Earthworm Community Density in Treated DIRT Plots

Vanessa Kiefer, Brittany Nofz, Rae Sansom

University of Michigan Biological Station

EEB 381- General Ecology

June 14, 2019

Clara Shaw

ABSTRACT

Nutrient cycling, a process that exchanges nutrients between the living and nonliving part of an ecosystem, plays a vital role in the growth and survival of organisms, especially plants, which require large amounts of nutrients that may otherwise remain inaccessible or unusable. Earthworms are common detritivores that live in forests with dense leaf litter, which contribute significantly to the cycling of nutrients. Worms were collected from soil plots at the UMBS DIRT project site, in order to study the effects of various treatments on worm abundance. In this study we found that the abundance of worms collected was significant between the epigeic and anecic worm types, and the biomass of the two worm types was marginally significant. Additionally, there was marginal significance for the amount of worms found in our extreme cases, the No Input compared to Double Litter and No Input compared to Double Litter Fertilizer. Because of earthworms' importance in nutrient cycling and their preference for dense leaf litter, they can have a large impact on the types of plants that are able to grow in a forest ecosystem.

INTRODUCTION

Nutrient cycling is the process through which nutrients are exchanged between the living and nonliving part of an ecosystem. Cycling in forest environments involves the input of nutrients into the system through the atmosphere and the weathering of minerals, uptake and storage of nutrients by plant life, the production of litter, the decomposition of litter, and the transformation of soil by the organisms living within it (Foster and Bhatti, 2006). This process plays a vital role in the growth and survival of organisms, and for plants in particular, which require large amounts of nutrients that may otherwise remain inaccessible or unusable. Without decomposers and detritivores to break them down, animal waste and plant litter would accumulate on the forest floor. The nutrients within these waste products would remain in forms that are biologically unavailable to plants, leading plants to “starve” to death, which could cause a chain reaction, with catastrophic results for the food chain (Harwood et al., 2018).

In forest ecosystems, trees support their own nutrient uptake by depositing leaf and root litter onto the forest floor. An estimated 90% of plant matter enters the detrital pool (Gessner et al., 2010), where it is broken down by detritivores. The decomposition of this litter allows nutrients to be returned to the soil, where they can be reabsorbed by the surrounding plant life (Foster and Bhatti, 2006). Detritivores both remove decaying organic material from the ecosystem and recycle it, which prevents the ecosystem from running out of the nutrients its organisms rely on. Therefore, detritivores are an essential part of the forest ecosystem, turning normally limiting resources, such as nitrogen and phosphorus, into soluble, accessible forms. Some typical detritivores found in a forest ecosystem include woodlice, millipedes, and earthworms, among many others. The diversity within a community of detritivores has consequences on the nutrient cycles within an ecosystem, with each species using resources differently. Detritivore diversity is associated with the concept of transgressive overyielding, which is when diverse communities of different decomposer species contribute to higher rates of decomposition than a population of a single highly efficient decomposer species (Gessner et al., 2010). In other words, the more species of decomposers there are in a community, the faster detritus is broken down. This suggests that there is resource partitioning occurring within the community. For example, previous studies have suggested that isopods and earthworms display facilitative interactions when introduced to high quality detritus, leading to a high decomposition rate (Gessner et al., 2010). Therefore, detritivore diversity impacts the availability of normally limiting resources, which may or may not prove useful to the surrounding environment, depending on the adaptations the system has developed.

Earthworms are common detritivores that are often used in various growing systems to improve nutrient cycling. Although these organisms can be helpful to gardeners and are widespread throughout the state, they are actually an invasive species in the northern parts of North America, where glacial periods eradicated all native species about 10,000 years ago. The earthworms now found in these parts of North America were brought overseas from Europe in the 1700s (Frelich et al., 2006). Earthworms are known as “ecosystem engineers,” meaning they are able to alter the way an ecosystem functions, potentially changing the diversity of organisms, the soil composition, and the movement of water or nutrients (Frelich et al., 2006). This engineering occurs between three major types of earthworms: epigeic, endogeic, and anecic. Each category is comprised of multiple species, grouped into these three categories based on where they feed, inhabit, and a few key outward characteristics. The epigeic worms commonly live on the leaf litter or very top layer of the nutrient-rich A horizon of soil without constructing burrows. They are relatively small and feed on the leaf litter without mixing much of the organic layer and mineral soil (Asshoff et al., 2010). Endogeic worms are also smaller in size but make permanent horizontal burrows in the soil. Because they spend most of their time below the surface they are responsible for the mixing of the mineral soil and help form a thicker A layer. These worms consume soil and any organic matter they find within it (Dabral et al., 2012). Anecic worms, commonly referred to as “nightcrawlers”, are the much larger worms that make vertical burrows up to 2 meters deep into the soil. Anecic worms only leave their burrows in order to feed on the leaf litter and they are able to consume a lot of organic matter over the duration of a year. These worms are also able to transfer organic material form the top layer into the mineral soil and vice versa (Asshoff et al., 2010). In addition to altering the structure of the soil with their burrows, earthworms are key contributors of nitrogen and phosphorous for their communities. Earthworms’ bodies break down quickly which, along with their excretions, provide a rich supply of organic nutrients to the surrounding soil (Lines-Kinley, 1970). While the presence of earthworms has been potentially beneficial for human agriculture, it is worth noting that they may be more detrimental in the old-growth forests of North America, which have adapted to a slower rate of decomposition, and may be harmed by the increased decomposition.

Previous studies have shown that the optimum temperature for an earthworm habitat is between 22-29°C, with temperatures significantly higher or lower leading to mortality and low rates of maturation (Viljoen et al., 2002). They also breathe through their skin and thus require a moist environment in order to avoid drying out. Leaf litter provides not only a vital food source for earthworms, but a protective cover as well. The dead leaves retain heat and water, protecting earthworms from harsh weather conditions that would otherwise kill them. Forests, with their dense floors of roots and leaf litter, provide an optimum habitat for worms to flourish.

