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Effect of Alliaria petiolata management on post-eradication seed bank dynamics

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Effect of Alliaria petiolata management on post-eradication seed bank dynamics

A Thesis Presented by

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EFFECT OF *ALLIARIA PETIOLATA* MANAGEMENT ON POST-ERADICATION SEED BANK DYNAMICS

A Professional Paper Presented

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<u>ABSTRACT</u>

EFFECT OF *ALLIARIA PETIOLATA* MANAGEMENT ON POST-ERADICATION SEED BANK DYNAMICS

SEPTEMBER 2023

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Alliaria petiolata (garlic mustard) is an aggressive non-native and invasive forb that negatively impacts native arbuscular mycorrhizal communities and inhibits or prevents the growth of native plant species. Invasive species mitigation and management strategies that use native revegetation vary in success. This study focuses on which species naturally regenerate in areas where *A. petiolata* has been mitigated to help inform restoration efforts.

Seedling emergence of species within the seed bank of four plot types (uninvaded, invaded, chemically treated, and mechanically treated) were observed two years post restoration efforts to determine which native species are likely to persist to seedlings following management. Species abundance and percent cover of all plant species were recorded to evaluate the presence of species in addition to plant health and physiological differences.

Native species abundance was significantly reduced within herbicide treated plots as compared to untreated and invaded plots (p=0.02). Plots treated with mechanical removal had the greatest percent cover of native plants as compared to all other plot types but were dominated by colonizing species which is typical of a disturbed habitat. Mechanical removal also resulted in a greater abundance and stability among functional groups of native species, than those treated with herbicide. Forb species dominated coverage of plots over other functional groups when treated with herbicide. *Alliaria petiolata* invasion and management methods significantly impacted forb and graminoid species, as they had significantly lower abundance in plots treated with herbicide.

The results demonstrate that the method of removal as well as the presence of *A. petiolata* affects emergence of plant species from the seedbank. The additional disturbance of mechanical removal may alter successional trajectories following invasion. Herbicide treatment resulted in the most similar species abundance as the uninvaded reference plots, which had the lowest seedling emergence and percent cover.

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CHAPTER 1
INTRODUCTION

Invasive plants are known to disrupt native ecosystems and have negative impacts on native species populations (Rejmánek 2000, Callaway and Ridenour 2004). An example of this is *Alliaria petiolata* (garlic mustard), an invasive forb native to Eurasia, first discovered in the U.S. in 1868 (Nuzzo 1998). The invasiveness of this species derives from several factors, one of which is its allelopathic characteristics. Sinigrin is a secondary metabolite produced by *A. petiolata*; it is released into the soil, where it disrupts the native microbe ecosystem (Stinson et al. 2006, Stinson et al. 2007, Wolfe et al. 2008, Lind and Parker 2010, Hale et al. 2011). *Alliaria petiolata* maintains invasion because sinigrin disrupts the formation of root symbiosis with arbuscular mycorrhizal fungi (AMF) associations (Barto 2008) and levels of this compound are found to decrease once individuals reach maturity (Evans et al. 2016). The invasiveness of this species in particular is a significant threat to deciduous forests (Nuzzo 1999). Previous research on *A. petiolata* invasion mainly consists of its' impacts on native species and response to mitigation techniques. However, there is little known about how the invasion of this species and its mitigation might affect future generations of native species.

Roughly 80% of all vascular plants have AMF associations, as they aid in nutrient absorption in the soil (Perotto et al. 2013). Disruption of this relationship is detrimental to the health of obligate (or highly dependent) species, to which separations from AMF will likely result in death or the inability to germinate (Stinson et al. 2006). Species less dependent on AMF associations are referred to as facultative species, and the separation or absence of this relationship can result in reduced growth, reduced root biomass, and low germination rates (Shannon et al. 2014). The symbiotic AMF associations within plant populations become more abundant when insufficient nutrients or imbalanced nutrient cycling exists within the soil (Cavagnaro 2008), therefore, plants may be dependent upon AMF associations regardless of whether the species dependence is obligate or facultative in the presence of irregular soil chemistry and low nutrient availability. This physical reaction caused by the altered fungal population may facilitate invasion of non-native species (Reinhart and Callaway 2006).

Mitigation of invaded areas has been attempted using a variety of management techniques, with variations in success (Kettenring and Adams 2011) even multiple years after implementation (Slaughter et al. 2007, Stylinski and Allen 1999, Harms and Hiebert 2006). Contributing factors include a lack of understanding of the appropriate species as well as variation in the duration between mitigation efforts and associated replanting events.

