THE ESTABLISHMENT, CONTROL, AND POST-CONTROL RESPONSE OF AMUR HONEYSUCKLE (*LONICERA MAACKII*)

by

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A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science

Department of Forestry and Natural Resources

West Lafayette, Indiana

August 2021

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This work is dedicated to all those whom I love and love me back, especially my father, who I miss more and more every day.

ACKNOWLEDGMENTS

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ABSTRACT

Amur honeysuckle (*Lonicera maackii*) is a shrub endemic to East Asia which has become invasive and nearly ubiquitous to the forest ecosystems of eastern North America. Through its extended growing season, competitive ability, and potential allelopathy, Amur honeysuckle alters native herbaceous-layer plant communities and inhibits the regeneration of native tree species. As such, it is representative of a range of invasive shrub species imported from East Asia. My thesis contributes to questions fundamental to the understanding this and other invasive shrubs: 1) How do species become invasive? 2) How can the invasions of the species be effectively controlled? 3) How does the ecosystem respond to treatment of the invasive species?

First, I examined the role of self-compatibility within Amur honeysuckle. I compared the berry production, seed production, and germination rates between closed-pollinated and open-pollinated flowering branches of Amur honeysuckle individuals across multiple types of invasions (heavy, light, and sprouting). I found that Amur honeysuckle not only possesses the ability to self-pollinate, but that it can produce viable self-pollinated seed sets. This ability may help explain how Amur honeysuckle able to invade isolated forest patches far removed from the main invasion.

Second, I evaluated the effectiveness of a novel herbicide adjuvant in reducing the amount of herbicide needed in the 'cut-stump' method of controlling Amur honeysuckle. Combining various concentrations of the most common herbicide in the world, glyphosate, with concentrations of cellulases derived from fungi, 2XL, I examined whether the cellulases improved the effectiveness of glyphosate, potentially by increasing glyphosate movement into the vascular tissue of Amur honeysuckle through the degradation of cell walls. While 2XL was not an effective adjuvant, glyphosate concentrations of less than half the recommended dosages were equally effective as higher concentrations in preventing sprouting of treated stumps. The ineffectiveness of 2XL may imply a need for protein-mitigated diffusion of glyphosate across cell walls and into the vascular tissue of the plant, which would be inhibited by the breakdown of cell walls.

Finally, I tested how deeper intensities of mulching-head treatments affected the sprouting response of Amur honeysuckle and the response of the herbaceous-layer plant community after treatment. I found a negative relationship between the volume of sprouting Amur honeysuckle and increasing depth of mulching-head treatment. Additionally, increasing mulching-head intensities were correlated with increased herbaceous layer diversity and conservation value as represented

by Floristic Quality Index. Mulching-head treatments are a promising tool in controlling heavy invasions of non-native shrubs.

CHAPTER 1.   INTRODUCTION

Invasive plant species are a global issue that harm the health of native ecosystems, disrupt ecosystem services and cost the global economy upwards of $120 billion annually, and their negative effects will likely accelerate in the future (Sakai et al. 2001, Pimentel et al. 2005, Oswalt et al. 2015). Invasive shrubs are a particularly serious threat to eastern forests in North America (Webster et al. 2006). Because Pleistocene glaciers did not advance as far south in East Asia compared to North America (Ehlers and Gibbard 2007), shrubs originating from East Asia did not experience thousands of years of shortened growing seasons and are better able to utilize shoulder seasons during the longer growing seasons of the Holocene (Fridley 2012). This contributed to the invasive of these shrub species when introduced to eastern North American forests (Ricklefs et al. 2007, Iannone et al. 2015, 2016). Controlling invasive shrubs can not only be expensive (Bailey et al. 2011), but it can also be time consuming. Between 1995 and 2004, Great Smoky Mountains National Park invested over 17,000 worker hours in the control of invasive woody plants (Webster et al. 2006). The need to prevent and control the spread of invasive shrubs is only growing.

Amur honeysuckle (*Lonicera maackii* [Rupr.] Herder, Caprifoliaceae) is the archetypal East Asian shrub that became invasive once introduced to forests in the eastern United States (Luken and Thieret 1995, 1996). Amur honeysuckle was brought to the United States for a myriad of reasons. Its copious flowering made the shrub a desired ornamental (Luken and Thieret 1995). Perhaps most responsible for Amur honeysuckle's widespread introduction was the USDA Soil Conservation Service which between 1960 and 1984 recommended using it for soil stabilization and as a food source for wildlife (Luken and Thieret 1996). While the species would prove not particularly effective for either purpose due to its shallow roots (Belcher and Donald W 1982, Frank et al. 2018a) and low-nutrition fruit (Ingold and Craycraft 1983), the species would prove excellent at escaping and expanding its range. Now nearly ubiquitous in forest ecosystems in the eastern United States, there is great need to understand and develop techniques to control the invasion of Amur honeysuckle, as well as other invasive shrub species.

