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The Effect of Callery Pear (Pyrus calleryana) on Soil Macrofauna (Diplopoda and Oligochaeta)

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Joseph M. McGee

Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology

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We hereby approve the thesis of

The Effect of Callery Pear (*Pyrus calleryana*) on Soil Macrofauna (Diplopoda and Oligochaeta)

Joseph McGee

Candidate for the degree of Master of Science

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Dr. Christine Mutiti Committee Member	Date
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Dr. Allison VandeVoort Committee Member	Date
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Dr. Eric Tenbus Dean of College of Arts and Sciences	Date

PREFACE

This thesis has been written in journal format and conforms to the style appropriate to my discipline. This manuscript will be submitted for publication in *Applied Soil Ecology*, a peer reviewed interdisciplinary scientific journal, and therefore reflects the required formatting for this publication. Tables and figures are integrated into the thesis body as required by my thesis committee.

Acknowledgements

This project could not have been completed by me alone. First and foremost, I would like to give a special shoutout to my thesis advisor, Dr. Bruce Snyder. His advice and critiques were fundamental and out of everyone mentioned here he had to put up with me the most. He is one of the most patient people you'll ever meet. A sincere thank you goes to every other person who contributed to this projected, presented in no particular order.

Dr. Christine Mutiti, for advice on soil and plant data, and Dr. Allison VandeVoort, for advice on soil analysis and allowing me the use of her lab and soil texture equipment. Both agreed to sit on my thesis committee and oversee my project and growth as a researcher.

James T. Vogt (USDA Forest Service), for his assistance with setting up in his old study site and for helping find a reference site to add onto the project.

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Lance Andrew, Nick Sides, Rafal, Rebecca Recinos, Sophia DeSantis, Sydney Brown, Sydney Irons, and Wyatt Goldman.

And finally, all my friends and family who supported me from the sidelines. My technical explanations sometimes went over their heads but they encouraged me fully nonetheless.

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Abstract

Callery pear (*Pyrus calleryana*) is a relatively recent invader of North America. Its ecological effects are still being explored, including those affecting soil macrofauna such as millipedes (Diplopoda) and earthworms (Clitellata: Oligochaeta). These animals play important roles in many soil processes so understanding how they respond to invasive species is vital to soil health. A previous study exploring potential herbicide control was completed in 2019, however as of 2022 there are still treatment zones with little to no Callery pear alongside fully invaded plots, providing an optimal habitat mosaic for comparison. This allowed us to pursue two research goals: To determine the medium-term (3–4 years) effects of chemical control and to determine how soil macrofauna biodiversity is affected by the local plant community in the presence of invasive Callery pear. Soil fauna were sampled using four methods for thorough investigation. 1) Leaf litter was processed in Berlese funnels to draw out small millipedes. 2) Large millipedes were collected on sight by hand. 3) Earthworms were collected by digging soil monoliths and sorting through the soil by hand. 4) Additional earthworm collections were done via the octet method, using an electroshock machine to drive fauna out of the ground. Surveying the plant communities and collecting soil for texture, pH, and C:N ratios also helped achieve the goals of this study. We found that Shannon plant diversity indices were lower in the Callery pear plots but statistically similar between the herbicide and reference plots, suggesting the effectiveness of chemical control. Additionally, millipede diversity (Shannon index) was positively correlated with basal area of live Callery pear but was not statistically different between treatments. Nonnative earthworm abundance had no correlation with Callery pear. These findings will help land managers make informed decisions when creating management plans for invasive pear.

1. Introduction

Callery pear (*Pyrus calleryana*) is a common horticultural tree originating from southeast Asia. Although advertised as self-incompatible, Callery pear cultivars can still reproduce if within 90 m of a genetically compatible individual (Swearingen et al., 2014). This includes cross-pollination between rootstocks and scions as well if they are allowed to flower (Culley et al., 2011). The first and most notable cultivar, the 'Bradford', originated as a thornless individual found in Frank Reimer's disease-resistance trial plots (Creech, 1973). Grafted clones were found to do well in urban settings and became popular street trees due to their showy flowers and compact form (Culley and Hardiman, 2007). Once it has escaped cultivation, however, Callery pear proliferates and outcompetes native plants thanks to an extended leaf phenology (Maloney et al., 2022), freeze tolerance (Maloney et al., 2022), reduced herbivory (Morewood et al., 2004; Hartshorn et al., 2021), fire tolerance (Warrix and Marshall, 2018), and a potentially extensive seedbank (Serota and Culley, 2019). Despite its ability to invade, however, effective management is not fully explored and the impacts of Callery pear on soil ecology are not well known. Fire has been reported to have potential for removal of near-surface seeds and one-year saplings but trees older than one year are only top-killed (Warrix and Marshall, 2018). Even so, several common herbicides have been found to be effective short-term for foliar, basal bark, and soil applications (Vogt et al., 2020). Callery pear has also been reported to change soil pH and C:N in an Ohio grassland (Woods et al., 2021), but Boyce (2022) found that its leaf litter is similar to red maple (*Acer rubrum*) in composition and thus not likely to have a major effect in forests.

One major aspect of soil ecology that has not been addressed with this species is the soil macrofauna, which includes soil animals 2–20 mm in diameter. We focused on two major taxa,

the millipedes (Diplopoda) and earthworms (Clitellata: Oligochaeta). Millipedes and earthworms play prominent roles in soil nutrient cycling, fragmenting large particles like leaf litter and woody debris so smaller decomposers can break them down more efficiently (Lavelle et al., 2006). Understanding how they respond to invasive species is crucial to soil health and management, however such research is currently lacking. Stašiov et al. (2021) explored relationships between European millipedes and nine tree species, four native (*Picea abies*, *Taxus baccata*, *Quercus cerris*, and *Carpinus betulus*) and five nonnative (*Picea orientalis*, *Pinus nigra*, *Pinus ponderosa*, *Thuja occidentalis*, and *Castanea sativa*). They found that millipede richness and activity-density were higher under native tree stands than nonnative, however this was in a Slovakian arboretum with human-planted monocultures. Earthworms, on the other hand, have had more ecologically-relevant research regarding invasive species in North America. Madritch and Lindroth (2009) found that nonnative earthworm abundance was high in sites dominated by either European buckthorn (*Rhamnus cathartica*) or Bell's honeysuckle (*Lonicera x bella*) but that abundance went down after the invasive plants were removed. A similar pattern was found for Chinese privet (*Ligustrum sinense*), but not only did nonnative earthworm abundance go down following removal but native earthworm abundance went up as well (Lobe et al., 2014). The exception to this pattern for Chinese privet removal were *Amynthas* spp., which were most abundant where privet had not invaded.

Prior to our study, Vogt et al. (2020) performed their herbicide efficacy study near Milledgeville, GA, allowing us to compare conditions under varying amounts of Callery pear. Having access to their site gave us the ability to pursue two research goals: 1) To determine the medium-term (3–4 years) effects of chemical control and 2) To determine how soil macrofauna biodiversity is affected by the plant community in the presence of invasive Callery pear.

Although herbicides have been found to be effective within one to two years, there have been no long-term studies, and so we aimed to assess the dominance of Callery pear several years after the applications done by Vogt et al. (2020). Additionally, macrofauna relationships with the surrounding plants in GA are not well understood, especially if those plants include invasive pear.

