a significant decrease in the outwash soil (t-statistic = -6.960, d.f. = 2, $p = 0.02$), but not in the moraine soil (t-statistic = -0.035, d.f. = 2, $p > 0.9$). The average difference in nitrogen was -0.005 (± 0.01) percent in moraine soil and -0.028 (± 0.012) in outwash soil. Both of these were not significant changes (moraine: t-statistic = -0.866, d.f. = 2, $p > 0.4$ and outwash: t-statistic = -4.25, d.f. = 2, $p = 0.051$). Independent t-tests were used to show that the average difference between moraine and outwash soil for carbon was significant (t-statistic = 4.25, d.f. = 4, $p = 0.013$). The average difference between the soils for nitrogen was nearly significant (t-statistic = 2.646, d.f. = 4, $p = 0.057$). Earthworms decrease carbon in the outwash plain soil, but did not show a significantly different effect on nitrogen between moraine and outwash plain soil. This is consistent with previous research by Frelich (2006) that also did not find a significant change in total soil nitrogen from earthworm invasions, but did find that nutrients, specifically nitrogen and phosphorus, experienced increased leaching and were less available in the soil.

In the second experiment, carbon and nitrogen differences were tested between anecic worm burrows and corresponding non-burrow samples. Nitrogen differences between burrow and non-burrow yielded a mean of -0.002 (± 0.016). Carbon differences between burrow and non-burrow samples gave a mean of 0.009 (± 0.277). A paired t-test showed there was no significant difference between the pairs (t-statistic = -0.408, d.f. = 9, $p > 0.6$ and t-statistic = 0.103, d.f. = 9, $p > 0.9$), indicating earthworms either did not have an effect or the zone of influence was larger than anticipated. Although there

was no significant difference between pairs, there was a significant positive correlation between burrow and non-burrow carbon ($p = 0.008$) and nitrogen ($p = 0.002$) levels. The paired samples correlation gave a value of 0.780 for carbon (see Figure 3) and 0.846 for nitrogen (see Figure 4). The range in carbon and nitrogen values was very large between pairs. Carbon within anecic burrows ranged from 0.78 to 2.08 percent (a 1.3 percent difference) and in non-burrows from 0.73 to 2.12 percent (a 1.39 percent difference). Nitrogen within burrows ranged from 0.03 to 0.12 percent and in non-burrow samples from 0.04 to 0.13 percent, a difference of 0.09 percent in both.

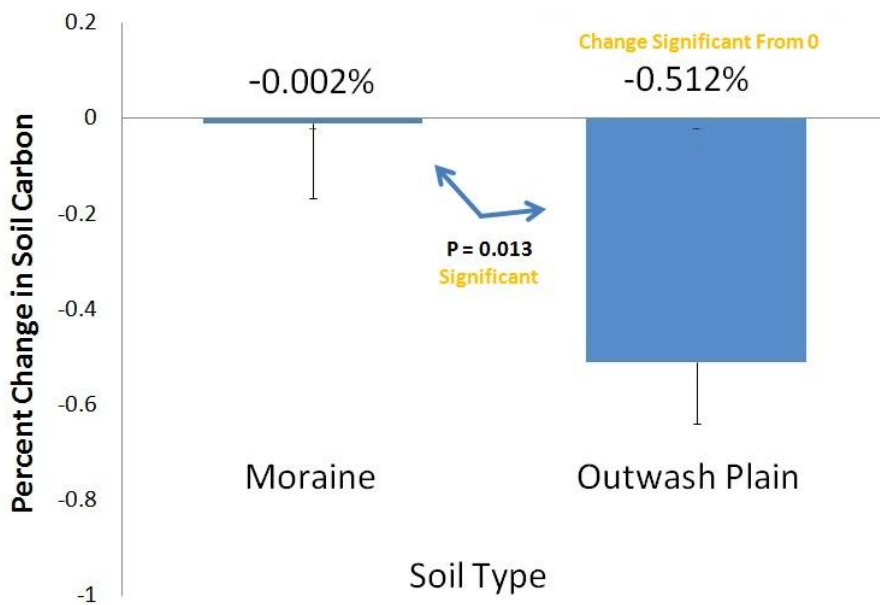


Figure 1. Average reduction in percent carbon in moraine soil and outwash plain soil samples.

