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ORIGINAL ARTICLE

Limited Evidence of Native Woody Plant Recovery and Changes in Millipede and Earthworm Communities Following Herbicide Applications on Callery Pear (*Pyrus calleryana*)

Joseph M. McGee[1] · James T. Vogt[2] · Bruce A. Snyder[1]

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Abstract

There is little published data available on herbicidal control for invasive Callery pear (*Pyrus calleryana*) or its ecological effects in North American forests. A previous field study exploring herbicide control for Callery pear was completed in 2019, providing an opportunity to (1) quantify reinfestation of Callery pear in the medium-term (3–4 years after treatment) along with changes in the woody plant community, and (2) determine how millipedes (Diplopoda) and earthworms (Clitellata: Oligochaeta) are affected by changes in the woody plant community following herbicide applications on Callery pear. Native woody plant abundance was not statistically greater in treated versus untreated plots and Callery pear was present throughout. These results suggest that the forest understory has not recovered following the initial herbicide applications. However, we found no evidence of any negative effects on millipedes or earthworms. Our study suggests that active management may be needed for forested ecosystems to recover following Callery pear invasion.

Study Implications

Woody invasive plants such as Callery pear threaten ecosystem processes, can convert open habitats to closed-canopy monocultures, impede restoration efforts, and in some cases represent a growing cost to production forestry. Soil organisms play important roles in nutrient cycling and soil health. This study demonstrated a slow recovery rate of the native woody vegetation and the continued presence of Callery pear, suggesting that initial herbicide treatments alone were not sufficient. However, we did not find evidence of a response from millipede or earthworm communities

✉ Bruce A. Snyder
bruce.snyder@gcsu.edu

1 Department of Biological and Environmental Sciences, Georgia College & State University, Milledgeville, GA, USA

2 USDA Forest Service, Southern Research Station, Athens, GA, USA

from any changes in the woody plant community following herbicide applications. Additional studies are needed to quantify and characterize Callery pear reinfestation along with changes in understory and soil macrofauna over the longer term.

Keywords Invasive species · Diplopoda · Oligochaeta · Soil Fauna

Introduction

Callery pear (*Pyrus calleryana*) is a tree originating from temperate East Asia and invasive in North America. Although advertised as self-incompatible, Callery pear cultivars can still reproduce if within 90 m of another cultivar (Culley and Hardiman 2009). 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). Callery pear has been known to escape cultivation and, although few studies have assessed its effects on native plants, it has traits that make it a potentially invasive species such as 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). Callery pear is currently present in 38 US states (EDDMapS 2024) and is common in neglected fields where, if left uncontrolled, it can form a closed-canopy monoculture (Vogt, pers. obs.). Callery pear has been noted to be an obstacle in pine reforestation (Sundell et al. 1999; Clabo and Clatterbuck 2019) making it a potentially serious economic pest. Despite its potential to invade, effective management is not fully explored.

Regarding impacts on soils, Boyce (2022) found that Callery pear 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 Callery pear invasion is the soil macrofauna, which includes soil animals 2–20 mm in diameter. Millipedes (Diplopoda) and earthworms (Clitellata: Oligochaeta) 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 and five nonnative. 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 been the subject of much research on invasive species in North America. Madritch and Lindroth (2009) found that nonnative earthworm abundance was higher in sites dominated by either European buckthorn

(*Rhamnus cathartica*) or Bell's honeysuckle (*Lonicera x bella*) than the uninvaded controls but that abundance decreased after the invasive plants were removed. However, Mahon and Crist (2019) found that earthworm densities were driven more strongly by white-tailed deer (*Odocoileus virginianus*) than Amur honeysuckle (*Lonicera maackii*). Chinese privet (*Ligustrum sinense*) control has also been found to lower earthworm abundance, but not only did nonnative earthworm abundance decrease following removal but native earthworm abundance increased as well (Lobe et al. 2014). The exceptions to this pattern for Chinese privet removal were Asian jumping worms (*Amynthas* spp.), which were most abundant where privet had not invaded.