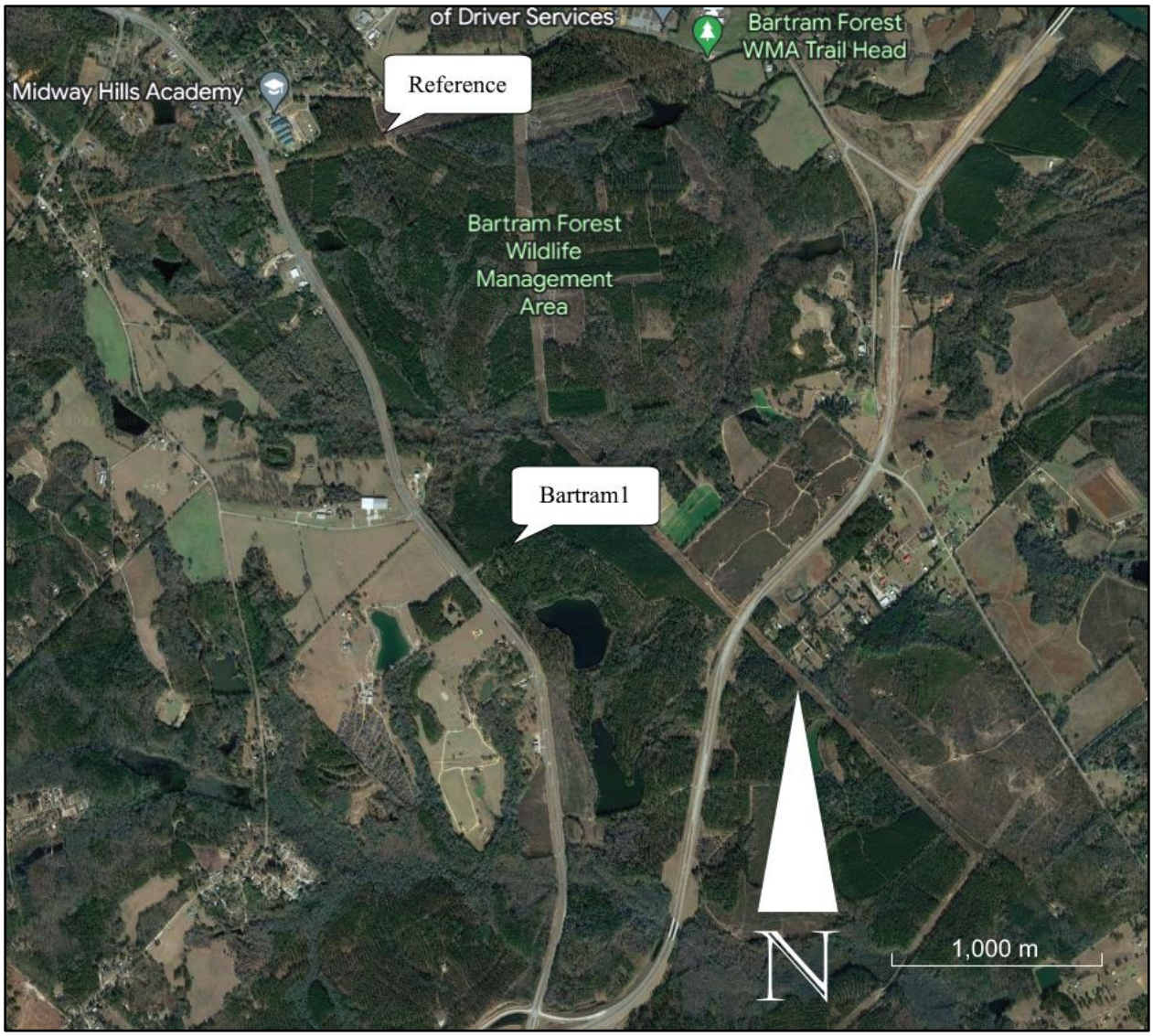
2. Methods

2.1. Study Site

This study was conducted at "Bartram1", one of two sites used for a Callery pear herbicide efficacy study by Vogt et al. (2020) at Bartram Forest Wildlife Management Area (WMA) in Milledgeville, GA (33.00101°N, 83.21450°W, see Fig. 1). Of the two sites used, Bartram2 was excluded due to soil treatments not killing Callery pear whereas Bartram1 had no such complications. This site is dominated by an overstory of slash pine (*Pinus elliotti*) with an understory of Callery pear, oak (*Quercus* spp.), sweetgum (*Liquidambar styraciflua*), black cherry (*Prunus serotina*), beautyberry (*Callicarpa americana*), and *Rubus* spp. Soil type is Norfolk loamy sand (fine-loamy, kaolinitic, thermic typic kandiudults) (NRCS 2019). Vogt et al. (2020) assigned thirty-six plots to Bartram1, arranged in six rows of six and separated by tree lines. Each row had a plot with one each of six randomly selected treatments, those being glyphosate, hexazinone, imazapyr, triclopyr, triclopyr + aminopyralid, or no herbicide. All herbicides were applied 18 Sep 2018 and deemed effective with Callery pear mortalities between 70–100% after 2 years. Each plot measured about 7 x 8 meters in size, their corners marked by metal stakes and the centers with PVC pipe. For our study we randomly selected three plots from every treatment except triclopyr + aminopyralid due to its similarity to triclopyr alone in terms of both chemical composition and effectiveness of control. Three additional plots were set up at

another site within the WMA (33.01850°N, 83.22166°W) to serve as an uninvaded reference, bringing the total number of plots to 18. The reference area is primarily loblolly pine (*Pinus taeda*) with an understory of oak, sweetgum, black cherry, and *Rubus* sp.

Figure 1. Map of Bartram Forest WMA. Our two study sites are highlighted with callouts. Map taken from Google Earth (https://earth.google.com/web) with imagery from 18 Dec 2022.



The reference site is on a soil type of Lakeland sand (thermic, coated typic quartzipsamment) (NRCS 2019). Bartram1 was previously on a 3-year burn cycle (Vogt et al., 2020) but neither

study site had been burned within the past five years of our study (J. Morgan Cook, personal communication).

2.2. Soil Macrofauna

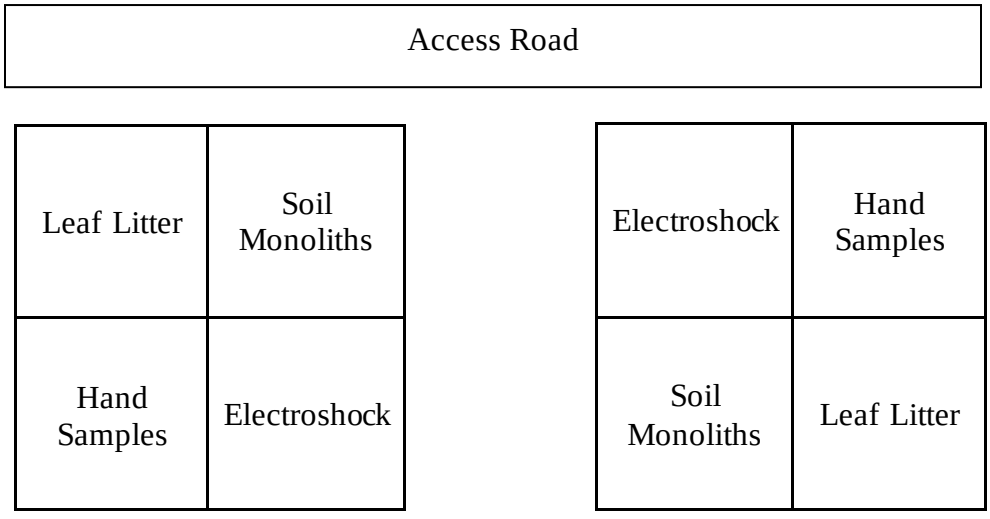
We had two sampling expeditions, one in the spring (22–24 Apr 2022) and in the fall (14–17 Oct 2022). Millipedes were collected using two collection methods: hand collecting and *a priori* leaf litter collection with Berlese funnel extraction (Snyder et al., 2006). Hand collecting involved searching for fauna at ground level through leaf litter, vegetation, and woody debris for 0.5 person-hours per plot. For *a priori* leaf litter collection, leaf litter along with surface soil was collected from each plot and transported to the laboratory in a canvas bag. Extraction was done using the Berlese funnel (#2831 Berlese Funnel, BioQuip Products, Inc. Rancho Dominguez, CA), a device comprised of a plastic bucket with a metal funnel lined with a metal screen. Litter was placed in the funnel for forty-eight hours while fauna were driven downward by the heat and light of a 25-watt incandescent light bulb, through the screen, falling into a cup of 70% ethanol.

