Relationships between Native Woody and Herbaceous Understory Vegetation Species Diversity and Invasive Earthworm Density and Biomass at UNDERC

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Catherine Graff

Advisor: Dr. Rose-Marie Muzika

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ABSTRACT

Northern temperate hardwood forests of North America are experiencing a gradual invasion of European earthworms. Earthworm-induced changes within these forests (which have developed earthworm-free since the last period of glaciation) are shifting the delicate ecosystem balance and becoming a growing conservation concern. Conducted at the University of Notre Dame Environmental Research Center in Wisconsin, this study examined potential relationships between invasive earthworm density and biomass and both native woody and herbaceous understory vegetation species diversity in even-aged sugar maple forests. Additionally, this study examined whether or not a significant difference exists between native woody and herbaceous understory species diversity in the presence of invasive earthworms. Lastly, this study compared the effectiveness of different mustard extraction solutions used to sample earthworms. The data suggest no statistically significant relationships between invasive earthworm density and biomass and native woody and herbaceous understory vegetation species diversity. Additionally, the data suggest no statistically significant difference between native woody and herbaceous understory species diversity in the presence of invasive earthworms. Lastly, the data suggest that there is a significant difference in the effectiveness of the two mustard extraction solutions, and that non-organic mustard extraction solution yields higher average earthworm density and biomass values by approximately 321.1% and 1096.7%, respectively when sampling a 35 cm by 35 cm area. This study suggests that the current variability observed in earthworm density and biomass does not seem to relate to the current variability of native woody or herbaceous understory vegetation species diversity in even-aged sugar maple forests at UNDERC.

INTRODUCTION

As the human population continues to expand, anthropogenic environmental disturbances increase in severity and prevalence. Conservation and restoration efforts must constantly adapt to effectively prevent and mitigate environmental damage. Although anthropogenic environmental disturbances such as deforestation, water contamination, and fossil fuel combustion can be managed through reduction practices and technological refinement, the introduction of non-native species (which may later become invasive) may require eradication or biological, chemical, or environmental control or manipulation. Invasive species are non-native species that become well-established in unfamiliar environments and may eventually lead to economic harm, harm to human health, and/or the decline or extirpation of native species via competitive exclusion or other processes (National Invasive Species Council, 2006). Therefore, studying the behavioral habits and ecological effects of invasive species can contribute to the development of future invasive species removal or reduction processes and thus support ecosystem conservation and restoration.

Because there are no known native earthworm species to regions of North American temperate hardwood forest, the introduction of non-native earthworm genera of European origin, especially *Dendrobaena*, *Lumbricus* and *Aporrectodea*, has become a serious conservation concern. Since the glaciers receded from these regions approximately 11,000 to 14,000 years ago, the Northern hardwood forest ecosystems have developed without native earthworms (Callaham et al., 2006; Holdsworth et al., 2014; National Resources Research Institute, 2011). Non-native earthworms were likely brought to the Northern hardwood forests in the late 1800s in European soils and plants, as well as in ship ballasts composed of rocks and soil. Additionally, non-native earthworms used as fishing bait are frequently dumped into lakes today, and earthworms can easily be transported in compost, landscape plants, and vehicle treads (Bohlen et al., 2004; Callaham et al., 2006; National Resources Research Institute, 2011).

These non-native earthworms have earned the title “invasive,” as they have been shown to fundamentally alter soil composition by increasing soil porosity, mixing soil horizons and mineral layers (leading to deeper penetration of carbon), increasing decomposition rates, depositing nitrogen-rich casts, and ingesting and repositioning seeds, causing seed bank alteration (Bohlen et al., 2004; Görres 2014; Loss et al., 2013; Nuzzo et al., 2015; Wironen and Moore, 2006). A complex cascade of negative ecological effects ensues from such activity. Native vegetation is unable to adjust to such shifts in soil composition while grasses and sedges thrive, leading to thinning of native understory vegetation and diversity loss of both woody and herbaceous understory species (Hale 2008a; Loss et al., 2013). Lack of habitat, particularly vegetation cover for salamanders and ground-nesting songbirds, leads to declining populations of these organisms (Görres 2014; Loss et al., 2013; Nuzzo et al., 2015). A study by Wironen and Moore (2006) conducted at the Gault Nature Reserve near MonAréal, Quebec suggests that as few as ten invasive earthworms per square meter may cause statistically significant increases in carbon and nitrogen in soil (Wironen and Moore, 2006). Additionally, recent data suggest that invasive earthworm colonization may promote non-native plant species invasion (Clause et al., 2015; Nuzzo et al., 2015). These invasive earthworms have specifically been detected in hardwood forests of Eastern Minnesota and Northern Wisconsin (Hale et al., 2005b; Loss et al., 2013), as well as in the Upper Peninsula region of Michigan and at the University of Notre Dame Environmental Research Center (UNDERC) in Wisconsin.

