



**An Investigation on Worm Abundance in Church Woods,
regarding pH, Tree Cover, and Soil Content
*Shelburne, Vermont***

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December 11, 2019

ABSTRACT

Worms are typical species to find in conjunction with farming practices as they have typically helped aerate the soil and recycle nutrients for farmers. However, they are a non-native invasive species that results in nutrient degradation in various habitats in the Northeastern United States, particularly in forest habitats. This study took place in Shelburne farms, a historically active agricultural area where worms are observed in patches. The goal was to determine if there were worms in an area of old growth forest called Church woods, and what parameters resulted in the persistence of worm populations outside of agricultural settings. By measuring two plots with ten holes excavated in each for soil and worm samples, worms were concluded to be in Church woods. The findings showed that worms prefer deciduous tree cover to coniferous, pH readings between 5 and 8, and that worm abundance increases as the organic soil layer thickness decreases. Efforts to control the spread of worms were discussed and appropriate action is still to be discussed. Future studies regarding the carbon sequestration capabilities of worms should be determined and compared with such capabilities of vegetation in an effort to weigh earthworms pros and cons within natural forest environments.

INTRO

Approximately 2.6 million years ago, glacial retreat shaped the land now known as Vermont. Because of the ice, all native earthworm species were extirpated from the area ("Earthworms", 2013). When settlers first chartered the town of Shelburne a little over 200 years ago they found it to be excellent land for agriculture, especially for livestock and orchards. It is thought that earthworms were introduced to the area unintentionally, likely through ship ballast or the soil and roots of plants brought over from Europe

People don't often think of earthworms as harmful or invasive, but actually in the northern forests of the United States they are both. Worms consume decaying organic matter and transform the chemistry of soil. This can be beneficial in an agricultural setting however, in the forest the worms destroy the duff layer, which is extremely important for forest soil ecology. The duff layer is made up of decomposing leaf litter and contains the vast majority of nutrients that will be deposited into the soil. When worms are present in the forest they consume the duff layer very quickly, leaving the forest floor uninhabitable for many plants and animals that depend on a thick duff layer (Knowles, Ross, & Gorres, 2016). Once the duff layer has been consumed by worms, the forest floor is left dry and cracked, scattered with tufts of the last bits of leaf litter that worms have dragged into their burrows. Duff layer destruction can also lead to an increase in invasive species who may be more tolerant of the lower quality conditions than native species ("Earthworm Invasion", 2013).

The goal of this study was to determine if worms were present in church woods, and if so, what is the abundance of the worms on both the east and west sides of the road. In addition, this study explores the relationships between worm abundance, pH of soil, tree cover type, depth of organic soil horizons, and % organic carbon in the soil.

STUDY AREA

Church woods is one of the few old-growth forests found on Shelburne Farms. It is comprised of a mix between both large deciduous trees as well as conifers. The soils in Church woods are primarily clay and silty clay (Lapin et al., 2001). The forest is well divided by a dirt road, creating a natural barrier between the two study sites. The eastern side of the road is populated by large maples, beeches, oaks, and a few hemlocks. The undergrowth is thick with saplings as well as numerous buckthorns and dogwoods. There was prevalent and deep layers of deciduous leaf litter with no obvious signs of worm invasion. The west side of the road was a much more uniform forest of hemlocks, with a few large oaks and young beeches mixed in. There is little to no undergrowth, but there was a very dense coniferous leaf litter layer and again no clear signs of worms. East and west study areas were set up mirroring each other across the road. Each study area was 100x100 foot square and directly bordering the road; containing 10 50x50x50cm holes per study area, totaling 20 holes.