Research into invasive plants typically tries to answer one of the following three questions: 1) How does the species become invasive (Bauer et al. 2012)? 2) How can the invasions of the species be effectively controlled (Bailey et al. 2011)? 3) How does the ecosystem respond to

treatment of the invasive species (Frank et al. 2018b)? My thesis, in part, answers each of these three questions in regard to Amur honeysuckle with three corresponding prepared manuscripts.

My first prepared manuscript (Chapter 2) examines the reproductive biology of Amur honeysuckle. Due to a discrepancy in the literature, it is unclear whether Amur honeysuckle possesses the ability self-pollinate and produce viable seeds (Goodell and Iler 2007, Barriball et al. 2014). Amur honeysuckle reaches the expansion of phase invasion more quickly than many other invasive woody plant species (Shigesada and Kawasaki 1997, Wangen and Webster 2006, Shields et al. 2014). Autogenous reproduction is known to be a common trait among weedy or invasive plant species (Baker 1974), but relatively uncommon among woody plant species (Duminil et al. 2009). Amur honeysuckle possessing the ability to self-pollinate and produce viable seed sets may shed light on how invasions from this species grow particularly quickly compared to other invasive woody plants.

The second prepared manuscript (Chapter 3) examines the effectiveness of Amur honeysuckle control with a novel herbicide adjuvant. There are many techniques to control invasive shrubs, and each have their own advantages and disadvantages (Bailey et al. 2011). More traditional treatment techniques include types of manual removal such as root wrenching, lopping, or root severing (Oneto et al. 2010). The use of fire through prescribed burns or propane burners has become increasingly common (Ward and Williams 2011, Mandle et al. 2011). Goats have even shown promise as a technique to biologically control invasive shrubs (Rathfon et al. 2021). While these techniques are often applied individually and in combination with each other, the most common form of treatment is chemical applications of herbicides (Webster et al. 2006). While effective, scientific (Busse et al. 2001, Tsui and Chu 2003, Gill et al. 2018) and public (Howle et al. 2010, Ma et al. 2018) concerns about the non-target effects of herbicides have been growing. The potential to reduce herbicide usage motivated us to evaluate the ability of a novel adjuvant to increase the efficacy of glyphosate, thus lowering the required concentration of the herbicide needed to control Amur honeysuckle. This adjuvant was derived from fungi and consists of a combination of cellulases that breakdown woody tissue. Conceptually, the cellulases should damage the integrity of cell defenses to the herbicide, allowing increased uptake of the herbicide.

The final prepared manuscript (Chapter 4) of my thesis follows up on a previous study (Frank et al. 2018b) by examining how intensity of mulching-head treatments affected the sprouting response of Amur honeysuckle and the response of herbaceous-layer species composition and

diversity. Mulching-heads have the ability to shatter the root collar of Amur honeysuckle individuals, which can prevent the stump from sprouting new stems after treatment. The deeper into the ground the mulching-head is applied, the more likely that the root collar is heavily damaged, and sprouting is reduced. However, a trade-off may exist in that the deeper intensities of treatment may disturb the soil and damage belowground tissue of forest perennials. In addition, soil disturbance is often related to the establishment of invasive plant species (Kulmatiski et al. 2006). Understanding the role intensity plays in the plant community response to mulching treatments can guide land managers in how to control Amur honeysuckle and other invasive shrubs while protecting or even promoting native plant diversity.

Understanding fundamental concepts of species invasion is critical to building broader understanding of what makes a species invasive and finding generality across systems in the long term. However, remaining grounded in application can provide critical information that allows land managers better conserve and protect native communities in the present day. My thesis makes contributions to both discussions, practical and conceptual, in each chapter to help better understand and control invasions of Amur honeysuckle.

CHAPTER 2. THE SELF-COMPATIBILITY OF AMUR HONEYSUCKLE (*LONICERA MAACKII*)

2.1 Abstract

Amur honeysuckle (*Lonicera maackii*) poses a dire threat to the health of forests throughout the eastern United States. While self-pollination has been identified as an important trait of invasive plant species, this trait is understudied, and Amur honeysuckle is anecdotally described as lacking this characteristic. To examine the ability of Amur honeysuckle to self-pollinate, we selected 171 individual shrubs distributed across nine sites. Each site was grouped into one of three invasion types: heavy, light, and sprouting (sites which a basal cutting treatment previously occurred, but Amur honeysuckle was allowed to reestablish). We compared the number of berries, seeds per berry, and seed germination rates of self- and cross-pollinated flowers by pairing branches covered with pollination bags prior to flower emergence with uncovered branches on the same individual shrub. Out of 171 individuals, 48 produced berries from self-pollination within pollination bags (28%), with 48% of bagged branches exhibiting some degree of necrosis or chlorosis due to increased temperature and humidity. Berries from self-pollination produced 1.5 ± 1.4 (mean $\pm$ 1 SD) seeds per berry, whereas berries resulting from cross-pollination produced 3.3 ± 1.5 seeds per berry. In a germination trial, 47.3% of self-pollinated seeds have germinated compared to 41.7% of cross-pollinated seeds. This study has shown that Amur honeysuckle can self-pollinate and set viable seed, providing the species with an important mechanism to increase population abundance during early stages of invasion.