The goal of this study was to see how worm communities vary across different environmental conditions within a forest ecosystem. To do this we analyzed worm type and abundance in plots within the DIRT Project at the University of Michigan Biological Station. Carbon-nitrogen analysis was also performed to determine if the types of treatments changed the ratio of carbon and nitrogen within the epigeic worms. We predicted that earthworms would be more abundant in plots that had more organic matter, because there would be a larger available food source, and that those worms would also have a higher C-N ratio due to the increased amount of carbon and nitrogen within the organic layer and A-Horizon. On the other hand, we expected to see fewer worms in the No Input plots, due to a lack of resources. We also expected to see a difference in the abundance of earthworms in the Double Litter Fertilizer plots, due to the increased quantity of nitrogen within the plot.

METHODS

We analyzed worm total abundance and the abundance of worms per niche group across five field treatments: Control (no treatment applied), No Input (all detritus and plant life removed), Wood (three years of carbon is added every three years via aspen wood chips), Double Litter (a second layer of leaf litter is added), and Double Litter Fertilizer (a second layer of leaf litter and nitrogen-based fertilizer are added). The treatments had been applied for 15 consecutive years by the start of this experiment. We tested a total of 15 plots from three blocks, with each treatment represented in all blocks. We collected worms from a 0.25 square meter area within each plot.

To collect the worms, we temporarily removed the organic layer from the surface. We applied four liters of mustard solution (1.06% ground mustard) to the plot using a watering can. After collecting, identifying, and recording earthworms for ten minutes, another four liters of mustard solution were applied to the plot. Earthworms were collected for another ten minutes. The organic layer was replaced. We collected earthworms and separated them by niche group, block number, and treatment type while in the field. We identified worms as one of three niche groups: anecic, endogeic, or epigeic (Figure 1). We repeated these steps for each plot across all tested blocks.

We measured the organic horizon in each plot, using a ruler for those plots with leaf litter, and measuring in centimeters (plots with no leaf litter had an organic horizon of 0 cm). We then used an Oakfield Corer to take a soil sample 20 cm in depth from each plot. The ruler was again used to measure the A-horizon and E-horizon of each plot, in centimeters (Figure 2).

We organized the earthworms by niche group, block, and plot, and dried them at 55-65 degrees Celsius over 48 hours. After drying, we took the dry weight of each type of earthworm within each plot. We set aside a portion of the epigeic earthworms from each plot, ground these portions into a fine powder using a mortar and pestle, and placed them into microcentrifuge tubes, each labeled with the plot type. One milligram of powder from each group was placed into an individual tin capsule, which was crimped shut and placed into an elemental analyzer. The elemental analyzer was dropped into a furnace at 950 degrees Celsius, with the addition of a blast of oxygen. This process used metal catalysts to oxidize our samples, turning them into CO_2 and N_2 gasses. Through chromatography, we separated the gasses. Then, the carbon-nitrogen ratio was found using a pre-calibrated thermal conductivity detector.

We used RStudio to analyze species distributions in the treatments with a Chi-Squared test, to determine if the earthworms (or particular niche groups) created larger communities in specific plots, or whether they were evenly distributed across plots. We compared earthworm frequency in the different niche groups across treatments with an ANOVA test. Afterward, we used a Tukey Test to determine which plots had differing earthworm ratios.

The dry mass data was run through an ANOVA test to determine whether there was a significant difference in the dry mass of each worm type across treatments when data from all blocks was collated.

Additional ANOVA tests were run using the carbon to nitrogen ratio data, to determine if there were any significant differences in the carbon percentages, nitrogen percentages, or carbon-nitrogen ratios in the epigeic worms from each treatment type.

RESULTS

In this study, we caught a total of 113 earthworms. Notably, the worms caught included epigeic and anecic worms, but none from the endogeic niche group. Epigeic worms made up the vast majority of our collected worms (Figure 3/Table 1). Across all replicates, we collected 99 epigeic worms versus 14 anecic worms. It is also clear that worms of all species appeared in much fewer numbers within the No Input plots, with only four epigeic worms and no anecic worms collected. We found that there was not a significant difference between the distribution of worms across the five different treatments (Chi-Squared test p-value = 0.4371). Despite the insignificance of the distribution of worms, we found a significant difference between the average number of anecic and epigeic worms found across the different treatment types (Figure 4, ANOVA, Worm Type F-value= 28.20, p-value < 0.001; Treatment F-value = 2.45, p-value =

0.074). Within the treatment types we found that there was a marginally significant difference between the number of worms found in the No Input and Double Litter (Tukey p-value = 0.089) and the No Input compared with the Double Litter Fertilizer (Tukey p-value = 0.089).

We found that there was not a significant difference between worm dry mass and the different treatments (Table 2, ANOVA p-value = 0.3708), but there was a marginally significant difference between dry mass from the different worm types (Table 2, ANOVA p-value = 0.0944). (See also: Figure 5).

We analyzed the carbon to nitrogen ratio between epigeic worms from the Double Litter Fertilizer, Control, No Input, Wood, and Double Litter plots between all blocks. We found that there was no significant difference in the ratio between the different types of treatments (Table 3, F value = 1.368, p-value = 0.326). (See also: Figure 6). When analyzing each element alone, we found that there was no significant difference between treatments for bodily percentage of carbon (Table 4, F-value = 2.155, p-value = 0.165) or nitrogen (Table 5, F-value = 0.286, p-value = 0.879). (See also: Figure 7 and Figure 8).

DISCUSSION

Earthworm type and quantity of litter can change the optimal habitat and amount of resources. Because of this, we predicted that different litter treatments in a forest ecosystem would impact the types of worms and their abundance found in each plot. We found that there was not a significant difference in the distribution of worms across the different treatments, but there was a significant difference in the number of epigeic worms that were found compared to the anecic across the five treatments. Additionally, our results show that there was marginal significance in the difference in worm abundance between No Input and Double Litter as well as between No Input and Double Litter Fertilizer, but that the number of worms found across all other plots had no significance. This lack of significance may be due to a lack of replicates and/or the comparison between the two extremes that either had no leaf litter or more litter than normal. The No Input plots were expected to have very few worms, since these plots contained no organic material for the worms to eat, whereas the Double Litter plots with and without the fertilizer provided plenty of food for the worms. In addition to serving as a food source, leaf litter provides shelter for the worms, retaining moisture and heat and thus protecting the worm community from drying out or getting too cold. However, it was hypothesized that, despite having an over-abundance of leaf litter, the Double Litter Fertilizer plots would have a lower abundance of worms. Due to the addition of nitrogen-based fertilizer, it was thought that earthworm populations would grow to carrying capacity, leading to overuse of the available nutrients and an eventual decline in populations to well below carrying capacity.

