The Benefits of Vermiculture in Agroecology and the Detriment of Invasive Earthworms to Northern Michigan Forest Ecosystems

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Abstract

Earthworms are often thought of as beneficial ecosystem engineers, but always in the context of agroecology. Vermicomposting is an incredibly efficient method for fertilizing crop fields, but the things that make them such a benefit to agricultural systems makes them a detriment to the health of native forest environments. Invasions of introduced exotic earthworms, driven by human activities that facilitate dispersal, have been having devastating effects on Northern Michigan forests. This study looks at which forests are the most vulnerable to invasion and what factors make them ideal habitats for exotic species, as well as how much organic matter invasive earthworms can remove from leaf litter and topsoil layers of forest environments in a given amount of time. Methods for conservation and invasion containment are also suggested, with a focus on spreading awareness that earthworms are not native to Michigan.

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Earthworms are often thought of as beneficial ecosystem engineers, but always in the context of agroecology. Vermicomposting is an incredibly efficient method for fertilizing crop fields, but the things that make them such a benefit to agricultural systems makes them a detriment to the health of native forest environments. Invasions of introduced exotic earthworms, driven by human activities that facilitate dispersal, have been having devastating effects on Northern Michigan forests. This study looks at which forests are the most vulnerable to invasion and what factors make them ideal habitats for exotic species, as well as how much organic matter invasive earthworms can remove from leaf litter and topsoil layers of forest environments in a given amount of time. Methods for conservation and invasion containment are also suggested, with a focus on spreading awareness that earthworms are not native to Michigan.

Introduction:

Earthworms have long been used in agriculture, but despite having a long-standing knowledge of their agroecological benefits, we have only just recently become aware of the negative impact they can have when they are introduced to new environments. Much to the surprise of many, there are no species of earthworms native to Michigan. All of the previously native species were extirpated by the southward movement of the Wisconsinian glaciers, and they have yet to return due to their slow expansion rate (Bohlen *et al.* 2004, Tiunov *et al.* 2006). European and Asian exotic earthworm species were introduced to this area through human activity, starting back during European settlement, when settlers brought soil over with plants, and soil used as ballast for ships was discarded all along the Great Lakes shorelines. Since then, worldwide agricultural commerce, movement of horticultural materials and landfill waste, logging roads, and recreational fishing have served as modes of dispersal for exotic species (Bohlen *et al.* 2004, Tiunov *et al.* 2006, Hendrix and Bohlen 2002, Bal *et al.* 2017). Exotic species have become widespread since their introduction to the point where most think of them as native, and this is due to their ability to spread more rapidly than truly native species and are much more tolerant of varying ecosystems and environments (Bohlen *et al.* 2004, Tiunov *et al.* 2006).

The most familiar role of earthworms is their beneficial relationship with agroecology. Vermicomposting is widely used to create nutrient-rich fertilizer, as worms are able to make numerous nutrients, such as phosphorous and nitrogen, more available from compost by turning dead organic matter over very rapidly (Gliessman and Engles 2007, Hendrix and Bohlen 2002, Bal *et al.* 2017, Bhat *et al.* 2017, Sharma *et al.* 2017). They are able to turn over a large quantity of material over a short period of time due to their low assimilation efficiency (Sharma *et al.* 2017, Bhat *et al.* 2017). Earthworms also increase soil porosity which facilitates drainage of the soil as they move through the soil horizons (Sharma *et al.* 2017, Hendrix and Bohlen 2002).

The movement of earthworms through the soil also changes the composition of the soil. The mucus left behind in their tunnels changes the texture of the soil, by helping it clump, which enhances water retention (Gliessman and Engles 2007). Earthworms modify the bulk density of the soil through cast production, which incorporates organic matter throughout varying levels of the soil horizons (Sharma *et al.* 2017, Frelich *et al.* 2006). Through this process, they also mix the organic layers of the soil with the mineral soil horizons (Bohlen *et al.* 2004, Hendrix and Bohlen 2002, Frelich *et al.* 2006).

The addition of minerals to the organic layers facilitates the mineralization of carbon, nitrogen, and phosphorous, making them rapidly available for uptake. Carbon is retained in the soil through this process, as it is stabilized and prevented from degrading into carbon dioxide or methane. Nitrogen is increasingly released as nitrogen dioxide, and phosphorous is made even more available because its biogeochemical status is changed in burrow linings (Sharma *et al.* 2017, Bhat *et al.* 2017). Past studies have shown that the rapid release of soil nutrients facilitated by vermicomposting increased crop yield by 25% and aboveground biomass by 23% (Sharma *et al.* 2017).