Once an ecosystem is subject to invasion by this plant species, complete eradication and ecosystem restoration to its original state is difficult. Restoration activity is a disturbance itself and can increase the likelihood of further invasion (D'Antonio and Meyerson 2002, DeMeester and Richter 2009). The feasibility of complete eradication may also be influenced by constraints determined in cost-benefit analyses (Anderson et al. 1996). Restoration of ecosystems that have been invaded by *A. petiolata* can be especially difficult due to the degraded and altered fungal community (Anthony et al. 2017), which does not naturally recover after removal of this species (Anthony et al. 2019). The reestablishment of fungi populations within the soil is essential to the success of restoration efforts due to the allelopathic characteristics of this species. Simply removing *A. petiolata* does not significantly improve native species (Stinson et al. 2007); therefore additional effort is necessary for restoration success.

Pesticide use has varying results in removing or diminishing populations of *A. petiolata* (Carlson and Gorchov 2004), but if used, chemicals are deposited and remain within the soil, which may inhibit native species germination and growth.

Physical (mechanical) removal of invasive plants results in soil disruption and likely contributes to difficulty in native seed germination (Thompson et al. 2001, Minchinton and Bertness 2003) and may cause dormant buried seeds to resurface and germinate. *A. petiolata* have high seed production and dispersal allowing for high seedling germination, survivorship and growth (Anderson et al. 1996). Seeds that to do not undergo germination within the first growing season are deposited into the seed bank and can remain dormant for up to four years (Baskin and Baskin 1992). These contributions to the seed bank that exist prior to removal create an empty niche to which seeds within the soil are more likely to germinate in the presence of newly available resources.

Alvarez and Cushman (2002) found 86% and 85% increases in native and non-native seedlings, respectively, following mitigation of invasive *Delairea odorata*; Asteraceae (Cape ivy), demonstrating mechanical removal of individuals may facilitate further invasion of non-targeted species. To combat multiple invasions by different non-native species, native revegetation can be implemented into the restoration efforts, where native species are planted post-removal of the invasive species to fill the newly vacant niche (Tu et al. 2001). Viable native propagules may also be limited, thus hindering the reestablishment of native species (Masters and Sheley 2001, Hartman and McCarthy 2004), especially when relying on natural revegetation alone. This control method is widely used and recommended; however, variation in success is still present. Replanting of native species is necessary, as the ecosystem may still be experiencing disturbance, and thus limiting the dispersal of native propagules (Galatowitsch and van der Valk 1996). This can be attributed to several factors such as the lack of research and public data concerning the native species best suited for replanting efforts, and of those indicated species, at which developmental stage to initiate replanting (i.e., seedling, sapling, etc.). Planted species need to be carefully chosen for attributes reflected in the resources currently available to result in establishment. This method has demonstrated success, but may also facilitate natural inhabitance of non-planted native species (Bay and Sher 2008). Invasive species that damage the fungal communities such as *A. petiolata,* among others *(Lonicera maackii and Ligustrum vulgar)* (Shannon et al. 2014) create complex issues with restoration that need to be accounted for in management plans. Overall, there is a demand for additional research to better inform control methods of invasive plant species (D'Antonio 2002).

This study intends to determine the relative effectiveness of different management techniques for areas successfully invaded by *A. petiolata* by observing the natural germination and growth of propagules within the seed banks of four plot types; (1) currently invaded by *A. petiolata,* (2) invaded by *A. petiolate* and removed chemically, (3) invaded by *A. petiolata* and removed mechanically, and (4) uninvaded by *A. petiolata.* Removal treatment in the plots began in 2014 and was conducted annually until two growing seasons prior (2017) to the study beginning in the spring of 2019. This study analyzed the residual effects of both manual and chemical eradication to determine which species persist the seedling stage following the disturbances ensued during mitigation. To further understand the patterns of species composition among treatment types, analysis was broken into measurements of abundance and percent cover.

CHAPTER 2
METHODS

2.1. STUDY SITES AND PLOT TYPES

This study focused on one of the four study sites from a previous study conducted by the Stinson Lab in the Environmental Conservation Department at the University of Massachusetts Amherst (Coates-Connor 2018). This experiment targeted *A. petiolata* populations within a land reservation in Tyringham, southwestern Massachusetts. Manual pulling and chemical application of 3% glyphosate were the two eradication methods implemented. Manual removal occurred during the spring seasons from 2014 to 2017 while herbicide was applied in the summers of 2014 to 2017. In total there were 12 plots with three replicates of conditions (or treatment) levels: uninvaded, invaded with *A. petiolata*, mechanically removed, and chemically removed. The location of each plot was chosen using observations of established vegetation. Invaded and treated plots are defined as having more than 20% occurrence of *A. petiolata*.

