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Potential Effects of Amynthas Agrestis Invasion on Woody Understory Flora in the CVNP

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Abstract
Ohio forests are under attack from a relentless march of invasive worms. Invasive worms are ecosystem engineers that dramatically alter soil characteristics and impact flora. Invasive worms, such as *A. agrestis*, occur simultaneously with the ecosystem engineer, *Odocoileus virginianus*, where their impacts may be synergistic. To determine the direct effects of *A. agrestis* invasion, this study utilized fenced plots that excluded deer. This study used nine plots across the Cuyahoga Valley National Park from August 2021 through October 2021. In each plot, the species richness, Shannon diversity and evenness of woody understory flora was measured, and mustard seed extraction was used to determine the abundance of *A. agrestis*. Correlations between abundance and measured variables were analyzed using a Pearson correlation coefficient, a t-test with $\alpha \leq 0.05$, and statistical power. These correlations were used to highlight the potential direct effects of *A. agrestis* invasion on woody understory flora. *A. agrestis* abundance and species richness was found to have a significant moderately positive correlation ($p = 0.042$, $r = 0.67$, $R^2 = 0.45$). Abundance and diversity were not significantly correlated but showed a moderate, positive trend ($p = 0.16$, $r = 0.5$, $R^2 = 0.25$). The correlation between abundance and evenness was not significant, but showed a moderately negative trend ($p = 0.22$, $r = -0.48$, $R^2 = 0.23$). The abundance of *A. agrestis* ranged from 0 to 15 worms present per plot. Invasion by *A. agrestis* may facilitate an increase in species richness in the woody understory, while potentially having no significant impact on plant diversity and species evenness. These results contradict those of previous studies on other worm species and on *A. agrestis*. However, these results match those obtained in a previous study in the Cuyahoga Valley National Park that also utilized fenced plots. This suggests that the direct effects of *A. agrestis* on woody understory flora may be different than those observed in the presence of White-tailed Deer and this may have impacts on conservation strategy as White-tailed Deer populations become better managed.

Introduction
There are many threats facing the forests of North-east Ohio. These threats include climate change, habitat destruction and disease, all of which are predicted to have dramatic effects on the forest ecosystem and biodiversity (Adams et al. 2001, Gandhi et al. 2018, Bonello et al. 2018, Ilkka 2011). An additional significant threat to Ohio forests is the ongoing phenomenon of invasive species (Barnard et al. 2009). Invasive species have major impacts on the forests they invade, leading to dramatic changes in biodiversity, productivity and causing millions of dollars in economic damage (Barnard et al. 2009). Invasive species come in all forms, from fungi, flora, to vertebrates and invertebrates (Barnard et al. 2009). Invasive species compete with native species, in some cases out-competing them and endangering native populations, especially those already threatened by other factors (Barnard et al. 2009). Their spread throughout an environment causes decreases in native populations and a reduction in biodiversity (Barnard et al. 2009).

Invasive species can dramatically alter the biotic and abiotic environment of forest ecosystems, which can have dramatic effects on native plant species (Beugnon et al. 2021). Species that dramatically affect a habitat, destroying or maintaining it, are known as ecosystem engineers (Bohlen et al. 2004, Jalilvand et al. 2008). While ecosystem engineers can play an important role in maintaining native ecosystems and allowing other organisms to thrive, invasive ecosystem engineers can cause massive alterations to native habitats that negatively impact native ecosystems (Beugnon et al. 2021, Bohlen et al. 2004).

An ecosystem engineer common within Ohio forests is the earthworm (Beugnon et al. 2021. Trimbath 2014). However, earthworms are not native to Ohio, with earthworms believed to have been absent from the region since the last glacial period up until the arrival of European settlers in the area, roughly 400 years ago (Beugnon et al. 2021, Trimbath 2014, Bohlen et al. 2004). These ecosystem engineers have been introduced to Ohio through human activity, such as through the transport of soil and the dumping of live bait (Trimbath 2014, Davalos et al. 2016, Bartz et al. 2021). As ecosystem engineers, earthworms dramatically alter both the physical and chemical properties of the soil they inhabit (Beugnon et al. 2021, Trimbath 2014, Craven et al. 2017, Davalos et al. 2016). Earthworms physically disturb the soil, consuming the soil organic layer and mixing the soil organic and mineral layers through their burrowing behavior (Trimbath 2014, Davalos et al. 2016, Bartz et al. 2021). Earthworms alter the nutrient cycling of forests as they consume and transform the organic matter on and within the soil, typically increasing the rate of nutrient cycling in forests, which when combined with the mixing of different soil layers caused by earthworms can have large impacts on soil microbial, invertebrate, and vertebrate communities (Trimbath 2014, Davalos et al. 2016, Bartz et al. 2021). In native environments, this activity of earthworms allows nutrients to be available to plants, but in ecosystems not adapted to the presence of earthworms, the sudden release of nutrients stored in the litter layer is detrimental (Davalos et al. 2016, Bartz et al. 2021, Jalilvand et al. 2008). The mixing of soil layers and transformation of soil organic matter by earthworms leads to changes in soil carbon and soil nitrogen storage and distribution, with total soil carbon typically decreasing (Davalos et al. 2016, Bartz et al. 2021). Changes in soil structure and nutrient dynamics have dramatic impacts on soil microbial communities, typically decreasing the size of soil fungal communities while enhancing soil bacteria (Davalos et al. 2016, Bartz et al. 2021, Baskin and Baskin 1998). These changes, along with the disappearance of the duff layer, can have large impacts on native soil organisms and plants (Trimbath 2014, Davalos et al. 2016, Bartz et al. 2021).