While all of these traits may work extremely well in agricultural environments, they have devastating effects in natural forest ecosystems that were previously undisturbed by earthworm activity. Primarily, the invasion of exotic earthworms changes the soil microbiome from a slow-cycling, fungus dominated system to one that is much faster-cycling and dominated by bacteria more that fungi. This happens because the disturbances created by the burrowing of the worms and flood of soil nutrients decreases fungal diversity and richness (Bohlen *et al.* 2004).

The same mixture of organic soil layers and mineral soil horizons that was so positive in agricultural environments is severely damaging to the soil ecology of forest environments. While the mineralization of carbon, phosphorous, and nitrogen makes them more readily available in the short term, it makes them extremely prone to erosion and leaching in the long term (Bohlen *et al.* 2004, Bal *et al.* 2017, Frelich *et al.* 2006). Agricultural systems are designed to withstand high rates of nutrient loss, and are usually supplemented with fertilizers and crop cycling, but forest ecosystems are not.

Some earthworm species, such as *Lumbricus terrestris*, dry out the topsoil and leave it vulnerable to erosion by burrowing up to 1-2 m into the soil and the pulling leaf litter and topsoil down with them (Sharma *et al.* 2017, Bohlen *et al.* 2004, Bal *et al.* 2017, Frelich *et al.* 2006, Hendrix and Bohlen 2002). In some northern sugar maple forests, the forest floor decreased from 10 cm to 0 cm in distances of as little as 75 m along a visible invasion front. The removal of the forest floor and leaf litter decimates the plant communities in forest systems, and the flood of nutrients encourages a monoculture to form, which is desirable in agricultural systems but not in forest systems (Bohlen *et al.* 2004, Bal *et al.* 2017, Frelich *et al.* 2006, Hale and Host 2005, Hendrix and Bohlen 2002).

The abundance of native plant species and tree seedlings drops significantly after invasion of exotic earthworm species (Bohlen *et al.* 2004, Bal *et al.* 2017). This is because seeds are either ingested or buried during earthworm activity, and the loss of the protective forest floor exposes seedlings to desiccation and predation. This also causes the understory to become much more vulnerable to grazing. (Bohlen *et al.* 2004, Frelich *et al.* 2006, Hale and Host 2005).

With all of these things in mind, we were interested in what kind of forest ecosystems were most vulnerable to invasion by exotic earthworms and what features of these environments would be the best indicators of vulnerability. Alongside this, we were also interested in just how much organic material exotic worm species were capable of cycling into lower soil horizons. For our field surveys, we chose two forest types: moraine and outwash. We hypothesized that we would find more earthworms at the moraine sites than we would at the outwash sites. Moraine habitats generally have more deciduous trees, lending to a rich layer of topsoil and leaf litter, as opposed to outwash habitats that are typically comprised of pines that don't provide as much leaf litter. The soil at moraine sites is also generally less acidic than at the outwash sites.

For our terrarium surveys, we looked at two exotic earthworm species, *Lumbricus terrestris* and *Eisenia fetida*. *L. terrestris* is an anecic species, which means it is a deep burrowing species, often tunneling up to 1-2 m. They cross many soil horizons and move a large quantity of organic matter and leaf litter from the surface into the deeper horizons (Bohlen *et al.* 2004, Bal *et al.* 2017, Frelich *et al.* 2006, Hendrix and Bohlen 2002). *E. fetida* is an epigeic species that resides mainly in the upper organic layer of the soil and causes very limited mixing of the soil horizons. It is the most common earthworm used for vermicomposting, however, because it is a very efficient composter (Bohlen *et al.* 2004, Hale and Host 2005, Gliessman and Engles 2007). We hypothesized that the earthworms would integrate more carbon into a sand soil horizon when given a carbon source to cycle, in this case garden compost, than would be present in a control that was not subject to worm activity. We also hypothesized that *L. terrestris* would be more efficient at cycling carbon than *E. fetida.*

Materials and Methods:

Field Surveys:

We conducted our field surveys at four different locations. All the locations were near Pellston, MI on University of Michigan Biological Station property, and were chosen based on cover type information from the ArcGIS Forest Ecology maps compiled by the University of Michigan Biological Station (Tallant 2017). We selected two sites that were moraine habitats, which had more deciduous trees and richer soil, and two that were outwash habitats, which had more coniferous trees and sandier soil. The two moraine sites were a true moraine and the Colonial Point old growth forest moraine, which we visited on 7/23/18 and 8/5/18 respectively. The two outwash sites were an outwash plain and an outwash channel, both of which we visited on 7/30/18.