Several common herbicides have been found to be effective in the short-term for foliar, basal bark, and soil applications for killing Callery pear but peer-reviewed studies are lacking. Prior to our study, Vogt et al. (2020) performed a short-term (2 years) herbicide efficacy study in a pine plantation near Milledgeville, GA, providing an opportunity for a follow-up study to evaluate medium-term (3–4 years) efficacy of chemical control, and to determine how millipedes and earthworms respond to changes in the forest community. Although herbicidal control has been found to be effective for one to two years, there have been no long-term studies, and so we aimed to assess the dominance of Callery pear three to four years after the applications done by Vogt et al. (2020). Additionally, macrofauna relationships with woody understory plants and trees in Georgia are not well understood, especially in a Callery pear control setting. In past studies such as those mentioned above, differences in fauna communities were attributed to changes in the soil caused by shifting plant communities through either pH or rates of litter decomposition. In this study we hypothesized that control of Callery pear would release woody understory plants, with corresponding increases in diversity and abundance. We aim to 1) determine forest community response to Callery pear and its chemical control over the short- to medium-term, and 2) determine if there are differences in the soil macrofauna community that will reveal how millipedes and earthworms respond changes in the woody plant community caused by controlling Callery pear. We expect that any change in the ground cover, such as plants or woody debris, caused by Callery pear would result in a response from the millipedes and earthworms.

Methods

Study Site

This study was conducted at "Bartram1," a site 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). 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 blackberries/dewberries (*Rubus* spp.). The 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 (foliar glyphosate, soil-applied hexazinone, foliar imazapyr, basal bark triclopyr, basal bark triclopyr + aminopyralid, or no herbicide). Each plot measured about 7× 8 m in size, their corners marked by metal stakes and the centers with PVC pipe. All herbicides were applied 18 Sep 2018 and deemed effective with Callery pear mortalities between 70–100% after 2 years. 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 2 km north (33.01850°N, 83.22166°W) to serve as an untreated and uninvaded reference, bringing the total number of plots to 18. The reference area was visually assessed for similarity to Bartram1 in terms of canopy, mid- and understory structure and was the closest geographically to the main study site. The reference area was dominated by loblolly pine (*Pinus taeda*) with an understory of oak, sweetgum, black cherry, and blackberries. Both sites had sandy soils. 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 five years before our study (J. Morgan Cook, personal communication).

Soil Macrofauna

We sampled soil macrofauna in the spring (22–24 Apr) and the fall (14–17 Oct) of 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, approximately 4 L 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-W incandescent light bulb, through the screen, falling into a cup of 70% ethanol.

We used two methods to sample earthworms. To hand-sort earthworms, we dug a 30 ×30 ×30 cm soil monolith (block of soil) in each plot and sifted through the contents by hand on a plastic tarp based on methodology by Anderson and Ingram (1993). 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. 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).

Woody Plant Community, Ground Cover, Soil, and Statistical Analyses

To assess woody plants and overall plant cover, we counted the number of trees and shrubs of each species greater than 30 cm tall within each 7 × 8 m plot in July 2022. Trees we defined as species that are generally single-stemmed at the rootstock and can attain heights of over 5 m at maturity, such as pines, oaks, black cherry, and Callery pear. Shrubs are generally multi-stemmed and usually less than five meters at maturity, such as blackberry and beautyberry. 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, 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, 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%. Percentages were calculated by measuring the area of ground covered and dividing that area by the total plot size. Any gaps (irregularly-shaped or otherwise) in ground cover were measured and subtracted from the area covered. Total basal area per plot (cm^2) was calculated each for trees from DBH and shrubs for DRC. Callery pear was differentiated by mortality (live or dead) due to differences in how they contribute to soil nutrients (fallen leaf litter vs. decomposing woody material).

