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## Garlic Mustard (*Alliaria petiolata*) Management Effectiveness and Plant Community Response

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Garlic mustard (*Alliaria petiolata*) management effectiveness and plant community response

A Thesis Presented

by

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## ABSTRACT

### GARLIC MUSTARD (*ALLIARIA PETIOLATA*) MANAGEMENT EFFECTIVENESS AND PLANT COMMUNITY RESPONSE

MAY 2019

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The control and eradication of the invasive biennial herb garlic mustard (*Alliaria petiolata*) and the restoration of invaded forest habitats present important linked challenges to land managers in North America. Removing garlic mustard by hand and by glyphosate herbicide application have both been used as eradication strategies with mixed results. Each method has advantages and disadvantages, but they are rarely compared for effectiveness and community impact across multiple years of management. Some previous studies have shown improvements in species diversity and plant community composition following management, while others have found no differences. To better understand both garlic mustard population and native plant community responses to these two methods across a broad geographic range, we tested these two management methods for four years in seven northern hardwood forests in Massachusetts and New York State. We found that pulling juvenile and adult garlic mustard plants for four years significantly reduced adult abundance, while spraying had no effect compared to invaded control plots. In the plant community, we found no negative impacts of garlic mustard on species diversity nor increased diversity in managed plots following three consecutive years of management. Our results suggest that increased diversity should not be the primary goal

of garlic mustard management at these sites and plant community monitoring at the site-specific scale should be explored. This study highlights how complicated decisions can be for managers when deciding which invasions to prioritize and how to measure plant community recovery.

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## CHAPTER 1

### ASSESSING THE EFFECTIVENESS OF TWO GARLIC MUSTARD (*ALLIARIA PETIOLATA*) MANAGEMENT METHODS

#### 1.1 Introduction

Invasive plant populations have well-known negative impacts on ecosystems, and their eradication or control is a pressing issue for land owners (Vitousek 1990; Vilà et al. 2011), but management can be expensive (Pimentel et al. 2005; Panetta 2009; Pyšek and Richardson 2010), and efforts are not always successful (Rejmánek and Pitcairn 2002; Kettenring and Adams 2011; Prior et al 2017). While eradication may, in theory, be more cost-effective than other forms of control (Panetta 2009), eradication is not always possible (Rejmánek and Pitcairn 2002). Weighing the costs and benefits of management options is complicated (Panetta 2009) and often missing from control experiments (Kettenring and Adams 2011). One key piece to consider when weighing management options is to understand how an invasive plant's population responds to different management types across a range of environmental conditions or locations.

Garlic mustard (*Alliaria petiolata*) is one invasive plant that has been difficult to manage. Numerous studies using different management methods have found mixed results in their success (Nuzzo 1991; Carlson and Gorchov 2004; Slaughter 2007; Shartell et al. 2012; Dornbush et al. 2013; Corbin et al. 2018). Garlic mustard has been successful at proliferating in undisturbed forest communities where it has been found to reduce the diversity of the native understory community (Cavers 1979; McCarthy 1997, Nuzzo 1999, Waller et al. 2006; Stinson et al. 2007). Seeds can remain viable in the seedbank for many years (Nuzzo 1991, Pardini et al. 2008; Rogers et al. 2008; Redwood et al.

2018), making eradication a multi-year effort through removal of newly germinated plants until the seed bank is exhausted (Pardini et al. 2008; Pardini et al. 2009, Shyu et al. 2013; Corbin et al. 2017; Redwood et al. 2018). A population model created by Pardini et al. (2009) suggested that induced mortality must be over 95% for rosettes and over 85% for adults and must be repeated every year for many years in order to effectively reduce garlic mustard populations. Corbin et al. (2017) estimated that full eradication of their garlic mustard population would take 10 years if 100% effective and over 50 years if only 90% effective. Understanding the most efficient and effective use of resources for controlling invasions and restoring the native plant community is arguably the most important challenge in garlic mustard management.

Preventing additions to the seed bank is critical for eradicating or reducing the abundance of a garlic mustard population. Small plants may produce only about 20-40 seeds, while large plants living in favorable conditions can produce about 2,400 – 3,000 seeds (Cavers et al. 1979). Garlic mustard populations have been estimated to produce a variable range of seeds, from around 9500 seeds per m<sup>2</sup> in less dense stands (Nuzzo 1993b) to more than 100,000 seeds per m<sup>2</sup> in dense stands under favorable conditions (Cavers et al. 1979). The majority of seeds typically germinate the first spring following production (Baskin and Baskin 1992), but a small number can remain viable in the soil for up to 10 years (Rodgers et al. 2008). Even if only a few viable seeds are left behind in the soil after eradication, a re-infestation can occur (Baskin and Baskin 1992).

Pulling and clipping adult garlic mustard plants in the spring has been found in some studies to significantly reduce adult garlic mustard populations (Nuzzo 1991; Pardini et al. 2008), but it is also labor and time intensive. Garlic mustard plants must be

bagged, removed from the site, and disposed of in a landfill after removal in order to avoid re-rooting or seed dispersal (Chapman et al. 2012). When plants are located in forests far from trails or roads, removal adds extra challenges. In one study, adult garlic mustard density actually increased over 5 years of annually pulling adult plants (Corbin et al. 2017).

Spring herbicide spraying has been found in some studies to be more effective than hand clipping or pulling at reducing garlic mustard populations (Nuzzo 1991, Shyu et al. 2013), while fall herbicide treatments have been successful in other studies at reducing only adult garlic mustard cover (Carlson and Gorchov 2004; Slaughter et al. 2007). Spraying in the spring targets both juvenile and adult plants but risks unintended damage to non-target early spring flora such as spring ephemerals (Nuzzo 1991; Slaughter et al. 2007; Pardini et al. 2008). On the other hand, spraying in the fall impacts fewer native plants, but it only targets juvenile rosettes, allowing any that are missed to become seed-producing adults in the following spring (Nuzzo 1991; Slaughter et al. 2007; Pardini et al. 2008).

Additional negative impacts from eradication methods must be weighed in order to inform effective management strategies. Hand pulling plants can cause disturbance to both above- and below-ground communities (Druille et al. 2013, Guido et al. 2015). A number of studies have found little or no negative effect of glyphosate on soil microbial communities (Roslycky 1982, Wardle and Parkinson 1990, Busse et al 2001), but Druille et al. (2013) found reduced arbuscular mycorrhizal fungi colonization in mycorrhizal-associated plants grown in glyphosate-treated soil.

Most studies that have sought to understand effective garlic mustard population control have only assessed one method of management at a time (Carlson and Gorchoff 2004; Hochstedler et al. 2007; Slaughter et al. 2007; Corbin et al. 2017), while other studies comparing different management methods have occurred over only one or two years (Nuzzo 1991; Shartell et al. 2012). Such studies have all occurred within one forest or across neighboring forest stands. There is a compelling need to better understand garlic mustard population responses to different management methods across larger geographic areas and across multiple years of management. Here, we compare two methods of garlic mustard management in seven northern hardwood forests in the Northeastern United States. We explore whether hand removal or glyphosate application can effectively reduce garlic mustard density after four years of annual treatments.

## **1.2 Methods**

### **1.2.1 Study Species**

Garlic mustard is native to Eurasia and was introduced into the United States in the 1800s. Since its introduction, garlic mustard has become an invasive threat to forests in the Northeastern and Midwestern United States and Southern Ontario and Quebec (Nuzzo 1999, Roberts and Anderson 2001). Garlic mustard is a biennial forb of the family *Brassicaceae*. It has a three-stage life cycle – seed, rosette, and adult – spanning two years (Nuzzo 1999). Basal rosettes form in the first summer after seeds germinate in the spring. The rosettes overwinter and mature into adults in the second spring and summer, producing flowers that mature to siliques in midsummer. Flowers are primarily pollinated by small bees and flies but can also self-pollinate (Callaway et al. 2008, Pardini et al. 2009). After summer seed dispersal, the plants die.

Due to its biennial life cycle, garlic mustard populations tend to follow a stage-structured interannual density pattern (Pardini et al. 2009, Shyu et al. 2013). In the first year of the life cycle, density of juvenile garlic mustard plants is high while density of adult plants is low. In the second year, density of adult plants is high while density of juvenile plants is low. Density in the second year is never as high as the density from the previous year because survival rates from the juvenile stage to the adult stage are relatively low (Cavers et al. 1979). This two-year cycle must be considered when evaluating management of stage-structured plants because effective management depends on controlling both stage classes and evaluating all patterns over two-year cycles (Pardini et al. 2008, 2009). Management resulting in low induced mortality (<20%) may not alter this two-year cycle, while moderate mortality (40-70%) may result in an increased complexity of the population dynamics (Pardini et al. 2009). High induced mortality (>85%) can produce noncyclic population dynamics and reduce the total population density (Pardini et al. 2009). Different management options for garlic mustard include targeting different stages; herbicide generally targets rosettes in the early spring or late fall, while pulling generally focuses on adult plants in the spring (Pardini et al. 2009). In this study, we targeted both stages of garlic mustard growth with spring pulling and spraying, while we targeted only first-year rosettes with fall spraying.

### **1.2.2 Study Area**

Our study area comprised seven forested sites spanning the area from central Massachusetts, west to the western Berkshire mountains, and southward to the Mid-Hudson Valley of southeastern New York State (Figure 1). The sites extended over

regional temperature and precipitation gradients, with an elevation range of 40-404 m above sea level. Mean soil moisture ranged from 19.1% to 35.6%.

We chose the study sites in 2013 after engaging with a variety of land management stakeholders across the region, including private, non-profit, state, and federal properties (Haines et al. 2018). All sites were located within intact forest canopy with active garlic mustard invasions at a baseline density of at least 20 adult garlic mustard plants per m<sup>2</sup> when established in 2013, which was a cut-off used in prior work (Stinson et al. 2007). In 2014, garlic mustard density in invaded, pulled, and sprayed plots varied between 6 and 250 plants per m<sup>2</sup>, with an average density of 91 plants per m<sup>2</sup>. From the start of the study in 2014 through the end in 2018, density in invaded control plots averaged 30 plants per m<sup>2</sup>, with juvenile plants averaging 29.18 plants per m<sup>2</sup> and adult plants averaging 0.82 plants per m<sup>2</sup>. We selected forested sites with similar canopy composition and site history. Study forests were dominated by sugar maple (*Acer saccharum*) and white ash (*Fraxinus americana*), which indicates early successional regrowth following land use and disturbance (Hall et al. 2002; Thompson et al. 2013). The methods used for controlling for site history included verifying with landowners/stakeholders that there was at least a two-decade history of garlic mustard invasion, excluding sites with clear evidence of past agricultural cultivation (i.e. choosing plots with a shallow and disorganized soil Ap horizon), and reviewing historical cover maps (Haines et al. 2018).

### **1.2.3 Experimental Design**

At each site, we established 3-meter square plots; there were three replicate plots for each of the following treatments: “invaded” (control, at least 20 garlic mustard plants

per plot, n=21), and “pulled” (invaded, with all garlic mustard plants removed by hand annually in the spring, n=21). Additionally, four of the sites included an additional treatment with three replicate plots per site: “sprayed” (invaded, with all garlic mustard sprayed with glyphosate annually in the spring or fall; n=12). Two sites in the Berkshires were sprayed in the fall (McLennan and Questing) and two sites in the Mid-Hudson Valley were sprayed in the spring (Black Rock and West Point; Figure 1). We applied experimental treatments to the entire plot and conducted vegetation surveys in the interior 2 x 2-meter area, leaving a 50 cm treated area around the edge as a buffer between the censused plant community and the untreated garlic mustard population surrounding the plots (Figure 2). The buffer acted to prevent seeds and allelopathic chemicals from dispersing into the survey area.

#### **1.2.4 Data Collection**

We completed surveys of garlic mustard abundance each spring (between May and June) from 2014 through 2018 by counting the number of individuals in each plot (recording juvenile and adult plant abundance separately). In 2014, we completed baseline surveys of the garlic mustard population. Immediately following the baseline survey, we applied the initial eradication treatments of pulling and spraying (except for the spray plots at two of the sites, McLennan Reservation and Questing Forest, which were sprayed in the early fall). In the four years following the initial eradications (2015, 2016, 2017, and 2018), we conducted a survey each spring, followed by the same eradication treatments as the initial eradication. At the two sites that we sprayed each fall, we did not apply spray treatments in the fall of 2017, but rosette abundance was very low that year.

Some environmental variables had previously been collected at each site by Haines et al. (2018). Environmental data included slope and aspect at the site level, and canopy closure at the plot level. Haines et al. (2018) quantified aspect using a magnetic compass, slope using a clinometer, and canopy closure using a spherical densiometer.

### **1.2.5 Data Analysis**

To compare the effectiveness of eradication methods between pulled, sprayed (spring and fall spraying combined) and invaded control plots, we used generalized linear mixed models (GLMMs) to test for the effects of treatment, year, and their interaction on 1) juvenile garlic mustard abundance, 2) adult garlic mustard abundance, and 3) total garlic mustard abundance. Garlic mustard abundance followed non-normal distributions. We included site as a random effect to control for expected differences between sites and nested plot within site to control for autocorrelation. We used Akaike information criterion (AIC) to compare models including and excluding different environmental variables to test if any environmental variables improved model fit. We considered both model fit and parsimony when determining if any variable should be included in the GLMMs listed above. We conducted Chi-square difference tests using the anova function to compare model fit. When the Chi-square test showed non-significant results and AIC differed by less than 2 between two models, the model with the fewest parameters was chosen (Bolker 2008).