Earthworm invasion of forests is associated with large-scale changes in the composition of understory flora and tree seedlings (Beugnon et al. 2021, Davalos et al. 2016). Increasing abundance of earthworms is associated with decreasing diversity and abundance of understory flora (Davalos et al. 2016). Many species of plants in Ohio forests rely on mycorrhizal fungi and so the decrease in fungal communities gives non-mycorrhizal plants a competitive advantage, potentially facilitating the invasion of species such as *Allia petioloata* (garlic mustard) (Davalos et al. 2016, Hale 2004). The presence of earthworms decreases the number of viable seeds in the seedbank and reduces the chance of successful seed germination, as the earthworm-associated reduction of the soil duff layer makes seeds and seedlings more vulnerable to predators and environmental factors (Beugnon et al. 2021, Davalos et al. 2016).

The effects of earthworms on soil properties, soil biota and forest flora are not equal between earthworm species (Davalos et al. 2016). The specific life history of the invading earthworm and the ecological group it belongs to influences the effects an earthworm will have on the forest (Bohlen et al. 2004). Earthworms are broadly grouped into three ecological groups, epigeic, endogeic and anecic, based on their feeding, burrowing and soil layer preferences (Davalos et al. 2016, Bartz et al. 2021). Epigeic worm species reside primarily near the soil surface in the organic layer and feed on soil litter, endogeic worms live in the mixed organic-mineral layers of the soil and anecic worms are those that form vertical burrows through the soil layers and feed on the soil surface (Davalos et al. 2016, Bartz et al. 2021). Each of these ecological groups interact with the soil in different ways, thereby causing different levels of soil layer mixing and how the soil layers are mixed, with epigeic worm species causing less soil mixing than endogeic and anecic worms (Bartz et al. 2021, Bohlen et al. 2004). Due to these differences in ecology, the different ecological groups have differing effects on the soil and on soil biodiversity (Bartz et al. 2021, Davalos et al. 2016).

There are several invasive worm species in Ohio, including European species from the genus *Lumbricus* and asian species from the genus *Amynthas* (Bartz et al. 2021). Species of the genus *Lumbricus* are typically long-lived endogeic worms while those of the genus *Amynthas* are typically epigeic worms with an annual life cycle (Bartz et al. 2021). The different life histories of these two genera indicate that these species may have different impacts on Ohio forests (Bartz et al. 2021). While much attention has been given to studying the impacts of *Lumbricus* species on forests, from which much of the above information has been obtained, less has been given to those of the *Amynthas* genus and so some of the specific effects of *Amynthas* invasion are not known (Bartz et al. 2021, Anthony et al. 2016). A common species from this genus found in northern forests is *A. agrestis*, though this identification is also often wrongly given to *A. tokioensis* and *Metaphire hilgendorf* (Bartz et al. 2021).

Invasive *Amynthas* worms feed on the soil litter layer, leading to a decrease in the thickness of the soil organic layer (Bartz et al. 2021, Davalos et al. 2016, Anthony et al. 2016). They produce granular castings just below the soil organic layer that alter soil thermal properties and impact nutrient cycling (Bartz et al. 2021, Davalos et al. 2016, Anthony et al. 2016). Endogeic lumbricid earthworms mix dead organic matter deep into the soil, leading to better soil carbon storage then when compared to the epigeic *Amynthas* worms whose activity nearer to the surface is believed to increase carbon mineralization and carbon loss from the soil (Bartz et al. 2021, Davalos et al. 2016). Like all earthworms, *Amynthas* worms cause changes in nutrient cycling, causing nutrients to cycle faster in an ecosystem and making them more available for uptake by plant life (Bartz et al. 2021). However, the availability of nutrients in earthworm invaded soil does not match with plants' growth periods or ability to uptake nutrients, leading to increased leaching of nutrients from the soil (Bartz et al. 2021).