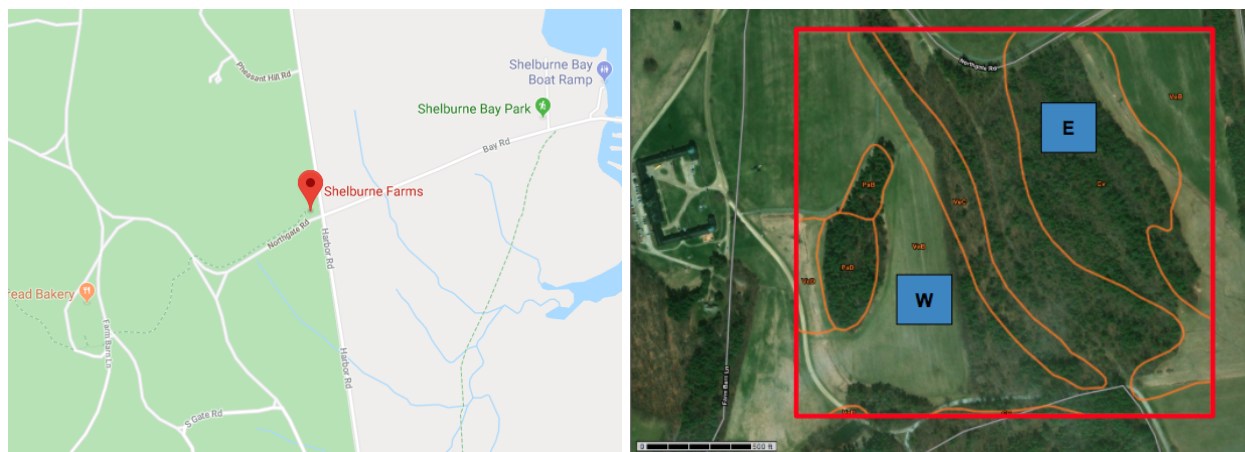


Figure 1. Map of Shelburne Farms, Shelburne, Vermont (LEFT) and map of Church Woods study area within Shelburne Farms (RIGHT) with the maple dominated (E) and hemlock dominated (W) designated along with accompanying soil types; VeB is Vergennes Clay (2-6% slope), VeC is Vergennes Clay (6-12% slope), and Cv is Covington Silty Clay.

METHODS

Sampling took place over a three month period from September 2019 through November 2019. A 100x100 foot area was marked by flags on the East (E) and West (W) side of the site

adjacent to one another within Church Woods. From then on, the dig sites were chosen through selective random sampling. For the first samples, a disc was thrown from the road into the 100x100 foot area. Where it landed was the dig site. The disc was then thrown from that site to mark another. If the disc was within 10 feet of a previous dig site, it was thrown again. Each site was labeled with the number it was as well as the side it was found on (i.e. 2E, 3W, etc). At each 100x100 foot site, a hole was dug 50 cm deep in an area of 50x50cm². Ten holes were dug per 100x100 foot area, totaling 20 sample sites, where worm abundance was calculated at each site. Carbon soil samples and pH samples were taken from the mixed up soil layers, the organic layer thickness was measured in centimeters with a ruler, and the excavated soil was sieved to find the number of worms. Using an identification key ("OPAL", n.d.) and the help of Josef Gorres, worm species were identified. pH levels were tested for all dig sites. For each soil sample collected, 5 mg was taken and diluted in 10 mL of distilled water. The soil was mixed thoroughly, allowed to settle for 10 minutes, and then measured using a pH meter until the meter reached stabilization. The process to measure the percent of carbon present in the soil started by letting two soil samples sit in a cool, dry place (Jeffords basement) for approximately one week. Once dried, the soils were sieved through a 2 mm sieve. This discarded non-soil particulate like twigs or leaves. A subsample of the sieved soil was then taken and ground with a mortar and pestle until it was able to pass through a 0.25 mm sieve. This was then put in a loosely capped vial, set in an oven at 80°C, and let sit for at least four hours. Once thoroughly dried, approximately 30 mg of the soil sample was measured out and placed in a small, flat aluminum foil container. The samples, along with duplicates, were run through an elemental analyzer to determine the percent of carbon in the soil. This was done for sites 6E and 7W which represented each side. With all of the required data established, tests for significance, correlation, and summary were performed. pH was split into two categories; above 5 pH or below 5 pH. Worm abundance and organic layer thickness were put together on a scatter plot to determine any correlation. Worm abundance was also compared against tree cover in a box plot. Lastly, percent of carbon in the soil was shown on a bar graph comparing the two sites with the duplicates.

RESULTS

Of the 20 sites that were sampled, 10 for each side of Church Woods, a total of 87 individual worms were found and identified. A little less than 70%, or 60 of the 87 individuals, of the total worm abundance were found on the maple dominated (east side). Statistical analysis yielded significant differences ($p=0.0463$)(Figure 2) between worm abundance and tree cover and these results were seen both during analysis and in the field. The hemlock dominated (west) side, where the least worms were found, contained a single outlier at site 6, which contained a total abundance of 12 worms identified in the 50cm³ plot. Both contained variable spreads that were about the same, and while the whiskers overlapped the actual boxes and medians did not (Figure 2).