Čoja et al. (2008) and Pelosi et al. (2021) both recommend a combination of hand-sorting and chemical extraction for lumbricid earthworms, however chemical extraction was not practical due to the large amount of solution we would need. We used the octet method instead, which Pelosi et al. (2021) concluded was a viable alternative even though it detected lower abundance. Čoja et al. (2008) also noted a bias for juveniles compared to other methods. To hand-sort earthworms, we dug a 30 x 30 x 30 cm soil monolith in each plot and sifted through the contents by hand on a plastic tarp. When finished, the soil was mixed and resorted for any earthworms left behind, but this extra step was halted halfway through the spring sampling due to the thoroughness of the first sift. Both sifts were used throughout the fall sampling, but two plots were half-depth and two three-fourths depth due to dry, compacted clay. For the octet

method, we used a commercial electroshock machine (DEKA 4000 W, DEKA Gerätebau, Marsberg, Germany), a device with eight metal probes driven into the ground equidistant from each other to send an electrical current through the soil (Schmidt, 2001; Snyder et al., 2011). We consecutively applied 300, 350, 400, 500, and 600 V each for two minutes in each plot, stopping occasionally to collect surfaced earthworms.

To prevent disturbances between different sampling methods, each plot was divided into quadrants with each quadrant assigned a specific collection method (Fig. 2). Collection methods were done only in their respective quadrants and quadrants were kept constant in every plot but were rearranged between spring and fall sampling. All fauna were euthanized and preserved in 70% ethanol. Additionally, earthworms were fixed in 5% formalin for at least twenty-four hours before long-term preservation in ethanol. All millipedes and earthworms were identified down to the lowest taxon possible using keys and descriptions by Shear (1999, unpublished), Shelley (1984; 2002), and Chang et al. (2016).

Figure 2. Sampling quadrants relative to the access road for each site. The left was used April 2022 and the right October 2022 for all 18 plots.



2.3. Plant Community

To assess the plant community, we counted the number of trees and shrubs greater than 30 cm tall in each plot in July 2022. We measured the diameter at breast height (DBH) at the standard 137 cm above the ground for all trees in each plot and diameter at root collar (DRC) for all shrubs. Any tree that had a significant amount of foliage over the plot was treated as inside even if its trunk was outside the boundaries due to its contribution to the leaf litter. We also recorded species, basal area (calculated from DBH or DRC), whether each tree or shrub was alive or dead, percent canopy cover over each plot, percentage of ground covered by leaf litter, percentage of ground covered by woody debris, whether each plot had logs greater than 1 m long (yes or no), percentage of ground covered by grass (including both grasses [Poaceae] and sedges [Cyperaceae] due to the dominance of sedges), and percentage of ground covered by broadleaf plants under 30 cm tall. All percentages were rounded to the nearest 5%.

2.4. Soil

Five soil cores (10 cm deep by 2.2 cm diameter) were taken from each plot, one at each corner and a final from the center using an AMS step probe soil sampler (AMS 401.42, model number 77636, Forestry Suppliers, Jackson, MS). Each set of five was combined and homogenized in the field to create a bulk soil sample. All bulk samples were returned to the lab in plastic bags and oven-dried at 40°C for forty-eight hours. Once dry, we ground the samples by hand with mortar and pestle to remove aggregates and sieved to a particle size of 2 mm.

Soil texture was measured using the hydrometer method (Day, 1965) with readings taken 30 sec and 8 hr after the suspension was mixed. For pH measurements, we made a 1:1 solution of soil and distilled water from each bulk sample and took the average of three readings using a

Fisher Scientific accumet AE150 pH benchtop meter with Automatic Temperature Compensation. To obtain percent total nitrogen, percent total carbon, and C:N ratios, we ground all samples to a fine, homogenous powder using a Spex SamplePrep 8000D Mixer/Mill. Elemental analysis was done by the U.S. Forest Service Southern Research Station Analytical Chemistry Lab (Research Triangle Park, NC) using a Thermo Fisher Scientific FlashEA 1112 NC Analyzer.

2.5. Statistical Analysis

All statistical analyses were done using R v4.2.2 (R Core Team, 2022) to compare fauna, flora, and soil characteristics. Shannon Diversity Indices were calculated using the vegan package (Oksanen et al., 2022). The stats package (R Core Team, 2022) was used for the following: Shapiro-Wilk normality tests to ensure data meet parametric assumptions, Pearson's product-moment correlations to correlate fauna community indices with plant community indices and soil data, analyses of variance (ANOVAs, Kruskal-Wallis rank sum tests, and post-hoc Tukey Honestly Significant Difference tests) to test differences in community indices and soil data between plots, and differences of mean (Welch two-sample t-tests and Wilcoxon rank-sum tests) to compare fauna community indices plots with large and small woody debris. We also used the FSA package (Ogle et al., 2022) for post-hoc Dunn Kruskal-Wallis multiple comparison tests to follow-up the Kruskal-Wallis tests. Data that did not meet parametric assumptions were either log- or square-root-transformed to meet assumptions if possible.

3. Results

3.1. The Plant Community, Soil, and Callery Pear Control

The following were found to be significantly different between treatment via analysis of variance ($p < 0.05$; exact test results are presented in Table 1): Shannon diversity index of woody plants, abundance of woody plants, evenness of woody plants, basal area of live Callery pear, basal area of dead Callery pear (although a post-hoc Dunn test shows no difference if corrected using the Holm method), basal area of live and dead Callery pear, and C:N. Mean diversity of woody plants (Fig. 3) was lowest in No Herbicide. Glyphosate, Hexazinone, and Triclopyr had higher diversity and were significantly different from No Herbicide. Reference and Imazapyr were statistically similar to No Herbicide as well as Glyphosate, Hexazinone, and Triclopyr. Mean abundance of woody plants (Fig. 4) was highest in No Herbicide. Glyphosate and Triclopyr were lower and statistically different. Hexazinone and Imazapyr were statistically similar to Glyphosate, Triclopyr, and Reference, but not No Herbicide. Reference was statistically similar to No Herbicide, Hexazinone, and Imazapyr, but not Glyphosate and Triclopyr. Mean evenness of woody plants (Fig. 5) was lowest in No Herbicide. Glyphosate and Triclopyr had higher evenness and were statistically different from No Herbicide. Reference, Hexazinone, and Imazapyr were statistically similar to No Herbicide, Glyphosate, and Triclopyr.

Mean basal area of live Callery pear (Fig. 6) was highest in No Herbicide. Hexazinone was lower and statistically different from No Herbicide but statistically similar to Glyphosate, Imazapyr, and Triclopyr. Imazapyr and Triclopyr were statistically similar to No Herbicide. Reference had a value of zero and was statistically similar to Glyphosate. Mean basal area of dead Callery pear (Fig. 7) was highest in Triclopyr but statistically similar to Glyphosate, Hexazinone, and Imazapyr. Reference and No Herbicide both had values of zero but were

statistically similar to Glyphosate. Mean basal area of live and dead Callery pear combined (Fig. 8) was highest in No Herbicide. Imazapyr and Triclopyr were lower and statistically different from No Herbicide but statistically similar to Hexazinone. Reference had a value of zero and was statistically similar to Glyphosate. Glyphosate was statistically similar to Hexazinone.

Mean soil C:N was highest in Reference (Fig. 9). Glyphosate, Hexazinone, Imazapyr, and Triclopyr were statistically similar to each other but statistically different from Reference. Neither soil texture nor pH were found to statistically differ among treatments. Soil texture was sandy loam to sandy clay loam and soil pH ranged from 4.3 to 4.6.

Table 1. Analysis of Variance Test Results. All tests done using ANOVA except for Basal Area of Dead Callery Pear, which was a Kruskal-Wallis test. †Log-transformed data ‡Square-root-transformed data.