In order to explore differences and similarities between regional and site-specific analysis, we also compared the effectiveness of eradication methods at the site level. At each site, we used generalized linear mixed models (GLMMs) to test for the effects of treatment, year, and their interaction on 1) juvenile garlic mustard abundance, and 2)

adult garlic mustard abundance. We included plot as a random effect to control for autocorrelation.

We conducted all statistical analyses in R 3.5.1 (R Development Core Team, 2008), with significance across all tests set at  $P \leq 0.05$ . We used the glmmTMB package (Brooks et al. 2017) to test all GLMMs. Garlic mustard abundance data followed negative binomial and zero-inflated quasi-Poisson distributions. The top models did not include any environmental variables. We used the Anova function in the car package (Fox and Weisberg 2011) to run ANOVAs. We performed post-hoc testing on significant variables with Tukey's HSD tests using the lsmeans function in the lsmeans package (Lenth 2016).

### **1.3 Results**

There was no effect of treatment on juvenile garlic mustard abundance. There was an effect of year on juvenile garlic mustard abundance ( $P < 0.001$ , Table 1), but no effect of the year X treatment interaction (Figure 3). As expected, juvenile abundance decreased in the pulled and sprayed plots one year after the first eradication treatments in 2014. Unexpectedly, juvenile abundance in the invaded control plots also decreased. Juvenile abundance remained low across all treatments for all four years following initial eradications. Juvenile abundance was higher in 2014 than in the other four years (Tukey post-hoc,  $P < 0.001$ ), while the four post-eradication years did not differ from each other.

There was an effect of both year ( $P < 0.001$ ) and the treatment X year interaction ( $P < 0.001$ ) on adult garlic mustard abundance (Table 1). Adult abundance differed by treatment only in 2015 and 2017 – where densities in invaded and sprayed plots were higher than in pulled plots (Figure 4). In those two years, adult abundance did not differ

between sprayed and invaded plots. In the other three years, adult abundance was similarly low among all treatments.

There was an effect of both treatment ( $P=0.03$ ) and year ( $P<0.001$ ) on total garlic mustard abundance, but no effect of the treatment X year interaction (Figure 5). Total abundance in pulled plots was lower than invaded plots (Tukey post-hoc,  $P=0.02$ ), but there were no differences between sprayed and pulled plots or sprayed and invaded plots. Across all treatments, total abundance was higher in 2014 than in the other four years (Tukey post-hoc,  $P<0.001$ ), and total abundance in 2016 was lower than in 2015 (Tukey post-hoc,  $P=0.01$ ) and 2018 (Tukey post-hoc,  $P=0.002$ ).

When we excluded the pre-eradication data from our garlic mustard abundance models, year and the treatment X year interaction affected juvenile garlic mustard abundance. Juvenile abundance was higher in 2018 than in the previous three years (Tukey post-hoc,  $P=0.001$ ), with invaded abundance in 2018 higher than pulled and sprayed abundance in 2016 (Figure 6). The results for adult abundance in post-eradication years (2015-2018) were similar to that across all five years, with the addition of the effect of year on abundance ( $P<0.001$ , Table 2). Total garlic mustard abundance in the post-eradication years was also similar to that across all five years, showing abundance in pulled plots as lower than invaded plots.

The random factor of site was a significant effect in all of the above models, with the exception of adult abundance when we excluded pre-eradication data ( $P=0.056$ ). When we included pre-eradication data, there was a significant effect of site on adult garlic mustard abundance ( $P=0.04$ ). We found a lack of convergence in most of our site-specific models for adult abundance. This was likely due to high proportions of zeros in

the data combined with too few observations at the site level. Therefore, we only presented the results of site-specific analysis for juvenile abundance here.

In the site-specific analysis for juvenile plants, results between most sites were similar to each other and results for five of the seven sites were similar to those found in the regional-scale analysis. At all sites except for West Point and River Road, there was an effect of year ( $P < 0.001$ ) but no effect of treatment or the treatment X year interaction (Table 3). At West Point, where we applied both pulling and spraying treatments, there was a significant effect of year ( $P < 0.001$ ) and of the treatment X year interaction ( $P < 0.001$ ). In 2018, juvenile abundance in invaded plots was higher than in pulled or sprayed plots at West Point, but abundance didn't differ by treatment in any other year at that site (Figure 8B). At River Road, where we applied only the pulling treatment, there was a significant effect of treatment ( $P < 0.043$ ), year ( $P < 0.001$ ), and the treatment X year interaction ( $P < 0.001$ ). When averaged across years, juvenile abundance in pulled plots was significantly lower than in invaded control plots (Tukey post-hoc,  $P < 0.001$ ) at River Road (Figure 7A). At that site, juvenile abundance didn't differ by treatment in 2014, but it was lower in pulled plots than in invaded control plots in the other four years (Figure 8A). At all seven sites, juvenile abundance declined after 2014, in a similar pattern as seen at the regional scale (Figure 8A and Figure 8B).

#### **1.4 Discussion**

We found that pulling was more effective than spraying at reducing the abundance of adult plants. Adult plants in both the invaded and sprayed plots followed the interannual variation that we would expect to see in unmanaged populations (Pardini et al. 2008; Pardini et al. 2009; Shyu et al. 2013), and abundances in those plots did not

differ from each other in any year. In the two years when unmanaged abundance was expected to be high (2015 and 2017), abundance in pulled plots was significantly lower than invaded and sprayed plots (Figure 4).

Juvenile garlic mustard abundance in invaded plots did not follow the expected pattern of inter-annual abundance variation. We expected juvenile abundance to increase in invaded plots in 2016 and 2018. Instead, juvenile abundance remained low across all treatments in all four years after 2014 (Figure 3). We found this same pattern on the regional scale and across all individual sites (Figure 8). Therefore, we didn't find any effect of treatment on juvenile plants. It is likely that juvenile abundance was abnormally high in 2014 and the abundance difference between years is part of a normal population fluctuation and reaction to changing biotic and abiotic conditions (Nuzzo 1999; Pardini et al. 2009). This suggests that no effect of pulling or spraying garlic mustard can be seen in the juvenile population within four years of management, highlighting the long amount of time it may take to exhaust the seed bank (Corbin et al. 2017; Redwood et al. 2018). While some studies have found that targeting one stage of garlic mustard can cause an abundance-dependent rebound effect in the other stage – where higher survival and/or fertility rates of surviving individuals overcompensate for management-induced mortality (Pardini et al. 2008; Pardini et al. 2009; Shyu et al. 2013) – here we saw no such rebound effect when we targeted both stages.

When pre-eradication data from 2014 was excluded from the model, juvenile abundance in invaded control plots still did not increase in 2016 as expected, but it did significantly increase in 2018 (Figure 6). These results indicate that other factors may have confounded the patterns we found in juvenile plants, such as regional drought

conditions that occurred from the spring of 2015 through the spring of 2017 (NIDIS 2019). Garlic mustard is drought sensitive, so drought conditions can lead to lower than normal germination rates (Meekins and McCarthy 2001; Pardini et al. 2009). The slight increase we saw in juvenile abundance from 2016 to 2018 in invaded plots may have been driven by recovery from that drought.

Other garlic mustard management studies of similar length to ours have also found a persistence of new rosettes annually after multiple years of management (Slaughter et al. 2007; Corbin et al. 2018). Slaughter et al. (2007) sprayed garlic mustard rosettes with glyphosate every fall for five years, while Corbin et al. (2018) pulled adult garlic mustard plants every spring for 5 years, and both studies found new juvenile rosettes germinating every year. Slaughter et al. (2007) believed that new juvenile plants were coming from seeds dispersed into their plots from outside the buffer zone. In our study, it's also possible that some of the juvenile rosettes found in our plots each spring were from seeds dispersed from plants outside the plots rather than from adult plants within the plots or from germination from a long-lived seedbank. Although we created a managed buffer between our survey area and the surrounding unmanaged garlic mustard population, it was only 50 cm wide. If there were adult garlic mustard plants taller than 50 cm along the edge of our plots, they could have dropped seeds into our survey area. Garlic mustard seeds are ballistically dispelled from siliques (Nuzzo 1991) and disperse an average of about 50 cm, but can have a maximum dispersal distance of about 1.15 m (Loebach and Anderson 2017),

However, new seeds could also have entered the plot if only a small number of rosettes within the plot survived treatments. Slaughter et al. (2007) targeted juvenile

rosettes with fall spraying and found a significant decrease in adult plants in sprayed versus control sites, but the small number of juvenile rosettes that survived spraying were able to set seed in their second summer before the next fall spraying, thus replenishing the seed bank. Both timing and type of management are important considerations for eliminating seed production. Repeating control twice within the same year – such as in the early spring and late fall – may help to further target individuals that were missed by the previous season's treatment and help to eliminate seedbank input.

One reason why pulling may have been more effective than spraying in this study could be due to the targeting of both juvenile and adult plants in the pull treatment. Although both juvenile and adult plants were also targeted by spring herbicide treatments, the simple mechanics of pulling may have made it easier to find and remove a greater proportion of juvenile rosettes. The juvenile rosettes were sometimes very small and partially obscured by other plants or by leaf litter at the time of spring treatment application. When pulling plants by hand, we had to be close to the ground to remove rosettes and were therefore more likely to find any partially-obscured individuals. When spraying, the person applying herbicide stood next to the plot and applied the glyphosate with an herbicide applicator wand, with their eyes a further distance from the area of application compared to pulling. Additionally, spraying occurred at a time in the spring when many native plants were already leafed-out. In an effort to target only garlic mustard plants and avoid spraying other plants, some garlic mustard leaves may have been missed. If a few small juvenile rosettes were missed, then some of them would have survived until the following spring and been counted as adults during that year's survey.

In practice, pulling efforts are targeted at only adult plants (Shartell et al. 2012; Corbin et al. 2017). Adult plants are easier to identify and pull because they are taller and more distinct. This makes garlic mustard an ideal invasive plant for organizing community volunteers to help with management (Chapman et al. 2012). Pulling short juvenile rosettes would be much more labor intensive and impractical in practice. Therefore, future studies comparing pulling and spraying treatments may consider only pulling adult plants, but continuing treatments for at least a decade to see if germination rates slow and show signs of a diminishing seedbank. If we only pulled adult plants at our sites, then adult plants would likely have remained in higher abundance each spring for a few more years after initial eradications than what we found here, so monitoring and analysis of the population would look different.

Due to garlic mustard's prolific seed production and extended dormancy potential, full eradication of a population will take at least a decade in some cases or may be impossible in others (Meekins and McCarthy 2001; Corbin et al. 2017; Redwood et al. 2018). Annual management and monitoring are necessary for many years after initial eradication efforts to keep garlic mustard from moving into the newly created open space (Baskin and Baskin 2002; Rodgers et al 2008). In a seed dormancy and survival experiment, Redwood et al. (2018) extrapolated that garlic mustard reproduction would need to be fully suppressed for 10 or more years to reach full eradication. Corbin et al. (2017) used two decision making tools that are available to land managers, the Invasive Plant Management Decision Analysis Tool (IPMDAT) and WeedSearch, to estimate the likeliness of eradicating a garlic mustard population and found that eradication would need to be 100% effective for 11 years or 90% effective for over 50 years to reach

permanent eradication. They recommended containment of the invasion and elimination of subpopulations instead of attempting full eradication of the main population (Corbin et al. 2017). Future studies could combine a longer management period (>10 years) with different approaches to managing a large invaded area (e.g. managing a main population vs. a subpopulation) with different management techniques.

Since glyphosate was less effective than pulling and has known negative impacts on non-target plant species (Hochstedler et al. 2007; Pardini et al. 2008) but pulling is more labor intensive and unfeasible in many cases, future studies may consider combining off-season spraying (late fall or early spring) with mid-season pulling. When combined, off-season spraying could drastically reduce the time and labor of pulling, while mid-season pulling could eliminate adult plants that survived the previous spraying. In cases where pulling is not a management option at all, studying the effects of spraying multiple times during the year may be considered. Late fall spraying would target first-year rosettes that germinated that spring, while early spring spraying would target any second-year rosettes that survived fall spraying. Longer-term data that includes different management arrangements such as these would further improve our understanding of the most efficient management options and would better inform the management community in their decisions.

## **1.5 Conclusion**

Numerous studies on garlic mustard's phenology, seed longevity, and response to management have established that a robust garlic mustard population will likely take upwards of a decade to eradicate, if ever, and will require high rates of mortality and averted seed production and dispersal. Here, we found that manual removal of juvenile

and adult garlic mustard plants is more effective than glyphosate application at reducing adult plant abundance, but four years of management was not enough to exhaust garlic mustard's seedbanks at these sites. We found an unexpected decline in juvenile plants after the first year of the study that was not caused by management but was possibly caused by drought or natural fluctuations in the populations. This decline impacted the strength of our conclusion that one management type is more effective than the other. In our regional-scale analysis, we lumped spring and fall spraying into one management type, making it impossible to decipher whether timing of herbicide application impacted the effectiveness of the management. When broken down by site, results indicated that timing of spraying did not affect juvenile abundance, but analysis of adult abundance at the site level was not possible given the characteristics of our data. This is the first study to compare the effectiveness of spraying and pulling over the span of five years and across many separate forested sites spanning a large geographic area in the Northeastern United States.