Amynthas earthworms impact plant species, with generally negative effects on most native plant species, though some individual native and non-native species may benefit from *Amynthas* invasion (Bartz et al. 2021). Some effects on plants are species-dependent, with *Amynthas* earthworms shown to impact the growth of tree seedlings in species dependent ways, with common buckthorn and sugar maple seedlings showing increased growth in their presence and white oak saplings showing decreased growth (Bartz et al. 2021). Like other earthworms, invasion by *Amynthas* earthworms has been demonstrated to affect species diversity (Bartz et al. 2021, Bohlen et al. 2004).

In forest ecosystems, it is very rare that the flora present is only being affected by earthworm invasion. The abundance and diversity of understory flora is often being affected by the invasion of multiple different species at once, and this is true of Ohio forests (Hutchinson et al. 2005, Barnard et al. 2009). The effects of multiple invader species can potentially be compounding and synergistic, causing changes to the nature of the effect a particular invader would have on an ecosystem (Burns et al. 2021). It is likely that the effects of multiple ecosystem engineers are also synergistic and likely lead to dramatic changes in understory flora abundance, species composition and diversity. An additional ecosystem engineer that is having a dramatic impact on Ohio forests is the White-tailed Deer (*Odocoileus virginianus*), whose populations have dramatically increased due to human activities that have led to the loss of their natural predators (Trimbath 2014). The large populations of White-tailed Deer present in Ohio forests have a significant impact on understory flora, selecting for plant species that can better resist pressure from herbivory (Trimbath 2014). Many native plant species are unable to resist pressure from herbivory, while many exotic plant species can, leading to potential changes in species composition facilitated by White-tailed Deer (Trimbath 2014). Many studies on the impacts of invasive earthworms have done so in the presence of deer species, as invasive worms and deer species often co-inhabit the same ecosystems (Trimbath 2014). In order to isolate the effects of *Amynthas* earthworms on understory flora, fenced plots that exclude deer from the area under study were utilized in this study.

This study sought to examine the direct effects of *A. agrestis* abundance on the species richness, evenness and diversity of woody plants in the forest understory. This study sought to isolate the impacts *A. agrestis* abundance has on these properties from the impacts herbivory by White-tailed Deer has on these properties. This paper clarifies the potential direct impacts of *A. agrestis* abundance on the diversity of woody plants in the forest understory of the Cuyahoga Valley National Park (CVNP) and gives insight into what an Ohio forest understory may look like in the absence of overgrazing by White-tailed Deer.

Methods

This study was conducted in forested areas across the Cuyahoga Valley National Park with sampling taking place between August 2021 and October 2021. Sampling occurred at previously established long-term fenced plots within the CVNP that prevented deer from grazing on flora within the plots. The plots were established in 2016 and each plot measured 10m by 10m and fencing was 3m high. The fencing was composed of metal rebar/T-post supports with plastic fence mesh suspended between them. Plots were located within different heavily forested areas of the CVNP, each being within 50m of a hiking trail. The local topography and forest type of the plots varied based on location.

In total, 9 plots were sampled. The location of each plot is displayed in **Figure 1**.

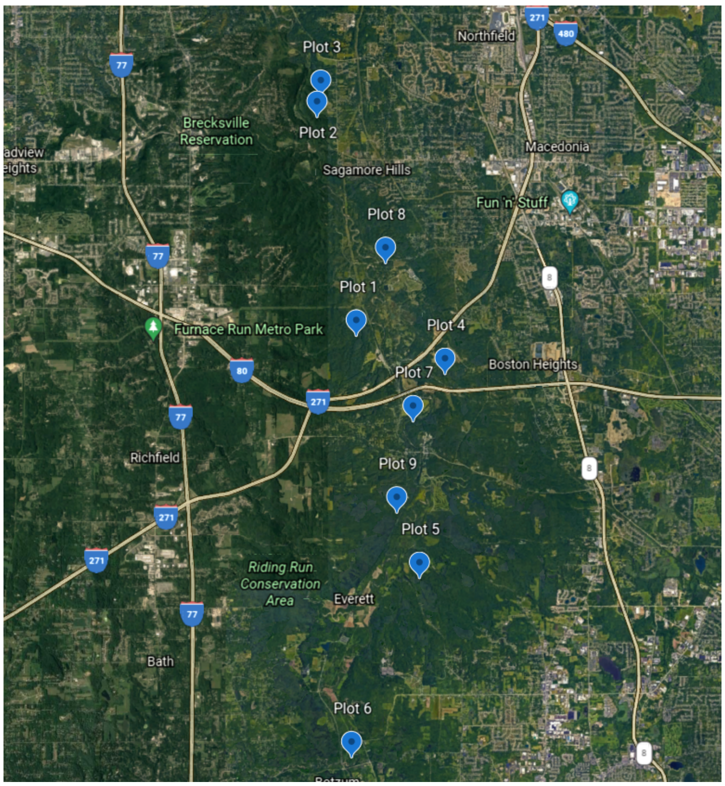

Figure 1: Each plot sampled is marked on the map using a labeled blue pin. Plots were numbered randomly. Plots were sampled between August 2021 and October 2021. Locations are accurate to within approximately 30m. The map was created using Google Earth.