The data showed significance in the number of epigeic and anecic worms found across all treatment types, while the dry mass results showed marginal significance between worm types. The difference between epigeic and anecic worms could potentially be due to the differences in worm type sizes, as anecic worms grow to be quite large, while epigeic worms remain smaller. In the early stages of experimentation, we noted an expectation to see a difference in the number of worms collected at Block 1 compared to the other two areas, as Block 1 is closest to the road. In previous studies, a correlation between road proximity and earthworm distribution has been observed (Shartell et al., 1970). Earthworms tend to be found in more abundant numbers further away from roadways, likely because of the vibrations created by passing vehicles. Such vibrations may stimulate earthworm movement, as seen in “worm grunting,” a method of earthworm extraction that uses vibrations to bring worms to the soil surface. In both cases, the earthworms may recognize the vibrations either as falling rain or approaching predators (Mitra et al., 2009).

Because leaf litter contributes a large amount of nutrients to the soil, we also tested the carbon and nitrogen levels of the collected worms through carbon-nitrogen ratio (C:N) analysis. We predicted that different treatments would result in worms with different C:N, due to different plant matter in the litter containing different levels of each nutrient. For example, wood has more carbon than leaves while leaves are more nitrogen-rich than other parts of the plant. However, we found that the C:N was not significantly different between the treatment types. We hypothesize that this could be due to similar foliage surrounding the blocks, such as the Big Tooth Aspen, which dominates the collection site, and may lead to similarly proportioned nutrient inputs around each plot. The nutrient concentration in Aspens is higher when compared to the species that generally coexist with it (Alban et al., 1993). All of the plots that had any organic input from the Aspen leaves could have had an abundance of nutrients - particularly nitrogen, phosphorous, calcium, and potassium (Alban et al., 1993) - available. This does not explain the composition of C:N that was found for the No Input plots, where root blockers were used to decrease the chances of organic matter input. Despite a sandier A-horizon, which we expected to contain significantly fewer nutrients, the No Input plot worms showed no significant difference in C:N when compared to the worms from other plots. However, the carbon and nitrogen make up of the No Input worms from Block 1 was significantly lower than that of the worms from other plots, although the C:N ratio was comparable. Further research is likely needed to determine whether it is common for No Input worms to have normal amount of carbon and nitrogen, or much lower amounts than normal, as well as the reasons behind this. Our data also showed that, despite the use of a nitrogen-based fertilizer, worms found in the Double Litter Fertilizer treatment plots did not have a significantly higher percentage of nitrogen in their remains. The reason for this could be that the nitrogen-based fertilizer contains a form of nitrogen that is biologically unavailable to the worms, but could enter the worms' body through plant consumption. The increased amount of nitrogen in plants would cause their C:N to decrease, which would in turn be reflected in the C:N of the earthworms that consumed them. The nitrogen-based fertilizer could also be important in plant growth in the plots, but part of the maintenance of the plots is removing all plant life, so the nitrogen would only increase in the worms if they were able to eat the roots and bacteria that used the nitrogen-based fertilizer. Further research over a longer time span is needed to determine whether the addition of fertilizer affects the worm abundance and/or C:N within fertilizer-treated plots.

Mistakes made with our samples, in addition to other variables, may have influenced our results. For example, our research relied in part on the consistent upkeep of the UMBS DIRT plots by the project team, as well as our own consistency in the experimentation process. Both processes may have contained human error. One notable example of a potential confounding variable is caused by the age of the root tarps surrounding some of the tested plots. Because upkeep of the root tarps is nearly impossible, there is no way to ensure that these tarps are still preventing root growth into certain plots. Therefore, there may be an outside source of nutrients contributing to the soil within our testing areas. Or, conversely, invading roots may be taking up what little nutrients were in the soil. This could be especially confounding for the data collected from our No Input plots.

A key component of this study was the confident identification of earthworms as either epigeic, endogeic, or anecic. Our samples ultimately included only epigeic and anecic worms. This could be due to the types of treatments, soil in the Great Lakes region, or human error. Endogeic worms specifically may have been misidentified due to their transparency. After eating, endogeic worms may have appeared to be epigeic worms, due to the visibility of dirt within their bodies. Additionally, endogeic worms are known to be scattered throughout the Great Lakes region, but are not as widely distributed as epigeic and anecic worms. Therefore, it is possible that our experiment was carried out in a region with fewer endogeic worms. Most of the collected worms were juveniles (had not yet acquired clitellum) and were slightly more difficult to identify. This is especially relevant as earthworms reproduce in the spring and summer months and collection took place in late May and early June (Holmes, 2000).

Collection of worms was completed over the course of three different days, each of which brought slightly different weather conditions, which may play a role in altering earthworm abundance. Plots with higher amounts of leaf litter may not have been as heavily impacted, since leaf litter retains moisture. The blocks were also in different places in the forest, with differing shade and sun exposure creating conditions that are either more moist or more dry, across treatments.