Though much research exists regarding how invasive earthworms are affecting native understory species diversity in the Northern hardwood forests, not much research exists regarding how invasive earthworms are affecting native woody understory species diversity in comparison to native herbaceous species diversity. Thus, in this investigation, I will examine potential relationships between: (1) native woody understory vegetation species diversity and invasive earthworm density and biomass; and 2) native herbaceous understory vegetation species diversity and invasive earthworm density and biomass. Additionally in this investigation, I will examine the possibility that a statistically significant difference exists between the diversities of native woody and herbaceous understory vegetation species in the presence of invasive earthworms. Lastly, I will compare the effectiveness of two types of mustard extraction solution used for sampling earthworms. Since invasive earthworms have previously been shown to affect standing vegetation species diversity (Bohlen et al., 2004; Callaham et al., 2006; Clause et al., 2015; Hale 2008a; Loss et al., 2013; Nuzzo et al., 2015), I hypothesize that:

I. There is a statistically significant relationship between native woody understory vegetation species diversity and invasive earthworm density and biomass, and

II. There is a statistically significant relationship between native herbaceous understory vegetation species diversity and invasive earthworm density and biomass.

Furthermore, since invasive earthworms have been shown to promote the establishment of grasses and sedges (Loss et al., 2013), which are herbaceous understory species, I hypothesize that:

III. There is a statistically significant difference between native woody and native herbaceous understory vegetation species diversities in the presence of invasive earthworms.

Lastly, since non-organic ground mustard seed used in an earthworm extraction solution may contain additional ingredients that organic ground mustard seed may not contain (such as salt, MSG, artificial color and/or preservatives), I hypothesize that:

IV. There is a statistically significant difference in the obtained earthworm density and biomass values between organic mustard extraction solution and non-organic mustard extraction solution.

METHODS

The investigation was conducted at the University of Notre Dame Environmental Research Center (UNDERC), a 7500-acre preserve located in Land O'Lakes, WI bordering Michigan's Upper Peninsula and the Ottawa National Forest.

To control overstory composition and sunlight penetration of the canopy, eleven sites located in even-aged sugar maple forests with similar proximities to lakes were haphazardly selected at various locations and elevations throughout the UNDERC property. At each site, one plot (2 m by 2 m) was established, and within each plot, the earthworm mustard extraction technique (National Resources Research Institute, 2011) was used in a 35 cm by 35 cm area to estimate earthworm density of each plot. All earthworms that surfaced in a 10-minute period were collected, weighed, and identified to genus, and all sampling was completed at least 12 hours after heavy precipitation to limit variability of soil moisture. Sampling utilizing an extraction solution of organic ground mustard seed (40 grams of ground seed mixed in 1 gallon of water) was completed within a two-week period in June 2015 on days ranging in temperature from 17 to 24 °C, and sampling utilizing an extraction solution of non-organic ground mustard seed (40 grams of ground seed mixed in 1 gallon of water) was completed within a one-week period in July 2015 on days ranging in temperature from 19 to 24 °C. Organic and non-organic mustard extraction solutions were poured in separate 35 by 35 cm areas within the same 2 m by 2 m plots.

All understory vegetation species were identified and classified as native woody or native herbaceous within each plot, and visual examination was used to approximate percent abundance of each identified species. For each plot, native understory woody and herbaceous vegetation species richness and evenness were calculated using the Shannon diversity index (using natural log in the formula).