The forest canopy consisted of predominantly *Acer saccharum* (sugar maple) with *Prunus serotina* (black cherry), *Fraxinus americana* (white ash), and *Betula alleghaniensis* (yellow birch).

2.2. SOIL COLLECTION

To test the effects of the eradication treatments on emergence and performance of native species, soil was collected from each of the four plot types. In April of 2019, samples were randomly extracted to a depth of 4 inches from each of the 12 plots, placed into trays, and transported to an 18.5m^2 shade house for observation at Harvard Forest in Petersham, Massachusetts. In total there were 96 soil sub-samples (N=48).

To minimize disturbance of the collected samples, the soil was carefully removed from the plot using a shovel and directly placed into nursery trays. Each sample was placed on an even surface within the structure. All trays were rotated in a sequential manner each week to account for any light exposure variation within the structure.

2.3. DATA COLLECTION

Species identification was confirmed with (Haines et al. 2011). The native status of each species was determined using the USDA PLANTS database (USDA, NRCS 2019). Each emerging individual was identified and counted in three separate sessions from June to August 2019.

Pictures of each tray were recorded and analyzed with IMageJ to calculate percent cover of each species. The entire foliage of each plant was outlined, including overlapping leaves and vegetation growing outside of the tray. Individuals that were dead or less than 1cm^2 were not recorded for percent cover. Physiological differences and growth characteristics were accounted for through the evaluation of both abundance and cover of each species. This comparative analysis of abundance and percent cover attempted to properly account for species of a larger foliage area with few individuals, as well as those who expand their population with a surplus of germinants.

2.4. Data Analysis

Abundance counts from each census were analyzed with the use of multiple ANOVA tests, and the Tukey HSD (Honest Significant Differences) for post-hoc analysis. Average species abundance was analyzed for differences between plot replicate and treatment types.

Percent cover data was analyzed using an ANOVA of log-transformed data. Normality of this data was determined through the Shapiro-Wilk test. Data was also analyzed with the nonparametric Kruskal Wallis test. Post-hoc analysis for this was conducted through a Dunn test. The Tukey HSD (Honest Significant Differences) test was used for post-hoc analysis of the ANOVA.

All data was analyzed using R version 4.2.2 (R Core Team 2022) and the following R packages: dunn.test v. 1.3.5 (Dinno 2017), knitr v. 1.42 (Xie 2014, 2015, 2023), reshape2 v. 1.4.4 (Wickham 2007), rmarkdown v. 2.20 (Xie, Allaire, and Grolemund 2018; Xie, Dervieux, and Riederer 2020; Allaire et al. 2023), ggplot2 v. 3.4.2 (Wickham 2016), magrittr v. 2.0.3 (Bache, Stefan Milton and Wickham, Hadley 2022), dplyr v. 1.1.2 and dbplyr v. 2.2.3 (Wickham et al. 2023), and tidyverse v. 2.0.0 (Wickham et al. 2019).

CHAPTER 3
RESULTS

3.1. ABUNDANCE

Uninvaded plots and plots treated with herbicide had significantly lower abundance of species than untreated invaded plots (p<0.02) (Fig. 1).

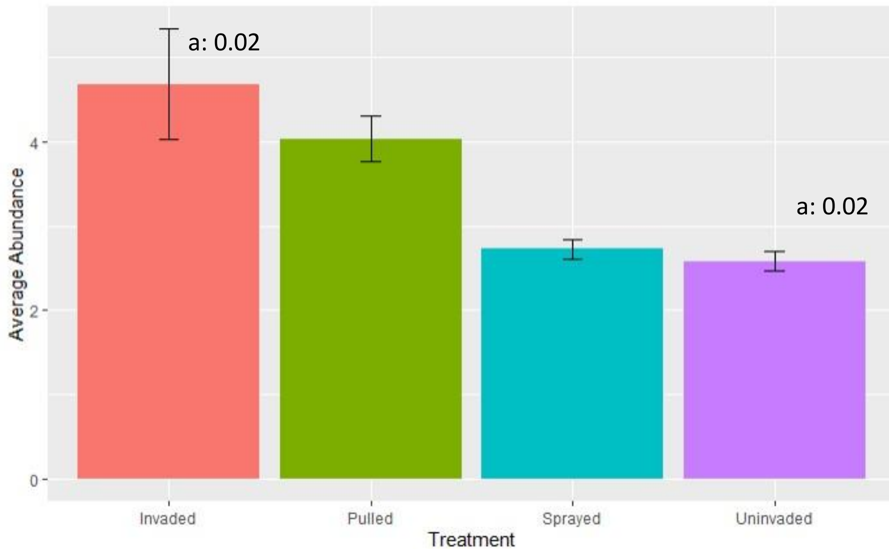

Figure 1. Average species abundance among the four plot types (treatment). Error bars denote standard error.