Mustard seed extraction was used to sample each plot for worm species richness and abundance. Mustard seed extraction was conducted within a 30.5 cm by 30.5 cm frame using 750 ml of a solution consisting of 2.5 tablespoons of mustard seed powder dissolved in water. Roughly half of the solution was poured into the area within the frame and allowed to soak into the soil for 5 minutes, with any adult *A. agrestis* that surfaced within the area of the frame being removed, identified and counted. *A. agrestis* that surfaced outside of the framed area were removed, but not counted. Juvenile *A. agrestis* were also not counted as I was unable to accurately identify juvenile worms in the field. The remainder of the solution was then poured into the area and another 5 minute collection period was conducted. At each plot, mustard extraction was conducted at 3 locations within the plot, with the first location being at the plot's center and then points 1.5 meters directly north and south of the center point. The total number of *A. agrestis* found between the three sampling locations was used as the abundance of *A.*

agrestis within each plot. The relationships between A. agrestis abundance and Species Richness, Evenness and H' were analyzed using simple linear regression and determining the Pearson Correlation Coefficient (r). The significance of r was evaluated using a t-test with $\alpha \leq 0.05$.

Next, all understory vegetation (defined as vegetation rooted to the forest floor that measured less than 1.5 meters in height) within a 1.5 meter radius of the center of the plot was identified and the species richness and abundance was recorded. Abundance was determined by counting the number of stems of each species present within the sampling area. These values were used to determine the Shannon Diversity Index of each plot, which was used as the measure of the diversity of understory woody species.

Results

I found that *A. agrestis* was widespread across the CVNP, with live *A. agrestis* found at nearly every plot surveyed and evidence of *A. agrestis* (distinct castings and reduced litter layer) found at every plot. *A. agrestis* abundance varied greatly between sites, ranging from 0 to 15 total worms per plot. Though evidence of *A. agrestis* activity was present at every site, mustard extraction failed to find any worms at 2 of 9 surveyed plots (Plots 2 and 4. See **Appendix**). These "0" results were included to maintain consistency and avoid biasing the data, but the effects of excluding these points from analysis are discussed later to allow evaluation. Understory flora species richness, species evenness and Shannon diversity index (H') varied between each site. At each plot, there were 2 to 8 plant species present. Species evenness varied from 0.682 to 0.946 and Shannon diversity index varied from 0.562 to 1.77.

I found that the abundance of *A. agrestis* had a significant, positive and moderate correlation ($p = 0.042$, $r = 0.67$, $R^2 = 0.45$) with woody plant species richness (**Figure 2**).

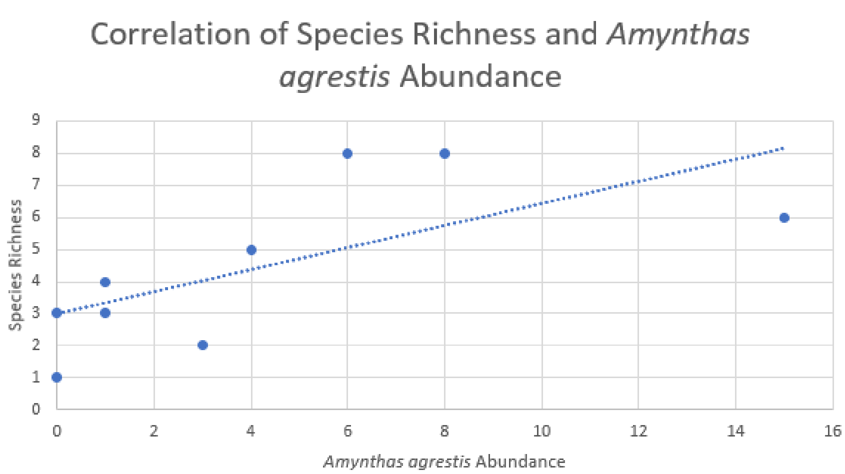

Figure 2: The species richness of the various plots within the CVNP and their correlation ($p = 0.042$, $r = 0.67$, $R^2 = 0.45$) to the abundance of *A. agrestis*. This result contradicts that found in other studies, where the correlation was negative. The statistical power of this analysis was found to be 0.5475.

Additionally, I found that the abundance of *A. agrestis* and Shannon Diversity (H') followed a similar pattern to Species Richness, with a positive and moderate correlation, though it was not significant ($p = 0.16$, $r = 0.5$, $R^2 = 0.25$). The results are displayed in **Figure 2**.