To collect soil data, 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℃ 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 s and 8 h 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 USDA Forest Service Southern Research Station Analytical Chemistry Lab (Research Triangle Park, NC) using a Thermo Fisher Scientific FlashEA 1112 NC Analyzer.

All statistical analyses were done using R v4.2.2 (R Core Team 2022) to compare fauna, flora, and soil characteristics. To determine if Callery pear or any other variable was driving patterns, we first calculated Shannon Diversity of native woody plants using the vegan package (Oksanen et al. 2022). We then used the stats package (R Core Team 2022) to run Pearson's product-moment correlations between woody plant diversity, richness, Pielou evenness, and abundance with Callery pear (living and dead pear were calculated both individually and together to test for additive effects) basal area and abundance. P-values were adjusted using the Benjamini–Hochberg procedure. The same was done for soil texture, C:N, and pH. Shapiro–Wilk normality tests ensured data met parametric assumptions. Any data that were not parametric were log- or square-root-transformed to meet assumptions, if able. To compare how the woody plants differed between plots, significant difference ($\alpha = 0.05$) between plots was tested using two-way ANOVA and Kruskal–Wallis rank sum tests. Post-hoc Tukey Honestly Significant Difference tests (stats package) were run on ANOVAs and Dunn Kruskal–Wallis multiple comparison tests [FSA package (Ogle et al. 2022)] on Kruskal–Wallis tests with significant differences to identify which pairs of treatments differed. To determine if there were any differences in the fauna community, they underwent the same analyses but were also correlated with all native plant diversity metrics mentioned above as well.

Results

The Woody Plant Community, Soil, and Callery Pear Control

Treatment had a significant effect on the basal area of Callery pear (live, dead, and combined), abundance of woody plants, and C:N (ANOVA; $P < 0.05$). Mean basal area of live Callery pear (Fig. 1A) was highest in No Herbicide and significantly higher than Glyphosate and Hexazinone. Imazapyr and Triclopyr were the next highest and not significantly different from No Herbicide. None of the four herbicide treatments were statistically different from each other, and Reference had no Callery pear. Glyphosate and Reference were statistically similar. Mean basal area of dead Callery pear was highly variable (Fig. 1B). Reference and No Herbicide had the lowest basal area and were similar to Glyphosate; all other treatments had significantly more dead basal area than No Herbicide but were not statistically different from Glyphosate. Mean basal area of live and dead Callery pear combined