Native species abundance was significantly higher than non-native species abundance across plot types and significantly higher in invaded plots versus herbicide plots (p = 0.02). Native species abundance was slightly greater in plots treated with manual removal as compared to the uninvaded plots. (Fig. 2).

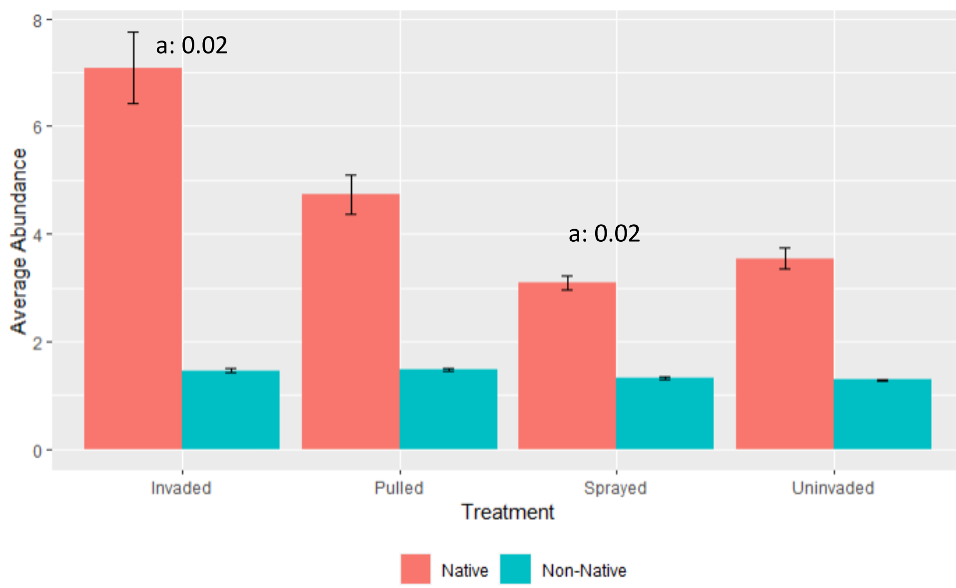

Figure 2. Origin of species abundance across all four treatment types. Error bars denote standard error.

Of the plot types that were disturbed (invaded, pulled, sprayed) there were differences between sprayed and invaded plots, where plots treated with herbicide had a lower average abundance.

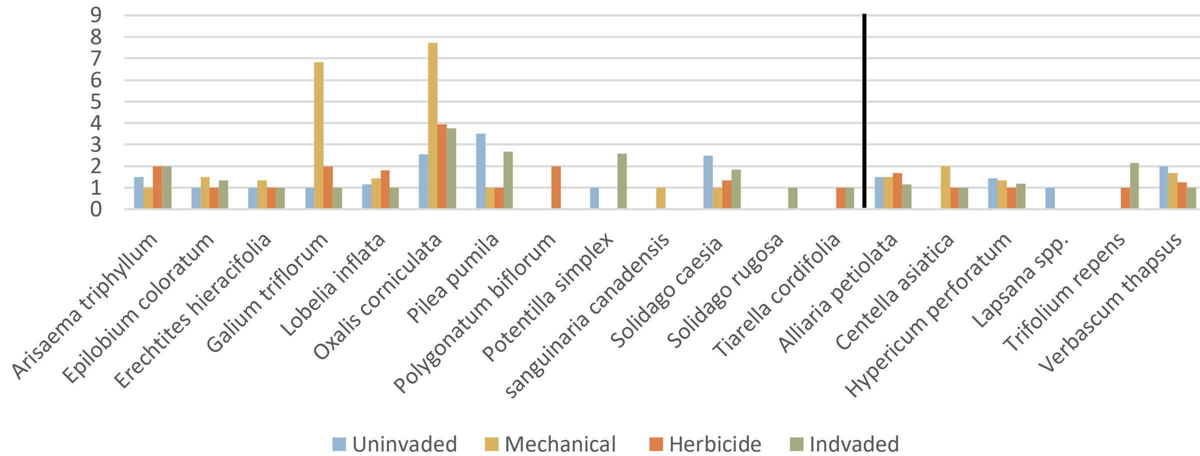

Figure 3. Average abundance of forb species. Native species are on the left side of the vertical bar, and non-native species to the right.

Native forbs had the highest populations in mechanically treated plots (75.3%), which was mostly composed of *Galium triflorum* (22.5%) and *Oxalis corniculata* (25.5%). This abundance was significantly higher than that of invaded, uninvaded and herbicide treated plots. Non-native forbs, including *A. petiolata*, did not vary significantly among treatment type. *Trifolium repens* was only present in invaded and sprayed plots. *Pilea pumila* abundance was highest in uninvaded (3.5) and invaded plots (2.7), and lowest in treated plots (1). *Potentilla simplex* was only present in invaded and uninvaded plots.