**CHAPTER 2**

**ASSESSING RESPONSE OF NATIVE PLANT COMMUNITIES TO  
DIFFERENT GARLIC MUSTARD (*ALLIARIA PETIOLATA*) ERADICATION  
METHODS**

**2.1 Introduction**

Non-native invasive plant species can negatively impact the composition, structure, and function of the communities they invade (Vitousek 1990; Simberloff and von Holle 1999; Callaway and Ridenour 2004; Vilà et al. 2011; Ricciardi et al. 2013). Invasive species have economic impacts around the world by causing biodiversity loss, ecosystem degradation, and impairment of ecosystem services (Pimentel et al. 2005; Pyšek and Richardson 2010). Eradicating or controlling invasive species from an area can also be costly, and management strategies are not always guaranteed to lead to ecosystem recovery (Rejmánek and Pitcairn 2002; Kettenring and Adams 2011; Guido et al. 2015; Prior et al. 2017). The goals of invasive species management often include eradicating or controlling the invasive species to the extent that it alleviates impacts and leads to recovery of native biodiversity (Panetta 2009; Andreu and Vilà 2011; Prior et al. 2017). However, goals and outcomes do not always align, and management actions are often more focused on successful removal of an invader than on native revegetation (Kettenring and Adams 2011; Guido et al. 2015; Prior et al. 2017). Therefore, determining the best approach to invasive plant management and monitoring in invaded communities poses a difficult but important challenge to land managers.

In order to effectively and efficiently manage or eradicate invasive plants, land managers must consider the impacts of management on native communities and non-

native target species and how communities reassemble after management efforts (Heleno et al. 2010; Andreu and Vilà 2011; Guido et al. 2015). Invasive plant management projects should involve treating the target invasive species as well as monitoring the response of the plant community (Galatowitsch 2012). Monitoring should occur before, during, and after management efforts so that results can be compared to a pre-restoration baseline (Andreu and Vilà 2011; Galatowitsch 2012). Reference and invaded control communities can be used in the monitoring phase to compare invaded and uninvaded communities and can be used as a benchmark against which to measure response and recovery after management (Noss 1990; White and Walker 1997; Andreu and Vilà 2011; Galatowitsch 2012).

Garlic mustard (*Alliaria petiolata*) is an invasive biennial forb common in temperate forest edges and understories in North America. It has well-documented impacts on native plant and fungal communities (Nuzzo 1999; Meekins and McCarthy 2001; Roberts and Anderson 2001; Stinson et al. 2006; Waller et al. 2006; Stinson et al. 2007; Hale et al. 2016). As of 2019, garlic mustard has invaded 37 states in the United States and much of southern Canada (USDA NRCS National Plant Data Team, 2019). Garlic mustard is of particular concern to land managers because of its negative impact on the soil microbial community and, in turn, the native plant species that rely on mutualisms with soil microbiota (Nuzzo 1993a; Roberts and Anderson 2001; Stinson et al. 2006). Studies have found reduced plant species richness and diversity, changes in species abundances, and shifts in community composition with garlic mustard invasions (McCarthy 1997; Nuzzo 1999; Waller et al. 2006; Stinson et al. 2007; Rodgers et al. 2008; Haines et al. 2018).

The most common and effective methods for managing garlic mustard include removing the entire plant by pulling its roots and shoots from the ground and applying the herbicide glyphosate (Nuzzo 1991; Rodgers et al. 2008). The method of management that is used determines both the seasonal timing of management and the garlic mustard life stage that is targeted (Shyu et al. 2013). Herbicide spraying typically occurs in the early spring or late fall. When applied in the spring, herbicide targets first-year seedlings and rosettes as well as second-year adults. When applied in the fall, herbicide targets only first-year rosettes. Pulling typically occurs in the spring and targets only adult plants before they are able to set seed (Rodgers et al. 2008). The different timings and methods of management can also impact the plant community in different ways. Spraying in the spring can impact sensitive spring ephemeral plants, while spraying in the fall will impact fewer non-target plants (Nuzzo 1991; Slaughter et al. 2007; Pardini et al. 2008). Pulling can cause disturbance of the soil and the surrounding plant cover (Guido et al. 2015).

A few garlic mustard management studies have assessed the impacts of management on the plant community. Some management studies have looked at diversity indices or community composition before and after removing garlic mustard roots and shoots (McCarthy 1997; Stinson et al. 2007; Dornbush and Hahn 2013) while other studies have looked at plant community response to spraying with glyphosate (Hochstedler et al. 2007). These studies found mixed results, with some measurements indicating recovery and others showing no change. Not all garlic mustard management studies have used uninvaded reference communities to compare against managed communities, but instead compared managed communities before and after treatment or against only invaded control communities. Further, none of these studies compared the

impacts of different management methods or timing. Additionally, all of these studies occurred within a single forest or neighboring forest stands, confining implications to a particular area that may not necessarily be useful in a broader regional application.

Thus, there is a need for a better understanding of how different garlic mustard eradication methods impact plant communities over a regional scale in order to inform management options across geographic variation. Here, we compared the plant community before, during, and after three consecutive years of garlic mustard removal and glyphosate application in 7 northern hardwood forests in the Northeastern United States. We explored the impacts of invasion and management on the plant communities and tested whether impacts varied by management type.

## **2.2 Methods**

### **2.2.1 Study Area**

We established our study sites in 2013 at seven forested sites as described in Chapter 1.

### **2.2.2 Experimental Design**

At each site, we established 3-meter square plots as described in Chapter 1, above. Briefly, we applied experimental treatments to the entire plot and conducted vegetation surveys in the interior 2 x 2-meter area, leaving a 50 cm treated area around the edge as a buffer between the censused plant community and the untreated garlic mustard population surrounding the plots (Figure 2). The buffer acted to prevent seeds and allelopathic chemicals from dispersing into the survey area. At each site, there were three replicate plots for each of the following treatments: “uninvaded” (control, no garlic mustard present, n=21), “invaded” (control, at least 20 garlic mustard plants per plot,

n=21), and “pulled” (invaded, with all garlic mustard plants removed by hand annually in the spring, n=21). Additionally, four of the sites included an additional treatment with three replicate plots per site: “sprayed” (invaded, with all garlic mustard sprayed with glyphosate annually in the spring or fall; n=12). Two sites in the Berkshires were sprayed in the fall (McLennan and Questing), and two sites in the Mid-Hudson Valley were sprayed in the spring (Black Rock and West Point; Figure 1).

### **2.2.3 Data Collection**

We completed vegetation surveys of the abundance (total number of individuals) of all understory plants (<1 m tall) each spring from 2014 through 2017. We identified plants to the species level where possible, and to the genus level where species identification was not possible. Species nomenclature followed Haines (Haines 2011). Trees recorded were all seedlings <1 m in height. In 2014, we completed a baseline plant survey in the spring (between May and June). We applied management efforts immediately after data collection in the spring (except for the spray plots at two of the sites, McLennan Reservation and Questing Forest, which were sprayed in the early fall). In the three years following the initial eradications (2015, 2016, and 2017), we conducted a survey each spring, followed by the same eradication treatments as the initial eradication.

Some environmental variables had previously been collected at each site as described in Chapter 1.

### **2.2.4 Data Analysis**

#### **2.2.4.1 Plant Diversity and Density**

To compare the response of the plant community in eradicated plots to the plant communities in invaded control and uninvaded reference plots, we compared three diversity indices. We calculated species richness, Shannon diversity, and Pielou's evenness at the plot level each year using all plants in each annual census except garlic mustard. Species richness ( $S$ ) was calculated as the total number of species present in each plot. Shannon Diversity ( $H'$ ) was calculated as:

$$H' = - \sum_{i=1}^S p_i \ln p_i$$

where  $p_i$  = the number of individuals of species  $i$  / the total number of individuals in the community (May 1975). Pielou's evenness ( $J$ ) was calculated as:  $J = H' / \ln S$  (Pielou 1975). We also compared these three diversity indices at the functional group level by calculating species richness, Shannon diversity, and Pielou's evenness at the plot level in the same way as above but within each functional group. We grouped plants by the following functional groups: forbs, trees (seedlings <1 m tall), shrubs (which includes woody vines), ferns (which includes fern allies), grasses (which include sedges and rushes), and non-natives (which excluded garlic mustard). The forbs, trees, shrubs, ferns, and grasses groups included only native species. Additionally, we calculated relative abundances of each functional group at the plot level. Relative abundance was calculated as the total number of individuals in a functional group within a plot divided by the total number of individual plants across all groups within a plot.

We used general linear mixed models (GLMMs) (and generalized linear mixed models for data following non-normal distributions) to test for the effects of treatment, year, and their interaction on 1) species richness, 2) Shannon diversity, 3) Pielou's evenness, and 4) functional group relative abundance. Species richness followed non-

normal distributions and Shannon diversity and Pielou's evenness followed normal distributions. We included site as a random effect to control for expected differences between sites and we nested plot within site to control for autocorrelation. Garlic mustard abundance significantly improved the fit of the forb species richness and Shannon diversity models and was added as an additional parameter in those models, using the formula: Treatment + Year + GM abundance + Treatment\*Year + (1|Site/Plot).

#### **2.2.4.2 Species Level Response**

There are certain changes in species composition that are not reflected in measures of diversity or richness (Stinson et al. 2007), so we also looked at changes in the representation of some individual species. We compared the response of the top five most abundant species across the study by comparing species abundance between treatments. We used GLMMs as specified above to test for the effects of treatment, year, and their interaction on the total abundance of each species at the plot level each year.

We conducted all statistical analyses in R 3.5.1 (R Development Core Team, 2008), with significance across all tests set at  $P \leq 0.05$ . We used Akaike information criterion (AIC) to compare models including and excluding garlic mustard abundance and different environmental variables to test if any other variables improved model fit. We considered model fit and parsimony when determining if any variable should be included in the GLMMs listed above. We conducted Chi-square difference tests using the anova function to compare model fit. When the Chi-square test showed non-significant results and AIC differed by less than 2 between two models, the model with the fewest parameters was chosen (Bolker 2008). After model comparison, garlic mustard

abundance was only included in two of our models (forb species richness and diversity), and environmental variables were not included in any model.

Shannon diversity and Pielou's evenness followed a normal distribution, while species richness followed a COM-Poisson distribution (Table 3). Species abundance data followed negative binomial, quasi-Poisson, and zero-inflated quasi-Poisson distributions (Table 4). We used the `lmer` function in the `lme4` package (Bates et al. 2015) to test all normally-distributed GLMMs. For data that were not normally-distributed, we used the `glmmTMB` package (Brooks et al. 2017) to test GLMMs. We used the `anova` function in the `lmerTest` package (Kuznetsova et al. 2017) to run ANOVAs associated with the `lme4` GLMMs, and we used the `Anova` function in the `car` package (Fox and Weisberg 2011) to run ANOVAs associated with the `glmmTMB` GLMMs. We performed post-hoc testing on significant variables with Tukey's HSD tests using the `glht` function in the `multcomp` package (Hothorn et al. 2008) and the `lsmeans` function in the `lsmeans` package (Lenth 2016).

## **2.3 Results**

### **2.3.1 Plant Diversity**

There was no effect of treatment on species richness, Shannon diversity, or Pielou's evenness, but there was an effect of year on all three indices ( $P < 0.001$ ,  $P < 0.001$ , and  $P = 0.027$ , respectively; Table 4). Species richness increased across all treatments after the initial eradication and was higher in 2015 through 2017 compared to 2014 (Tukey post-hoc,  $P < 0.001$ ). Diversity was higher in 2016 (but not 2017) than in 2014 across all treatments (Tukey post-hoc,  $P = 0.003$ ). While there was an overall effect of year on

Pielou's evenness, a Tukey post-hoc test showed no significant differences between any years.

When sorted by functional groups, ferns, grasses, shrubs, and nonnatives (excluding garlic mustard) had very low relative abundance. Forbs and trees seedlings comprised the majority (>85%) of the relative abundance in all treatments when garlic mustard was excluded in the pre-eradication year and averaged across the post-eradication years (Figure 9). Therefore, we only tested the effects of treatment and year on forb and tree functional groups.

We found no effect of treatment or year on forb or tree relative abundance. There was an effect of treatment on forb species evenness ( $P=0.033$ ), but a Tukey post-hoc showed no significant differences in evenness between treatments. The forb species diversity and richness models were both significantly improved by adding total garlic mustard abundance as a parameter. There were effects of treatment ( $P=0.02$ ), year ( $P=0.002$ ), and garlic mustard abundance ( $P=0.018$ ) on forb species richness (Table 4). Forb species richness was significantly lower in uninvaded compared to pulled plots (Tukey post-hoc,  $P=0.03$ ; Figure 10). There were no effects of treatment, year, or their interaction on forb species diversity, but there was a significant effect of garlic mustard abundance ( $P=0.043$ ). Forb Shannon diversity (Figure 11A) and species richness (Figure 11B) were positively correlated with garlic mustard abundance.

For tree species, we found an effect of year on diversity ( $P<0.001$ ) and richness ( $P<0.001$ ), but we found no effect of treatment. When averaged across all treatments, tree species richness was higher in all three post-eradication years than in the pre-eradication year (Tukey post-hoc,  $P<0.001$ ).

### 2.3.2 Species Level Response

Two tree species and three spring ephemerals in the forb functional group comprise the five most abundant species across the study (Figure 12; Table 5). There were effects of treatment and year on sugar maple (*Acer saccharum*) and white ash (*Fraxinus americana*) seedlings (<1m in height; Figure 13). Sugar maple abundance in pulled plots was significantly higher than in uninvaded plots when averaged across all years (Tukey post-hoc,  $P=0.03$ ). When averaged across all treatments, Sugar maple abundance declined significantly each year after the initial eradications (Tukey post-hoc,  $P<0.001$ ). We found an almost opposite trend in white ash abundance, for which there was also a significant treatment X year interaction (Figure 13). From 2014 through 2016, white ash abundance remained fairly steady, but increased in 2017. White ash abundance in 2017 was significantly higher than in 2014 (Tukey post-hoc,  $P<0.001$ ) and 2016 (Tukey post-hoc,  $P<0.001$ ). Differences in white ash abundance between treatments varied each year. In the first two years of the study, white ash abundance was slightly higher in uninvaded plots than the other three treatments. Average abundance in uninvaded plots didn't vary much over the four years, but increased in invaded, pulled and sprayed plots. In 2017, average white ash abundance in invaded, pulled, and sprayed plots was higher than in uninvaded plots, but not significantly so.