Our results suggest that earthworms have a preference for habitats with abundant amounts of leaf litter to serve as their food and shelter. Because of the large amount of organic matter and nutritional deposits from trees, particularly Aspens, in forest ecosystems, North American forests create a habitat ideal for earthworm colonization and, therefore, are more at risk of further invasion. While earthworms play a valuable role in nutrient cycling, it is important to examine other impacts they have on the environment. Invasive earthworms have been linked to the destruction of forests due to their alteration of the soil's physical properties, eating through and destroying the organic layer of the soil that various woodland plants and animals rely on to survive. This causes the forest soil to be dry and opens it up to be vulnerable to erosion which in turn reduces its capacity to retain carbon and nitrogen from the atmosphere (Groffman, 2019). This leads to soil remaining nutrient-poor, and reduces biodiversity levels of plant and animal communities. Seedlings and plants found in the forest understory are particularly vulnerable because earthworms bring down nutrients out of the reach of these plants' shallow root systems. Forests invaded by earthworms have drastically fewer plants growing in their understory compared to forests without earthworms (Knowles et al., 2016). The increased mortality rates of these plants leads to a lower overall plant population and increases the vulnerability of the remaining vegetation to grazing by herbivores (Hale et al., 2008). This deficient soil also causes the forest to be more susceptible to invasion by pioneer plant species that are better able to survive in poor quality habitats (Hale et al., 2008). Earthworms also compete with other decomposers in the community, leading to lower populations of fungi, including mycorrhizae, a symbiont of plants.

In our study, we found epigeic earthworms to be significantly more abundant in the treatment plots than anecic worms. There was a marginally significant difference between the treatments with dense leaf litter than treatments where litter was absent. Because of this, epigeic worms may have a larger impact on the woodland landscape than anecic worms. Areas with abundant leaf litter may also face further invasion from earthworms and associated ecosystem changes. Over time, it is likely that we will observe substantial changes to North American woodland ecosystems associated with the invasion of earthworms.

ACKNOWLEDGEMENTS

We would like to express our gratitude toward the people who made this study possible:

Dr. Knute Nadelhoffer, thank you for introducing us to the DIRT Project and allowing us to work in the plots. Thank you to John, Kate, and everyone who is a part of the DIRT Team for guiding us through the plots, aiding in experimental setup, and for giving us your patience. Thank you to Sherry, the stockroom manager, for setting us up with our supplies, no matter how odd the request. We would also like to thank Tim Veverica from the chemistry lab for providing the elemental analyzer results for this project. Finally, we would like to thank Dr. Clara Shaw for introducing us to Dr. Nadelhoffer and his team, helping us with this paper every step of the way, salvaging our RStudio code, and guiding us whenever we were confused. We would be nowhere without your patience and advice.

WORKS CITED

Alban, D. H., & Pastor, J. (1993, February 9). Decomposition of aspen, spruce, and pine boles on two sites in Minnesota. Retrieved June 12, 2019, from https://www.nrcresearchpress.com/doi/abs/10.1139/x93-220#.XQFVJohKiUm

Asshoff, R., Scheu, S., & Eisenhauer, N. (2010). Different earthworm ecological groups interactively impact seedling establishment. *European Journal of Soil Biology vol. 46* (5), doi:https://doi.org/10.1016/j.ejsobi.2010.06.005

Dabral, M., Joshi, N., Maikhuri, R., Joshi, A., & Dabral, S. (2012, August 16). Effect of diet on feeding and casting activities of earthworms (Drawida nepalensis) and response of crop growth. Retrieved June 12, 2019, from http://www.tropecol.com/pdf/open/PDF_54_3/10-Dabral%20et%20al.pdf

W Foster, Neil & Bhatti, Jagtar. (2006). Forest Ecosystems: Nutrient Cycling. *Encyclopedia of Soil Science.* Retrieved June 10, 2019, from https://www.researchgate.net/publication/268423754_Forest_Ecosystems_Nutrient_Cycling

Frelich, Lee E, et al. "Earthworm Invasion into Previously Earthworm-Free Temperate and Boreal Forests." *Biol Invasions*, vol. 8, 4 July 2006, pp. 1235–1245., doi:10.1007/s10530-006-9019-3. Retrieved June 12, 2019 from https://link.springer.com/content/pdf/10.1007%2Fs10530-006-9019-3.pdf

Gessner, Mark & Swan, Christopher & K Dang, Christian & Mckie, Brendan & D Bardgett, Richard & Wall, Diana & Hättenschwiler, Stephan. (2010). Diversity Meets

Decomposition. Trends in ecology & evolution. 25. 372-80. 10.1016/j.tree.2010.01.010. https://www.researchgate.net/publication/41622258_Diversity_Meets_Decomposition

Groffman, P. (2012, July 25). Earthworms are invading our forests. *Earth Wise podcast.* Retrieved June 12, 2019, from https://www.caryinstitute.org/discover-ecology/podcasts/earthworms-are-invading-our-forests

Great Lakes Worm Watch. (n.d.) Worm Identification: Earthworm Ecological Groups. University of Minnesota. Retrieved June 12, 2019, from http://nrri.d.umn.edu/worms/identification/ecology_groups.html

Great Lakes Worm Watch (n.d.) . [A diagram depicting the three major niche groups of earthworms, for the purpose of identification]. (n.d.). University of Minnesota. Retrieved May/June, 2019, from http://nrri.d.umn.edu/worms/identification/ecology_groups.html

Hale C.M., Frelich L.E., Reich P.B., & Pastor J. (2008) Exotic earthworm effects on hardwood forest floor, nutrient availability and native plants: a mesocosm study. *Oecologia vol. 155*, 509–518. Retrieved June 12, 2019, from https://link.springer.com/article/10.1007%2Fs00442-007-0925-6

Harwood, J., Wilkin, D., Kraus, D., Gray-Wilson, N., Brainard, J., Johnson, S., Willan, J., Karasov, C. (2018, August 13). Consumers and Decomposers. Retrieved June 12, 2019, from https://www.ck12.org/biology/consumers-and-decomposers/lesson/Consumers-and-Decomposers-MS-LS/

Holmes, K. J. (2000). *Earthworms.* Mankato, MN: Bridgestone Books.