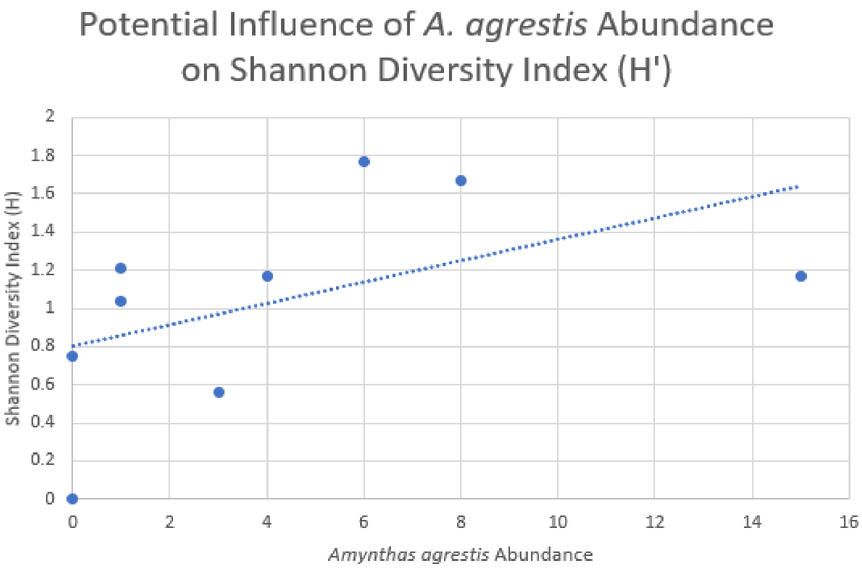

Figure 2: Shannon Diversity index of woody plant species in the forest understory of enclosed plots within the CVNP and its correlation ($p = 0.16$, $r = 0.5$, $R^2 = 0.25$) to the abundance of adult *A. agrestis*. Unlike previous studies, a positive (but not significant) correlation between H' and abundance of *A. agrestris* was found, rather than an expected negative correlation. The statistical power of this analysis was determined to be 0.263.

Finally, I found that the abundance of *A. agrestis* had a non-significant, moderate negative correlation ($p = 0.22$, $r = -0.48$, $R^2 = 0.23$) with woody plant species evenness (**Figure 4**).

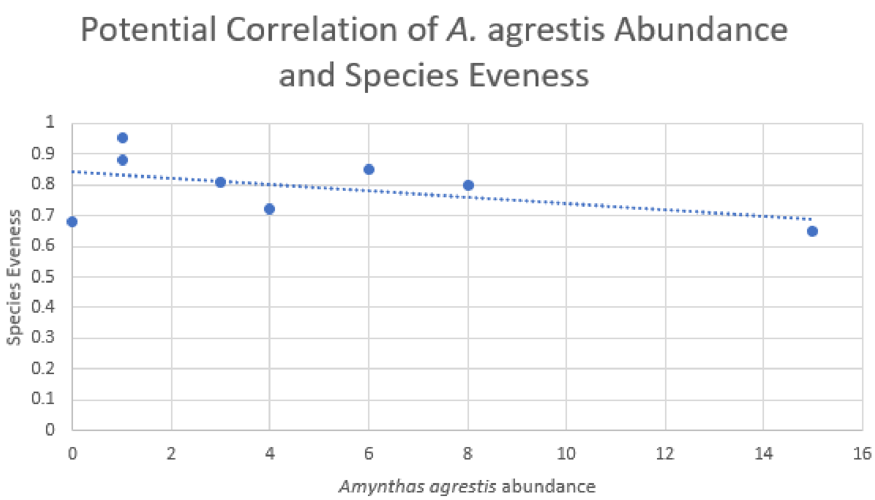

Figure 4: The species evenness of the various plots within the CVNP and their correlation ($p = 0.22$, $r = -0.48$, $R^2 = 0.23$) to the abundance of *A. agrestis*. One sample, plot 4 (see **Appendix**), was removed from the data set when determining the correlation between *A. agrestis* abundance and species evenness as the species evenness of Plot 4 could not be calculated due to only 1 woody plant species being present. The statistical power of this analysis was 0.2080.

Removal of plots 2 and 4 in which no worms were found during mustard seed extraction causes significant changes to the data. These changes affect whether some of the correlations found are significant or not, though the general directions of the trend lines remain the same. When these data points are removed from the correlation between *A. agrestis* abundance and species richness, the resulting correlation stops being statistically significant ($p = 0.17$). However, the direction and strength of the trend line remains relatively the same ($r = 0.57$, $R^2 = 0.44$). Removal of the data points in the correlation between abundance and H' results in significant changes to r and R^2 ($r = 0.29$, $R^2 = 0.086$), though the overall direction of the trend remains the same and the correlation found is still not statistically significant ($p = 0.52$). For the correlation between abundance and species evenness, if plot 2 is removed the correlation found becomes strong rather than moderate in strength and becomes statistically significant ($p = 0.028$, $r = -0.79$ and $R^2 = 0.63$). These changes affect the interpretation of the data in some ways.