At each location, we used a modified version of the sampling methods described in McCay 2013. Our quadrat was constructed from a large metal bucket from which the bottom was removed. Because it was 60.5 cm in diameter instead of the 20 cm quadrat described in McCay 2013, we altered our collecting process to accommodate for the size increase. We sampled two quadrats at each location. A center point was chosen at random and denoted with a stake. We then placed the two quadrats a meter in either direction from the center point so that they were 180° from each other.

A solution of mustard powder was used as a vermifuge to encourage the worms to surface within the quadrats. We used a concentration of 10 g/L in water. Due to the tripled size of our quadrats, we prepared two doses of 6 L of solution for each quadrat instead of the described two doses of 2 L of solution. The solution was prepared and transported in large 18 L jugs. We took two jugs to each location, each containing 12 L of solution for each quadrat.

The solution was applied using a 2 L measuring cup to ensure that each dose was 6 L. For each quadrat, 6 L were applied and then 5 minutes were allowed to pass before the next 6 L were applied, after which another 5 minutes were allowed to pass. During each 5-minute interval, all emerging worms were removed from the quadrat and placed into a collection cup to be counted at the end of the total 10 minutes. We compared the worm communities at the moraine sites with the worm communities at the outwash sites by combining and averaging the counts from them and running a Wilcoxon Test on the two subsequent mean values.

At the center point of each location, we used a temperature probe to measure the surface soil temperature as well as the soil temperature at 5 cm. We also used a pH testing kit to measure the soil pH at the center point of each location. We ran a linear regression on the surface soil temperature, 5 cm soil temperature, and the soil pH measurements from all four locations in comparison to the worm counts from all four locations.

Terrarium Surveys:

We constructed six terrariums out of duct tape and 2 L soda bottles. Each terrarium was made up of three bottles, where two of the three had both the tops and bottoms removed and the third only had the top removed and the bottom was left intact to act as the base. Each segment was attached using duct tape. We filled each terrarium with 35 cm of a 3:1 mixture of sand and vermiculite. The sand was collected from University of Michigan Biological Station main campus. On top of the sand mixture, we added 5 cm of compost from the University of Michigan Biological Station garden. Finally, we added 2 cm of leaf litter, which was also collected from main campus.

We purchased two different species of earthworms from a local bait shop. We obtained a container of "green worms," which were *Lumbricus terrestris,* and a container of red worms, which were *Eisenia fetida*. We used twelve specimens total, three terrariums per species and four species per terrarium. We added the worms to the terrariums on 7/24/18 after we added 0.5 L of water to each terrarium to increase the soil moisture and make it more habitable for the worms. We added an additional 0.5 L of water on 7/25/18 to maintain the soil moisture for the duration of the experiment. After this point, we checked the moisture daily, but it remained suitable for the rest of the time.

We allowed the worms to inhabit the terrariums for 12 days until 8/4/18. At this point, we added 2 L of water to each terrarium and placed all six of them in a -20° C deep freezer for two days. On 8/6/18, we removed them from the deep freezer and used a band saw to cut the terrariums into quarters. The first cut was made lengthwise to make two halves, and then each half was cut horizontally. Cutting the terrariums in quarters allowed them to thaw more quickly for examination. They were left to thaw for 36 hours, after which we were able to remove the compost and leaf litter layers.

We then homogenized the sand layer of each terrarium by dumping the four sections into a bucket and thoroughly mixing them together. We did this for all six terrariums. There was significant cross contamination between an *L. terrestris* sample and an *E. fetida* sample that led to two terrariums being discarded. We collected samples from each bucket in separate glass jars and added a control sample that consisted of only sand collected from main campus in a glass jar. We than placed all five sample jars into a 100° C drying oven for 12 hours to evaporate any water.

While the samples were drying, we took the mass of five crucibles. We then removed the samples from the oven, allowed them to cool, and then took a smaller sample from each one and placed them into separate crucibles. We took the mass of the crucibles as before, but while filled with the samples. We then placed the crucibles into a 500° C muffle furnace to burn off any carbon that was brought down into the sand layer. We allowed the samples to sit in the muffle furnace for four hours before turning it off. We allowed them to cool for several hours before removing them. Once we removed them, we took the mass of each crucible a final time to measure if there had been any loss of carbon mass.

We subtracted the masses of the crucibles from the pre- and post-burning masses to obtain the pre- and post-burning masses of the samples themselves. We then subtracted the post-burning mass from the pre-burning mass to obtain the carbon mass lost during the carbon burning process. We took the average of the values from the samples that had worms present and used the mean to run a one sample T-test with the value from the control sample. We also ran a paired T-test using the mean of the *L. terrestris* samples and the mean of the *E. fetida* samples.