After measuring Oe/Oi horizon depth (cm) for each 50cm³ plot, linear regression revealed a strong negative correlation ($r=-0.92$; $r\text{-square}=0.8467$, Figure 3) between this and the total worm abundance of each of the 20 sites. Data analysis illustrated a statistically significant relationship ($p<0.001$), with 84.6% of the variation in Oe/Oi horizon depth (cm) explainable by worm abundance. Data analysis also yielded a statistically significant relationship ($p=0.0005$; Figure 4) between pH and worm abundance. Only 2 of the 87 individual worms were found at a site that was tested to be at a pH lower than 5.00. These two individuals were found at site 7 of the hemlock dominated (west) side where the pH was measured to be 4.97 (Table 1). As it is noted in Table 1, any sites below these pH readings contained 0 worms, regardless of which side of the road they were on, noting that the significant cut off for worms preference was around a 5.00 pH reading.

Carbon analysis of site 6 of the maple dominated (east) side and site 7 of the hemlock dominated (west) side, both ran in duplicate, and compared with quality control samples, also yielded significant results (Figure 5). An average of the duplicates were taken to reveal a 6.29% total mass of carbon in the mixed soil sample from site 6 on the maple dominated (east) side. The average of the duplicates taken from the mixed soil sample from site 7 of hemlock dominated side yielded only a 1.63% total mass of carbon at the site.

Table 1. Raw data on the worm abundance for each site, thickness of the organic duff layer, pH level, and type of tree cover

Site Number	Dominant Tree Cover	Worm Abundance (Total Individuals per Site)	Oe/Oi Horizon Depth (cm)	pH Reading
1	Maple (East)	5	3.75	5.85
2	Maple (East)	2	8.50	6.23
3	Maple (East)	3	9.50	5.58
4	Maple (East)	8	2.50	5.83
5	Maple (East)	5	5.45	6.36
6	Maple (East)	8	3.05	6.63
7	Maple (East)	6	5.25	6.3
8	Maple (East)	5	7.55	6.01
9	Maple (East)	11	1.00	5.75
10	Maple (East)	7	5.75	6.23
1	Hemlock (West)	2	5.45	5.83
2	Hemlock (West)	8	2.30	6.03
3	Hemlock (West)	0	12.00	4.69
4	Hemlock (West)	0	10.50	4.93
5	Hemlock (West)	0	11.30	4.85
6	Hemlock (West)	12	2.25	6.12
7	Hemlock (West)	2	9.25	4.97
8	Hemlock (West)	0	10.75	4.54
9	Hemlock (West)	0	12.25	4.65
10	Hemlock (West)	3	7.75	5.92

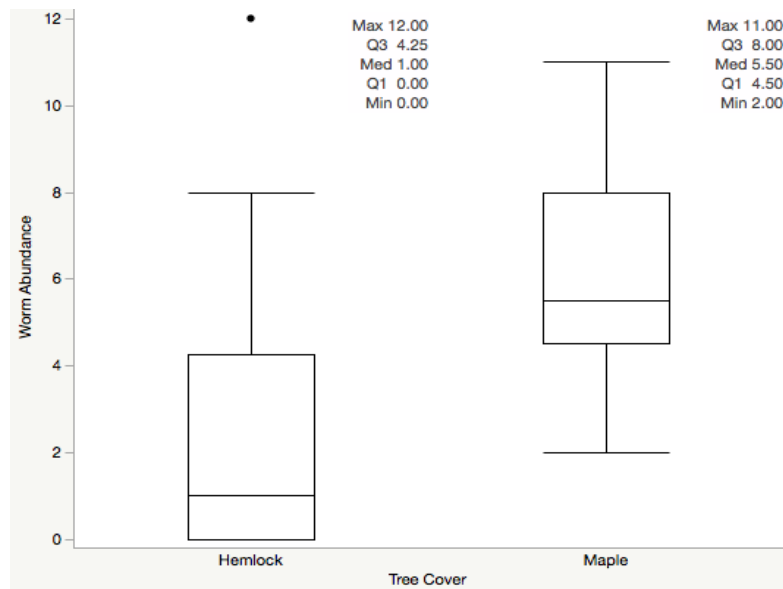


Figure 2. Box plot comparing the abundance of worms between the Hemlock (West) and Maple (East) treatments, representing the spread of variety across a sample size of 87, where statistical analysis yielded significant differences ($p=0.0463$) in abundance between the treatments.