There was an effect of year on Jack-in-the-pulpit (*Arisaema triphyllum*) abundance (Table 5). Jack-in-the-pulpit abundance in 2015 and 2016 was higher than in 2014 and 2017 when averaged across treatments, but there was no significant difference in abundance between the pre-eradication year (2014) and the third year after initial eradications (2017). For Canada mayflower (*Maianthemum canadense*) and American

trout lily (*Erythronium americanum*), there were effects of year and a significant treatment X year interaction (Table 5). The highest abundance of Canada mayflower occurred in uninvaded plots, but despite this, abundance in uninvaded plots was not significantly higher than in the other three treatments. Canada mayflower abundance was lowest in 2014 and highest in 2017 across all treatments. Similarly, the highest abundance of trout lily occurred in uninvaded plots but was only significantly higher than other treatments in two years (Figure 14). Abundance of trout lily was significantly higher in uninvaded plots than in sprayed plots in 2015 and was higher than both sprayed and invaded control plots in 2016. In the third year following the initial eradications (2017), trout lily abundance didn't differ between treatments and wasn't significantly different than the pre-eradication year (2014).

## **2.4 Discussion**

### **2.4.1 Plant Diversity**

There are numerous studies from around the world that show the negative impacts of invasive plants on species richness, diversity, and evenness, or composition (Vitousek 1990; Hejda et al. 2009; Vila et al. 2011). The scale at which we measure relationships between native and invasive species can impact whether that relationship is positive or negative (Levine 2000; Waller et al. 2016). We might expect to find positive relationships between native diversity and invasive species diversity at broader scales (Levine 2000; Tilman 2004; Waller et al. 2016), but we might expect those relationships to be negative at finer scales (Stohlgren et al. 2006; Fridley et al. 2007; Waller et al. 2016). Here, we looked at community response to garlic mustard management at a finer scale at multiple forested sites spanning a large geographic region of the Northeast. While

we collected data at the fine scale, we analyzed it at an aggregated larger scale. This approach to analyzing this data may have strengthened some fine-scale patterns found across multiple sites while possibly muting other fine-scale patterns that differed by site across the region.

Previous field studies have shown mixed effects of garlic mustard invasion on native plant communities. While studies have found negative impacts from garlic mustard on native plant richness, diversity, or species abundance in some instances (McCarthy 1997; Nuzzo et al. 1999; Meekins and McCarthy 1999; Stinson et al. 2007; Waller et al. 2006; Haines et al. 2018), they have also found little or no impact on diversity or composition of invaded areas in other instances (Nuzzo et al. 1999; Rodgers et al. 2008; Rooney and Rogers 2011; Davis et al. 2014; Davis et al. 2015; Haines et al. 2018). One study even linked community impact with the age of an invasion, finding fewer impacts on plant communities with older garlic mustard invasions due to decreased phytotoxic chemical production in older populations (Lankau et al. 2009).

Similarly, garlic mustard eradication studies have also found mixed impacts on the plant community (McCarthy 1997; Meekins and McCarthy 1999; Hochstedler et al. 2007; Stinson et al. 2007; Barto and Cipollini 2009; Dornbush and Hahn 2013).

McCarthy (1997) found significant increases of relative abundance of native annual plants, vines, and tree seedlings after one year of manual removal. Hochstedler et al. (2007) found no significant difference in species richness or diversity between invaded plots and plots sprayed with glyphosate for five years. Stinson et al. (2007) found significant increases in diversity and equitability after two years of partial removal of garlic mustard. Dornbush and Hahn (2013) found no changes in native plant richness or

cover following four years of garlic mustard removal in plots where no re-introduction of native plants occurred. Dornbush and Hahn (2013) also saw native plant richness increase to an even greater extent when deer were excluded from plots, reducing herbivore pressure. Our study is unique compared to these studies because it looks at plant community response to garlic mustard management across multiple sites.

Based on previous findings, we expected to see mixed impacts (negative or no impacts) of garlic mustard on species richness, diversity, and evenness in invaded plots compared with uninvaded plots. Here, we found no differences in richness, diversity, and evenness between invaded and uninvaded plots. These results are similar to what was previously found in some of these same plots by Haines et al. (2018), although that study analyzed data over a shorter time period. Additionally, we expected to see either no change or an increase in diversity indices in sprayed and pulled plots following eradication, where diversity in sprayed and/or pulled plots would either be the same as or higher in comparison to invaded control plots, and more closely resembling diversity in uninvaded plots. Diversity did not increase over time in sprayed or pulled plots. Given the lack of difference between invaded control and uninvaded reference plots, increased diversity in eradicated plots would not necessarily have been an indication of recovery from invasion.

It is possible that the densities of garlic mustard found in our study plots may not have reached a high enough threshold to impact species richness or diversity. In a field experiment in a western Massachusetts forest, Stinson et al. (2007) also found no effect of garlic mustard invasion on species richness, but they did find a decline in Shannon diversity and evenness with increasing *in situ* densities of garlic mustard. However, there

is no clearly defined threshold across previous studies that indicates at what point garlic mustard abundance might start to have a clear impact on the plant community. Here, garlic mustard abundance in invaded plots averaged 37.57 plants per m<sup>2</sup> from 2014 to 2017, with juvenile plants averaging 36.04 plants per m<sup>2</sup> and adult plants averaging 1.52 plants per m<sup>2</sup>. Garlic abundance varied between 0 and 250 plants per m<sup>2</sup>, but abundance was not included in our overall diversity, richness, or evenness models because it did not improve model fit. Therefore, it seems as though there is no relationship between overall diversity and increasing densities of garlic mustard at our sites.

Results of an experiment by Stinson et al (2006) found that more highly mycorrhizal-dependent tree seedlings may be more likely to experience negative impacts from garlic mustard than less mycorrhizal dependent plants, while McCarthy (1997) found increased richness and abundance of woody perennials after experimentally removing garlic mustard. Therefore, we expected lower relative abundance and diversity among the tree functional group in invaded plots compared to uninvaded plots, as well as higher relative abundance and diversity in pulled and sprayed plots after eradications compared to invaded control plots. Instead, we found no differences in tree relative abundance, diversity, richness, or evenness between treatments. Stinson et al. (2007) found that relative abundance of tree seedlings increased with only partial, but not full removal of garlic mustard, which is similar to our findings following full removal of garlic mustard in our plots. Full removal of garlic mustard may cause disturbance to the soil that is detrimental to new tree seedling growth. Sugar maple and white ash, the dominant tree species at our sites, are both intolerant to disturbance. Additionally, full

removal of garlic mustard may have opened space for new garlic mustard or other non-native or weedy species to germinate.

Within the forb functional group, treatment did not affect forb relative abundance or species diversity. Forb species richness was lower in uninvaded plots compared to pulled plots, but not different from the other treatments. Unexpectedly, species diversity and richness were both positively correlated with garlic mustard abundance (Figure 11). This result is the opposite of what Stinson et al. (2007) found in the relationship between overall species diversity and garlic mustard abundance and may point to a specific dynamic among forest forb species and their relationship to garlic mustard. Not all forest understory species are equally susceptible to garlic mustard invasion (Meekins and McCarthy 1999; Stinson et al. 2006). It's possible that many of the forb species are less mycorrhizal-dependent than some other species and therefore less impacted by garlic mustard (Meekins and McCarthy 1999; Stinson et al. 2006). Additionally, some forb species may be able to successfully compete with garlic mustard for other resources like light, nutrients, water, and space. Some of the forb species may be weedy and may even respond positively to disturbances like invasion. The relative abundance of forb species in plots was high (averaging from about 20% to 40%; Figure 9), so the high relative abundance of forbs may present effective competition to garlic mustard under these particular forested conditions.

Another potential explanation for our findings is that the conditions in our forested sites are not optimal for garlic mustard to create the dense monocultures that have been observed in other habitats. Forest edges and forest interiors with more open canopy structure are the most vulnerable to garlic mustard invasion (Meekins and

McCarthy 2001). Experimental growth of garlic mustard in different shade conditions, focusing on forest edge vs. forest interior conditions, have shown reproductive and physiological disadvantages to low light for garlic mustard plants originating from both the forest edge and in the forest understory (Stinson and Seidler 2014). Additionally, the lower reproductive success of garlic mustard in the forest interior can point to a potential source-sink propagule dynamic where the edge acts as a propagule source and the interior acts as a propagule sink (Stinson and Seidler 2014). Our plots were all located in the forest interior with canopy closure between 46% and 90%. It is possible that garlic mustard isn't able to out-compete native plant communities under these light conditions due to reduced growth and propagule pressure, and therefore it isn't causing the reduction in plant diversity that we expected to find. Rooney and Rogers (2011) also suggest that similar findings of a lack of native species abundance differences between invaded and uninvaded areas may be due to too few invasive plants to have measurable effects at such a small plot scale, and thus a larger scale may be a more effective monitoring parameter.

#### **2.4.2 Species Level Response**

Garlic mustard has been found to slow growth and reduce AMF root colonization in some tree seedlings including sugar maple and white ash as well as other species with strong AMF associations (Stinson et al. 2006, Barto et al. 2011). These tree seedlings have been shown to favor less invaded areas compared to areas with higher garlic mustard abundance (Stinson et al. 2007). Relative abundances of native tree seedlings have been found to increase after partial garlic mustard removal in as short a term as just two growing seasons (Stinson et al. 2007). We expected to find a reduced abundance of these tree species in plots invaded by garlic mustard compared to uninvaded plots. We

also expected to see increased abundance in these species in pulled and sprayed plots following eradication compared with invaded plots.

Here, we found that sugar maple tended to have the lowest abundance in uninvaded plots, with uninvaded abundance significantly lower compared to pulled plots (Figure 13a). Given the concern for garlic mustard's effects on maple seedlings, this result is counter to what we expected. Similarly, Haines et al. (2018) found that sugar maple was positively associated with garlic mustard abundance at their sites. Sugar maple abundance declined significantly every year across all treatments following initial eradications. This might be explained by relatively higher abundance of sugar maple at one site in addition to sugar maple's tendency to have mast fruiting years with high mortality rates in the following years (Frey et al. 2007, Cleavitt et al. 2014). We found the opposite pattern over time with white ash, with significantly higher abundance of white ash in 2017 compared to 2014 and 2016 (Figure 13b). This might also be explained by a white ash masting year in 2017. Both of these tree species have been documented to have large variation in annual recruitment, so annual variation is expected (Frey et al. 2007). Given this annual variation in tree seedling abundance, it's important to compare invaded areas to similar uninvaded areas within the same growing season when monitoring for impacts of garlic mustard and management, rather than to compare invaded or managed areas to themselves across years.

Haines et al. (2018) found that Canada mayflower (*Maianthemum canadense*) and trout lily (*Erythronium americanum*) had negative associations with garlic mustard presence, with densities of those two species lower in the invaded plots than in the uninvaded plots. In another study, jack-in-the-pulpit grew significantly less rapidly in

soils where garlic mustard was grown versus soils where only jack-in-the-pulpit was grown (Rodgers et al. 2008). In contrast, that same study found higher growth of wheat in garlic mustard soils vs. soils where other conspecifics were grown (Rodgers et al. 2008). Davis et al. (2015) assessed a number of individual species associations with garlic mustard and found only two species to be negatively correlated with garlic mustard, while many were positively associated. Therefore, different species may respond positively or negatively to garlic mustard, either in reaction to or regardless of garlic mustard's allelopathic chemicals.

Looking at the three most abundant forb species, we found no differences in jack-in-the-pulpit abundance between invaded, uninvaded, and eradicated plots. We also did not find significantly higher abundance of Canada mayflower and trout lily in uninvaded compared to invaded plots as expected. Trout lily was significantly lower in sprayed plots than in uninvaded plots during the first two years following initial eradications (Figure 14), suggesting that this species may be more sensitive to herbicide application than disturbance from pulling. However, trout lily abundance was measured each spring before herbicide was applied, so if herbicide did reduce trout lily abundance, then those impacts would have had to come from residual effects of spraying in the previous year.

### **2.4.3 Management and Monitoring Considerations**

One interesting observation to take from this study when considering management goals and monitoring approaches is that there can be a tradeoff between different plant community health indicators such as high species diversity and high abundance of some native species. We observed that some uninvaded plots at some of our sites had dense monocultures of spring ephemerals such as Canada mayflower and trout lily, while those

species abundances were low in other plots and completely absent from some sites. Such patterns at the plot level may have contributed to the lower species richness and diversity in uninvaded plots, while differences at the site level may have masked differences in species abundances between treatments. Further, such patterns are seasonally specific; had we conducted vegetation surveys later in the summer, the spring ephemerals would have been gone and other annual species may have occupied those spaces. When setting management goals or monitoring the impacts of invasive plants or management efforts, managers should consider such nuances in the community. For example, on its face value, a metric like low diversity may indicate a problem, but if that metric reflects a small dense patch of spring ephemerals, it may actually indicate a thriving forest understory.