Results:

Field Surveys:

We collected a total of 21 worms at the true moraine site. 9 were collected in the first quadrat and 12 in the second, which was an average of 10.5 worms. The soil pH was 5.5. The surface soil temperature was 20.5° C and the 5 cm soil temperature was 19.5° C. We had similar results for the worm count at the Colonial Point old growth forest moraine site, with a total of 28 worms. 14 worms were collected out of each quadrat, which was an average of 14 worms. The soil pH was 4.0. The surface soil temperature was 21.0° C and the 5 cm soil temperature was 19.0° C.

We collected a total of 1 worm at the outwash plain site. 0 were collected in the first quadrat and 1 was collected in the second, which was an average of 1 worm. The soil pH was 5.0. The surface soil temperature was 21.0° C and the 5 cm soil temperature was 20.0° C. We collected a total of 0 worms at the outwash channel. No worms were collected from either quadrat, so we collected an average of 0 worms. The soil pH was 4.5. The surface soil temperature was 22.0° C and the 5 cm soil temperature was 20.0° C. A visual representation of our collection results can be found in Figure 1.

The combined mean of the moraine sites was 13 and the combined mean of the outwash sites was 0. Using these values, we ran a two-sided Wilcoxon rank sum test with continuity correction. We used an alpha level of 0.05 and a confidence level of 0.95. The calculated p-value was 0.02558, which showed that there was a statistically significant difference between the worm communities at the moraine sites and the worm communities at the outwash sites. Because of this, we were able to reject the null hypothesis and our alternate hypothesis was supported.

The linear regression for soil pH and worm count was run with an alpha level of 0.05, a confidence level of 0.95, and 6 degrees of freedom. The p-value, however, was 0.6681 showing that there was no significant connection between soil pH and worm counts. The linear regression for surface soil temperature and worm count was run with an alpha level of 0.05, a confidence level of 0.95, and 6 degrees of freedom. The p-value was 0.09975, which showed that there was also no significant collection between surface soil temperature and worm counts. The linear regression for 5 cm soil temperature, run with an alpha level of 0.05, a confidence level of 0.95, and 6 degrees of freedom, was the only one that showed a significant connection with worm counts with a p-value of 0.000117. Least squares graphs for all three linear regressions are displayed in Figures 2, 3, and 4.

Terrarium Surveys:

The first *L. terrestris* sample had a mass of 36.5 g before carbon burning and a mass of 34.5 g after carbon burning. This was a 2.0 g, or 5.48%, loss of carbon. The second *L. terrestris* sample had a mass of 35.1 g before carbon burning and a mass of 32.5 g after carbon burning. This was a 2.6 g, or 7.41%, loss of carbon. The first *E. fetida* sample had a mass of 37.6 g before carbon burning and a mass of 35.3 g after carbon burning. This was a 2.3 g, or 6.12%, loss of carbon. The second *E. fetida* sample had a mass of 37.5 g before carbon burning and a mass of 34.4 g after carbon burning. This was a 3.1 g, or 8.27%, loss of carbon. The control had a mass of 44.0 g before carbon burning and a mass of 43.7 g after carbon burning. This was a 0.3 g, or 0.68%, carbon loss.

We ran a one sample T-test to compare the mean of the percent carbon lost from samples where worm activity was present, and the percent carbon lost from the control. We ran the test with an alpha value of 0.05, a confidence level of 0.95, and 4 degrees of freedom. The resulting p-value was 0.0205, which showed that there was a statistically significant difference between soil samples where there was worm activity and the control that did not have worm activity. This meant that we could reject the null hypothesis and our alternate hypothesis was supported. A visual representation of the grams of carbon lost can be found in Figure 5.

We ran a paired T-test to compare the mean carbon lost from the *L. terrestris* samples and the mean carbon lost from the *E. fetida* samples. We ran this test with an alpha value of 0.05, a confidence level of 0.95, and 1 degree of freedom. The resulting p-value was 0.09271, which showed that there was no significant difference in the amount carbon moved by the two species. We were unable to reject the null hypothesis.

Discussion:

Our results for our field surveys supported our hypothesis that we would find more worms in the moraine sites than in the outwash sites. The most surprising result was that pH had no significant connection to worm count, as previous studies have shown that worms, which are calciferous, tend to avoid acidic environments (Bal *et al.* 2017). This was not supported in our results at all as the most acidic environment was the Colonial Point moraine, which had a soil pH of 4.0, was where we collected the most worms. This could have been due to the heavy layer of predominately deciduous leaf litter that covered the forest floor, as well as the fact that the forest is on a point that is surrounded by heavily fished waters. There is more than likely a well-established, thriving earthworm community there from years of discarded fishing bait.