Knowles, M., Ross, D., Gorres, J., Wilmont, S., Danks, C., & Cogbill, C. (2016). Earthworms in Forests. Retrieved June 12, 2019, from https://fpr.vermont.gov/sites/fpr/files/Forest_and_Forestry/Forest_Health/Library/EarthwormsInForests_final.pdf

Lines-Kelly,, R. (1970, January 01). How earthworms can help your soil. Retrieved June 12, 2019, from https://www.dpi.nsw.gov.au/agriculture/soils/biology/earthworms

Mitra, O., Callaham, M., Smith, M., & Yack, J. (2009). Grunting for worms: Seismic vibrations cause Diplocardia earthworms to emerge from the soil. *Biology Letters, vol. 5* (1), 16-19. https://doi.org/10.1098/rsbl.2008.0456

Science Learning Hub. (2012, June 12). Niches within earthworms' habitat. Retrieved June 9, 2019, from https://www.sciencelearn.org.nz/resources/7-niches-within-earthworms-habitat

Shartell, L. M., Lilleskov, E. A., & Storer, A. J. (1970, January 01). Predicting exotic earthworm distribution in the northern Great Lakes region. Retrieved June 12, 2019, from https://www.nrs.fs.fed.us/pubs/45331

Viljoen, S., & Reinecke, A. (2002, December 10). The temperature requirements of the epigeic earthworm species Eudrilus eugeniae (oligochaeta) - a laboratory study. Retrieved June 12, 2019, from https://reader.elsevier.com/reader/sd/pii/003807179290116F?token=A67137444C3DE5AF6FC6233F2BAB63728BB0BB5A6F3BFA50A9B4AAAA5AECCE40143876C2018914EF3C4717A4621F0B56

APPENDICES

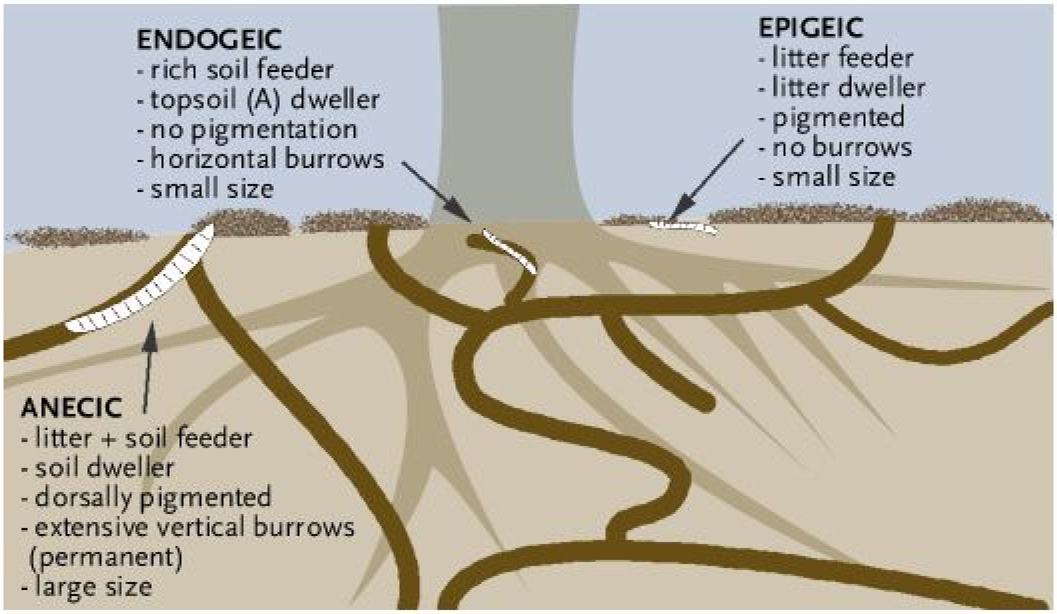

Figure 1. Figure depicting the habitats and key identification features of earthworms in three major niche groups.

Figure 2. Soil samples from various DIRT plots at the University of Michigan Biological Station.

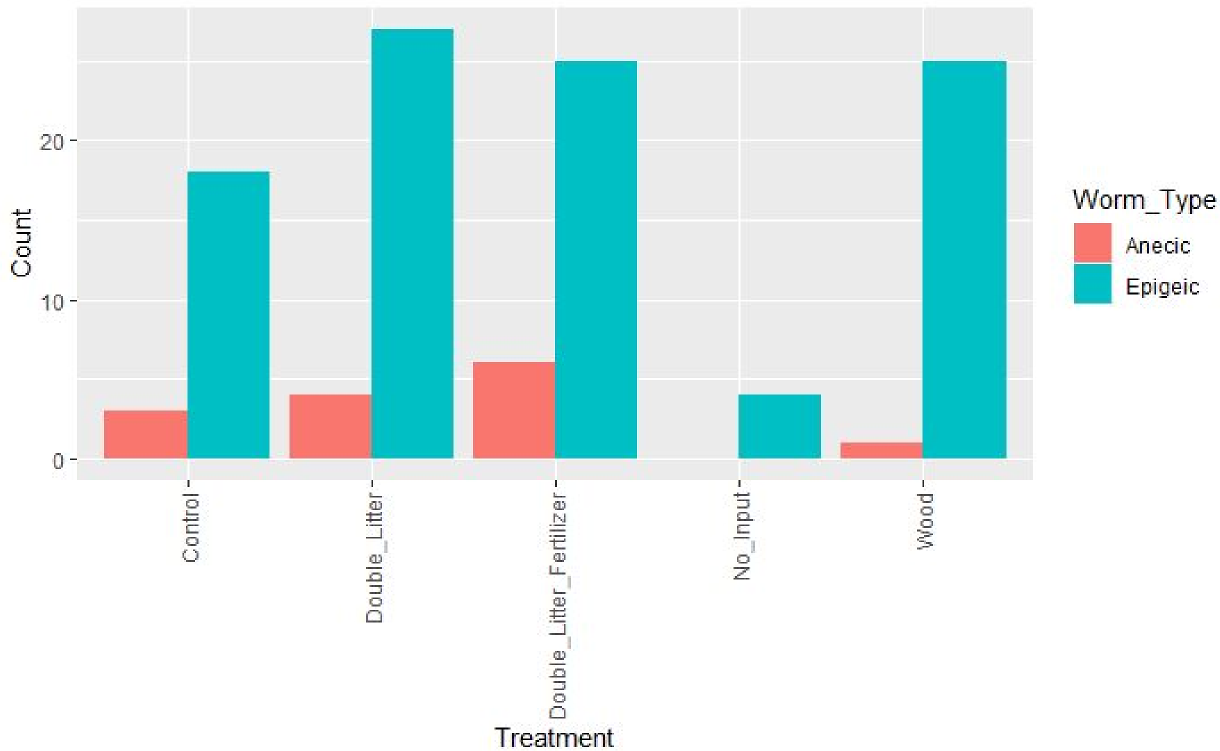

Figure 3. Counts of each worm type for each of the five different treatments across all three blocks. The blue bars representing epigeic worms shows that they were more abundant in all of the treatments but their density varied as well as the anecic worms.