Native woody species were most abundant in pulled plots, and lowest in uninvaded plots. *Prunus serotina* abundance was much higher in pulled plots and absent in uninvaded plots. *Prunus pensylvanica* also had a notable abundance as it was only present in mechanical removal plots. *Acer saccharum* was present in all treatments except for pulled.

Celastrus orbiculatus was highest in sprayed plots and absent in uninvaded plots. Abundance was the same in invaded and pulled.

Of the *Rubus* genus, most species did not vary in abundance among plot types except for *Rubus allegheniensis* and *Rubus setosus*, which were significantly more abundant in untreated invaded plots ($p<0.01$).

3.2. Percent Cover

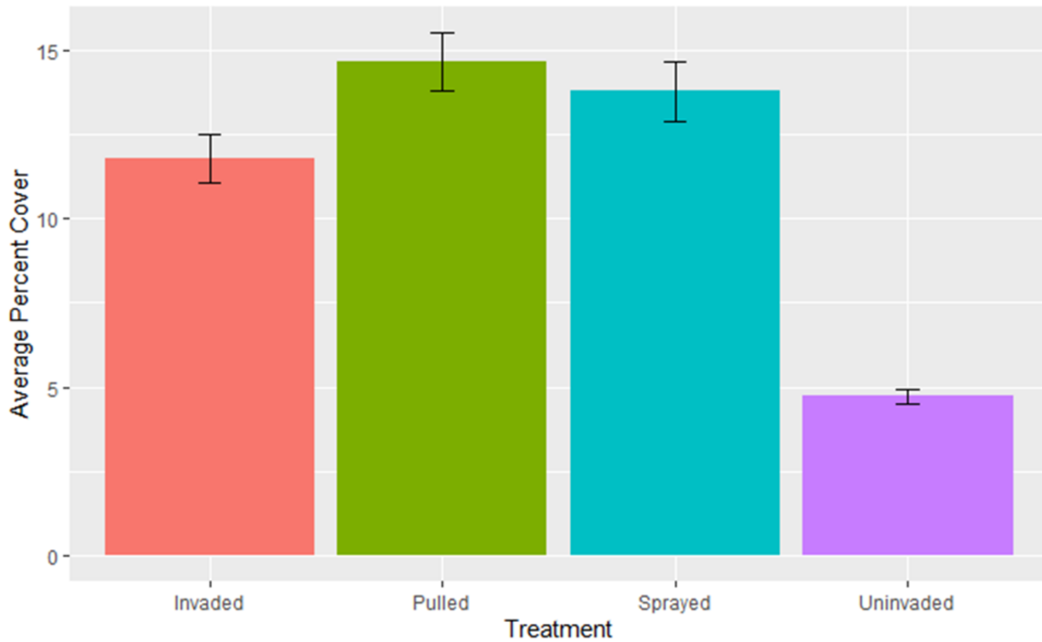

Figure 4. Average percent cover among the four treatment types. Uninvaded plots were significantly different from other treatments $p<0.001$.