Woody Plant Shannon Diversity Index (Fig. 3)					
	Df	Sum Sq.	Mean Sq.	F value	Pr(>F)
Comparisons	5	1.076	0.215	5.436	0.008
Residuals	12	0.4752	0.0396		
Woody Plant Abundance† (Fig. 4)					
	Df	Sum Sq.	Mean Sq.	F value	Pr(>F)
Comparisons	5	8.371	1.674	11.72	<0.001
Residuals	12	1.714	0.1428		
Woody Plant Pielou Evenness Index (Fig. 5)					
	Df	Sum Sq.	Mean Sq.	F value	Pr(>F)
Comparisons	5	0.333	0.067	3.534	0.034
Residuals	12	0.226	0.019		
Basal Area of Live Callery Pear† (Fig. 6)					
	Df	Sum Sq.	Mean Sq.	F value	Pr(>F)
Comparisons	5	78.78	15.756	15.27	<0.001
Residuals	12	12.28	1.032		
Basal Area of Dead Callery Pear (Fig. 7)					
	χ^2	df		P-value	
Comparisons	14.364	5		0.013	
Basal Area of Live and Dead Callery Pear‡ (Fig. 8)					
	Df	Sum Sq.	Mean Sq.	F value	Pr(>F)
Comparisons	5	1330.2	266.04	38.49	<0.001
Residuals	12	82.9	6.91		
C:N† (Fig. 9)					
	Df	Sum Sq.	Mean Sq.	F value	Pr(>F)
Comparisons	5	0.26	0.052	13.03	<0.001
Residuals	12	0.047	0.004		

Figure 3. Woody Plant Shannon Diversity Index by Treatment. Error bars represent standard error. Subjects sharing letters are statistically similar as per ANOVA with post-hoc Tukey test.

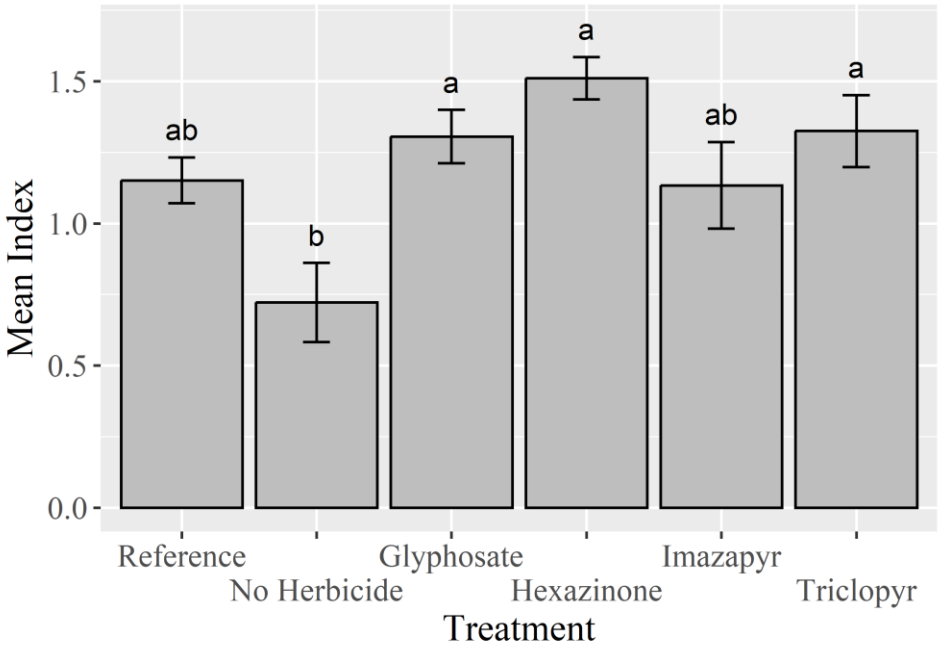


Figure 4. Woody Plant Abundance by Treatment. Error bars represent standard error. Subjects sharing letters are statistically similar as per ANOVA with post-hoc Tukey test using log-transformed data.

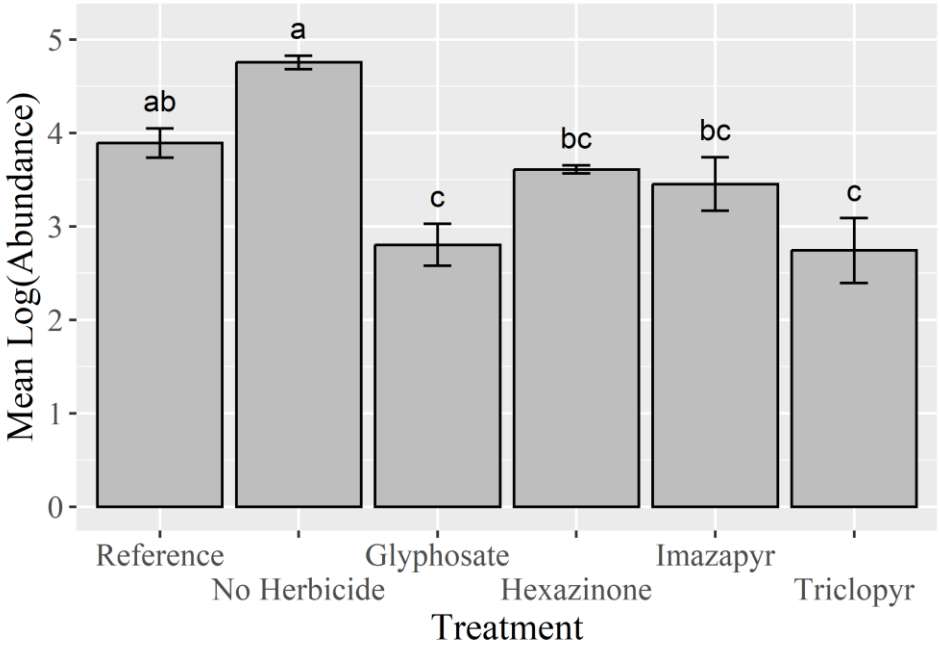


Figure 5. Woody Plant Pielou Evenness Index by Treatment. Error bars represent standard error. Subjects sharing letters are statistically similar as per ANOVA with post-hoc Tukey test.

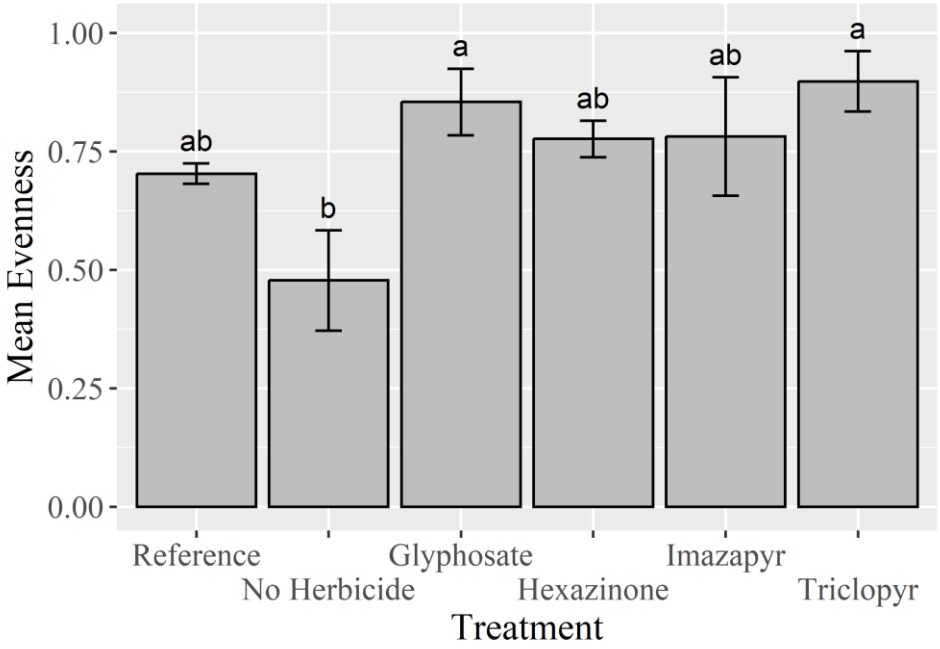


Figure 6. Basal Area of Live Callery Pear by Treatment. Error bars represent standard error. Subjects sharing letters are statistically similar as per ANOVA with post-hoc Tukey test using log-transformed data. Reference has a value of zero.