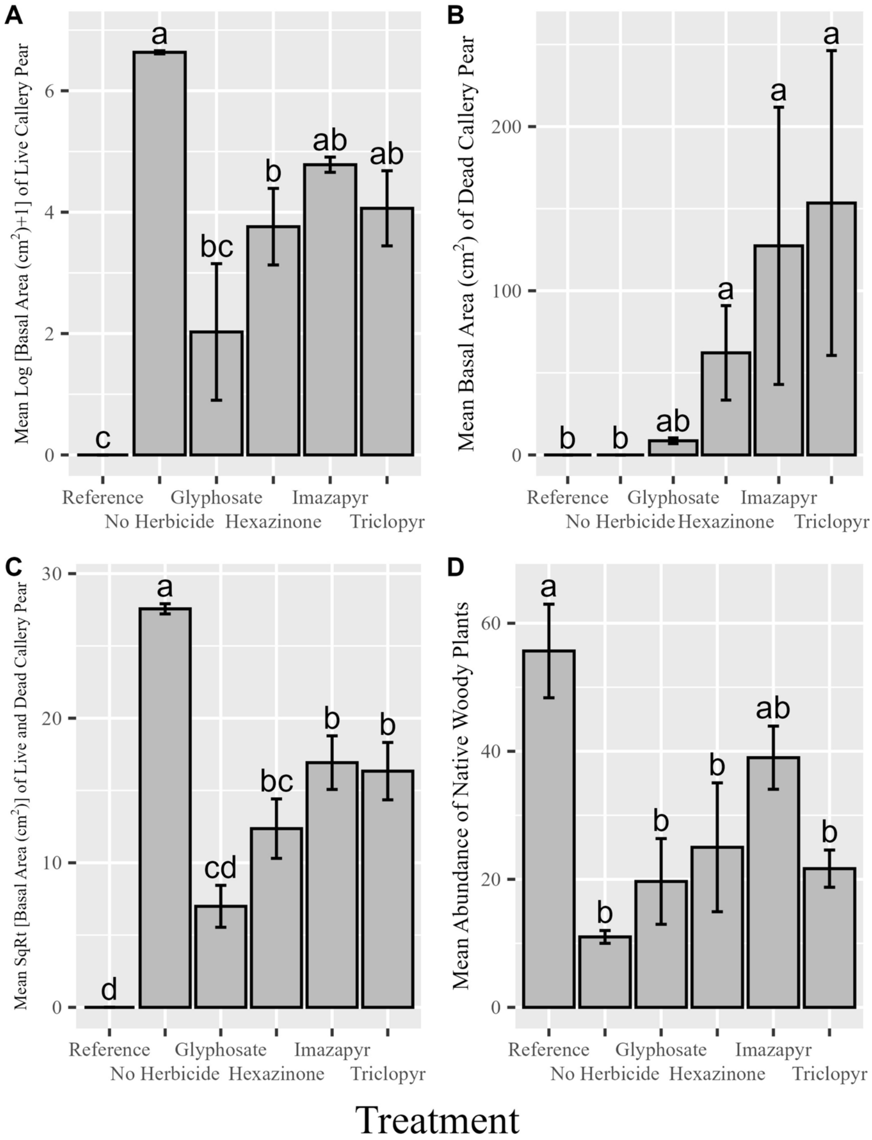

Fig. 1 Plant community metrics by treatment: basal area (cm^2) of live (**A**), dead (**B**), and total (**C**) Callery pear, and abundance of native woody plants (**D**). Reference in A–C has a value of zero. Data were transformed as necessary as stated in axes and methods. Error bars represent standard error. Subjects not sharing letters are statistically different as per ANOVA with post-hoc Tukey test (**A**, **C**, **D**), or Kruskal–Wallis test with post-hoc Dunn test and unadjusted p-values (**B**)

(Fig. 1C) was significantly higher in No Herbicide compared with all other treatments. Glyphosate and Reference were statistically indistinguishable.

The abundance of native woody plants (Fig. 1D) was statistically similar in all herbicide treatments and the no herbicide treatments. Total basal area of native plants did not differ between treatments. The Reference plots had greater native woody plant density than all treatments with the exception of Imazapyr. Mean soil C:N in Reference was higher than all other treatments, which were similar (Fig. 2). 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. There were no significant differences in ground cover in terms of grasses and sedges, broadleaf forbs, or woody debris.

Soil Macrofauna

One-hundred eighty-three 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 (Fig. 3): *Oxidus gracilis* (71.7%), *Pseudojulus paynei* (9.2%), *Polyxenus* sp. (7.6%), *Cleidogona* sp. (4.9%), Xystodesmidae (3.8%), *Abacion tesselatum* (2.2%), and immature Julida (0.5%). Earthworms were less diverse (Fig. 4): 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 mature *Amynthas minimus* (20–50 mm length by 1.5–2.0 mm width). *Oxidus gracilis* was less abundant in the No Herbicide plots but ANOVA/Kruskal–Wallis found no significant differences in millipede or earthworm communities among different treatments. Additionally, no macrofauna community metrics (Shannon diversity, richness, evenness, and abundance) were found to correlate with