2.2 Introduction

Plant invasions typically occur in three phases that mimic a logistic growth curve: establishment, expansion, and saturation (Shigesada and Kawasaki 1997). Most species invasions fail to advance beyond the establishment phase, a lag period in population growth that often lasts for decades (Wangen and Webster 2006). A new invasion moves into the expansion phase when founding individuals have reproduced enough to become self-perpetuating and exponential growth begins (Shigesada and Kawasaki 1997). Once the expansion phase is reached, it becomes much

more difficult control the spread of the invasion (Webster et al. 2006). Thus, many have suggested that focusing on control during the establishment phase is key to preventing negative effects caused by the spread of invasive plants (Radosevich et al. 2003, Webster et al. 2006).

Many traits contribute to the innate invasiveness of a plant species (Baker 1974, Sutherland 2004). One such trait common among many invasive plants is the ability to self-pollinate (Baker 1974, Barrett et al. 2008). Despite describing different events in the process, the terms self-pollination, self-compatibility, self-fertilization, and selfing are used interchangeably to describe this ability of plants to reproduce autogenously without the need for conspecific pollen. In an early assessment of the potential role of self-pollination in plant invasions, Baker (1955) stated that "With self-compatible individuals, a single propagule is sufficient to start a sexually-reproducing [sic] colony (after long-distance dispersal), making its establishment much more likely than if the chance of two self-incompatible yet cross-compatible individuals sufficiently close together spatially and temporally is required." Originally dubbed "Baker's Law" by Stebbins (1957), the concept that invasive plants more often possess this feature has been corroborated, but needs more rigorous testing (Barrett 1996, Van Kleunen and Johnson 2007).

More recent studies have examined Baker's Rule across a range of invasive species (Barrett et al. 2008). An analysis of 361 plant species native to Europe, but invasive in North America, found that self-compatible species had larger introduced ranges than species that are not self-compatible, leading the authors to recommend the screening of non-native species for self-pollination before allowing their import (Van Kleunen and Johnson 2007). An analysis of 11 invasive plant species in South Africa once again found support for Baker's Law, in that uniparental reproduction led to successful establishment and persistence (Thorne 2015). While evidence from multiple studies have supported Baker's Law, not all studies are unanimously supportive. An analysis of 19,960 plant species by Sutherland (2004) revealed that, while self-compatibility is more common in invasive plants compared to non-invasive plants, the author attributed the relationship to weeds being more likely to be annuals or biennials and those groups being more likely to be self-compatible. However, the datasets used in the study made an unclear distinction between non-native, invasive weeds and non-native, non-invasive weeds, and the author acknowledges this as a caveat that may have obscured relationships in the analysis (Sutherland 2004).

As a functional group, woody species have a tendency to be less self-compatible than non-woody species and experience more intense inbreeding depression compared to non-woody plant species (Duminil et al. 2009). However, studies have shown that that some invasive woody species do exhibit this trait. In an examination of 17 woody and non-woody invasive species in South Africa, Rambuda and Johnson (2004) found that all 13 woody species (trees, shrubs, and vines) examined displayed at least some self-compatibility (Rambuda and Johnson 2004). However, an in-depth study of a single invasive tree species, *Acacia dealbata*, that displayed self-compatibility found evidence of inbreeding depression in the form of fewer seeds per fruit, lower progeny survival, and decreased growth of offspring resulting from self-pollination, compared to those resulting from outcrossing (Rodger and Johnson 2013). Therefore, there may be a tradeoff between self-compatibility and fecundity.

Invasive woody plant species are particularly difficult and costly to control in the forests of eastern North America (Webster et al. 2006, Rathfon and Ruble 2007, Bailey et al. 2011). Amur honeysuckle (*Lonicera maackii* [Rupr.] Herder) is an archetypal example of an aggressive invasive shrub from East Asia (Luken and Thieret 1996). With its vigorous root growth, prolific sprouting, rapid maturation, high fecundity, and an extended leaf phenology, Amur honeysuckle can alter nutrient cycling (Schuster and Dukes 2017), and it may produce allelopathic chemicals (Bauer et al. 2012). These characteristics allow this species to suppress herbaceous-layer cover and species diversity (Hutchinson and Vankat 1997, Gorchov and Trisel 2003, Hartman and McCarthy 2008), and prevent the successful regeneration of overstory species (Hartman and McCarthy 2004, Shields et al. 2015b).