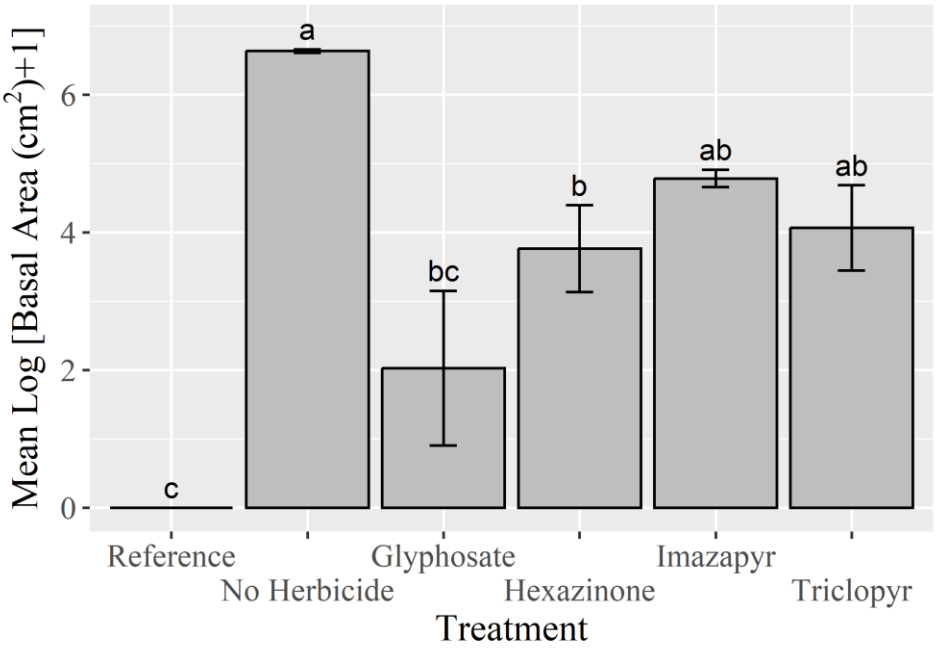


Figure 7. Basal Area of Dead Callery Pear by Treatment. Error bars represent standard error. Subjects sharing letters are statistically similar as per Kruskal-Wallis test with post-hoc Dunn test and unadjusted p-values. Reference and No Herbicide have values of zero.

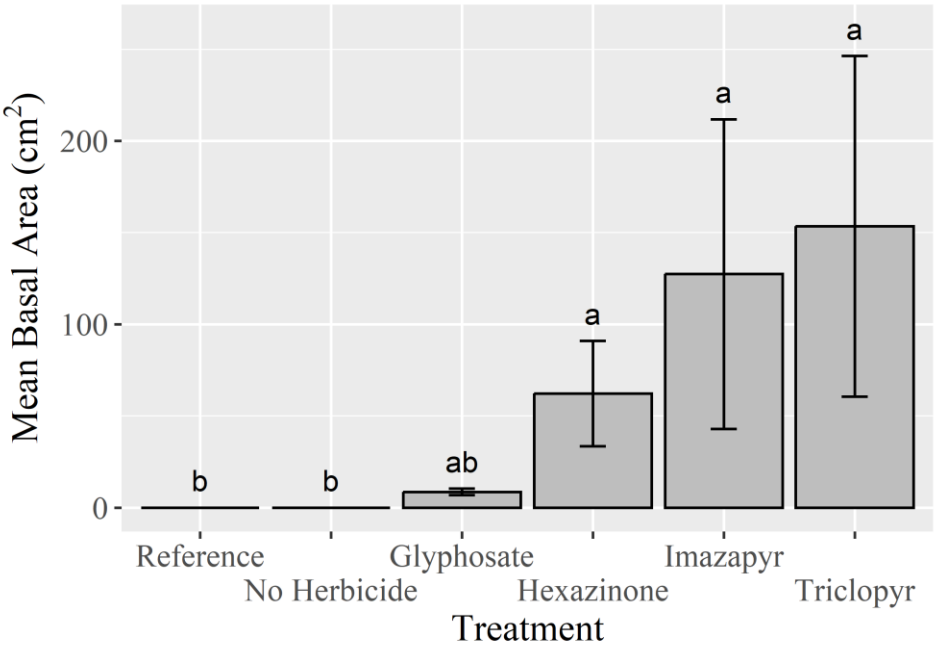


Figure 8. Basal Area of Live and Dead Callery Pear by Treatment. Error bars represent standard error. Subjects sharing letters are statistically similar as per ANOVA with post-hoc Tukey test using square-root-transformed data. Reference has a value of zero.

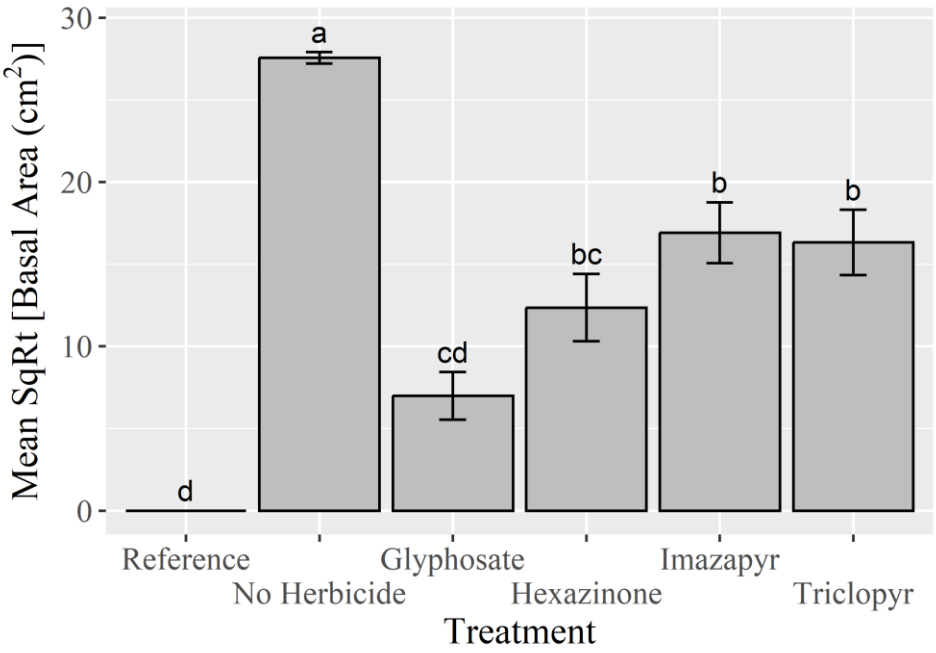
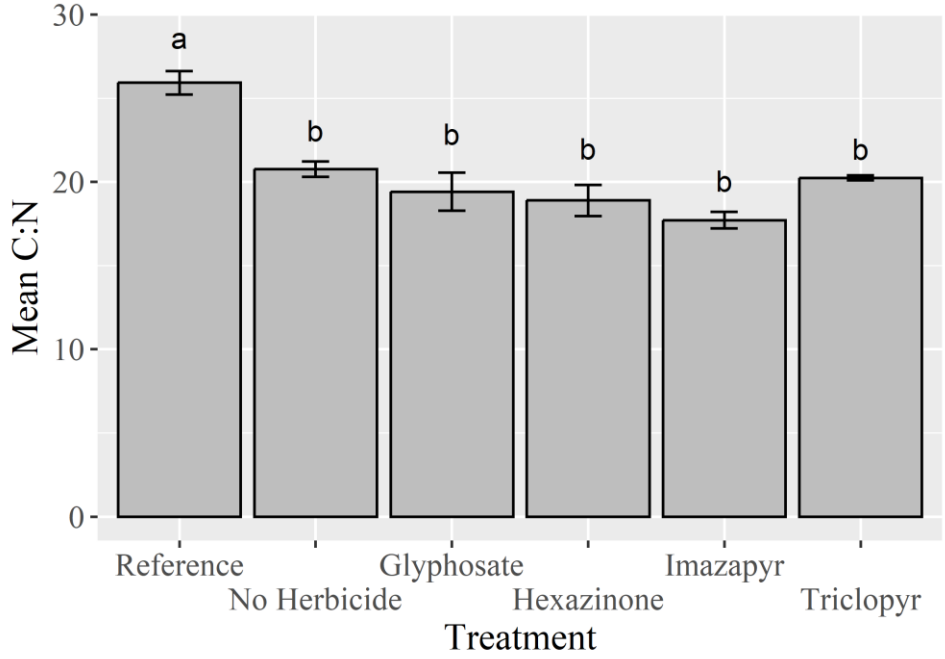


Figure 9. Soil C:N by Treatment. Error bars represent standard error. Subjects sharing letters are statistically similar as per ANOVA with post-hoc Tukey test using log-transformed data.