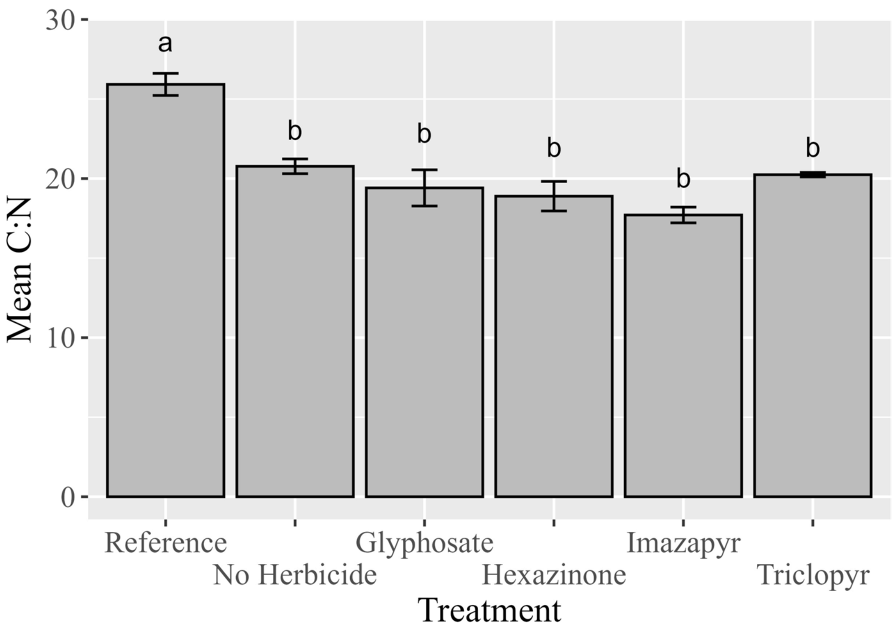

Fig. 2 Soil C:N by treatment. Error bars represent standard error. Subjects not sharing letters are statistically different as per ANOVA with post-hoc Tukey test using log-transformed data

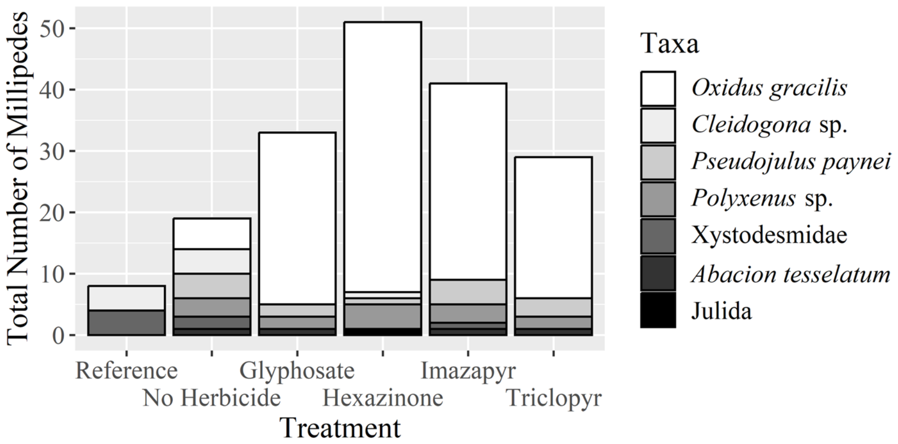

Fig. 3 Total abundance of millipede taxa by treatment. *Oxidus gracilis* was the only nonnative millipede species collected