Table 1. The number of earthworms found from each treatment across all three blocks.

Summary of Earthworm Counts found in all Blocks					
	Control	**Double Litter**	**Double Litter Fertilizer**	**No Input**	**Wood**
Anecic	3	4	6	NA	1
Epigeic	18	27	25	4	25

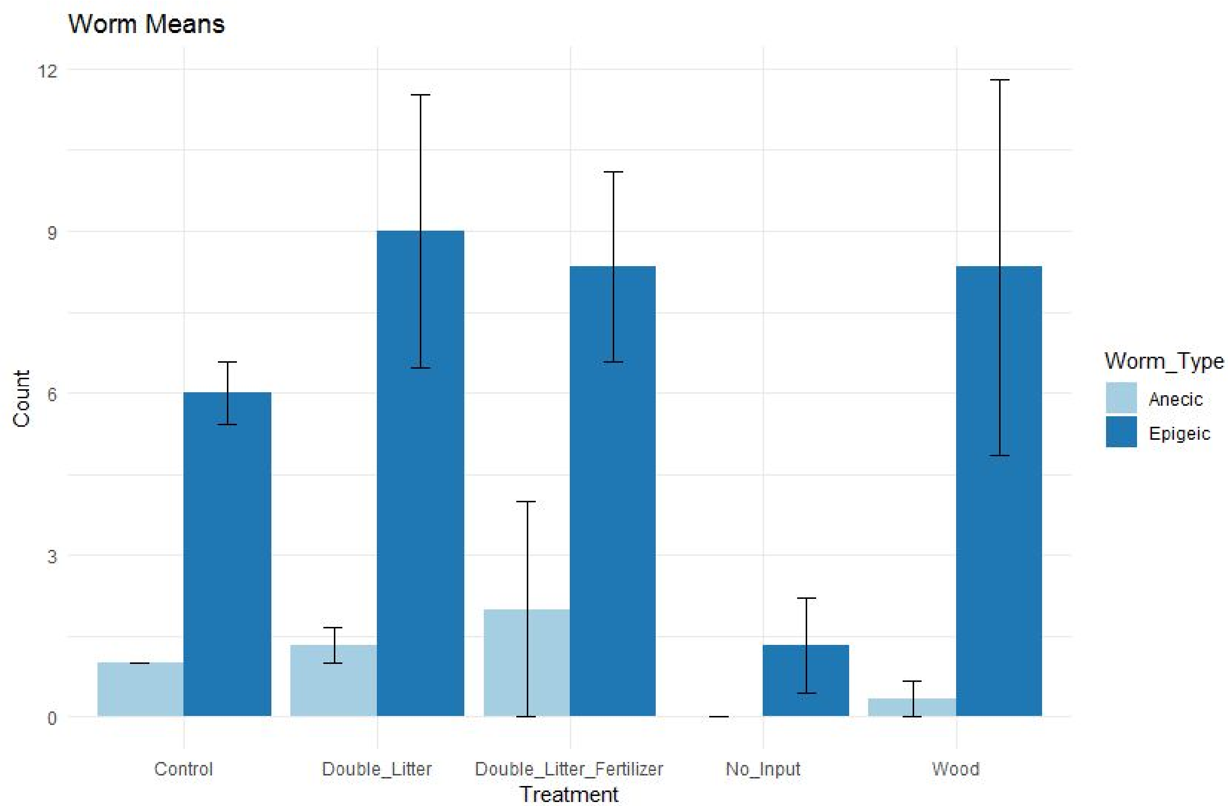

Figure 4 . Average number of worms found within each type of treatment across all three blocks and broken down into the two types of worms found. The cyan bars represent anecic worm counts and the darker blue bars represent epigeic worm counts.

Table 2. ANOVA results comparing significance of worm type and treatment on mean dry mass.

Significance of Worm Type and Treatment on Mean Dry Mass					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Worm Type	1	0.1181	0.11807	3.161	0.0944
Treatment	4	0.1712	0.04279	1.146	0.3708
Residuals	16	0.5976	0.03735	-	-