The only significant factor that correlated with worm count was 5 cm surface temperature. More worms were found in areas that had lower 5 cm soil temperatures. This could have been because the leaf litter layer was thicker at the moraine site, protecting the lower soil layers from being heated more by the sun. The outwash sites had a very thin leaf litter layer and therefore more solar radiation could reach the soil. Although we were unable to pinpoint any specific factor that contributed to the moraine sites being more vulnerable to invasion by exotic worms, they still had significantly higher numbers of them present than the outwash sites. Future studies could be done to survey more possible contributing factors.

The results for the terrarium survey supported our hypothesis for the comparison of carbon contents in worm disturbed soil and soil that was undisturbed by worm activity. The soil that the worms were allowed to incorporate with compost had a significantly higher amount of carbon lost during carbon burning than the control did. We only allowed the worms to cycle the compost for 12 day, and there was already a notable loss of organic matter from the compost layer. Future studies could replicate this process on a much larger scale, for a much longer period of time, to quantity just how much of an impact different exotic species have on the organic layers of forest ecosystems.

The results of the comparison between the two earthworm species were surprisingly insignificant. We expected there to be a noticeable difference between the amount of organic material moved by the two species. A possible explanation for the similarity between them could be that we didn't run the experiment long enough. It could take a much longer amount of time for them to start showing their typical movement patters. This is a great opportunity for larger scale studies in the future.

There are quite a few ways that this experiment could be improved upon. For the field surveys, doing more quadrats would provide more data and sampling from more than four locations, as well as more diverse locations, would provide more insight into possible factors of forest vulnerability. For the terrarium survey, there was a lot of room for error in the amount of time that we allowed each sample to dry. Allowing more time in both the drying oven and the muffle furnace would most likely give more reliable results. Extending the run time of the experiment would provide more data, as would doing it on a larger scale than three 2 L bottles taped together. A better method for cutting the terrariums and extracting the samples would also be beneficial.

Studies like this show that there is little being done to stop the spread of exotic earthworm species into native forests. Earthworm imports are regulated by the Animal and Plant Health Inspection Service (APHIS), but there is a dangerous lack of awareness in the public of the negative effects that earthworms are having on natural environments (Hendrix and Bohlen 2002). Agriculture represents a huge hurdle in the process of fighting the spread of introduced species. Use of native species in agroecology would provided a much better solution but has not yet been well-received in the farming community, as it would require a change in general practice (Hendrix and Bohlen 2002). The benefits that earthworms provide to agriculture also make it difficult to portray them as a dangerous invasive species. They're widely used in home gardens and in recreational fishing as well, so shifting public perception poses quite a challenge (Hendrix and Bohlen 2002).

The best methods for stopping the spread of exotic earthworms to any more vulnerable native forests would be to impose selective importation on a species by species basis, deciding which ones pose the greatest threat. Strong restrictions complete bans of certain species may be necessary in some cases (Hendrix and Bohlen 2002). Conservation efforts, such as local bans and quarantines, would be a good place to start on a small scale to help contain invasions and protect particularly vulnerable environments. Spreading awareness is equally as important. Discouraging the discarding of unused fishing bait as well as the use of exotic earthworms in home gardening and large-scale agriculture could help change the public perception of them and facilitate change that could help protect the forests of Northern Michigan (Hendrix and Bohlen 2002).

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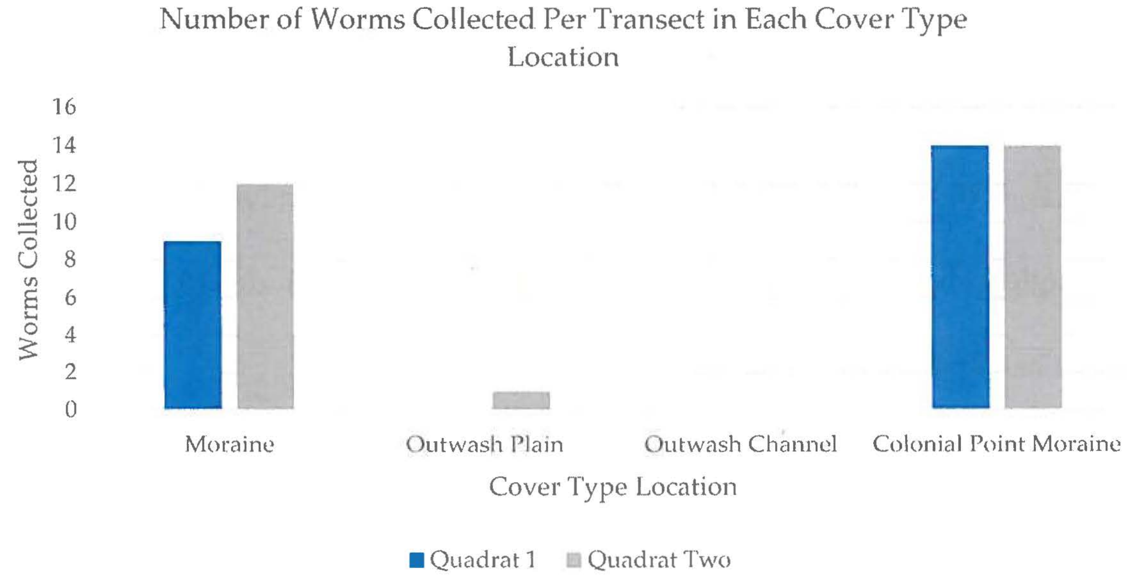