Relationships between native woody and herbaceous understory vegetation species diversity and earthworm density and biomass were examined visually by constructing correlation graphs. A paired t-test compared native woody and native herbaceous understory species diversities. Another paired t-test compared the earthworm density and biomass values obtained via organic mustard extraction solution to those obtained via non-organic mustard extraction solution. Lastly, bar graphs were constructed to compare the average earthworm density and biomass yields between organic and non-organic mustard extraction solutions.

RESULTS

The data reveal that when a solution of organic mustard solution is used, there is no relationship between the Shannon diversity index value for woody understory vegetation species in each plot and the estimated earthworm density in each plot (R^2 = 0.03789). However, when an extraction solution of non-organic mustard seed is used, it appears that there is a slightly positive relationship between the variables (R^2 = 0.10459) (Figure 1).

Additionally, it appears that there is no relationship between the Shannon diversity index value for herbaceous understory vegetation species in each plot and the estimated earthworm density in each plot regardless of the extraction solution used (R^2 = 0.00085 for organic, R^2 = 2E-05 for non-organic) (Figure 2).

Furthermore, when a solution of organic mustard solution is used, the data suggest that there is a slightly positive correlation between the Shannon diversity index value for woody understory vegetation species in each plot and the estimated earthworm biomass in each plot (R^2 = 0.12955). There also appears to be a slightly positive correlation between the variables when an extraction solution of non-organic mustard seed is used (R^2 = 0.12393) (Figure 3).

The data also suggest that there is no relationship between the Shannon diversity index value for herbaceous understory vegetation species in each plot and the estimated earthworm biomass in each plot regardless of the extraction solution used (R^2 = 0.01231 for organic, R^2 = 0.03953 for non-organic) (Figure 4).

A paired t-test comparing native woody and native herbaceous understory species diversity yielded a statistically insignificant p-value of 0.4987. Another paired t-test comparing the mean estimated earthworm density of plots sampled with organic mustard extraction solution and the mean estimated earthworm density of plots sampled with non-organic mustard solution yielded a statistically significant p-value less than 0.0001. An additional paired t-test comparing the mean estimated earthworm biomass of plots sampled with organic mustard extraction solution and the mean estimated earthworm biomass of plots sampled with non-organic mustard solution yielded a statistically significant p-value of 0.0005.

Lastly, it appears that per 35 cm by 35 cm area, non-organic mustard extraction solution resulted in an approximately 321.1% higher average earthworm density value (15.18 ± 1.843

worms per 35 cm X 35 cm area) than organic mustard extraction solution (4.727 ± 0.7757 worms per 35 cm X 35 cm area) (Figure 5). Additionally, sampling with the non-organic mustard extraction solution resulted in an approximately 1096.7% higher average earthworm biomass value (5.104 ± 1.059 g) than organic mustard extraction solution (0.4654 ± 0.1003 g) (Figure 6).

DISCUSSION

The data suggest that there is not a statistically significant relationship between native woody understory vegetation species diversity and invasive earthworm density and biomass, thus leading to a rejection of the first hypothesis. Additionally, the data suggest that there is not a statistically significant relationship between native herbaceous understory vegetation species diversity and invasive earthworm density and biomass, thus leading to a rejection of the second hypothesis. Because the p-value from the t-test between native woody and herbaceous species diversities was greater than the set confidence interval of 0.05 ($p = 0.4987$), the third hypothesis that a statistically significant difference exists between native woody and herbaceous species diversities was also rejected.

Although this study can only provide a snapshot in time of earthworm density and biomass in plots of even-aged sugar maple forests at UNDERC, the results do suggest that the current variability observed in earthworm density and biomass does not seem to be related to the current variability of native woody or herbaceous understory vegetation species diversity. Perhaps these results were observed because the effects of invasive earthworms are currently being outweighed by the relative stability of the soil composition, seedbank composition, microbial communities, and/or insect communities.