Overall, our results may be driven by patterns of spatial heterogeneity from site to site or by microsite variation. There was a significant effect of site on all diversity models except for forb evenness (Table 4), and on all species-specific models except for Canada mayflower (Table 5). This suggests there may be community variation between sites. Our regional approach to analysis may dampen any patterns that are important at the site-specific scale.

Biotic and abiotic factors such as topographic heterogeneity, microsite disturbances, light availability, and edaphic factors can cause spatial variation in species abundance and composition within a site (Stein et al. 2014; Davis et al. 2015). Some species, such as habitat specialists, may prefer specific microsite conditions, while habitat generalists and invasive species don't require such conditions but tolerate the same range of conditions in different microsites (Waller et al. 2006, Davis et al. 2015). Such

microsite variation confounds the problem of selecting reference communities to compare against managed communities when assessing the effectiveness of management efforts (White and Walker 1997). A major challenge to measuring community recovery is that a reference may not always be a realistic or useful reference, as they can change or differ from invaded or restored areas due to differences between or within sites (Stein et al. 2014; Prior et al. 2017). Such implicit differences between plots may be one factor driving our results.

These results suggest interannual and geographic variation that may make it difficult to identify any region-wide response in the plant community (White and Walker 1997; Galatowitsch 2012). We recommend site-specific monitoring of the plant community to better understand the impacts of management on the plant community, versus or in addition to such regional monitoring as we conducted here. Analysis at the regional scale may be muting or missing compositional or species-specific trends that are important at the local scale. Further, our results indicate that managing for increased species richness and diversity may not be the most appropriate goal, given the lack of difference in richness and diversity between invaded and uninvaded plots. Future monitoring efforts should involve other site-specific indicators such as responses of important species that are specific to each site. Here, some of the most abundant species across the study that we analyzed were not present at some of the sites.

The apparent lack of impact of garlic mustard on native plant richness, diversity, and abundance brings up the question of whether it is necessary to manage garlic mustard at these sites. While we didn't find impacts on the metrics we measured, garlic mustard could be impacting other aspects of the plant community which we did not measure in

this study, or the reference plots used may have been inappropriate for comparison. Additionally, we know that garlic mustard is currently having an impact on the soil fungal community at these sites. A related study of soil microbiota and soil properties in these plots found a shift in the dominant fungal guild in invaded compared to uninvaded plots that may directly impact plant disease response, soil nutrient cycling, and plant performance (Anthony et al. 2017). It is possible that there is a lag between impacts on the soil community and impacts on the plant community, and what Anthony et al. (2017) found in the invaded soil community may be indicative of future changes in the plant community. While we can't predict specific changes in the plant community, we can conclude that monitoring for changes would be prudent if management were to be paused at this time. Lastly, we did not find any clear negative impacts of management on the plant community, so we can presume that eliminating garlic mustard now is not harmful and may actually prevent changes before they are detectible or become irreversible.

## **2.5 Conclusion**

Management decisions should focus on relieving the impact invasive species have on the communities they invade (Hejda et al. 2009). It is therefore important to compare invaded communities against similar uninvaded communities in order to understand invasion impacts and inform management goals. Many studies have found negative impacts of garlic mustard on native species richness, diversity, and composition, while others have found improvements in richness and diversity following garlic mustard management. In this study, we found neither negative impacts of garlic mustard on species diversity, nor increased diversity in managed plots after three consecutive years of management. However, it is questionable whether garlic mustard is truly not having an

impact on the plant community, or whether we did not use the right scale or measure the right indicators in our monitoring efforts. The results of our study add to the growing body of literature that documents how a single invasive species can have varying degrees of impact under different conditions and at different scales. These results also highlight how decisions can be complicated for managers when determining which garlic mustard invasions to prioritize and how to measure community recovery.

Determining management goals, whether they are increasing diversity, eradicating garlic mustard, or reducing densities and preventing spread, can help drive decisions of management type and timing. Because we sprayed in the spring at two sites and the fall in the other two sites, we can't determine if season of glyphosate application drove our results. If analyzed at the site level, we would have been able to compare results from spring spraying against results from fall spraying. These results illuminate how difficult decisions about garlic mustard management methods and goals can be to make. More comparative studies on how treatment types and timing effect plant communities would provide more resources from which to make such decisions with more confidence, leading to effective and efficient management plans. Future studies should collect data at multiple times during the year to get a better understanding of how the garlic mustard population and the native plant community change throughout the year under different management strategies. It would also be especially interesting to see how garlic mustard reacts when management is applied multiple times during the year. Additionally, studies that combine different management types at different seasons, such as spraying in the fall and pulling in the spring, may provide good insight into maximizing management resources through multi-method approaches.

Table 1. Response of garlic mustard abundance to treatment and year, including pre- and post-eradication years (2014-2018). Abundance represents density per 4m<sup>2</sup>.

Response Variable	Model	Effect	DF	X <sup>2</sup>	P
Juvenile Garlic Mustard	Negative Binomial GLMM	Treatment	2	5.037	0.081
		Year	4	376.167	< <b>0.001</b>
		Treatment x Year	8	13.950	0.083
		Site (random)	18	31.9	< <b>0.001</b>
Adult Garlic Mustard	Zero-inflated Quasi-Poisson GLMM	Treatment	2	0.013	0.935
		Year	4	77.313	< <b>0.001</b>
		Treatment x Year	8	32.888	< <b>0.001</b>
		Site (random)	19	4.199	<b>0.04</b>
Total Garlic Mustard	Negative Binomial GLMM	Treatment	2	6.779	<b>0.03</b>
		Year	4	373.764	< <b>0.001</b>
		Treatment x Year	8	13.812	0.09
		Site (random)	18	30.271	< <b>0.001</b>

Table 2. Response of garlic mustard abundance to treatment and year for post-eradication years only (2015-2018). Abundance represents density per 4m<sup>2</sup>.

Response Variable	Model	Effect	DF	X <sup>2</sup>	P
Juvenile Garlic Mustard	Negative Binomial GLMM	Treatment	2	1.191	0.551
		Year	3	25.444	< <b>0.001</b>
		Treatment x Year	6	14.698	<b>0.023</b>
		Site (random)	15	33.244	< <b>0.001</b>
Adult Garlic Mustard	Zero-inflated Quasi-Poisson GLMM	Treatment	2	36.861	< <b>0.001</b>
		Year	3	57.892	< <b>0.001</b>
		Treatment x Year	6	20.012	<b>0.003</b>
		Site (random)	16	3.389	0.056
Total Garlic Mustard	Negative Binomial GLMM	Treatment	2	9.782	<b>0.008</b>
		Year	3	28.653	< <b>0.001</b>
		Treatment x Year	6	10.661	0.099
		Site (random)	15	31.135	< <b>0.001</b>

Table 3. Response of garlic mustard abundance to treatment and year by site. Abundance represents density per 4m<sup>2</sup>.

Sites with Pulling and Spraying Treatments					
Site	Model	Effect	DF	X <sup>2</sup>	P
Black Rock	Negative binomial GLMM	Treatment	2	2.7933	0.247
		Year	4	90.9069	<b>&lt;0.001</b>
		Treatment x Year	8	14.4726	0.070
		Plot	17	18.629	<b>&lt;0.001</b>
McLennan Forest	Zero-inflated negative binomial GLMM	Treatment	2	1.6852	0.431
		Year	4	161.8162	<b>&lt;0.001</b>
		Treatment x Year	8	4.9188	0.766
		Plot	18	17.452	<b>&lt;0.001</b>
Questing Forest	Negative binomial GLMM	Treatment	2	0.2697	0.874
		Year	4	179.3335	<b>&lt;0.001</b>
		Treatment x Year	8	9.2983	0.318
		Plot	17	21.625	<b>&lt;0.001</b>
West Point	Negative binomial GLMM	Treatment	2	0.1399	0.932
		Year	4	160.4280	<b>&lt;0.001</b>
		Treatment x Year	8	36.4481	<b>&lt;0.001</b>
		Plot	17	5.6696	<b>0.017</b>
Sites with only Pulling Treatments					
Site	Model	Effect	DF	X <sup>2</sup>	P
Harvard Forest	Negative binomial GLMM	Treatment	1	0.8772	0.349
		Year	4	238.7574	<b>&lt;0.001</b>
		Treatment x Year	4	1.0125	0.908
		Plot	12	12.157	<b>&lt;0.001</b>
Pittsfield State Forest	Quasi - Poisson GLMM	Treatment	1	2.5300	0.112
		Year	4	28.9081	<b>&lt;0.001</b>
		Treatment x Year	4	2.2433	0.691
		Plot	12	4.7751	0.029
River Road	Negative binomial GLMM	Treatment	1	4.1018	<b>0.043</b>
		Year	4	186.5828	<b>&lt;0.001</b>
		Treatment x Year	4	25.9725	<b>&lt;0.001</b>
		Plot	12	11.917	<b>&lt;0.001</b>

Table 4. Effects of treatment and year\* on species richness, Shannon diversity, and Pielou's evenness. Diversity indices exclude garlic mustard but include other non-native species.

Response Variable	Model	Effect	DF	F	P
Overall Shannon Diversity	Normal GLMM	Treatment	3	1.309	0.279
		Year	3	11.366	<b>&lt;0.001</b>
		Treatment x Year	9	0.707	0.703
		Site (random)	19	7.983	<b>0.005</b>
Overall Pielou's Evenness	Normal GLMM	Treatment	3	0.501	0.683
		Year	3	3.125	<b>0.027</b>
		Treatment x Year	9	0.746	0.667
		Site (random)	19	10.33	<b>0.001</b>
Forb Shannon Diversity	Normal GLMM	Treatment	3	2.401	0.078
		Year	3	2.463	0.065
		GM abundance*	1	4.152	<b>0.043</b>
		Treatment x Year	9	0.997	0.445
		Site (random)	20	0.24	0.624
Forb Pielou's Evenness	Normal GLMM	Treatment	3	3.137	<b>0.033</b>
		Year	3	0.429	0.733
		Treatment x Year	9	0.454	0.903
		Site (random)	19	0	1
Tree Shannon Diversity	Normal GLMM	Treatment	3	0.844	0.475
		Year	3	10.367	<b>&lt;0.001</b>
		Treatment x Year	9	0.690	0.718
		Site (random)	19	18.291	<b>&lt;0.001</b>
Tree Pielou's Evenness	Normal GLMM	Treatment	3	2.056	0.119
		Year	3	1.530	0.208
		Treatment x Year	9	1.040	0.409
		Site (random)	19	254.07	<b>&lt;0.001</b>
Response Variable	Model	Effect	DF	X <sup>2</sup>	P
Overall Species Richness	COM-Poisson GLMM	Treatment	3	6.032	0.110
		Year	3	88.733	<b>&lt;0.001</b>
		Treatment x Year	9	5.269	0.810
		Site (random)	19	5.401	<b>0.02</b>
Forb Species Richness	COM-Poisson GLMM	Treatment	3	9.827	<b>0.02</b>
		Year	3	15.328	<b>0.002</b>
		GM abundance*	1	5.624	<b>0.018</b>
		Treatment x Year	9	7.006	0.636
		Site (random)	20	38.16	<b>&lt;0.001</b>
Tree Species Richness	COM-Poisson GLMM	Treatment	3	1.001	0.801
		Year	3	78.204	<b>&lt;0.001</b>
		Treatment x Year	9	7.113	0.625
		Site (random)	19	14.588	<b>&lt;0.001</b>

\*Forb Shannon diversity and forb species richness were the only models for which fit was significantly improved by including total garlic mustard abundance.

Table 5. Effects of treatment and year on the abundance of the individual species with the highest overall abundance. Abundance represents species density per 4m<sup>2</sup>.

Species Latin Name	Functional Group	Mean Density (per 4m <sup>2</sup> )	No. sites	Model	Effect	DF	X <sup>2</sup>	P
<i>Acer saccharum</i>	Tree	27.32	7	Quasi-Poisson GLMM	Treatment	3	9.114	<b>0.028</b>
					Year	3	277.315	<b>&lt;0.001</b>
					Treatment x Year	9	4.068	0.907
					Site (random)	19	68.813	<b>&lt;0.001</b>
<i>Arisaema triphyllum</i>	Forb	9.73	7	Quasi-Poisson GLMM	Treatment	3	5.232	0.156
					Year	3	52.641	<b>&lt;0.001</b>
					Treatment x Year	9	9.403	0.401
					Site (random)	19	46.918	<b>&lt;0.001</b>
<i>Erythronium americanum</i>	Forb	25.8	4	Zero-inflated Negative Binomial GLMM	Treatment	3	0.230	0.973
					Year	3	91.606	<b>&lt;0.001</b>
					Treatment x Year	9	130.568	<b>&lt;0.001</b>
					Site (random)	20	8.958	<b>0.003</b>
<i>Fraxinus Americana</i>	Tree	14.16	7	Zero-inflated Negative Binomial GLMM	Treatment	3	8.891	<b>0.031</b>
					Year	3	17.245	<b>&lt;0.001</b>
					Treatment x Year	9	17.170	<b>0.046</b>
					Site (random)	20	62.177	<b>&lt;0.001</b>
<i>Maianthemum canadense</i>	Forb	9.49	5	Zero-inflated Poisson GLMM	Treatment	3	0.229	0.990
					Year	3	15.125	<b>0.002</b>
					Treatment x Year	9	28.355	<b>&lt;0.001</b>
					Site (random)	19	0.097	0.756

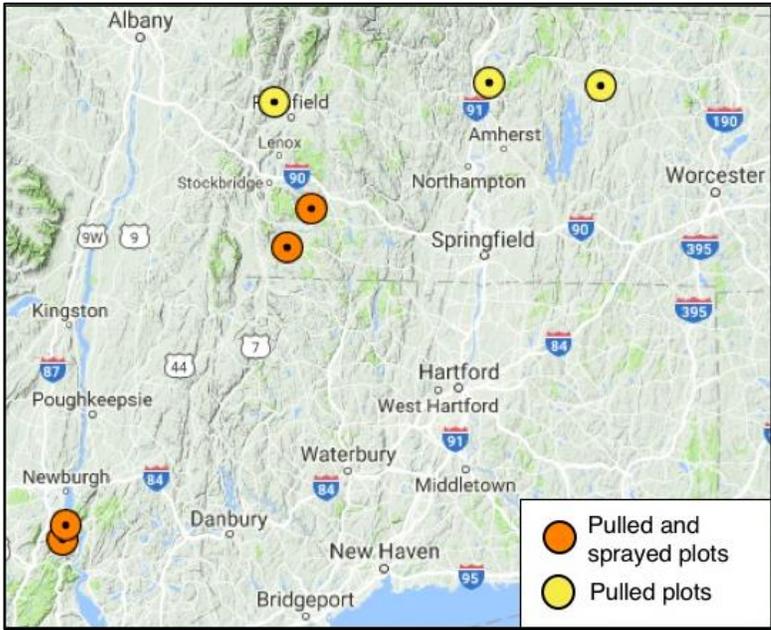


Figure 1. Locations of study sites. At the sites with yellow dots, only pulling occurred. At the sites with orange dots, spraying occurred in addition to pulling.