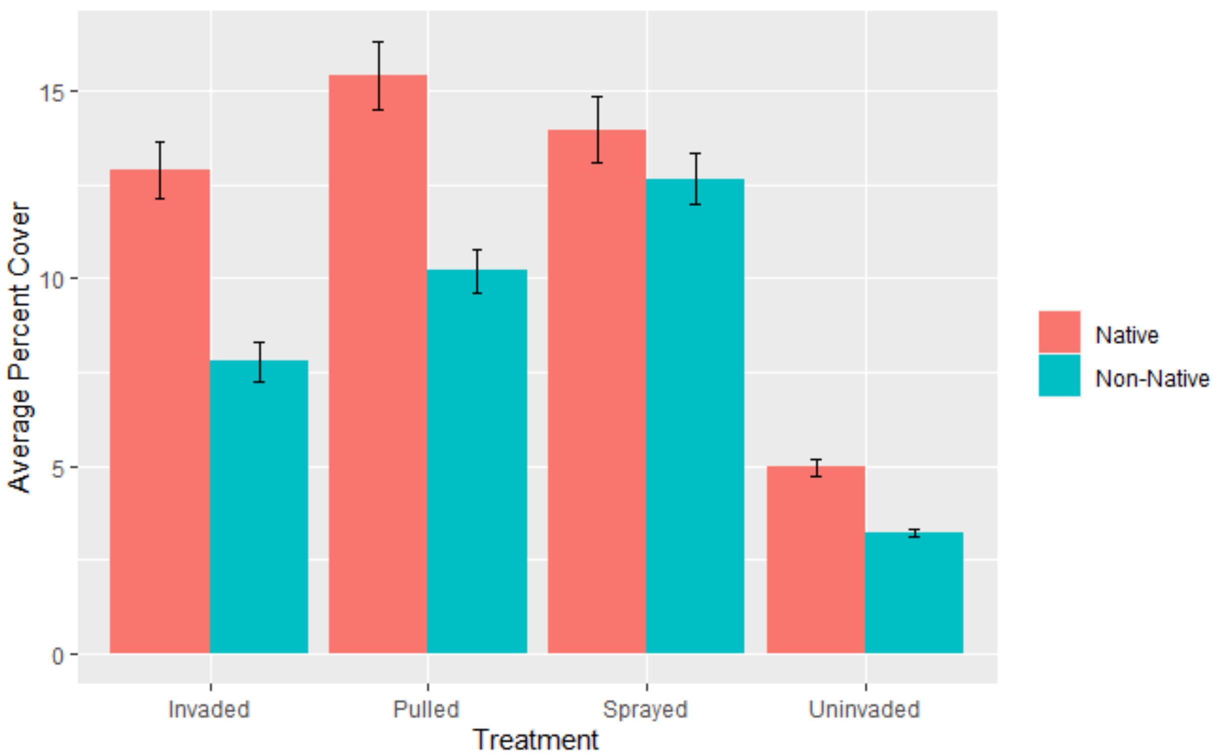

Figure 5. Average percent cover of native and not native species across all four treatment types.

The percent cover of all species within uninvaded plots was significantly lower than all other treatment types ($p<0.001$) (Fig. 4). For the remaining treatment types, there was no significant difference in cover. Native species percent cover was significantly lower in uninvaded plots compared to uninvaded and pulled plots ($p=0.01$), sprayed plots ($p=0.01$) and invaded plots ($p=0.001$) (Fig.5).

Forb species, especially native species, contained the highest percent coverage compared to other functional groups, and were significantly lower in uninvaded plots as compared to pulled plots ($p<0.05$) and spray plots ($p<0.001$) (Fig. 6). The non-native forb, *Verbascum thapsus* had the highest cover in sprayed plots and pulled plots but was scarce in both invaded and uninvaded plots. Other native forbs following this pattern were *Erechtites hieracifolia, Galium triflorum* and *Polygonatum biflorum. Galium triflorum* had the greatest coverage in pulled plots (37.2%) and less than 6% or less in the remaining plots. *P. biflorum.* had an expansive coverage in sprayed plots and was either absent or of trace amounts in the other three treatment types.

Cypersus strigosus was the graminoid species having the greatest coverage among all treatment types, with the sprayed plots reflecting the most similar to uninvaded plots. Pulled plot coverage had the most similar coverage to invaded plots. *Juncus dudleyi* was significantly greater in invaded plots (~12%) than the other treatment types, all with less than 5% coverage. ($p<0.05$).

The non-native woody species recorded were *Celastrus orbiculatus* and *Rubus spp. C. orbiculatus* had an average coverage of 7% in invaded plots and was scarce in the remaining plot types. *Rubus spp.* was absent in sprayed plots and of similar coverage among other plot types. *Prunus serotina* had significantly higher coverages in invaded and sprayed plots, with pulled plots and uninvaded plots much lower, and similar in coverage area.

The patterns of *C. orbiculatus* in the average abundance and percent cover results suggest that this non-native vine is likely to have fewer-healthier individuals before being exposed to remediation efforts but may have a higher number of individuals after the use of herbicide as opposed to a physical disruption of the soil. Prioritizing the preservation of native-primary growth species in conjunction with invasive species management will increase the likelihood of more resilient native population. (Galatowitsch 2012).