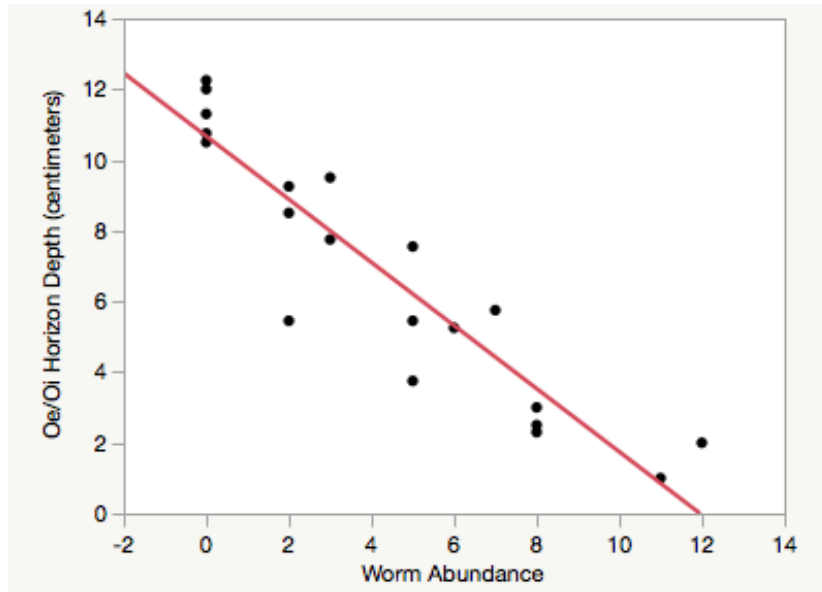


Figure 3. Scatterplot of the effect of worm abundance on Oe/Oi horizon depth (cm) across a sample size of 87, illustrating a statistically significant ($p < 0.001$) negative correlation ($r = -0.92$) between the two variables with 84.6% ($r\text{-square} = 0.8467$) of the variation in Oe/Oi horizon depth that is explainable by worm abundance.

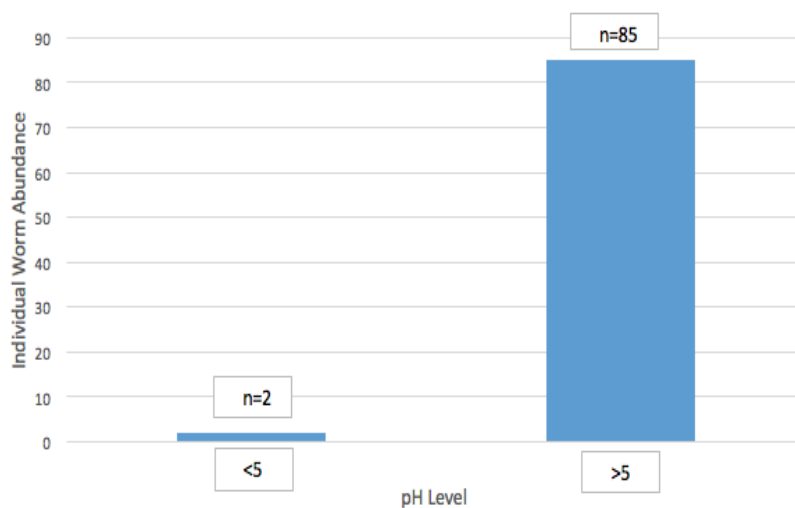


Figure 4. Bar chart showing the relationship between pH and abundance; a statistically significant indication that the cutoff for worm survival/preference in soil is a reading of pH=5.00 and above ($p = 0.0005$).