Discussion

The presence of invasive earthworms can have dramatic effects on understory flora (Trimbath 2014, Bartz et al. 2021). These effects have often been studied in ecosystems additionally under the influence of deer, ecosystem engineers that also have dramatic effects on understory flora (Trimbath 2014). In Ohio forests, White-tailed Deer are a major nuisance with large populations that overgraze on understory flora (Trimbath 2014). The effects of invasive *A. agrestis* earthworms on understory flora in the CVNP may possibly be masked or changed by the synergistic effects of White-tailed Deer (Trimbath 2014, Bartz et al. 2021, Burns et al. 2021). This study sought to isolate the effects on the diversity, species evenness and species richness of woody plants in the forest understory by *A. agrestis* abundance in the CVNP from the effects of herbivory by White-tailed Deer using enclosed plots. As management of White-tailed Deer populations improves and their impacts on Ohio forests are mitigated or reduced, the potential influence of *A. agrestis* may become more pronounced (Trimbath 2014). In order to better manage the effects of *A. agrestis* on Ohio forests, information regarding *A. agrestis* specific effects on flora will be critical. This study sought to take the first step in providing data that can be used to predict how *A. agrestis* will affect Ohio forests with reduced deer populations.

This study utilized fenced plots across the CVNP to isolate the effects of *A. agrestis* abundance on understory woody flora from those of White-tailed Deer. This study found that, unlike previous studies, worm abundance had a significant positive correlation with species richness (Bartz et al. 2021, Frelich et al. 2007). This result, which suggests that invasion by *A. agrestis* may facilitate an increase in species richness, contrasts with the results of other studies conducted on other species of invasive worms. Studies on another common invader of Ohio forests, *Lumbricus terrestris*, had found that their invasion was associated with a decrease in species richness (Bartz et al. 2021, Frelich et al. 2007).

Additionally, and also contrary to previous results, the abundance of *A. agrestis* did not have a significant correlation with the diversity (H') of the woody understory (Davalos et al. 2016, Beauséjour et al. 2017). Additionally, the correlation found, though not statistically significant, was positive rather than negative. These findings suggest that invasion by *A. agrestis* may not lead to changes in woody understory flora diversity. This finding differs from that found in previous studies on *Amynthas* species and other earthworms such as *L. terrestris*, where the presence of invasive earthworms and their increasing abundance was associated with decreasing floral diversity (Beugnon et al. 2021, Bohlen et al. 2004, Beauséjour et al. 2017). This difference could have resulted from the isolation of White-tailed Deer from the study area, as previous studies did not isolate the effects of grazing by deer from the impacts of worms. This finding corresponds with that of (Trimbath 2014), in which fenced plots were also used to isolate the effects of White-tailed Deer from that of invasive worms. This finding suggests that invasive *A. agrestis* may not directly reduce the diversity of understory flora, and has the potential to positively impact understory diversity, though further research would need to be conducted to test this hypothesis. It is important to note that the statistical power of this study was low ($\beta = 0.2630$), suggesting that there is a high chance that a statistically significant correlation was not found due to the small sample size. Statistical power analysis suggested that a sample size of $N = 18$ was the sample size required to find a potential correlation.

In addition to this finding, no significant correlation between *A. agrestis* abundance and species evenness of the woody understory was found. This result matches that found in the literature regarding other earthworm species (Craven et al. 2017). This result also suffered from low statistical power ($\beta = 0.2080$) and power analysis indicated that a sample size of $N = 20$ would have been needed to find a potentially significant correlation.

These results together differ from the expected results for earthworm invasion (Beauséjour et al. 2017, Frelich et al. 2007, Beugnon et al. 2021, Bohlen et al. 2004, Bartz et al. 2021). This could be the result of isolating the impact of *A. agrestis* from that of White-tailed Deer, or may suggest that the effects of *A. agrestis* on understory flora may be different from that of other earthworm species. These differing effects may potentially result from the different ecological group *A. agrestis* belongs to when compared to other invasive worms such as *L. terrestris*. *A. agrestis* has been previously demonstrated to have different effects on the soil environment than other earthworm species and I hypothesize that these different soil impacts lead to different impacts on plant flora than other earthworm species (Bohlen et al. 2004). These results additionally suggest that the effects of *A. agrestis* invasion observed in the presence of White-tailed Deer may be different than those observed without their presence, suggesting that the effects of White-tailed Deer on the woody understory are indeed synergistic with those of invasive worms such as *A. agrestis*.