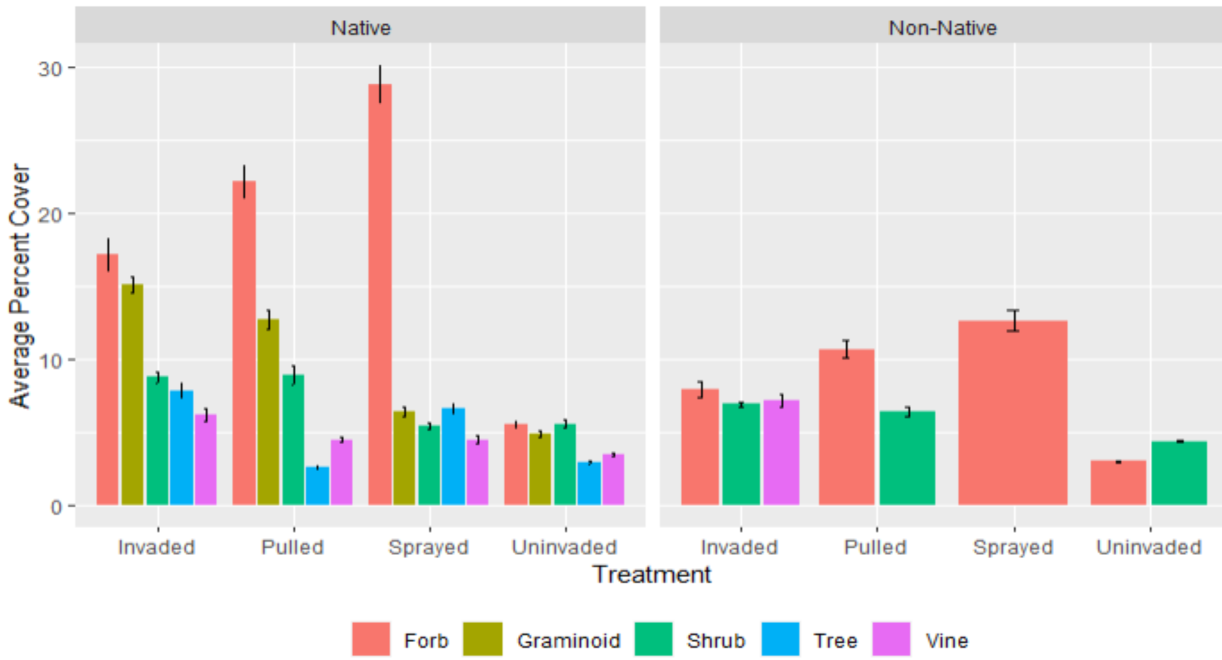

Figure 6. Percent cover of native and non-native species organized by functional group.

CHAPTER 4
DISCUSSION

4.1. ABUNDANCE

Herbicide treatment had a significant impact on overall species abundance as compared to untreated invaded plots but was also the most synonymous with untreated uninvaded plots (Fig. 1). This impact was mostly observed in the reduction of native species abundance, which was higher in uninvaded plots (Fig. 2). This suggests that herbicide usage is more effective in a densely invaded area with few native species, as the abundance of plants surrounding the treated area may be impacted.

All graminoid species identified were native, with abundance highest in invaded plots and lowest in herbicide plots with a 50% reduction or greater (Fig. 3). *Cypersus strigosus* and *Juncus dudleyi* abundances were most comparable to the uninvaded plots when treated with mechanical removal. This pattern of colonizing species demonstrates typical effects of disturbance on species composition in addition to supporting the implementation of mechanical removal when attempting to preserve native graminoid populations.

Physical disruption in the soil may cause an increase in abundance of native forbs as there were more individuals present in plots treated with mechanical removal than any other plot type, including the uninvaded plots. This suggests that the disruption of the soil may increase disturbance with a restoration area, thus facilitating a surge in the germination of colonizing species. Non-native forb abundance was not significantly impacted by treatment and was also not greatly affected from invasion as the abundance is consistent throughout all plot types (Fig. 3). However, there was variation among treatment type on a species level.

Alliaria petiolata removal can create a vacant niche, which may facilitate the growth or expansion of another non-native species adjacent or within the restoration area, posing a risk of co-invasion. An example of this may be present within the heightened abundance of non-native forb species *Verbascum thapus* in uninvaded plots. This species also had the lowest abundance in plots containing *A. petiolata.* Non-native *Trifolium repens* was absent in uninvaded and mechanical removal plots, but highest in invaded plots. These patterns suggest there may be a risk of a co-invasion, and management techniques should be properly structured to account for a potential shift in species composition.

Some native forbs such as *Potentilla simplex* and *Pilea pumila* were either absent or of a reduced abundance in treated plots as compared to the invaded and uninvaded plots (Fig. 3). Depending on the species of forb, the impact of treatment on abundance varies regardless of whether or not it is native or non-native without significant patterns. This suggests that treatment methodology should be tailored for specific species when attempting to preserve native forbs, and to account for a potential co-invasions of non-target species.

The fact that woody species were somewhat more abundant in mechanical removal plots suggests that soil disturbance and the removal of *A. petiolata* work synergistically to determine species dynamics at the seedling stage. For example, woody species composed of trees, shrubs and vines had a slightly higher abundance in mechanical removal plots, which was mostly attributed to *Prunus serotina.* This species is similar to *A. petiolata* in that it shares allelopathic characteristics and has been found to inhibit native species especially outside of its natural range (Halarewicz et al. 2021, Bączek 2022) and has a high reproductive potential especially in canopy gaps (Auclair and Cottam 1971) of which are a characteristic of disturbed forests. This behavior is further supported through the absence in uninvaded plots. This heightened abundance within mechanical plots could have impacted the presence and abundance of other plants due to the altered soil conditions caused by this allelopathic characteristic.