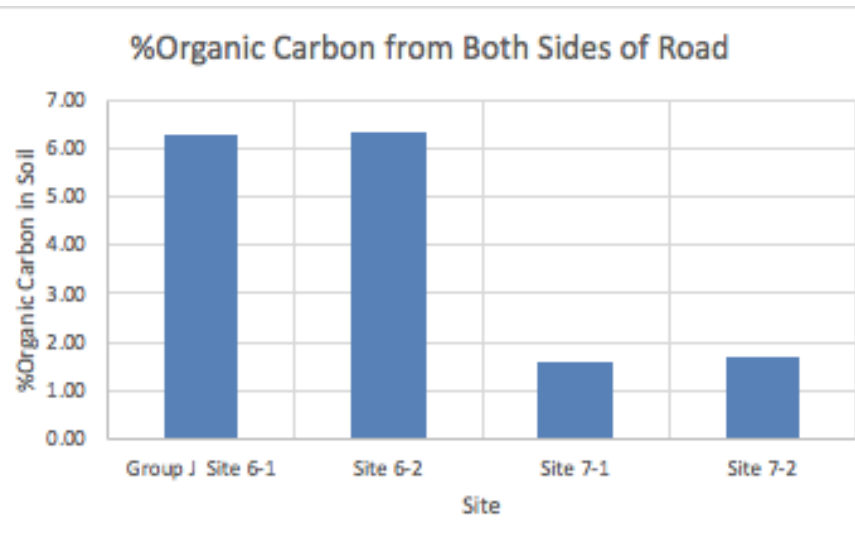


Figure 5. Bar chart of the %Carbon from mixed samples of Site 6 on the Maple (East) side and mixed samples of Site 7 of the Hemlock (West) side, with a duplicate run for each sample. Indicating much higher carbon levels for the Maple (avg.=6.29%) than for Hemlock (avg.=1.63%).

DISCUSSION

Main results yielded what was expected regarding a much higher abundance of worms found on the deciduous hardwood, maple dominated (east) side (Figure 2). This statistically significant relationship proves true as worms tend to have a higher preference for deciduous vegetation and the leaf litter that accompanies it, as it is easier to digest (Hough, 1960). Regarding soil that is predominantly covered by hemlocks and conifers, the upper soil horizons tend to be strongly acidic (hemlock source). The high acidity of hemlock litter along with the waxy coating that covers the accompanying needles makes this a less preferred environment for worms, as they tend to have a difficult time digesting this type of litter (Knowles et al., 2016).

Regarding this higher preference and easier digestion for deciduous forest cover, worms have also been found to have some sort of relationship with sugar maples, which was the predominant species of the maple dominated (east) side. Sugar maples tend to supply the soil with calcium, which is predominantly stored in their leaves when they drop during the fall. As their leaf litter breaks down, these nutrients get released into the surrounding soil. This in turn has been studied to be a key tool for earthworm survival, considering that these creatures “breathe out” calcite crystals that are made from the processes of combining carbon dioxide (CO₂) and calcium (Messenger, 1986). This would make sense as to why then nearly 70% of the individuals found were found on the maple dominated (east) side of the study area (Figure 2).

Deciduous cover also has a role to play in pH levels. As noted before, hemlocks and conifers tend to decrease the pH levels, by increasing the acidity of the surrounding soil.

Hemlock litter is both highly acidic and has a tendency to break down slower as it is covered in a waxy coating, which also makes it difficult for worms to break it down (Hough, 1960). In most sites that yielded a pH reading of less than 5.00 we noticed little to no earthworms present and found a significant relationship ($p=0.0005$) in the relationship of the cutoff at 5.00 readings (Figure 4). Worms not only tend to prefer and survive better at pH levels from 5.00 to 8.00 (“Earthworms”, 2013), but it has also been studied that they have the potential to increase soil pH (Burtelow et al., 1998).

It is possible for worms to have a positive effect on soil pH considering that they can reduce the acids of the organic layers. Considering they not only decompose these organic layers that contain trace amounts of acidity, but also based on the way they mix up the soil horizons and layers, the ability to redistribute, and in turn dilute, acidic soil is apparent (Burtelow et al., 1998). It is a situation where worms tend to prefer more basic soils, and with being able to survive there better, they have a tendency to make it even more suitable for themselves. The site with a pH reading of below 5.00 (4.97; Table 1.) still contained an abundance of 2 individuals. However, five other sites were measured at readings below 4.97 and none of these sites contained any worms, proving that they need some sort of a base layer or threshold to be able to survive.

As with breaking down the organic matter and layers of the soil, worms also have the ability to redistribute carbon throughout the soil. To ensure quality control, we took mixed soil samples from each site after we dug 50cm deep. As it is noted in the table (see Appendix), quality control samples Saugeen and Carscallen yielded sufficient recovery as compared with our samples. The duplicates run were similar to each other (Figure 5) and averages were taken for each.

Our results yielded that the maple dominated (east) side contained 6.29% carbon mass within the mixed sample of soil from site 6, compared with 1.63% carbon mass of the mixed soil sample from site 7 of the hemlock dominated (west) side. These results could be related to the fact that worms have the ability to store carbon in these aggregates that they create. Within these aggregates, carbon is harder to reach via microbial decomposition and in turn has the ability to be stored and sequestered within the soil for longer amounts of time.

While one would expect higher carbon levels under the hemlock dominated (west) side based on their productivity levels, it has been studied that worms have the potential to increase readily-mineralizable carbon mass, expressed per gram of soil organic matter, to increase 1.4-fold regarding worm abundance due to these aggregates (Burtelow et al., 1998). Therefore we concluded that the near 70% worm abundance that we found on the maple dominated (east) side had a correlation with the carbon levels measured. One could note that with more worms, comes a more productive carbon sequestering process within the aggregates in the soil, and in turn more carbon mass found in soils that contain these earthworms.