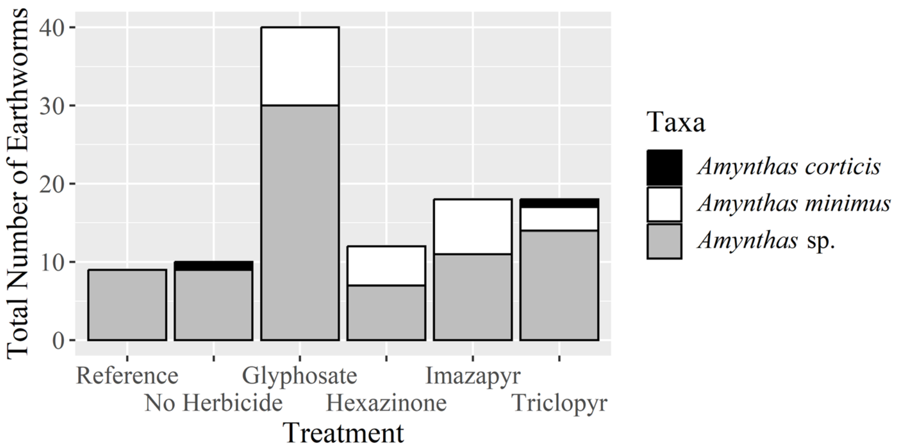

Fig. 4 Total abundance of earthworm taxa by treatment. All earthworms collected were nonnative invasive species

any plant, soil, or ground cover metrics after P-value adjustment (Online Resource 1).

Discussion

The Woody Plant Community, Soil, and Callery Pear Control

We expected to find the most Callery pear in the No Herbicide plots, however some of the treatment plots were statistically similar, suggesting recovery after the herbicide applications done by Vogt et al. (2020). This was most apparent in the Imazapyr and Triclopyr plots where the most Callery pear (aside from No Herbicide) was present. Additionally, only Glyphosate plots were statistically similar to the reference plots which had never been invaded. Dead Callery pear was also highest in the Imazapyr and Triclopyr plots (although not statistically different from the other herbicide treatments). Native plants were also highly abundant in the Imazapyr plots relative to the other treatments. There was very little difference between treatment plots in terms of the remaining native woody plants.

Native plant diversity, richness, and evenness were all statistically similar, and abundance differed between the treatment and reference sites more than between treatments. To our knowledge this is the first study examining the effects of Callery pear on woody plants in a pine-based ecosystem. Nebhut and Dukes (2023) did a similar study in early-succesional meadows around the same time. They found no significant impact on native woody plants, however they admit this is likely due to the low densities of Callery pear present in their meadows. We observed dense thickets of Callery pear where it was growing, so it is more likely that the lack of variance in abundance reflects what it was before herbicide treatments rather than a lack of effect from the pear trees.

Callery pear regeneration was present in most plots. These patterns suggest that the differences we see between plots are not a result of varying treatments but instead a result of some plots having naturally denser vegetation than others. Although we did exclude the smallest seedlings, our data still represent a significant portion of the regenerating vegetation. The fact that there is so little difference between plots is likely a factor of both time and the overall forest structure. The mature slash pines shade out much of the understory, and where light does penetrate, shrubs like beautyberry and blackberry prevail. With so little light reaching the ground, it's likely that 3–4 years isn't enough time to see any significant change. Vogt et al. (2020) did not assess the native plant composition, but had they done so our data would likely be similar. This is not likely a result of soil C:N, which differed only at the Reference site, nor soil texture