3.2. Soil Macrofauna

183 millipedes and 108 earthworms were collected across the spring and fall sampling periods. Millipedes were comprised of the following taxa, listed in order of dominance (See Fig. 10 for proportions of millipedes per treatment): *Oxidus gracilis* (71.7%), *Pseudojulus paynei* (9.24%), *Polyxenus* sp. (7.61%), *Cleidogona* sp. (4.89%), Xystodesmidae (3.80%), *Abacion tesselatum* (2.17%), and immature Julida (0.543%). Earthworms were less diverse (Fig. 11): immature *Amynthas* spp. (74.1%), *Amynthas minimus* (24.1%), and *Amynthas corticis* (1.9%). Of the immature *Amynthas* spp., only three exceeded the size range of *Amynthas minimus* (20–50 mm length by 1.5–2.0 mm width).

Millipede Shannon diversity was positively correlated with abundance of woody plants ($t = 2.604$, $df = 16$, $p = 0.019$, $r = 0.546$), basal area of live Callery pear ($t = 3.262$, $df = 16$, $p =$

0.005, r = 0.632), and basal area of living and dead Callery pear combined (t = 2.139, df = 16, p = 0.048, r = 0.472). There was no correlation with dead Callery pear (t = -1.625, df = 16, p = 0.124, r = -0.376).

Figure 10. Total Proportions of Millipede Taxa by Treatment. *Oxidus gracilis* was the only nonnative millipede species collected.

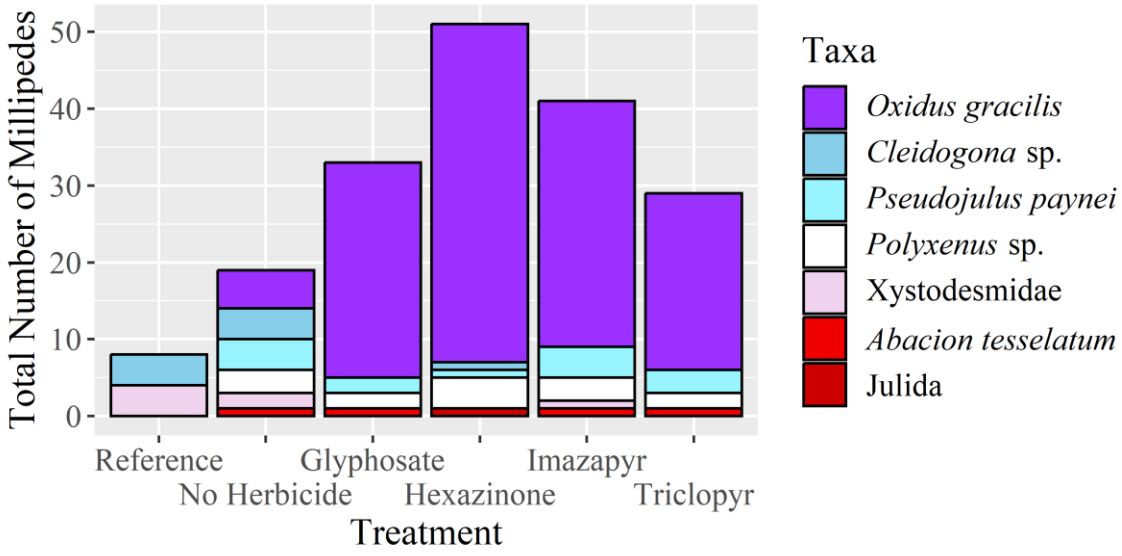
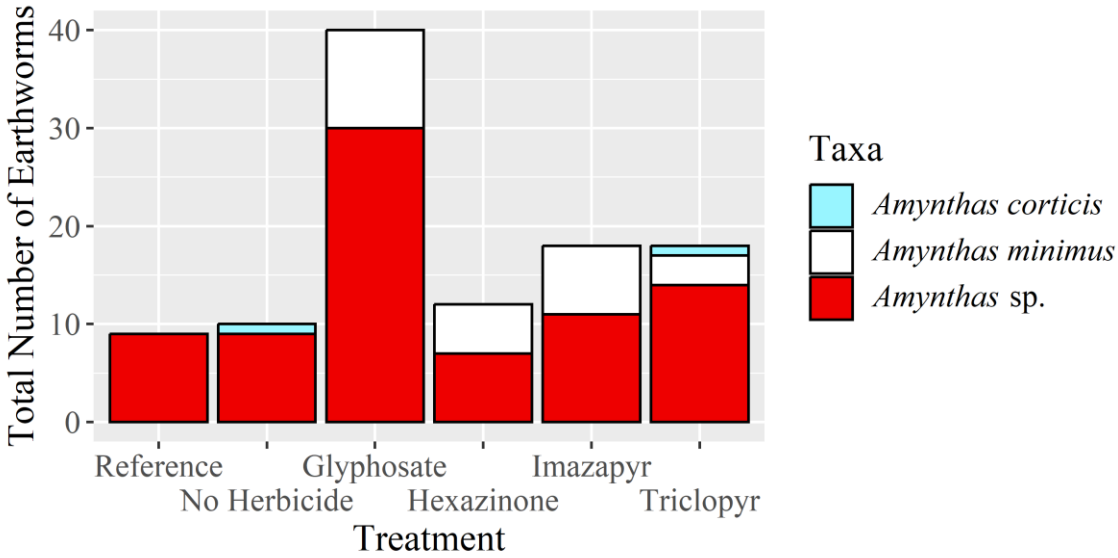


Figure 11. Total Proportions of Earthworm Taxa by Treatment. All earthworms collected were nonnative invasive species.



Millipede species richness was positively correlated with abundance of woody plants ($t = 2.736$, $df = 16$, $p = 0.015$, $r = 0.565$), basal area of live Callery pear ($t = 3.237$, $df = 16$, $p = 0.005$, $r = 0.629$), and basal area of living and dead Callery pear ($t = 2.648$, $df = 16$, $p = 0.018$, $r = 0.552$). Millipede richness was negatively correlated with the Pielou evenness index of woody plants ($t = -2.134$, $df = 16$, $p = 0.049$, $r = -0.471$).

Millipede abundance was positively correlated with percent wood cover ($t = 3.174$, $df = 16$, $p = 0.006$, $r = 0.622$) and negatively correlated with percent canopy cover ($t = -2.46$, $df = 16$, $p = 0.026$, $r = -0.524$). There was no significant difference in millipede abundance between plots with large and small woody debris (t-test, data log-transformed, $t = -0.310$, $df = 8.720$, $p = 0.742$). Millipede evenness was negatively correlated with percent wood cover ($t = -2.940$, $df = 12$, $p = 0.012$, $r = -0.647$). Wilcox tests showed no significant differences between Pielou evenness indices of plots with large or small woody debris ($W = 36$, $p = 0.487$). For all significant correlations for millipedes see Fig. 12.

There were no significant correlations for earthworm diversity or richness. Earthworm abundance was positively correlated with percent nitrogen ($t = 3.022$, $df = 16$, $p = 0.008$, $r = 0.603$), percent clay ($t = 3.240$, $df = 16$, $p = 0.005$, $r = 0.629$), and percent canopy cover ($t = 2.559$, $df = 16$, $p = 0.021$, $r = 0.539$). Earthworm abundance was negatively correlated with percent sand ($t = -2.934$, $df = 16$, $p = 0.01$, $r = -0.591$), percent broadleaf groundcover ($t = -2.251$, $df = 16$, $p = 0.039$, $r = -0.490$), and abundance of woody plants ($t = -2.204$, $df = 16$, $p = 0.043$ $r = -0.483$). Earthworm evenness was positively correlated with percent wood cover ($t = 2.684$, $df = 8$, $p = 0.028$, $r = 0.688$). Wilcox tests showed no significant differences between Pielou evenness indices of plots with large or small woody debris ($W = 25$, $p = 0.613$). For all significant correlations for earthworms see Fig. 13.