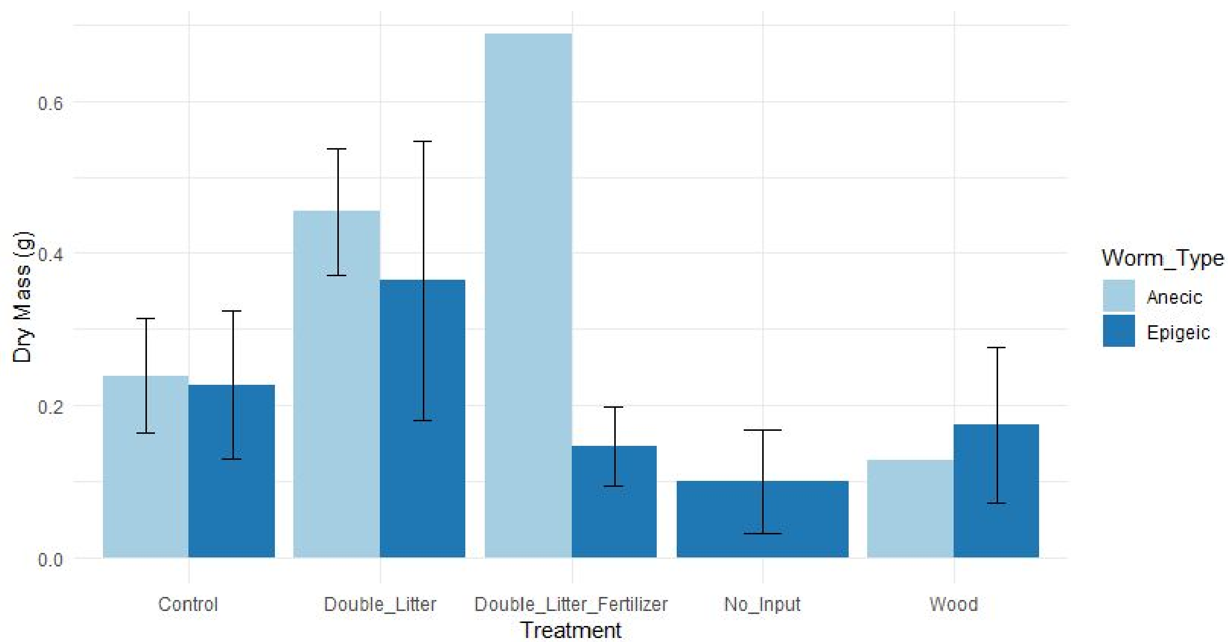

Figure 5. Average dry mass of worms per each treatment across all blocks. The cyan bars represent the average dry mass in grams of anecic worm and the darker blue bars represent the average dry mass in grams of epigeic worm counts.

Table 3. ANOVA results comparing the carbon to nitrogen ratios of epigeic worms across all plots.

Significance of Carbon to Nitrogen Ratio in Epigeic Worms					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
C:N to Treatment	4	9.343	2.336	1.368	0.326
Residuals	8	13.656	1.707	-	-

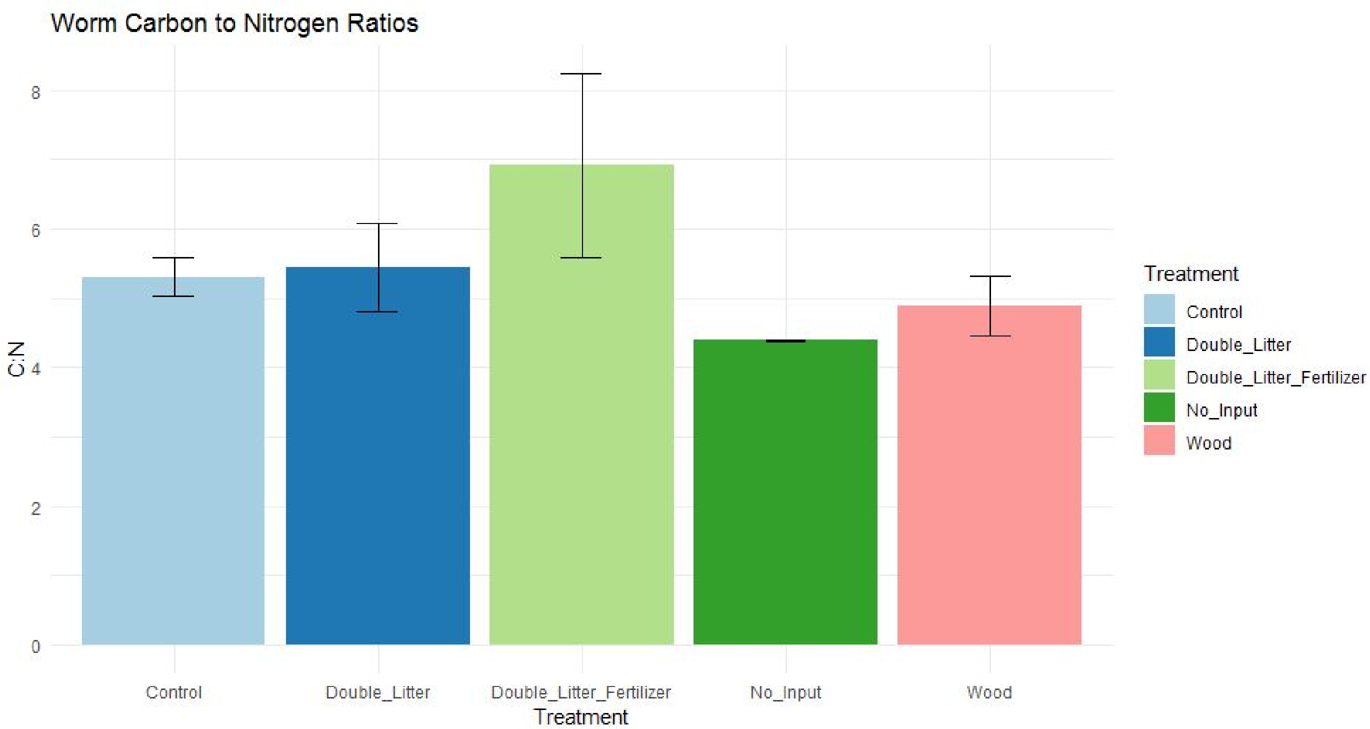

Figure 6. Ratios of carbon vs. nitrogen of earthworm dry remains. The cyan bar represents the C:N ratio of worms from the Control plots, blue represents that of the worms from the Double Litter plots, light green represents that of the worms from the Double Litter Fertilizer plots, darker green represents that of the worms from the No Input plots, and salmon represents that of the worms from the Wood plots.

Table 4. ANOVA results comparing the percentage of bodily carbon of epigeic worms across all plots.

Significance of Carbon Percentages in Epigeic Worms					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Carbon Percentage	4	449.1	112.3	2.155	0.165
Residuals	8	416.8	52.1	-	-

Table 5. ANOVA results comparing the percentage of bodily nitrogen of epigeic worms across all plots.