Certain *Rubus* species tend to have a greater abundance in understories that have experienced a recent disturbance, which typically persist for the first 2-3 years following an event. These species are typically replaced by *Prunus pensylvanica* given the presence of viable seeds (Marks, 1974). This pattern of succession could be present on this site as *Rubus allegheniensis* and *Rubus setosus* were most abundant on untreated invaded plots, and significantly less abundant on mechanical removal plots which was the only plot type where *Prunus pensylvanica* occurred.

The abundance results highlight the resiliency of non-native and invasive species, as the native species abundance experienced the most variation between both mechanical and chemical application while non-native species did not have a significant variation.

4.2. Percent Cover

Plots experiencing the least disturbance expressed such through the percent cover results of uninvaded plots, as they had least amount of vegetative cover across both native and non-native species (Fig. 4). This lesser disturbance within the uninvaded plots is also demonstrated by the comparable coverages among functional groups.

Pulled plots had the greatest amount of variation between functional groups (Fig. 6), suggesting manual removal will result in a more evenly distributed species composition than the use of herbicide. Herbicide application may be the most effective in the prioritization of native forb coverage over other functional groups, as sprayed plots were dominated by native forb species (~29%), with other functional groups covering only ~6% or less (Fig. 6). This may also suggest that the natural progression of succession is hindered or phasic replacement cycle is altered as this vast coverage of forbs is outcompeting the growth of woody vines, shrubs and trees as compared to the plots treated with manual removal. However, while the speed at which succession occurs may be essential to project management, herbicide application could still be effective to control areas with several invasive species as the only non-native species recorded for coverage area were forbs – all shrubs, graminoids, vines and trees were native. Other plot types did not have this same pattern.

Non-native *Verbascum thapsus* quickly outcompetes native species through its expansive coverage in early growth stages. This species was low in abundance but had notable coverage in sprayed and pulled plots, which suggests that this plant may allocate resources for well-established individuals rather than a high dispersal rate and population count.

There was a high coverage of *P. serotina* in invaded and sprayed plots, and low abundance, meaning this is attributed to few individuals. In pulled plots, the abundance was much greater than other treatments, but of a very low coverage. This could have resulted from the physical disruption of the soil which would resurface viable seeds previously deposited. The similarities between the invaded and sprayed plots suggest a lack of sensitivity to herbicide treatment.

4.3. Conclusion and Management Recommendations

Results of this study support the reasoning that further mitigation efforts may be necessary to ensure the replacement and establishment of a healthy native species community. Methodology should be chosen on a site-by-site basis, clearly outlining the goals for the native species present and how to support them through the restoration process. Project managers should implement different methodologies depending on their goals for the ecosystem trajectory, as there are varying degrees of impact for both herbicide application and manual removal among functional groups and individual species.

Exclusive to herbicide treated plots, there was a domination in percent cover of forb species over other functional groups, which was not reflected in abundance. This pattern suggests herbicide application may result an allocation of resources to remaining forb individuals. If a goal or methodology of a restoration effort involves the domination of native forbs, herbicide will be the most effective treatment. Non-native forbs did not have a significant difference in abundance or percent cover between herbicide application and mechanical removal. Invasion of *A. petiolata* resulted in an increase in graminoid populations, which was most heavily reduced when exposed to herbicide treatment of the target species. These populations were most synonymous with the abundance within uninvaded and untreated plots when mechanical removal was applied.

Herbicide treatment is the most effective method if the restoration goal is to return the impacted area to a mature forest in terms of species abundance, which most accurately resembles the species abundance of the untreated and uninvaded plots.

Mechanical removal of A. petiolata is recommended if the goal is to support native species throughout succession, especially if an altered ecosystem trajectory is expected, preventing the ecosystem from returning to its pre-existing conditions.

The patterns between non-targeted invasive species (such as *V. thapus* and *T. repens)* and *A. petiolata* demonstrate the need for species specific and adaptive management to account for potential co-invasions of non-target species.

The absence of non-native trees, shrubs, and vines could be due to the lack of propagule pressure or more of a reflection of the stage at which this ecosystem is recovering, supporting the importance of long-term monitoring. This extended observation period will enable the ability to implement adaptive management strategies as the ecosystem undergoes the altered phasic replacement cycle imposed by the novel characteristics of *A. petiolata* invasion.

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