Since this study is the first of its kind on the UNDERC property, there is no baseline data to which the data from this study can be compared. Thus, the data from this study alone cannot suggest that invasive earthworm density and biomass is or is not affecting native woody and herbaceous understory species diversity in the long term. If this study is replicated in the future to explore potential relationships between native woody and herbaceous understory species diversity and invasive earthworm density and biomass in more depth, perhaps my data could be used as baseline data.

It is important to note that earthworms were collected from every plot sampled in this study, which may be indicative of a widespread infestation of earthworms on the UNDERC property. Although earthworms are only able to spread approximately 5 to 10 meters per year, estimates suggest that approximately 80 percent of the Minnesota and Wisconsin landscape harbors invasive earthworms (Hale 2008). Therefore, if these worms continue to reproduce and expand in range at UNDERC, potentially leading to increased density and biomass in areas of even-aged sugar maple forest that are already infested, native woody and/or native herbaceous understory vegetation diversity in these forests may be at risk of decline or further decline if decline is already occurring. As mentioned previously, studies have shown that gradual invasive earthworm colonization is associated with gradual understory vegetation species diversity decline (Callaham et al., 2006; Hale 2008a; Loss et al., 2013).

Due to the time and material constraints of this study, it was not possible to locate any non-invaded, even-aged sugar maple forests on the UNDERC property. However, a future study comparing the native woody and herbaceous understory vegetation species diversities between invaded and non-invaded even-aged sugar maple forest plots could potentially reveal the true risk status of native woody and herbaceous understory species diversity in the presence of invasive earthworms and thus support ecosystem conservation and restoration efforts.

Relating to ecosystem conservation and restoration, invasive earthworms pose as a significant challenge to prevent, reduce, and eradicate, as there are currently no effective methods for large-scale control or extirpation (Hale 2008a). As mentioned previously, earthworms are only able to spread approximately 5 to 10 meters per year, but anthropogenic activities such as dumping leftover earthworm fishing bait into bodies of water, transferring earthworm eggs and cocoons in vehicle treads, and moving invasive earthworms in compost and landscape plants may be the most concerning methods of earthworm expansion (Hale 2008a). Callaham et al. (2006) suggest that materials potentially containing invasive earthworm species be regulated through policy and invaded areas be monitored and treated via land management practices and potential chemical ground treatments. Callaham et al. (2006) also suggest that public education regarding invasive earthworm disposal after recreational fishing and invasive earthworm presence in ornamental landscape plants is important to slowing current rates of invasion. Additionally, Keller et al. (2007) suggest that requiring bait stores to sell non-invasive earthworm species and the promotion of proper earthworm disposal by fishermen is essential to slowing invasive earthworm invasion. Thus, perhaps the best method of invasive earthworm control and management at this point is simply to limit the anthropogenic activities that spread invasive earthworms.

As for the different mustard solutions used in this study, the p-value yielded from the t-test comparing the mean estimated earthworm density of plots sampled with organic mustard extraction solution and the mean estimated earthworm density of plots sampled with non-organic mustard solution was less than the set confidence interval of 0.05 ($p < 0.0001$) and is therefore statistically significant. The p-value yielded from the t-test comparing the mean estimated earthworm biomass of plots sampled with organic mustard extraction solution and the mean estimated earthworm biomass of plots sampled with non-organic mustard solution is also statistically significant ($p = 0.0005$). Thus, the fourth null hypothesis that there is not a statistically significant difference in the obtained earthworm density and biomass values between organic mustard extraction solution and non-organic mustard extraction solution cannot be rejected.

It appears that the non-organic mustard extraction solution resulted in greater earthworm density and biomass values in comparison to the organic mustard extraction solution. Therefore, the data suggest that the non-organic mustard solution was more effective for earthworm extraction in this study than the organic mustard solution and might have produced more accurate estimates of earthworm density and biomass for each plot. Perhaps the combination of additional ingredients (or a single additional ingredient) in the non-organic ground mustard seed, such as salt, MSG, artificial color and/or preservatives, could be increasingly irritating to earthworms and thus result in higher surfacing rates. However, other factors could potentially be affecting earthworm response to extraction solution. Since the extractions with organic and non-organic mustard extraction solutions were completed approximately four weeks apart due to time and material constraints, the observed worm responses could have been influenced by differences in soil temperature and/or moisture or perhaps even by temporal reproductive cycles. To truly determine whether or not non-organic mustard solution is more effective for earthworm extraction, further experimentation is necessary.