Significance of Nitrogen Percentages in Epigeic Worms					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Nitrogen Percentages	4	4.381	1.095	0.286	0.879
Residuals	8	30.611	3.826	-	-

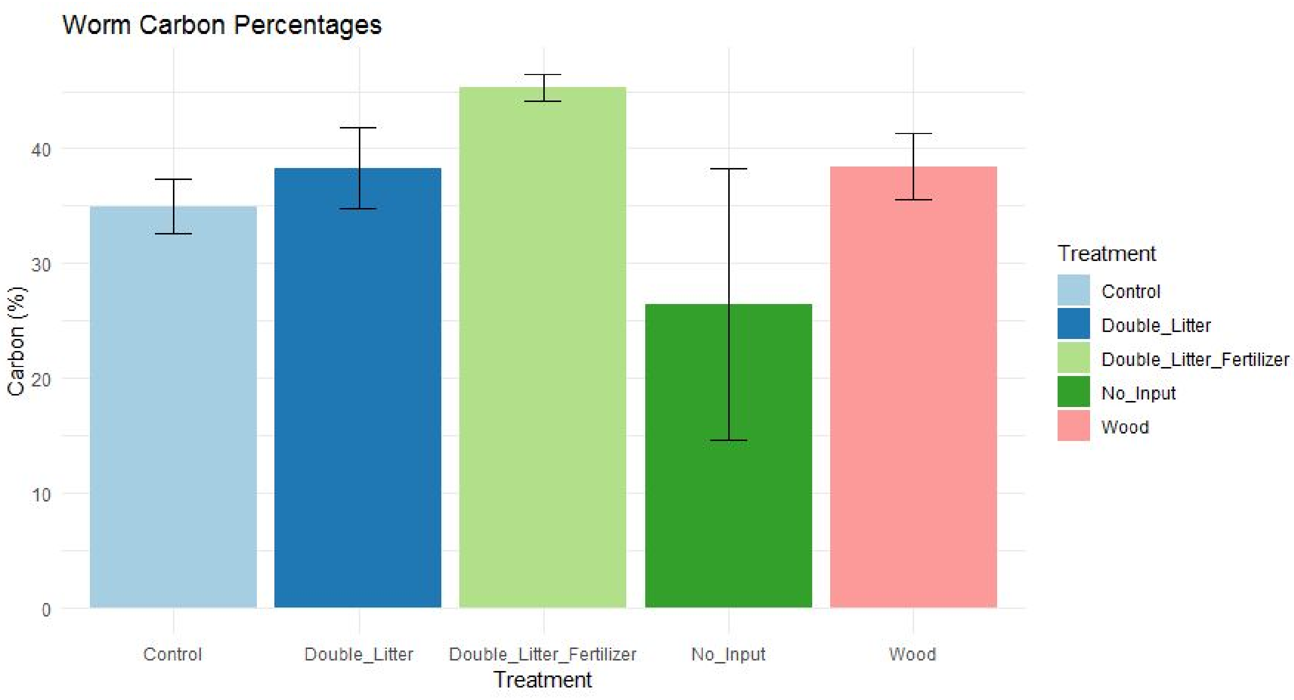

Figure 7. Percentage of carbon in earthworm dry body mass. The cyan bar represents the percentage of carbon of worms from the Control plots, blue represents that of the worms from the Double Litter plots, light green represents that of the worms from the Double Litter Fertilizer plots, darker green represents that of the worms from the No Input plots, and salmon represents that of the worms from the Wood plots.

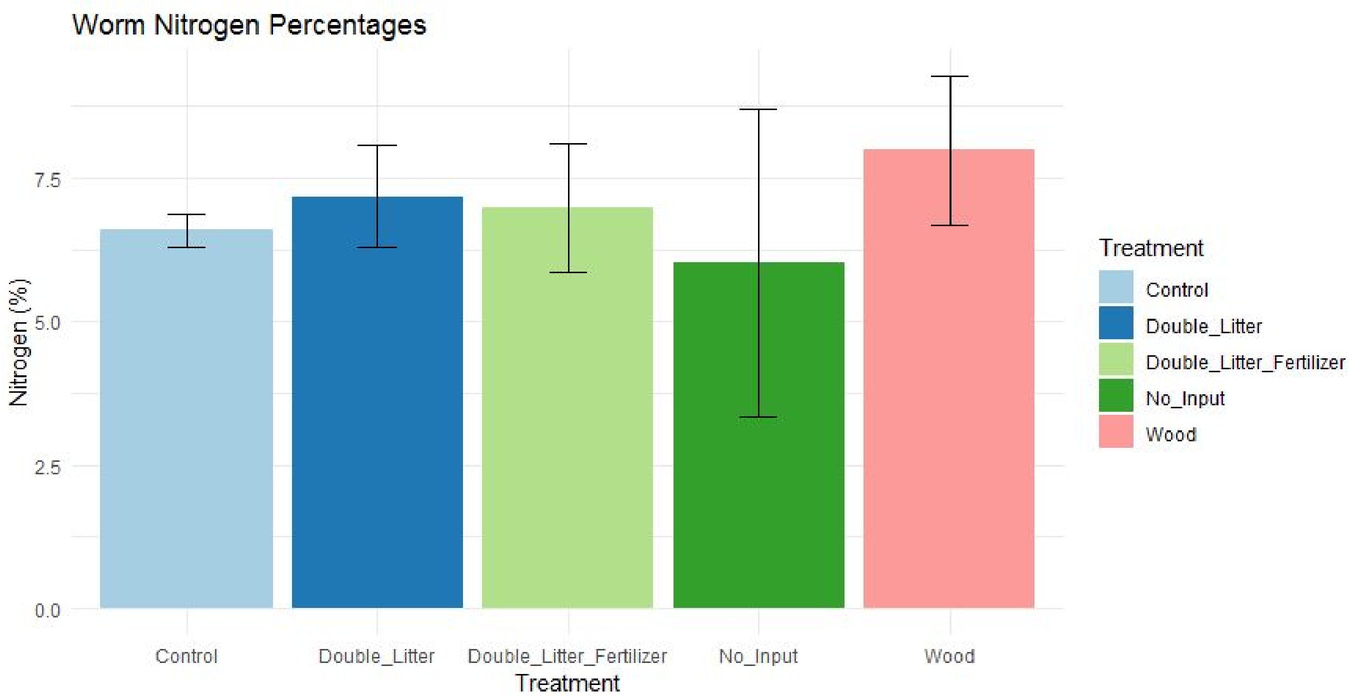

Figure 8. Percentage of nitrogen in earthworm dry body mass. The cyan bar represents the percentage of nitrogen of worms from the Control plots, blue represents that of the worms from the Double Litter plots, light green represents that of the worms from the Double Litter Fertilizer plots, darker green represents that of the worms from the No Input plots, and salmon represents that of the worms from the Wood plots.