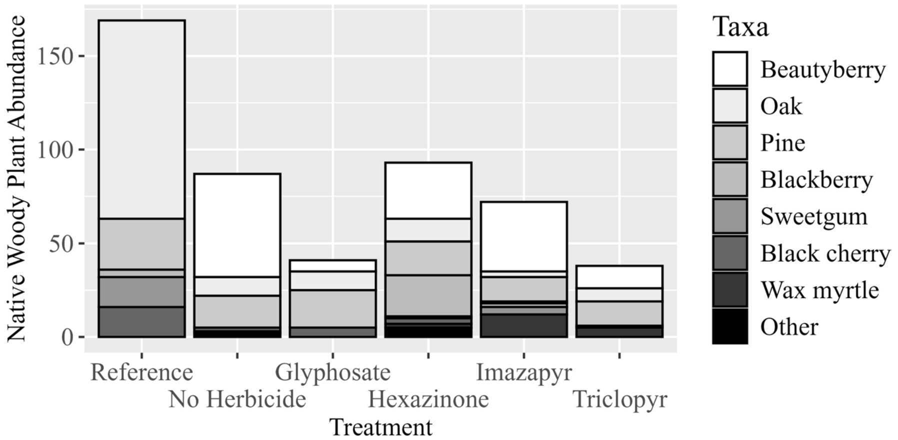

Fig. 5 Total abundance of native woody plants by treatment

or pH, which did not statistically differ at all. Oaks, the main understory tree in both sites (Fig. 5), have a mean leaf C:N of ~60 (Snyder, unpublished data), whereas Callery pear averages 36 (Boyce 2022), ergo Callery pear has a vastly different soil nutrient input. While Boyce (2022) suggested that Callery pear litter inputs may be similar to red maple, these systems have little, if any, maple. The soil C:N we see may be a reflection of Callery pear changing nutrient levels over time. However, more research is needed to determine if these changes are the result of Callery pear on the landscape or if it is simply a site difference, especially since soil C:N is not significantly different in No Herbicide from the herbicide plots.

The reference site, which had never been invaded by Callery pear, had a higher abundance of native plants but had similar soil texture and pH. Of those native plants, there was a greater proportion of pines and oaks than the treatment sites, which had a more even understory (although not statistically so) with larger amounts of sweetgum, black cherry, and shrubs. This greater evenness at the treatment site may have been an edge effect from the US Highway 441 roadside, which was just over 300 m away and infested with Callery pear. Although the initial herbicide applications were successful in killing Callery pear, the native plants have not fully recovered after 3–4 years. Our results do not necessarily imply that recovery cannot happen, however 3–4 years is likely not enough time for the forest to recover on its own. Significant change in this timeframe would require active management to further reduce Callery pear and promote forest regeneration.

Soil Macrofauna

Pairwise comparisons found no significant differences in diversity, richness, abundance, or evenness in millipedes or earthworms between treatments, nor any significant correlations after p-value correction. This suggests no effects from the herbicides or any subsequent changes in the plant community. This is a departure from other studies looking at plant-animal relationships (Madritch and Lindroth 2009; Lobe et al. 2014; Stašiov et al. 2021), which may help demonstrate that soil properties have changed little in our study system.. The most notable finding regarding the soil macrofauna was that *Oxidus gracilis*, a nonnative millipede from Asia, made up the greatest proportion of millipedes. Although not statistically significant, *O. gracilis* seemed to be more abundant in the herbicide plots while being less abundant in the No Herbicide plots, and they were absent in the Reference plots. These observations could be because soil C:N was reduced by Callery pear invasion, but there are additional soil differences between the herbicide treatment and reference sites that need further investigation. Soil C:N was not statistically different between No Herbicide and the herbicide treatments, so this does not explain the variability in *O. gracilis* abundance. Native woody plants are less abundant in the No Herbicide plots, but that alone does not explain the absence of nonnative millipedes in the Reference plots. The treatment site was closer to US-441 so they may be benefiting from an edge effect. In all, our data isn't enough to detect any effects from Callery pear or its control on millipedes or earthworms in a pine-dominated ecosystem. As of now there are also no clear mechanisms to explain the patterns in *O. gracilis* abundance.

Conclusion

We conclude that although initial herbicide treatments were successful, after 3–4 years the native woody plant community has not recovered and Callery pear is growing back where the herbicides were applied. We did not find any evidence that millipedes or earthworms were affected 3–4 years after Callery pear was treated. Therefore, when controlling Callery pear, land managers should prioritize a combination of active management techniques, which may include follow-up herbicide applications, prescribed fire (with caution—see Warrix and Marshall 2018), planting and seeding, and other actions that restore the native vegetation to a pre-pear state.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s44391-025-00030-y.

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Authors' Contributions JMM: Conceptualization, Methodology, Investigation, Data Analysis, Visualization, Writing—Original Draft Preparation, Writing—Review & Editing. JTV: Conceptualization, Methodology, Writing—Review & Editing. BAS: Conceptualization, Methodology, Investigation, Resources, Writing—Review & Editing, Supervision.

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Declarations

Competing interests The authors have no competing interests.

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