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FIGURES

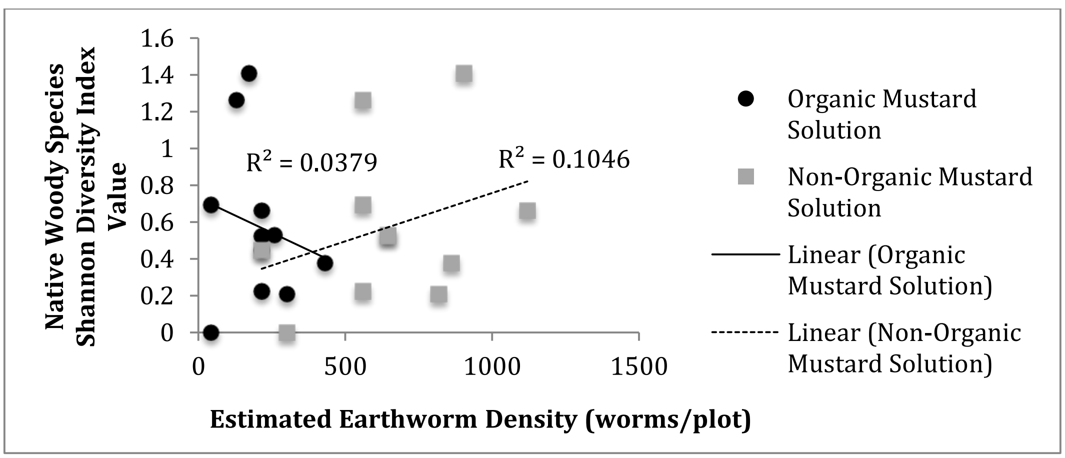

Figure 1. Shannon diversity index value (calculated using natural log) for woody understory vegetation species in each plot versus estimated earthworm density in each plot. When an extraction solution of organic mustard seed is used, it appears that there is no relationship between the variables (R^2 = 0.03789). When an extraction solution of non-organic mustard seed is used, there is a slightly positive relationship between the variables (R^2 = 0.10459).

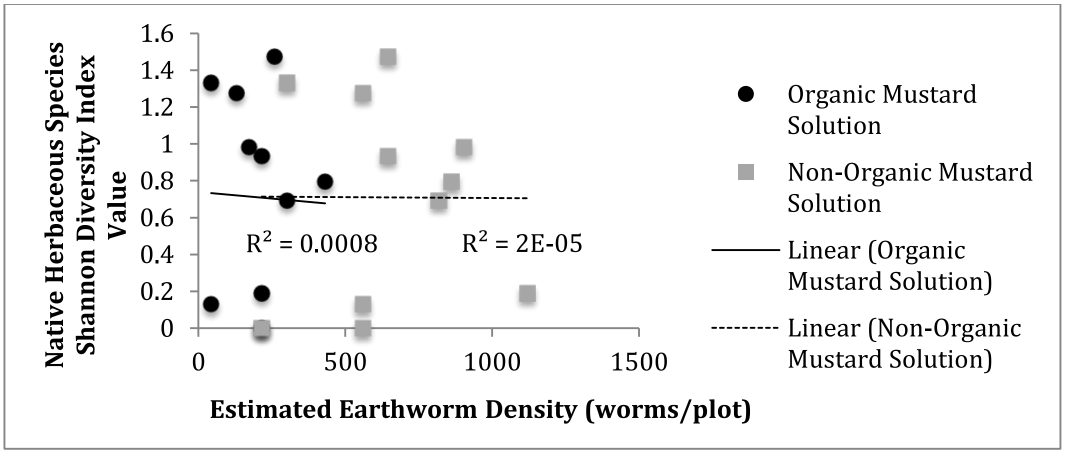

Figure 2. Shannon diversity index value (calculated using natural log) for herbaceous understory vegetation species in each plot versus estimated earthworm density in each plot. Regardless of the extraction solution used, there does not appear to be any signifcant relationship between the variables (R^2 = 0.00085 for organic, R^2 = 2E-05 for non-organic).