With the completion of this study, we are still left weighing the pros and cons of invasive earthworms in these types of environments. It is evident that they are efficient and productive when it comes to gardening, composting, and overall agriculture processes based on how they

mix up organic layers and nutrients within the soil. However, based on their ability to reduce essential leaf litter, their mixing of soil horizons, and overall depletion of organic layers where seedlings would initially develop, they are not ideal inhabitants in what should be a productive forest.

Regarding their populations, few measures can actually be carried out to control this invasion. More hemlocks and conifers can be planted to decrease soil pH and ward off the earthworms, but this would take a long time and might not prove effective. Biological control by introducing a fungus that kills worms could be effective enough, yet they don't kill off the cocoons of the worms where their eggs are, and they could possibly affect other organisms within the soil. Native moles could be introduced to control the population, as they tend to feed on earthworms, but the consequences of unleashing yet another species into this environment may also prove detrimental ("Moles" NWF, n.d.).

In an attempt to weigh the pros and cons of invasive earthworms, we are left contemplating how much of an impact they really make within the old growth forest of Church Woods. It is important to ask if the pro of them successfully sequestering carbon within their aggregates outweighs any of the previously addressed cons of them depleting the soil of vital nutrients. Future studies regarding this question should revolve around testing and measuring just how much carbon they sequester and for how long. These studies should also include measurements of how much carbon an invasive earthworm population can sequester compared with a sugar maple or a hemlock, and if this outweighs all the other benefits that these trees and plants provide, considering their reduced growth potential as a result of earthworms.

Sincere acknowledgements towards Dana Bishop, our consultant, Donald Ross, Josef Gorres, Jillian Sarazen, Maryam Nouri Aiin, and the University of Vermont for providing this research opportunity and all their help along the way.

LITERATURE CITED

- Burtelow, A., Bohlen, P., & Groffman, P. (1998). Influence of exotic earthworm invasion on soil organic matter, microbial biomass and denitrification potential in forest soils of the northeastern United States. (pp197-202). *Applied Soil Ecology*. [https://doi.org/10.1016/S0929-1393\(98\)00075-4](https://doi.org/10.1016/S0929-1393(98)00075-4)
- Earthworms. (2013). Retrieved December 2, 2019, from Penn State Extension website:
<https://extension.psu.edu/earthworms>
- Earthworm Invasion. (2013). Retrieved December 2, 2019, from
<https://www.uvm.edu/uvmnews/news/earthworm-invasion>
- Hough, A.I F. (1960). Silvical characteristics of eastern hemlock (*Tsuga canadensis*). (pp23). U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station.
- Knowles, M., Ross, D., Gorres, J. Earthworms In Forests. (2016). Retrieved October 10, 2019, from
https://fpr.vermont.gov/sites/fpr/files/Forest_and_Forestry/Forest_Health/Library/EarthwormsInForests_final.pdf
- Lapin, M., Webb, M., Cogbill, C. (2001). Church Woods Clayplain Forest Fragment. Ecological and Botanical Assessment with Ecological Management Recommendations
- Messenger S., (1986) Alkaline runoff, soil pH and white oak manganese deficiency (pp317-325), *Tree Physiology*. Retrieved November 3, 2019, from <https://doi.org/10.1093/treephys/2.1-2-3.317>
- Moles. (n.d.). Retrieved December 2, 2019, from National Wildlife Federation website:
<https://www.nwf.org/Home/Educational-Resources/Wildlife-Guide/Mammals/Moles>
- OPAL Soil and Earthworm Survey - Earthworm Identification Guide. (n.d.). Retrieved November 2019, from <http://www.opalexplornature.org/soilsurvey>.

Table 2. Appendix of Raw Data from Carbon Analysis:

Sample	Date	Mass (mg)	%N	%C	
Site 6E-1	11/14/2019	32.03	0.21	6.27	
Site 6E-2	11/14/2019	31.66	0.23	6.32	
Site 7W-1	11/14/2019	25.66	-0.62	1.57	
Site 7W-2	11/14/2019	28.32	-0.51	1.69	
QC Saugeen	11/14/2019	27.28	-0.36	2.64	98.1% rec
QC Carscallen	11/14/2019	29.6	-0.59	2.01	108.6% rec