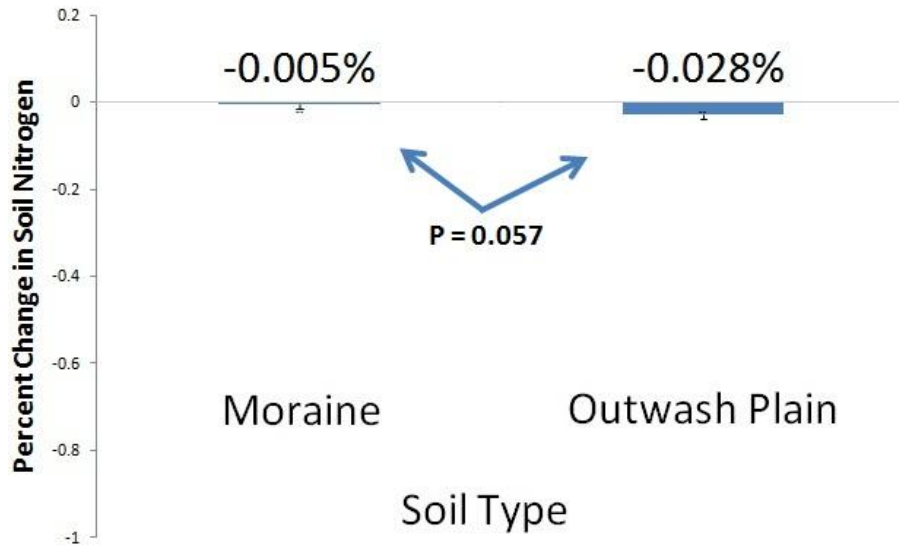


Figure 2. Average reduction in percent nitrogen in moraine soil and outwash plain soil samples.

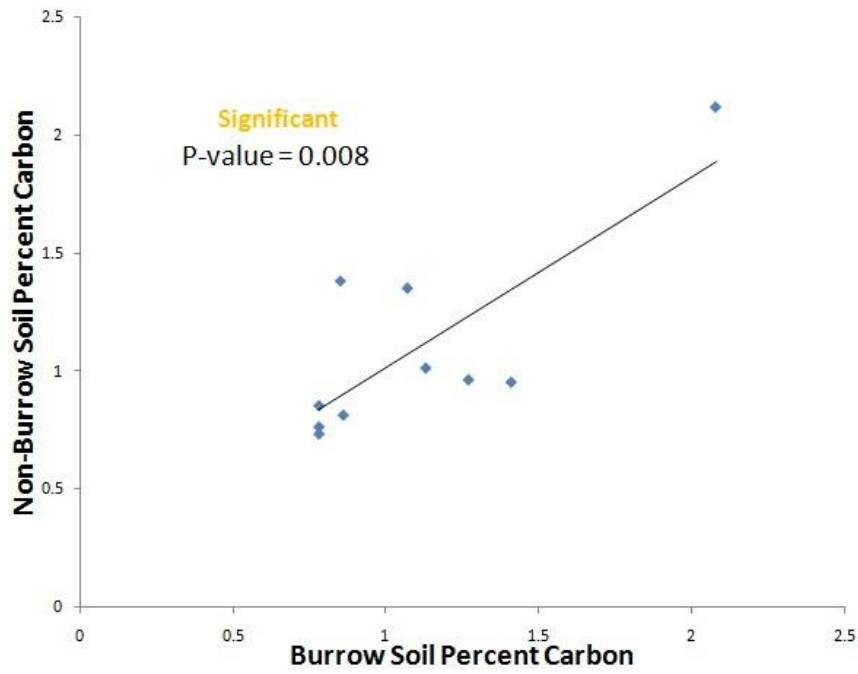


Figure 3. The significant positive correlation of carbon percentages between burrows and their corresponding non-burrow samples.

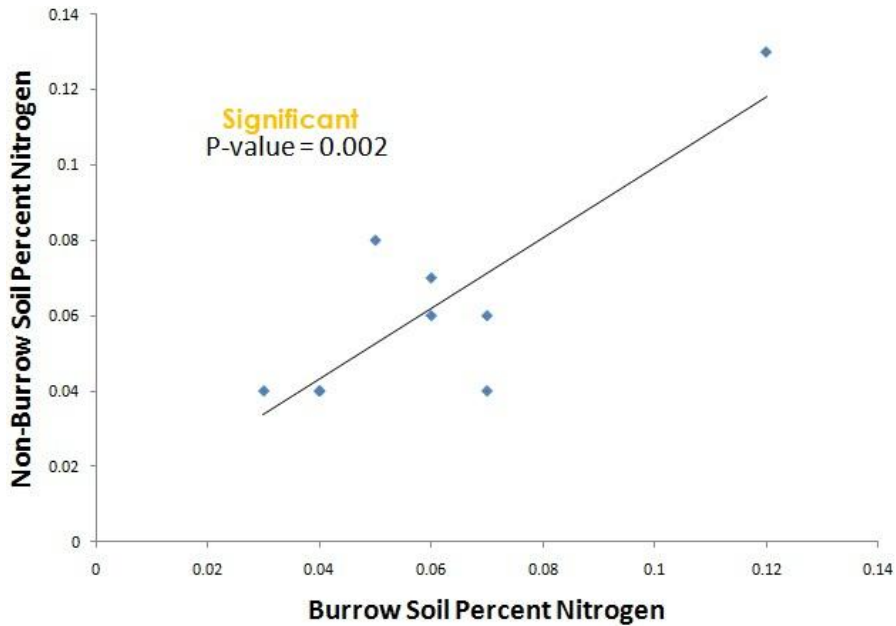


Figure 4. The significant positive correlation of nitrogen percentages between burrows and their corresponding non-burrow samples.