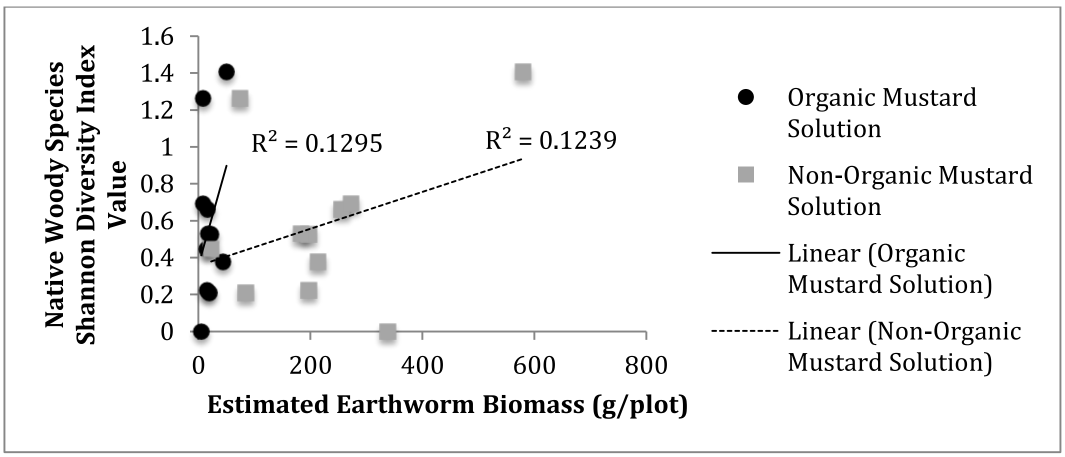

Figure 3. Shannon diversity index value (calculated using natural log) for woody understory vegetation species in each plot versus estimated earthworm biomass in each plot. When an extraction solution of organic mustard seed is used, there is a slightly positive relationship between the variables (R^2 = 0.12955). There is also a slighty positive relationship between the variables when an extraction solution of non-organic mustard seed is used (R^2 = 0.12393).

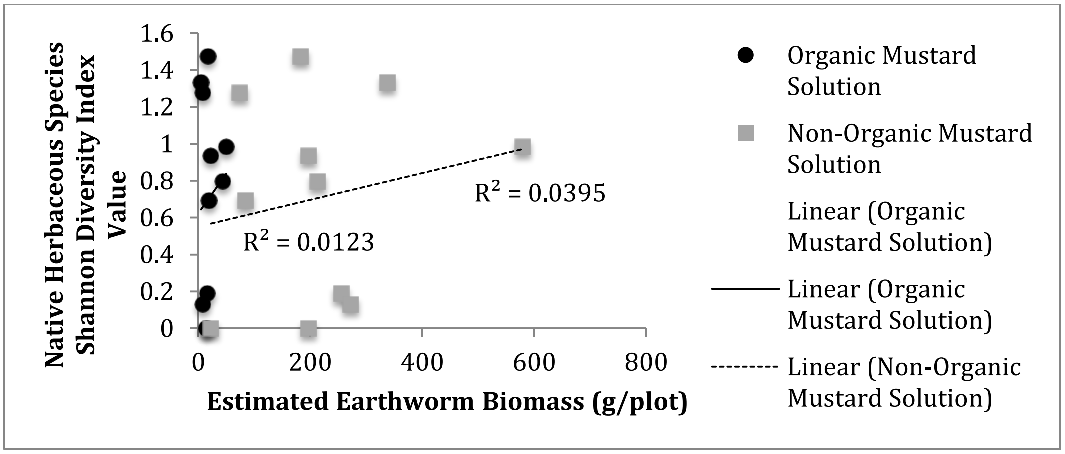

Figure 4. Shannon diversity index value (calculated using natural log) for herbaceous understory vegetation species in each plot versus estimated earthworm biomass in each plot. Regardless of the extraction solution used, there does not appear to be any significant relationship between the variables (R^2 = 0.01231 for organic, R^2 = 0.03953 for non-organic).