When the “0” points (plots 2 and 4) are removed from the data, the conclusions drawn from the results change somewhat. With their removal, I would be unable to conclude that the abundance of *A. agrestis* had any significant correlation or potential effect on species richness. I would be unable to conclude that invasion by *A. agrestis* may facilitate an increase in species richness and contrasts the results of other studies conducted on other species of invasive worms (Bartz et al. 2021, Frelich et al. 2007). The conclusions regarding *A. agrestis* abundance and H’ do not change when the “0” points are removed, though the potential correlation becomes weak rather than moderate. Another significant change to the conclusions of the data occurs when plots 2 and 4 are removed from the correlation of abundance with species evenness. Removing these points causes the correlation to be significant and strongly negative. This leads to the conclusion that an increase in *A. agrestis* abundance potentially causes a decrease in species evenness. The mechanism behind this effect could be the changes to soil structure and chemistry caused by *A. agrestis* invasion favoring certain plant species over others. This result differs from that found in the literature (Craven et al. 2017).

There is potential that the "0" points greatly underestimated the abundance of *A. agrestis* within the relevant plots. However, the choice to keep these points in the data comes from the potential that all mustard seed extractions greatly underestimated the abundance of *A. agrestis* within the plots. The same methodology was used between all sites and so it is likely that if mustard seed extraction greatly underestimated the worm abundance at plots 2 and 4, it likely greatly underestimated the worm abundance at all other plots as well. Mustard extraction could have failed to yield any worms in plots 2 and 4, despite evidence of *A. agrestis* activity being present, due to these plots having a much lower abundance and density of worms than the other plots. If this is the case and all of the performed mustard seed extractions greatly underestimated worm abundance, then finding zero *A. agrestis* likely fairly represents the abundance of *A. agrestis* in these plots when compared to the other plots.

An alternative reason the mustard seed extractions could have failed to yield any *A. agrestis* at plots 2 and 4 could have been the environmental conditions during sampling that day. The mustard seed extractions were conducted throughout the course of 4 weeks, meaning the conditions for each extraction were different. Prior to the extractions conducted at plots 2 and 4, the area had experienced rainfall and the soil was therefore moist. This could have affected the results of the extraction. Despite this, I chose to include these results as the conditions were different each time the extraction was done, and removing points because I feel the conditions were significantly detrimental may unfairly bias the data. I do not feel there is an objective reason to remove these data points.

This study is limited in its ability to be generalized due to its limited sample size. It is likely that with a larger sample size, a significant negative correlation between *A. agrestis* abundance and species evenness and a significant positive correlation between *A. agrestis* abundance and plant diversity could be found. These results would match with the expected results from similar studies (Craven et al. 2017, Trimbath 2014).

Further studies regarding the impact of *A. agrestis* on understory flora should focus on further illuminating the impacts of *A. agrestis* abundance on plant diversity and species evenness. Additionally, further studies should investigate potential changes in species composition caused by *A. agrestis* and whether the increase in species richness potentially facilitated by *A. agrestis* invasion is due to it facilitating invasion by non-native flora or if the increase consists of native species.

If I were to conduct this study again, I would make several changes to increase the quality of the study. First of all, I would sample all 25 fenced plots within the CVNP as this would allow weak trends to potentially become apparent in the data. It is possible that the small sample size caused some trends to not become apparent simply by chance or because they have a weak correlation. Secondly, I would take worms captured during mustard seed extraction back to the lab for identification because field identification of *A. agrestis* is difficult as *A. tokioensis* and *Metaphire hilgendorf* both look nearly identical to *A. agrestis* and so it is possible I misidentified many worms. The relationship between invasive epigeic worms and plant diversity is complicated further by multiple invasive species being present at the same site, as these worms commonly occur together (Bartz et al. 2021). Thirdly, I would use a larger volume of mustard seed solution during extraction, as most guides on mustard seed extraction utilize larger volumes of mustard seed solution than I was able to use, and so my reduced volume possibly was not sufficient to cause all epigeic worms present to surface. Additionally, I would survey more locations within the plot so that more surface area of the plot was sampled to give a better estimate of earthworm abundance. This would additionally allow earthworm density to be accurately estimated, which would have been a more useful definition of abundance than the simple number of worms found as used in this study. A fifth difference I would make is better organizing and labeling pictures of unidentified plants found within the sampling area so that they could be later identified, and unidentified plants shared between plots could be more easily recognized as the same plant so that data regarding species richness and abundance would be more accurate.

Overall, this study found that the direct effects of the invasion of Ohio forests by *A. agrestis* may be different than that of other worm species (Beauséjour et al. 2017, Frelich et al. 2007, Beugnon et al. 2021, Bohlen et al. 2004, Bartz et al. 2021). These different effects are a potential increase in species richness, while not significantly affecting species diversity and evenness. These represent effects independent of herbivory by the White-tailed Deer. However, it is important to note that the combined effects of these two ecosystem engineers may be synergistic and may be different than a simple combination of the direct effects of both species. As the White-tailed Deer population is brought under control and its impact on Ohio forests is reduced, it is possible the direct effects of *A. agrestis* will become more noticeable. Understanding the direct effects of *A. agrestis* is important for developing conservation strategies to help preserve Ohio forests.