Figure 12. Fitted Linear Models for Millipede Community Metrics. Only statistcially significant correlations are shown.

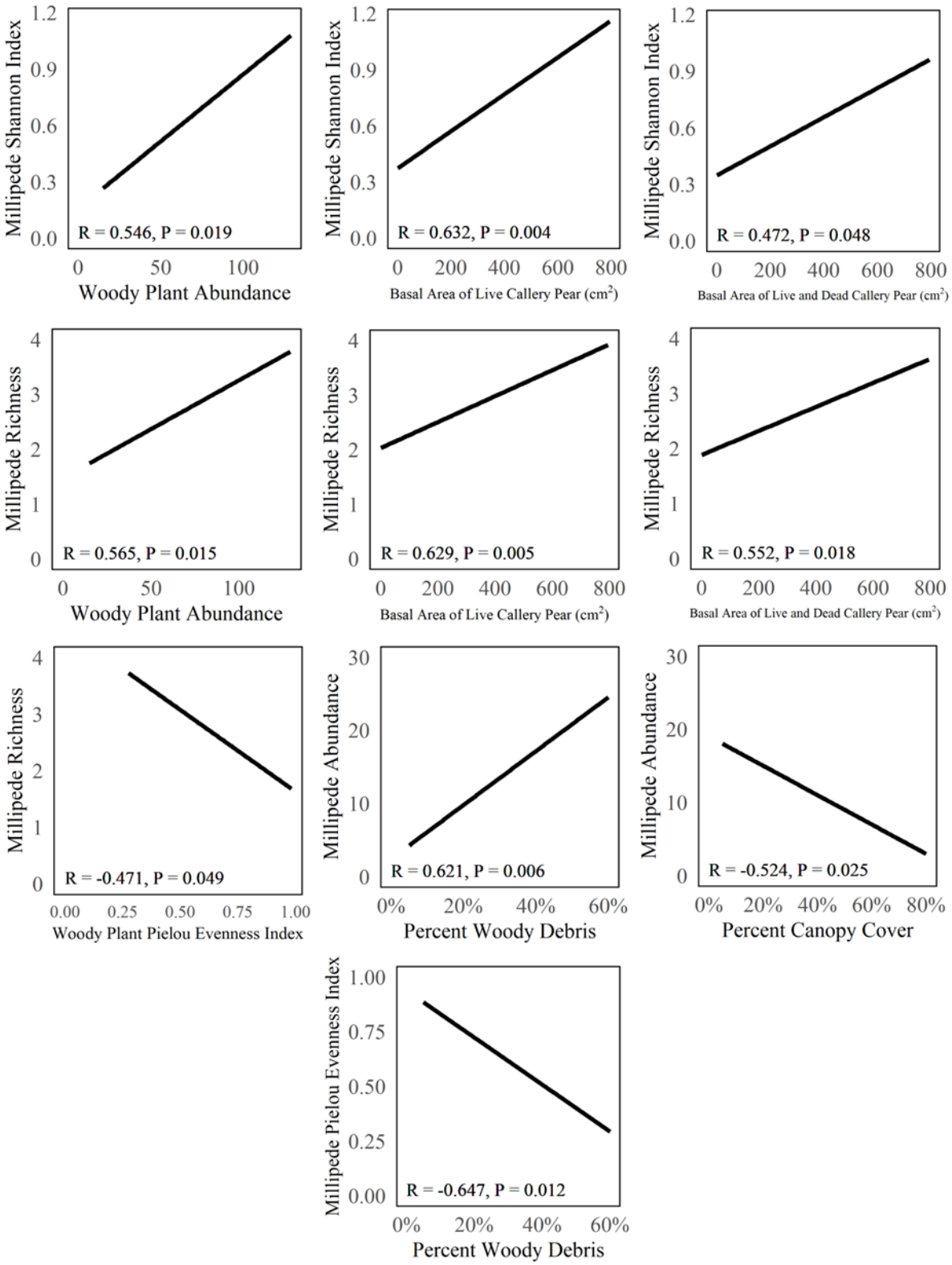
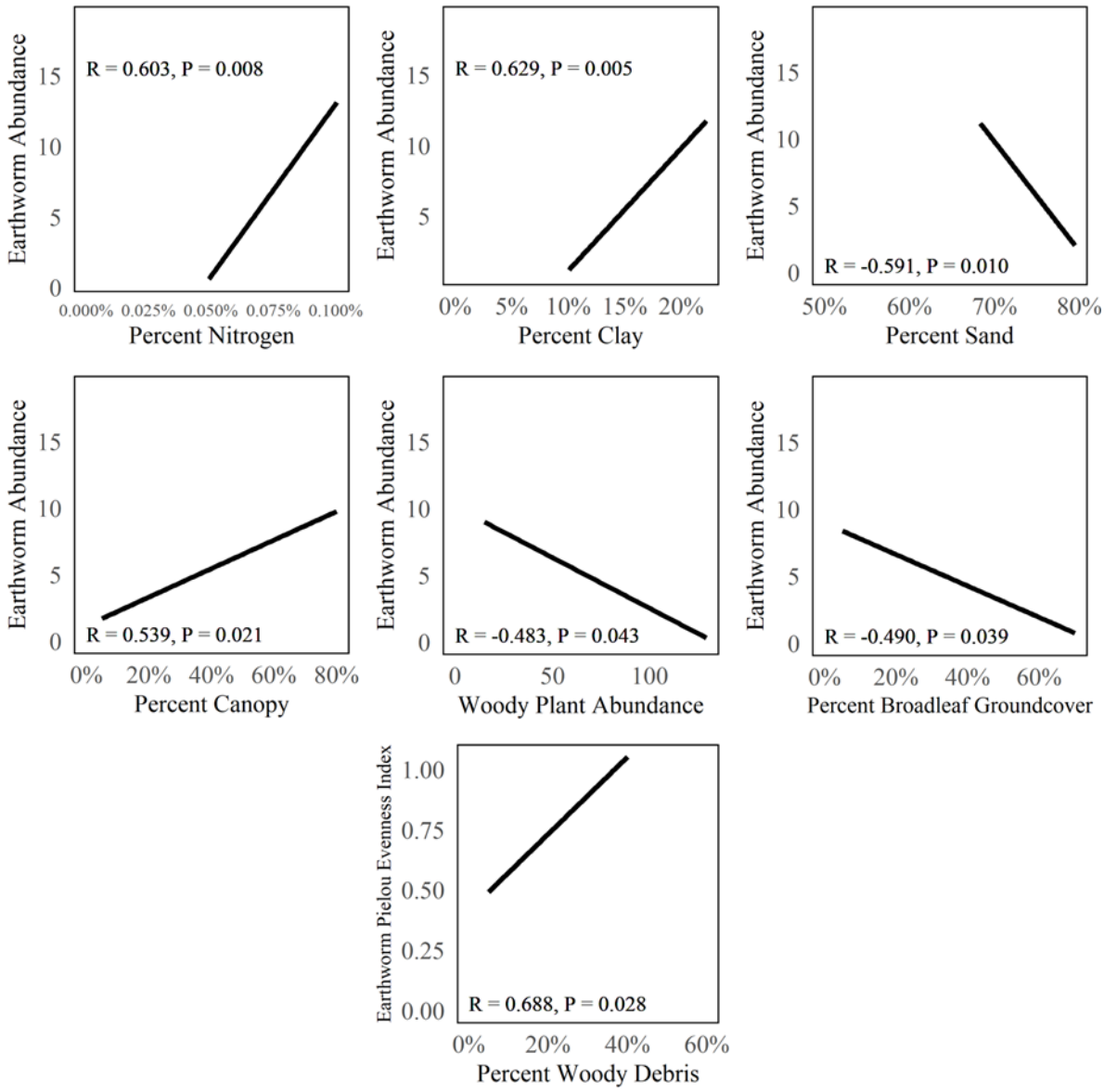


Figure 13: Fitted Linear Models of Earthworm Community Metrics. Only statistically significant correlations are shown.