Figure 1.) Worm collections were conducted at four different cover type locations on University of Michigan Biological Station near Pellston, MI. Samples were taken from two quadrats at each location. Moraine sites had richer soil with more deciduous tree species, whereas outwash sites had sandier soil and more coniferous tree species. The combined mean of the moraine sites and the combined mean of the outwash sites were used to run a Wilcoxon Test with an alpha level of 0.05 and a confidence level of 0.95. This test resulted in a p-value of 0.02558. This showed a significant difference between worm community counts in the two cover type groups, which is represented in this graph.

Least Squares Graph of the Linear Regression for Surface Soil Temperature (°C) and Worm Counts

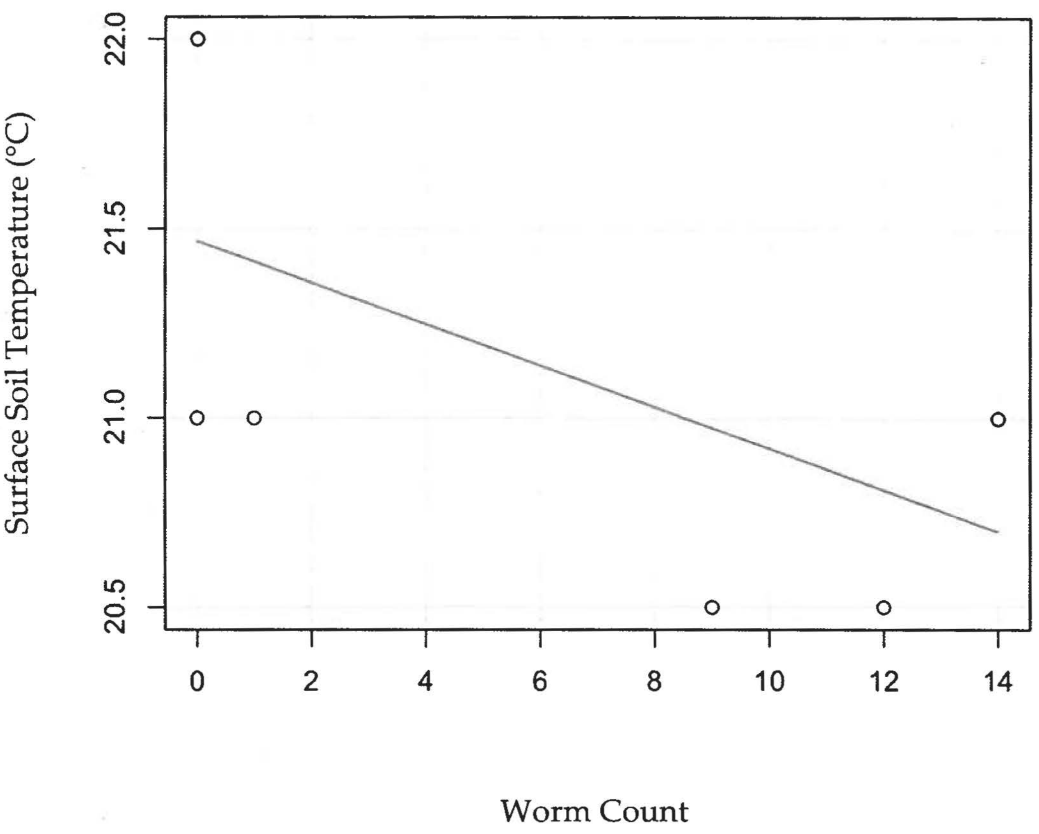

Figure 2.) The linear regression for surface soil temperature and worm count was run with an alpha level of 0.05, a confidence level of 0.95, and 6 degrees of freedom. The p-value was 0.09975, which showed that there was also no significant collection between surface soil temperature and worm counts.