4. Discussion

We found that worms were significantly affecting the soil differently in moraine versus outwash plain tanks (see Figure 1). Our results also showed that worms had no effect on the percent of carbon in the moraine. In summary, worms had a strong negative effect in the outwash, decreasing the carbon in the outwash plain by 0.5 percent. If earthworms were to expand their range into sandy outwash plains, there may be unexpected negative consequences. In terms of nitrogen, both moraine and outwash plain samples were not significantly different from zero, but nearly significant from each other (see Figure 2).

These findings show carbon and nitrogen leaving the outwash plain soil 250 times and five times faster, respectively, than in the moraine. One interpretation to explain the extreme carbon loss in the outwash plain soil can be attributed to increased activity. The outwash plain soil is nutrient deficient, encouraging the worms to find a more suitable environment. Increased movement would aerate the soil, causing an increase in nutrient leaching and earthworm respiration. A second interpretation might be that the worms have more accessible water in the outwash plain soil. The top layer

of organic material in the moraine, which was much thicker, may have prevented water from leaching to lower zones for earthworm respiration.

A third interpretation is that the worms may have had a disproportionately positive effect on the decomposers and respiration in the outwash plain soil. Research by Edwards (2004) shows that earthworms can affect soil respiration through their own respiration, mucus production, microbial turnover, processing of organic matter, and changing the soil's structure. If the worms were in a state of increased activity, they would be aerating the soil more and creating more casts. Over the short term, fresh earthworm casts increase respiration rates in the soil due to increased microbial activity. Dense earthworm populations can even directly contribute up to 30 percent of the soil system's respiration (Edwards 2004).

The outwash soil may also have natural decomposers that are specifically evolved to take advantage of sporadic resources due to the initial nutrient deficiency of the soil. A sudden influx of nutrients in earthworm casts could have caused a sharp increase in microbial respiration. Any of these explanations could account for the discrepancy in carbon loss exhibited in outwash versus moraine soils.

Our question about whether or not anecic earthworms will increase the amount of nitrogen and carbon in the soil was answered statistically with no discernable difference. When our results showed the opposite of what we predicted, we debated about what forces could be at work here. Either earthworms were having no effect at all or their range of influence on the surrounding soil was greater than we anticipated.

Subsequently, there was a strong correlation between burrow and non-burrow pairs. This strong correlation suggests that variations in carbon and nitrogen may be occurring over a greater distance than the three inches that separated our paired samples. This could provide evidence that the patchiness of resource distribution is the most significant determinant in the level of carbon or nitrogen in any given sample. Our results from Colonial Point correlate with the results from our tank experiment in that earthworms have no significant effect on moraine soil.

There are a number of confounding and noisy variables potentially affecting our results. Soil horizon emulation in the construction of the moraine and outwash plain tanks could have been inaccurate. Also, unintentional mistakes could have resulted

from multiple experimenters forming soil tanks, extracting soil samples, or preparing soil samples for analysis. Due to being in a non-preferred environment, we must also consider the possibility of the worms dying in our tanks or behaving unnaturally. In our second experiment, we may have incorrectly identified anecic worm burrows, or they may have been vacant.

We concluded that the presence of earthworms in the outwash soil had negative effects. Our samples had an average mass of 21.964 grams and about 0.112 grams of carbon exited each of them. If this is extrapolated to an acre, one acre would lose 5,544 grams of carbon in 12 days. Consequently, the soil is less nutritious and plant growth could be limited. If our outwash results are further extrapolated to the Great Lakes region, earthworms could cause the forests to change from carbon sinks to carbon sources (Sedjo 1992). However, this conclusion does not account for the effect of increased atmospheric carbon on plant growth.

At the forest community level, earthworms' impact below ground can negatively affect plant diversity above ground. Research by Bohlen (2004) shows that earthworms change the slower cycling, fungal-dominated decomposition system to a faster cycling, bacterial-dominated system. This is already contributing to the decline of the now endangered and threatened fern *Botrychium mormo* in the Great Lakes region. This fern depends on its mycorrhizal fungi as well as the leaf litter layer, which are both being diminished by earthworms (Gundale 2002). Reduction of leaf litter by earthworms is also believed to be responsible for the decline of some salamander populations. Young salamanders depend on prey within the leaf litter, therefore, earthworms are reducing their food source availability (Bohlen *et al.* 2004).

In order for a non-native species to be called invasive it must have a proven negative effect on the ecosystem. Our data clearly shows that earthworms have a negative effect on the soil. However, more research needs to be done to determine how plants are affected by this carbon loss. It would be interesting if future experiments could test the direct and indirect effects of earthworms on not only the soil but also over higher trophic levels. In the future, distinguishing between the different forms of carbon and nitrogen could give more conclusive insight into the earthworms' impact. Our experiment should be replicated on a longer time scale and with more samples to see

how detrimental earthworms are as an invasive species. Our Colonial Point experiment could show significant results, overcoming the effects of patchy resource distribution, if it was also performed in the outwash soil. In order to account for patchiness and isolate the earthworm zone of influence, we could test at increasing distances away from the anecic earthworm burrows.

Our research leads us to conclude that non-native earthworms, through a significant loss of carbon, have a negative effect on soil. This acts as evidence to designate earthworms as an invasive species in the outwash plain. However, in order to justify this, further experimentation needs to be done to test the long term effects on higher trophic levels.

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