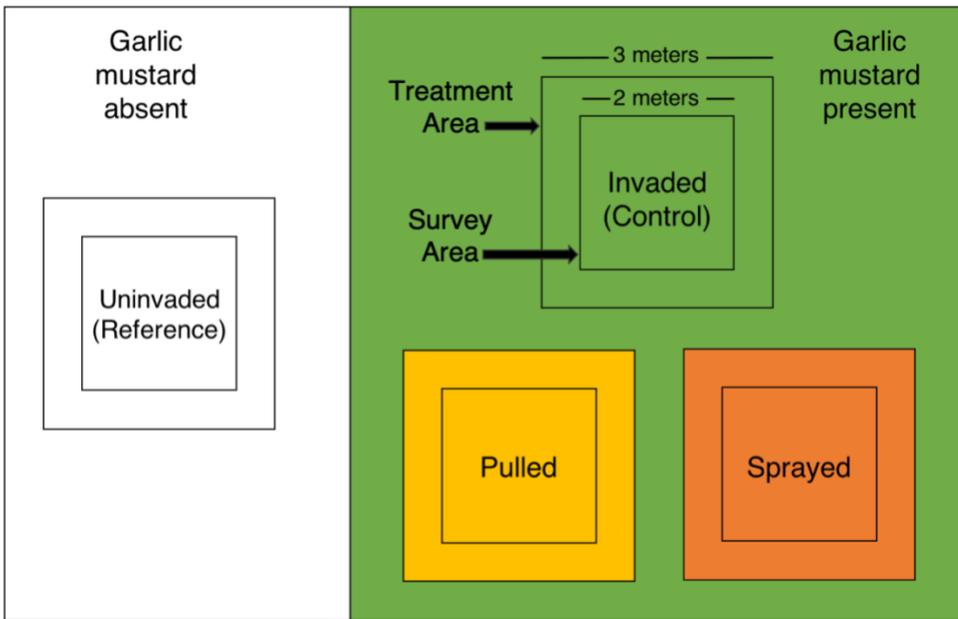


Figure 2. Example of plot design at each site. Plots were not arranged around each other in this exact pattern. Invaded, pulled, and sprayed plots were in an area of forest invaded by garlic mustard with a density of at least 20 plants per square meter. Uninvaded plots were established in an area not invaded by garlic mustard, but adjacent to the invaded area.

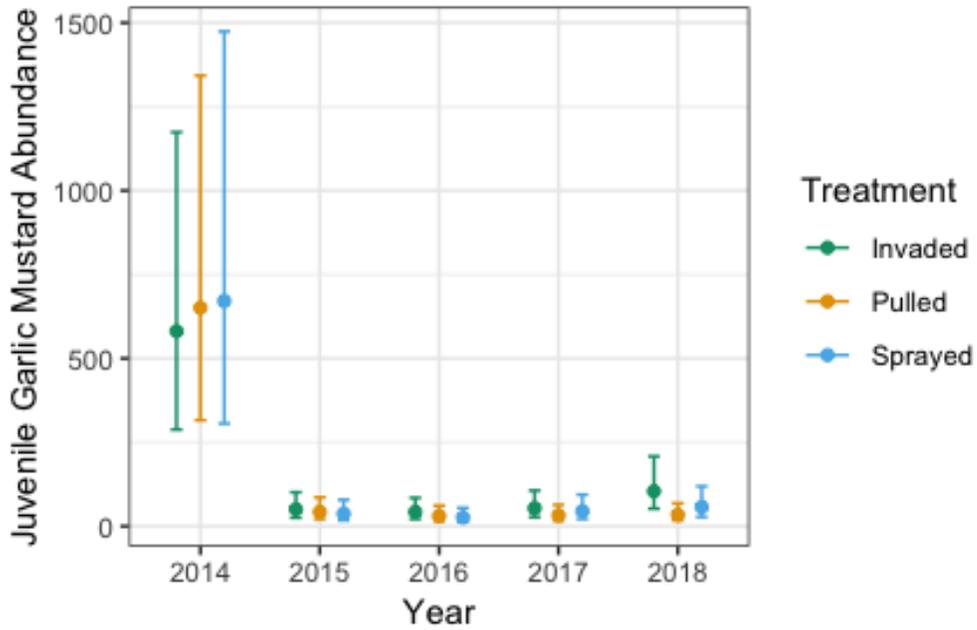


Figure 3. Juvenile garlic mustard abundance as a function of year and garlic mustard treatment. Abundance represents density per 4m<sup>2</sup>. Error bars indicate 95% confidence intervals.

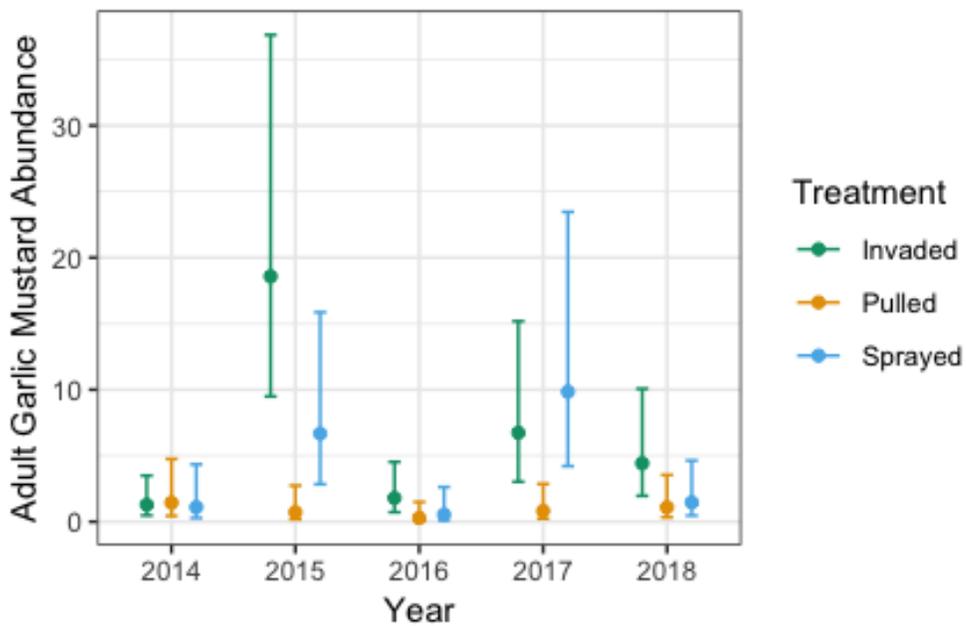


Figure 4. Adult garlic mustard abundance as a function of year and garlic mustard treatment. Abundance represents density per 4m<sup>2</sup>. Error bars indicate 95% confidence intervals.

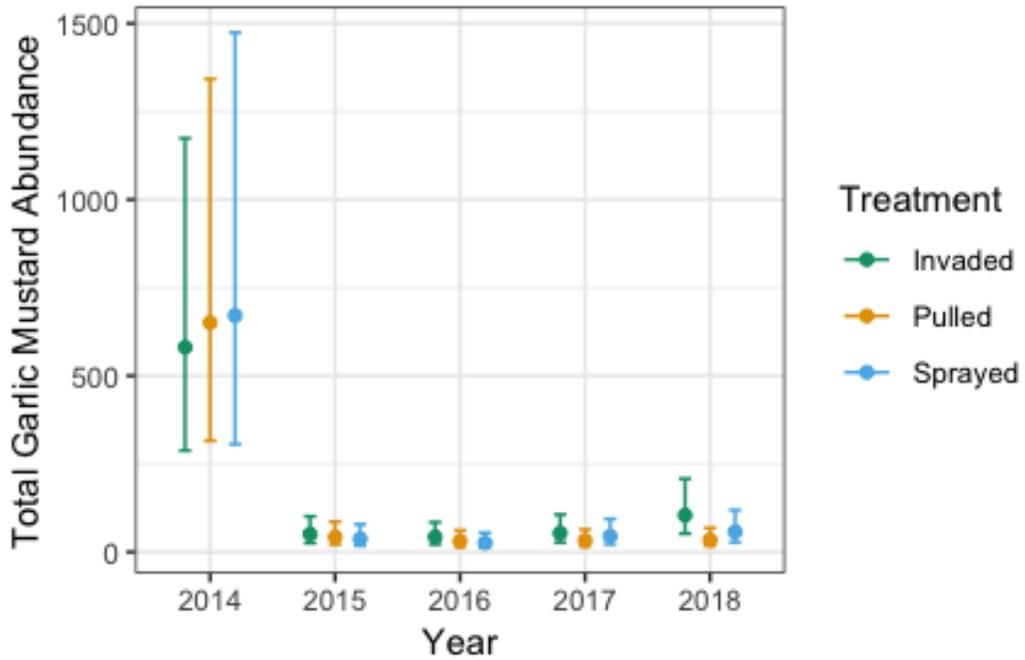


Figure 5. Total garlic mustard abundance as a function of year and garlic mustard treatment. Abundance represents density per 4m<sup>2</sup>. Error bars indicate 95% confidence intervals.

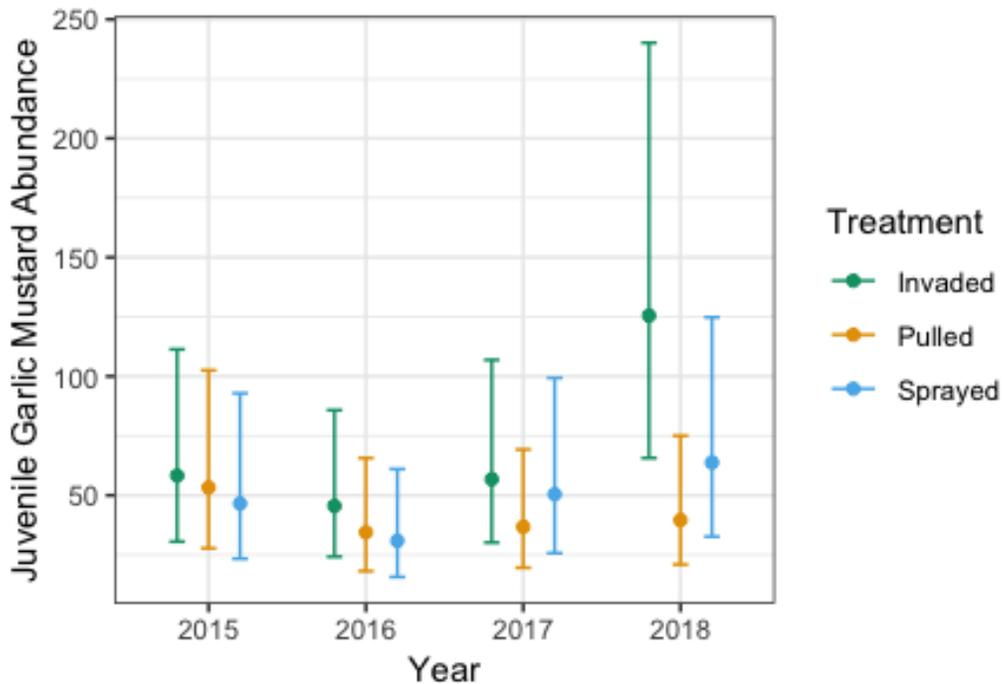


Figure 6. Juvenile garlic mustard abundance post-eradication (2015-2018) as a function of year and garlic mustard treatment. Abundance represents density per 4m<sup>2</sup>. Error bars indicate 95% confidence intervals.