Least Squares Graph of the Linear Regression for 5 cm Soil Temperature (°C) and Worm Counts

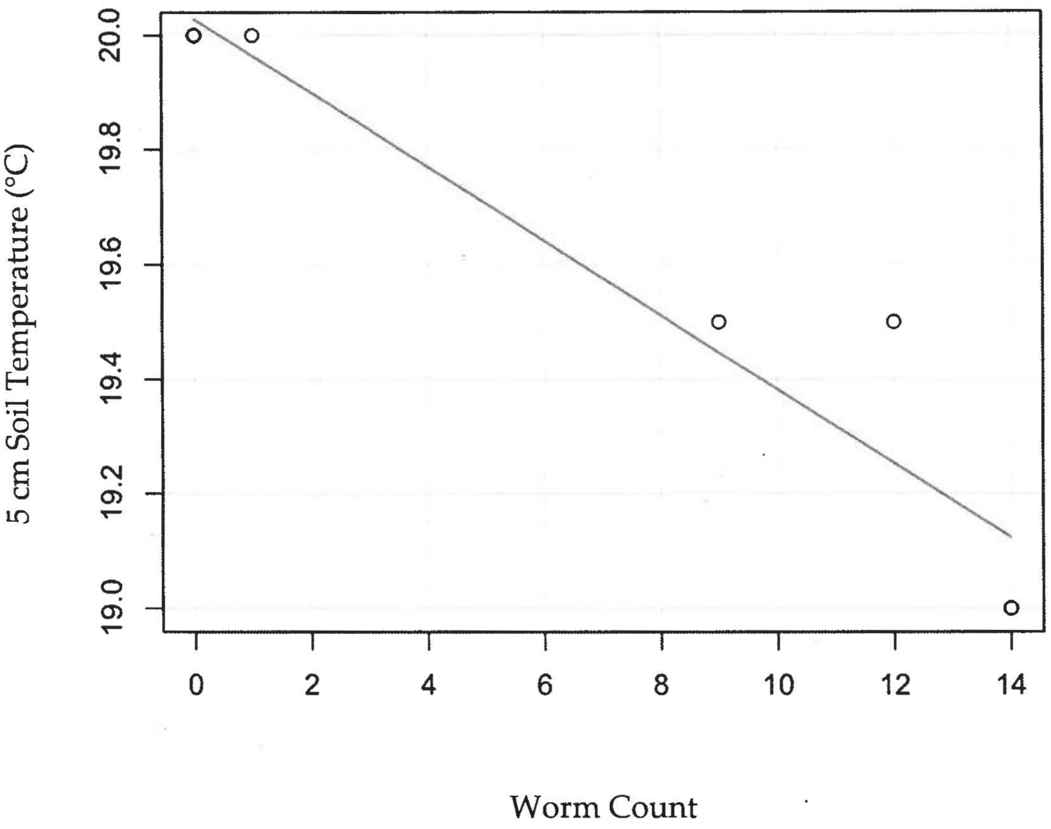

Figure 3.) The linear regression for 5 cm soil temperature, run with an alpha level of 0.05, a confidence level of 0.95, and 6 degrees of freedom, was the only one that showed a significant connection with worm counts with a p-value of 0.000117.