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Appendix

Summary on the effects on the data of choosing to include or exclude the "0" points.

Effect on Results of Including/Removing "0" Points				
Effect on Species Richness Analysis				
	r	R^2	p	β
"0" points included	0.67	0.45	0.042	0.5475
"0" points removed	0.57	0.44	0.17	0.2462
Effect on Shannon Diversity (H') Analysis				
	r	R^2	p	β
"0" points included	0.5	0.25	0.16	0.2630
"0" points removed	0.29	0.086	0.52	0.0874
Effect on Species Evenness Analysis				
	r	R^2	p	β
"0" points included	-0.48	0.23	0.22	0.2080
"0" points removed	-0.79	0.63	0.028	0.6402

Summary of the results calculated per plot.

Summary of Calculated Results per Plot				
Plot #	Worm Abundance	Species Richness	H'	Species Eveness
1	4	5	1.166449	0.724755
2	0	3	0.749331	0.68207
3	8	8	1.67259	0.804346
4	0	1	0	N/A
5	6	8	1.773751	0.852994
6	1	3	1.039721	0.946395
7	15	6	1.169308	0.652603
8	1	4	1.213008	0.875
9	3	2	0.562335	0.811278

Summary of the raw data collected at each plot.

Raw Data From Plot 1	
Flora Species Found	**Number of Each Species Found**
Ash	19
Hornbeam	12
Spicebush	1
Sugar Maple	3
Unknown 1	2
TOTAL:	**37**
Worm Sample Location	**Number of Worms Found**
1.5m North of Plot Center	0
Plot Center	4
1.5m South of Plot Center	0
TOTAL:	**4**

Raw Data From Plot 2	
Flora Species Found	**Number of Each Species Found**
Ash	20
Glossy Buckthorn	4
Red Birch	3
TOTAL:	**27**
Worm Sample Location	**Number of Worms Found**
1.5m North of Plot Center	0
Plot Center	0
1.5m South of Plot Center	0
TOTAL:	**0**

Raw Data From Plot 3	
Flora Species Found	**Number of Each Species Found**
Ash	11
Beech	3
Unknown 1	10
Unknown 2	3
Red Maple	1
Pricket	1
Buckthorn	1
Red Oak	2
TOTAL:	**32**
Worm Sample Location	**Number of Worms Found**
1.5m North of Plot Center	2
Plot Center	4
1.5m South of Plot Center	2
TOTAL:	**8**

Raw Data From Plot 4	
Flora Species Found	**Number of Each Species Found**
Ash	20
TOTAL:	**20**
Worm Sample Location	**Number of Worms Found**
1.5m North of Plot Center	0
Plot Center	0
1.5m South of Plot Center	0
TOTAL:	**0**

Raw Data From Plot 5	
Flora Species Found	**Number of Each Species Found**
Ash	7
Beech	1
Poison Ivy	2
Unknown Shrub1	2
Red Raspberry	1
Unknown Shrub2	7
Unknown Shrub3	1
Unknown 4	7
TOTAL:	**28**
Worm Sample Location	**Number of Worms Found**
1.5m North of Plot Center	3
Plot Center	0
1.5m South of Plot Center	3
TOTAL:	**6**

Raw Data From Plot 6	
Flora Species Found	**Number of Each Species Found**
Common Privet	5
Unknown Privet	10
Poison ivy	5
TOTAL:	**20**
Worm Sample Location	**Number of Worms Found**
1.5m North of Plot Center	0
Plot Center	1
1.5m South of Plot Center	0
TOTAL:	**1**

Raw Data From Plot 7	
Flora Species Found	**Number of Each Species Found**
Oak	3
Ash	1
Pricket	4
Unknown 1	22
Unknown 2	3
Unknown 3	1
TOTAL:	**32**
Worm Sample Location	**Number of Worms Found**
1.5m North of Plot Center	2
Plot Center	5
1.5m South of Plot Center	8
TOTAL:	**15**

Raw Data From Plot 8	
Flora Species Found	**Number of Each Species Found**
Wild Cherry	5
Unknown 1	10
Unknown 2	5
Unknown 3	20
TOTAL:	**40**
Worm Sample Location	**Number of Worms Found**
1.5m North of Plot Center	0
Plot Center	0
1.5m South of Plot Center	1
TOTAL:	**1**

Raw Data From Plot 2	
Flora Species Found	**Number of Each Species Found**
Ash	2
Unknown Shrub 1	6
TOTAL:	**8**
Worm Sample Location	**Number of Worms Found**
1.5m North of Plot Center	0
Plot Center	1
1.5m South of Plot Center	2
TOTAL:	**3**