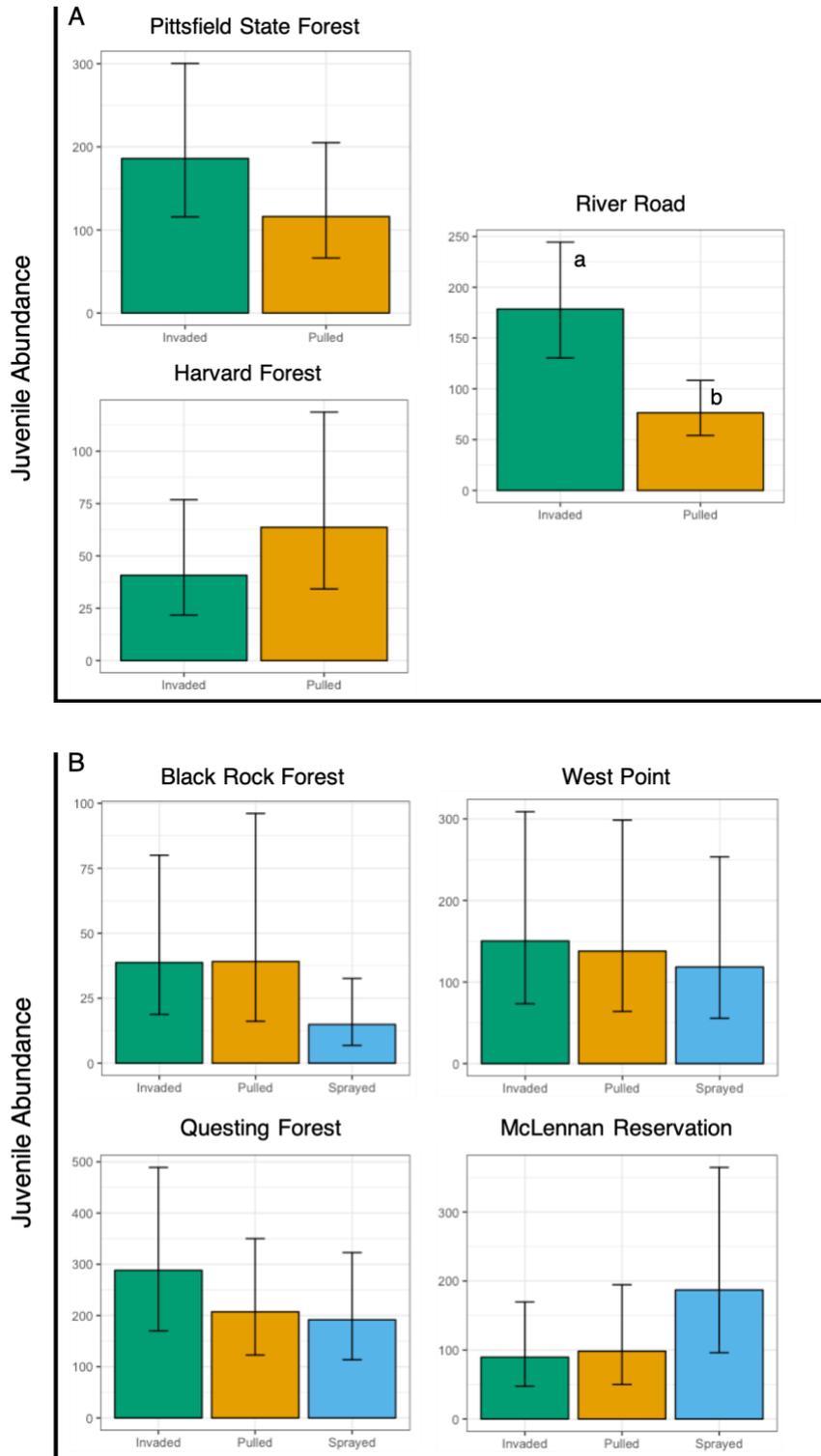


Figure 7. Juvenile garlic mustard abundance as a function of treatment by site in sites with just pulling (A) and sites with both spraying and pulling (B). Abundance represents density per 4m<sup>2</sup>, averaged across all years. Error bars indicate 95% confidence intervals of the mean.

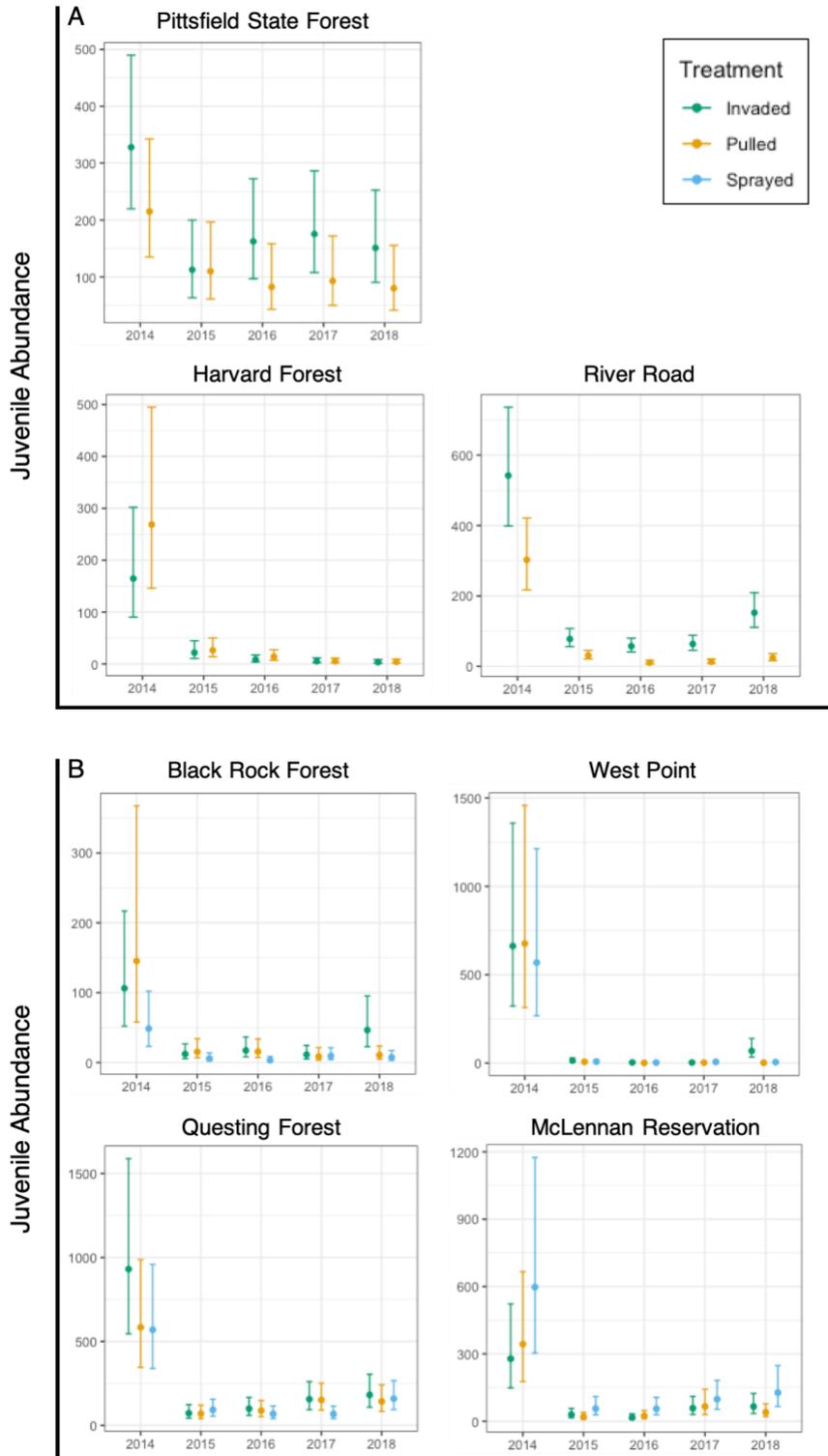


Figure 8. Juvenile garlic mustard abundance as a function of treatment and year by site in sites with just pulling (A) and sites with both spraying and pulling (B). Abundance represents density per 4m<sup>2</sup>, averaged across all years. Error bars indicate 95% confidence intervals of the mean.

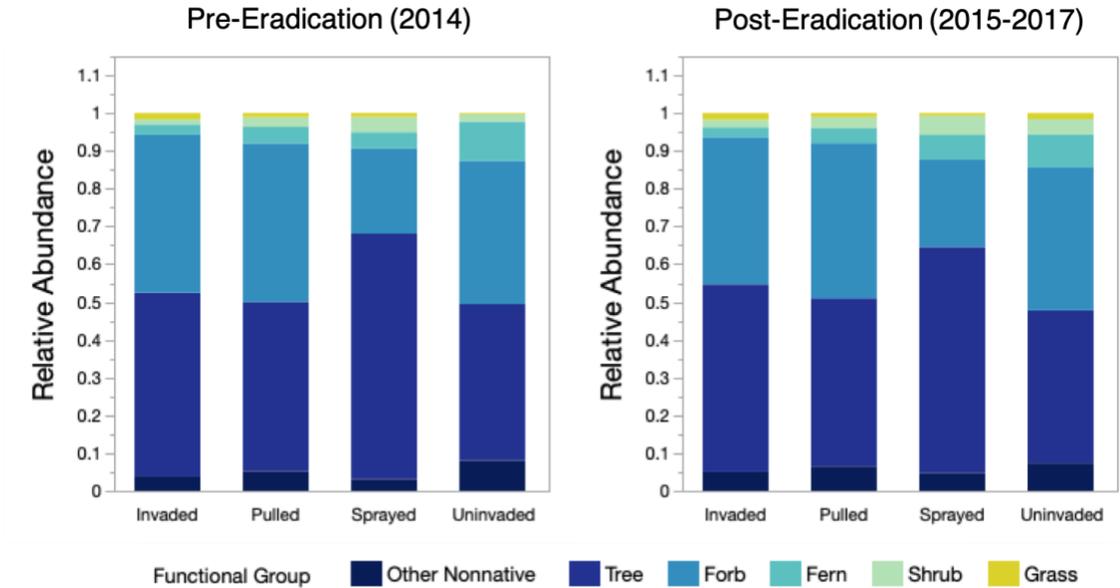


Figure 9. Relative abundance of functional groups by garlic mustard treatment, pre-eradication (2014) and post-eradication (2015-2017, averaged). The tree functional group includes only tree seedlings under 1 m in height. The “other nonnative” group includes all non-natives species aside from garlic mustard. Garlic mustard was excluded here.

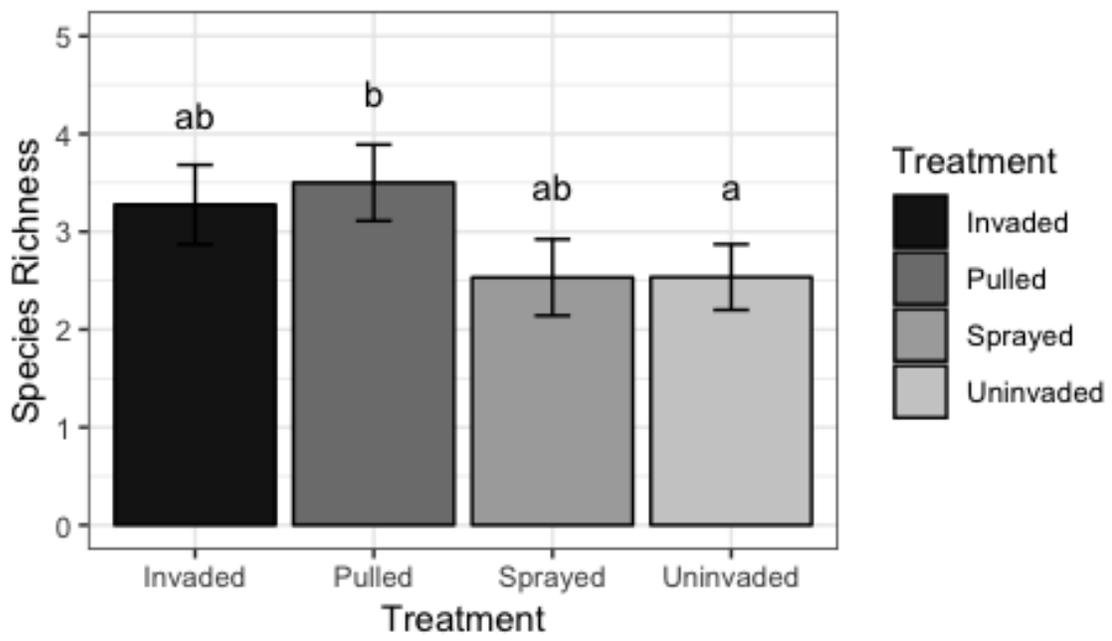


Figure 10. Forb species richness ( $S$ ) as a function of garlic mustard treatment, averaged across all years. Error bars indicate 95% confidence intervals.