Least Squares Graph of the Linear Regression for Soil pH and Worm Counts

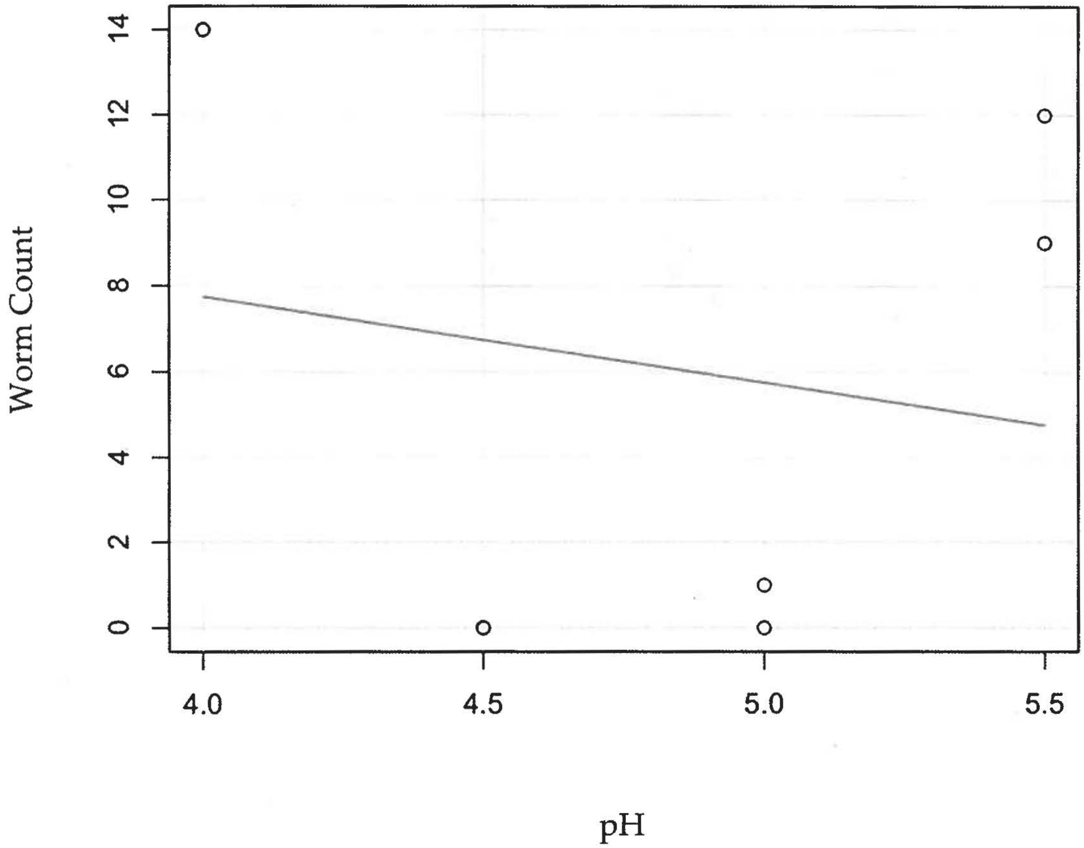

Figure 4.) A linear regression for soil pH and worm count was run with an alpha level of 0.05, a confidence level of 0.95, and 6 degrees of freedom. The p-value, however, was 0.6681 showing that there was no significant connection between soil pH and worm counts.

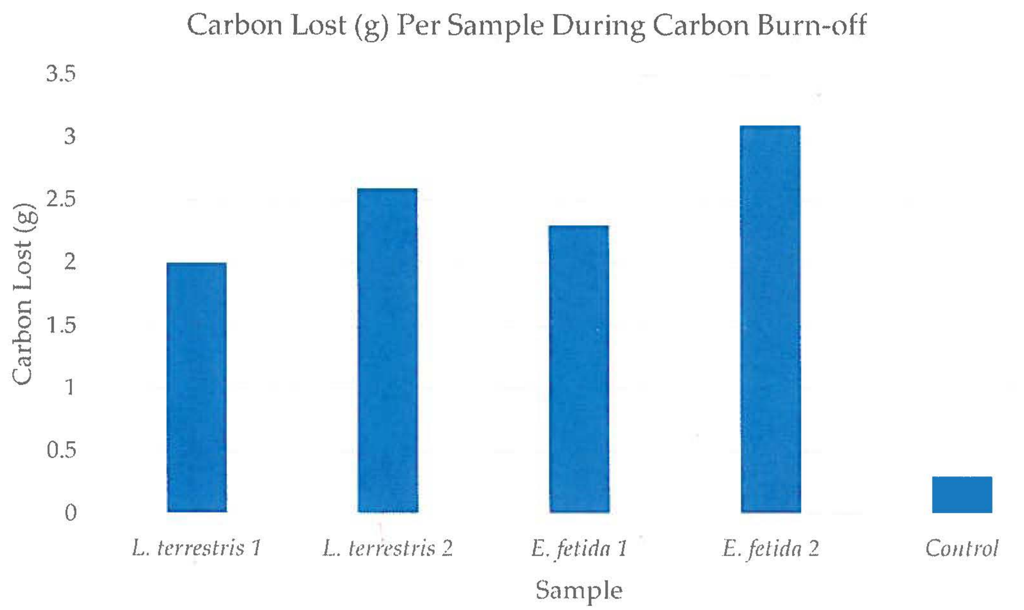

Figure 5.) Four terrariums of two worm species (*Lumbricus terrestris* and *Eisenia fetida*) were allowed to circulate compost into sandy soil for 12 days before the sand was homogenized and dried. Samples of the dried sand from each terrarium and a control were taken and placed into a muffle furnace at 500° C to burn off the carbon they contained. The masses of the samples were taken before and after carbon burn-off and the differences between those values are graphed above.