4. Discussion

4.1. The Plant Community, Soil, and Callery Pear Control

Although woody plant richness did not differ between treatments, diversity, abundance, and evenness did. Meta-analyses by Vilà and Weiner (2004) and Oduor et al. (2016) reveal that invasive species often adapt and compete well with native species, which we see in the No

Herbicide plots where Callery pear is most abundant and woody plant diversity and evenness are lowest due to Callery pear dominance. Odour et al. (2016) also found that self-incompatible invasives had higher frequencies of local adaptation than native self-incompatible plants. Considering the high rates of gene flow and genetic differentiation in Callery pear (Sapkota et al., 2022) it is likely that adaptability is a potential driver of invasion. Diversity and evenness overall were higher in the herbicide plots, but exact patterns were nuanced. Glyphosate, Hexazinone, and Triclopyr were statistically different from No Herbicide in terms of diversity, but Imazapyr was not. This is surprising considering that Vogt et al. (2020) found some of the highest pear mortality rates of Bartram1 in the Imazapyr plots, but it could just be a matter of how much was in there in the first place. In our survey we found that Imazapyr had the highest mean basal area for both live Callery pear and total (live and dead) Callery pear out of the four herbicides, so the high amount of pear that returned after removal are likely driving down diversity. Additionally, while we did see many native saplings, we did not include trees or shrubs <30 cm tall in our analyses. Those plots may not have been very diverse in the first place and will likely remain so for many years while the native plants reestablish following Callery pear removal. The uninvaded reference plots also have a higher diversity than No Herbicide but are not statistically different. We did notice that the understory trees in the reference plots were a little less diverse due to a greater dominance of oaks so that may be a contributing factor.

In terms of evenness, all herbicide plots were greater than No Herbicide but only Glyphosate and Triclopyr were statistically different. Again, there were high means of Callery pear basal area and many native saplings in the herbicide plots not included in our analyses. Both the herbicide site and the reference site also have only a few species and are largely dominated by pines and oaks so evenness likely wouldn't be very high in these sites regardless.

Despite the complexity of our results they show that the effects of the herbicide treatments can still be observed 3–4 years later. We argue that chemical control is effective in the medium-term due to the increased diversity and evenness of the native plant community. There was a relatively high basal area of dead Callery pear in the herbicide plots leftover from the chemical applications but there were still some living Callery pear as well, suggesting the need for additional treatments. Imazapyr had the second most live pear in terms of basal area, compared to No Herbicide, although the four herbicide plots were not statistically different.

Soil C:N was statistically lower at the herbicide site than the reference site, suggesting lingering effects of nutrient inputs by Callery pear to the topsoil even after it is removed. The tree that was most abundant and similar in size to Callery pear were the understory oaks, which have a mean leaf C:N of ~60 (Snyder, unpublished data), whereas Callery pear averages 36 (Boyce, 2022). However, although Callery pear could be affecting the soil nutrient cycle with labile, nitrogen-rich litter, it is difficult to thoroughly confirm without readings from the same site before invasions.

4.2. Soil Macrofauna

Millipede Shannon diversity, richness, abundance, and Pielou evenness were not statistically different between plot types, suggesting that treatments did not affect millipedes directly. Even so, correlations indicate that there may be indirect effects due to how the herbicides shaped the plant community. Diversity and richness were both positively correlated with live Callery pear, suggesting that Callery pear benefits millipede diversity. Stašiov et al. (2021) did not look at Callery pear specifically but did conclude that nonnative plants would not likely be as beneficial for millipedes as native plants. However, we found that our nonnative plant in question did appear to beneficial in that millipede diversity and richness were positively

correlated with live Callery pear basal area. Native millipedes in Madagascar have been shown to be more abundant in thicker leaf litter so long as they have enough nitrogen (Spelzhausen et al. 2020) and soil C:N was lower (enriched in N) at the pear site than the reference site so the millipedes may be benefiting from the extra nitrogen. Although we didn't measure it, anecdotally the leaf litter under the Callery pear was thicker in depth, so that may play a part as either food, moisture, shelter, or a combination of the three. Most taxa were found most consistently in the No Herbicide plots except for *Oxidus gracilis*, a nonnative millipede species from eastern Asia (Hoffman, 1999). If low C:N or deep leaf litter (through any mechanism) provide better habitat for millipedes, it would make sense that *Oxidus* would be abundant in the No Herbicide plots. Instead they are most abundant in the herbicide plots, somewhat abundant in the No Herbicide plots, and absent from the reference plots.

Being the dominant species in our sample (>70%), however, *Oxidus* drives overall millipede abundance. Millipede abundance is positively correlated with woody debris and negatively correlated with canopy cover. Millipede evenness is also negatively correlated with woody debris, suggesting that the *Oxidus* may be congregating where wood is abundant. Spraying Callery pear did result in dead pears and many of them had fallen by the time we had surveyed, but anecdotally much of the woody debris was from other trees (especially pine). Our results are consistent with Ulyshen and Hanula (2009) and Boggs et al. (2020) who both found that millipedes and other arthropods were more abundant near fallen logs in pine forests. Boggs et al. (2020) also observed that millipedes 1) were captured more often in traps placed farther away from logs than near and 2) were more abundant near hardwood than pine wood, however they grouped millipedes with predatory centipedes (Chilopoda) in their analyses. Neither Ulyshen and Hanula (2009) nor Boggs et al. (2020) identified millipedes beyond class (e.g.,

Diplopoda, Chilopoda). Although the death of the Callery pear could have contributed to the woody debris and thus created habitat for *Oxidus*, it is difficult to draw a solid conclusion without knowing how much of the woody debris came from treated Callery pear. In future studies it would be helpful to record wood species as well as amounts. Canopy cover may be correlated with how disturbed the habitat is, and thus how appealing it is to opportunistic nonnatives, as canopy cover did tend to increase with distance from the access road.

Earthworm diversity, richness, abundance, and evenness were not statistically different between plot types, suggesting no direct effect from the herbicides. However, finding no differences for any community metrices contradicts other studies that found invasive earthworms had greater abundance under invasive plants (Madritch and Lindroth, 2009; Lobe et al., 2014). Due to how labile Callery pear litter is, it does increase the amount of soil nitrogen immediately added to the soil but less so than other invasive species. The amount of change may not be great enough to elicit the response from earthworms seen with other invasive plants such as European buckthorn, which has a mean leaf C:N of ~13 (Heneghan et al., 2004). Additionally, Middle Georgia has historically been an agricultural region, including Baldwin County where Bartram Forest WMA is located (Murray 1935). Much of the region was intensively farmed, degrading the soil (Crawford 1988) and creating unfavorable conditions for native earthworms, which may explain the low diversity we found.

Evenness was positively correlated with woody debris, but considering we only identified one earthworm genus with two species this is difficult to interpret. Abundance, however, is more straightforward. In many systems nitrogen is a limiting factor so a positive correlation with nitrogen is a given. Abundance being positively correlated with clay and negatively with sand also makes sense since clay holds nitrogen and moisture better than sand does. A positive

correlation with canopy cover may also have to do with moisture levels in the soil, which we did not measure. A negative correlation with broadleaf groundcover may have to do with nitrogen competition due to much of the groundcover being fast-growing *Rubus* spp., however *Rubus* leaves tend to be high in nitrogen (Fan et al., 2015) and so should be a good source of food. Low-growing *Rubus* spp. such as dewberries may also form dense root masses that make the soil around them less desirable to burrow in. Earthworm abundance is negatively correlated with abundance of woody plants but not basal area of Callery pear, suggesting that although abundant, Callery pear does not drive this pattern. Considering that the dominant native trees—pines and oaks—have relatively low levels of nitrogen in their leaves, this pattern may be because a greater dominance of tree leaves in the leaf litter means less nitrogen and thus a less nutritious food source.

5. Conclusion

We can conclude that herbicide applications are effective even after several years, although follow-up treatments are needed. Callery pear also does not adversely affect total millipede diversity or nonnative earthworm abundance. Nonnative *Oxidus gracilis* was present in the No Herbicide plots in moderate numbers and extremely dominant in the herbicide plots, so it may have benefited from the Callery pear removal. Callery pear may also lower soil C:N and adversely impacts the plant community.

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