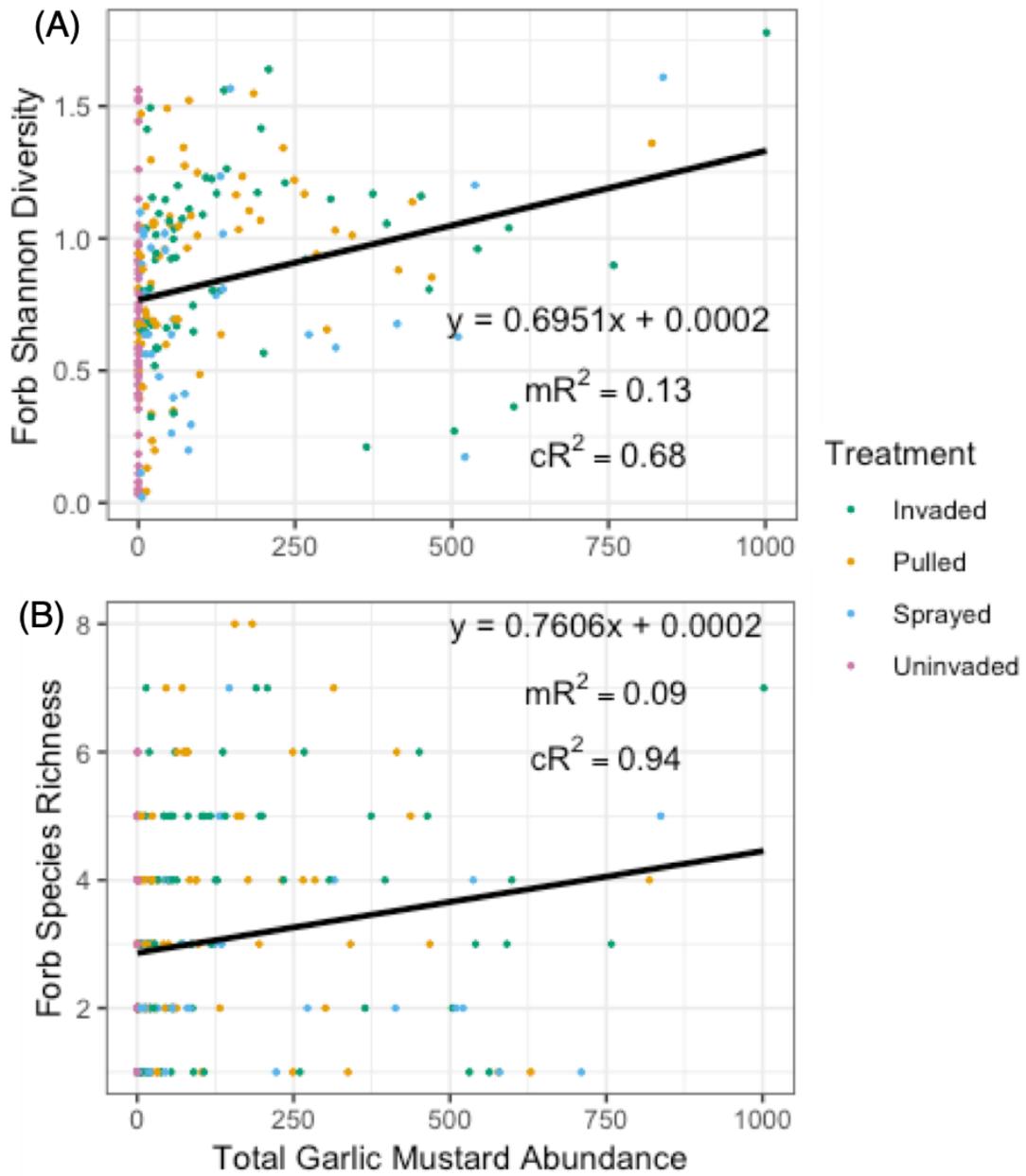


Figure 11. Forb Shannon diversity (A) and species richness ( $S$ ) (B) as a function of total garlic mustard abundance. Abundance represents density per  $4m^2$ .  $mR^2$  is the marginal R-squared value and  $cR^2$  is the conditional R-squared value.

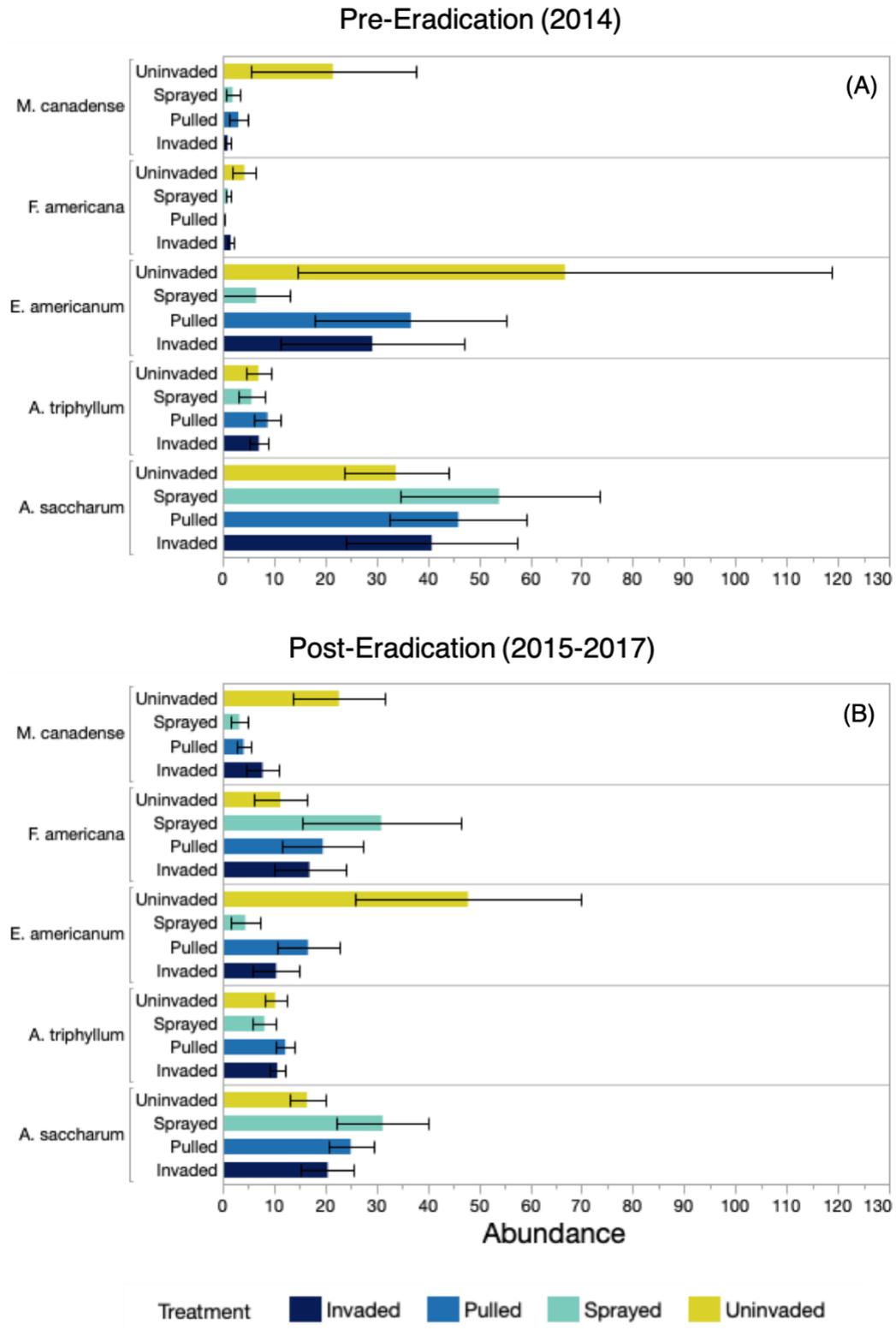


Figure 12. Average abundance of Top 5 most abundant species, pre-eradication (2014) and post-eradication (2015-2017, averaged). Error bars indicate  $\pm 1$  standard error of the mean

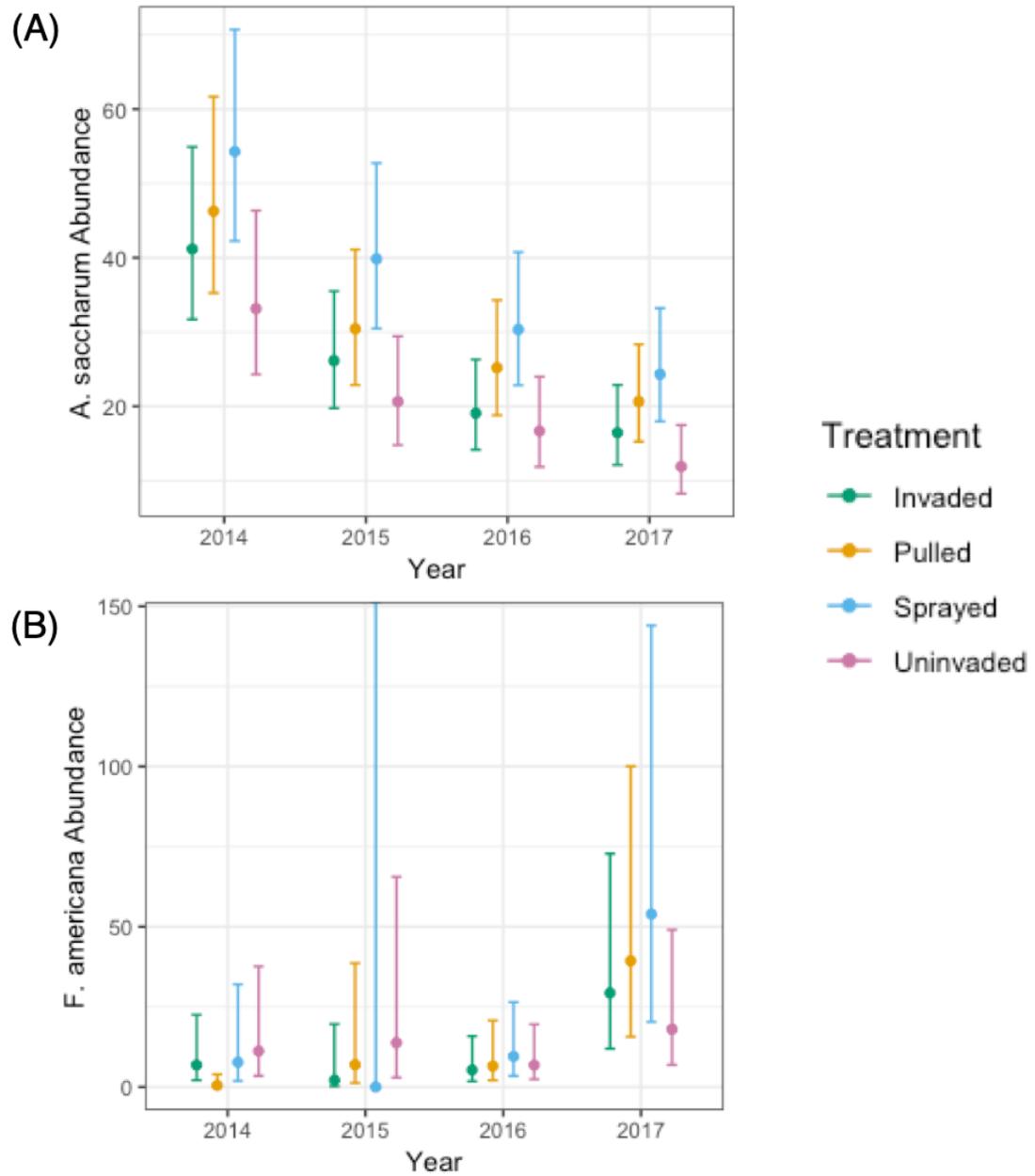


Figure 13. Sugar maple (*Acer saccharum*) (A) and white ash (*Fraxinus americana*) (B) abundance as a function of year and garlic mustard treatment. Abundance represents density per 4m<sup>2</sup>. Error bars indicate 95% confidence intervals of the mean.

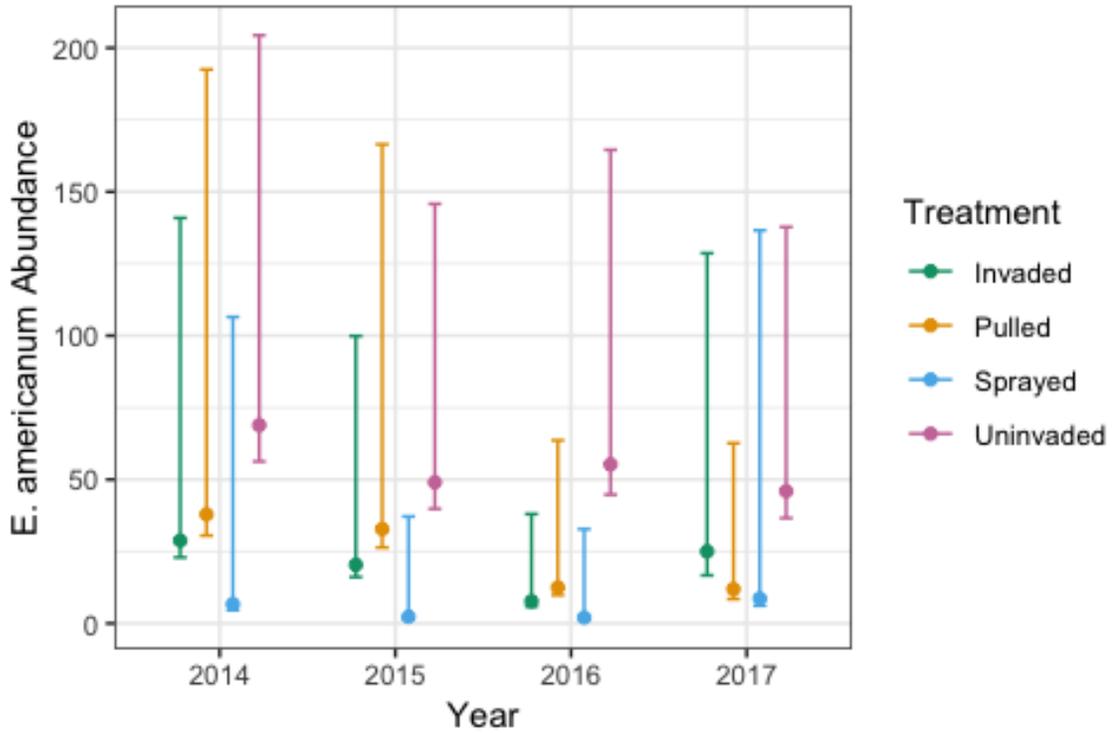


Figure 14. American trout lily (*Erythronium americanum*) abundance as a function of year and garlic mustard treatment. Abundance represents density per 4m<sup>2</sup>. Error bars indicate 95% confidence intervals of the mean.

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