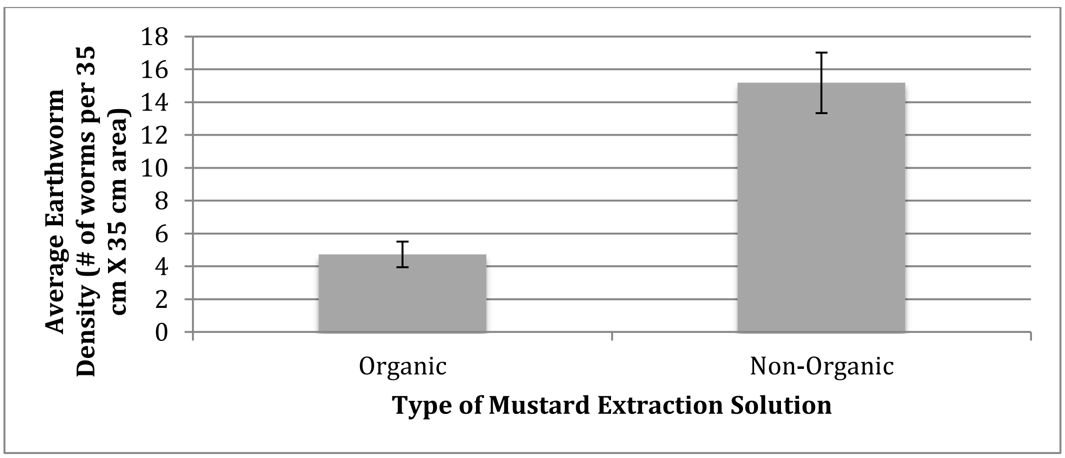

Figure 5. Average organic and non-organic mustard extraction solution earthworm density yields per 35 cm by 35 cm area in eleven plots. Non-organic mustard solution resulted in an approximately 321.1% higher average earthworm density value (15.18 ± 1.843 worms per 35 cm X 35 cm area) than organic mustard solution (4.727 ± 0.7757 worms per 35 cm X 35 cm area).

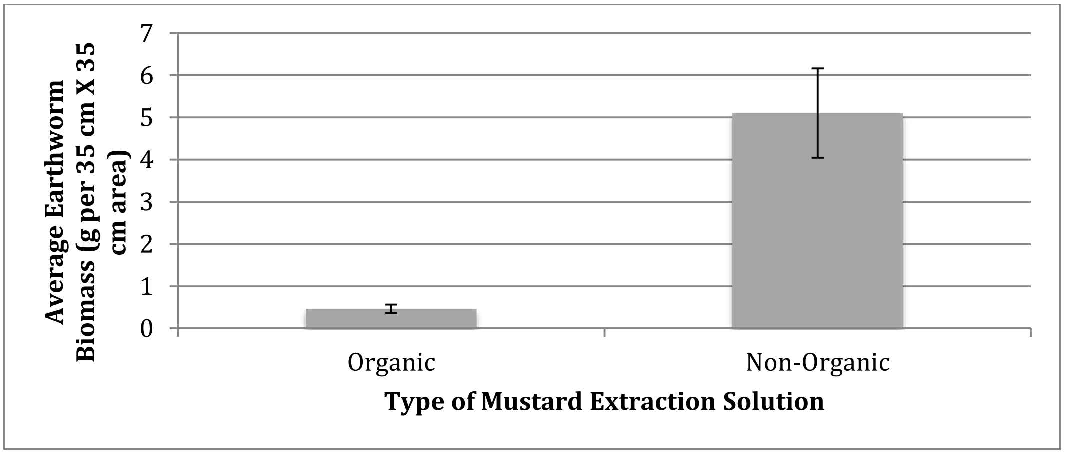

Figure 6. Average organic and non-organic mustard extraction solution earthworm biomass yields per 35 cm by 35 cm area in eleven plots. Non-organic mustard solution resulted in an approximately 1096.7% higher average earthworm biomass value (5.104 ± 1.059 g) than organic mustard solution (0.4654 ± 0.1003 g).