While much is known about the pattern and rate of invasion by Amur honeysuckle (Deering and Vankat 1999, Shields et al. 2014), its breeding system, which is a critical aspect of invasion biology, remains obscure in the literature. Specifically, there has been little study of whether Amur honeysuckle is self-compatible. While commonly described in the literature as being self-incompatible, these descriptions do not cite any primary literature. Examinations of selfing in Amur honeysuckle are limited; our review of the literature revealed two studies. Goodell and Iler (2007) examined selfing in Amur honeysuckle by isolating flowers with pollination bags. They detected a low, but non-zero (<3% of bagged flowers produced a berry) rate of self-pollination occurring in bagged flowers. They also manually supplied bagged flowers with pollen from neighboring flowers which increased the production of berries and seeds, but they produced far

fewer berries and seeds than open-pollinated flowers. While Goodell and Iler (2007) found clear evidence that honeysuckle possesses the ability to self-pollinate, the scale of the study was limited to just 24 individuals across three sites and the statistical power to isolate the effect of each site was limited.

Barriball et al. (2014) used genetic parentage analysis to compare outcrossing rates and mating structure of individuals on the edge woodlots to those in the interior. While their study was focused primarily on pollinator community behavior, their genetic tests revealed low, but varying, rates of self-pollination in this population (5.4% ± 3.4% on the edge and 6.7% ± 6.6% in the interior). Once again, this study was limited by sample size (36 individuals were sampled), and it only utilized a single site. Addressing the variability in the rate of self-pollination, the authors speculated that honeysuckle might have the ability to throttle its abortion mechanism of self-pollinated flowers or seeds, depending on access to conspecific pollen. Both of these studies found evidence the Amur honeysuckle possesses the ability to self-pollinate, but both these studies downplayed the role this trait may play in the invasiveness of the species (Goodell and Iler 2007, Barriball et al. 2014).

Amur honeysuckle invasions can remain in the establishment phase for up to 15 years before progressing to the expansion and then saturation phases (Shigesada and Kawasaki 1997, Shields et al. 2014). Barriball et al. (2014) found a higher rate of selfing in the interior of the forest compared to the edge where access to heterospecific pollen was higher. While the difference was not examined statistically, it is possible that the rate of selfing differs between invasion intensities and stages. Age and size of individuals, and access to heterospecific pollen, could all contribute to the rate of selfing in Amur honeysuckle. Self-compatibility helps advance an invasion from the establishment phase to the expansion phase (Baker 1974), so within younger, less-dense Amur honeysuckle invasions – which would have less access to heterospecific pollen due to lower densities of plants – selfing may occur at a greater rate to speed establishment. In well-established, heavy invasions, abundant access to heterospecific pollen promotes rapid population growth and the development of a genetically diverse population. Selfing may not only be an important mechanism in low-density invasions early in the establishment phase but may also be an important mechanism of establishment in populations that sprout back after mechanical treatment. These sprouting "re-invasions" or populations would consist of individuals in similar life history stages as heavy invasions but have lower densities of reproductive individuals, and thus may have less

access to heterospecific pollen, similar to light invasions. All three invasion types could have similar rates of self-pollination, which would indicate that the trait is immutable within the species and does not respond to different population densities or developmental stages.

The objectives of our study were two-fold: (1) determine the rate of self-pollination in Amur honeysuckle and how seed production and viability differ between self- and open-pollinated plants, and (2) determine if the rates of self-pollination, seed production, and seed viability differ between invasions of different population density and developmental stage. To address these objectives, we established nine replicate sites evenly distributed across three types of Amur honeysuckle invasion varying in density and developmental stage (heavy density, light density, and sprouting after mechanical treatment). Pollination bags covered a branch on each individual plant for closed-pollination to occur with another branch left unbagged for open-pollination. Berries were collected and the seeds were used in a germination trial. We predicted that bagged branches (closed-pollinated) would produce a lower number of berries and seeds compared to open-pollinated branches, but that their germination rates would not differ. We also predicted that the rate of selfing would be inversely proportional to the availability on conspecific pollen; the light invasion sites would see highest rates of selfing, followed by sites where post-treatment sprouting occurred, with the lowest rate of self-pollination occurring in the heavy invasion sites.

2.3   Methods

We established nine total replicates between Martell Forest (40.43231700591411 N, -87.03881814433798 W) and the Richard G. Lugar Forestry Farm (40.42903302540403 N, -86.95382160836958 W; henceforth referred to as 'Lugar Farm') in north-central Indiana, near West Lafayette (minimum of 4.8 km apart). The sites were evenly distributed into one of three types of Amur honeysuckle invasion based upon visual inspection: heavy invasion, light invasion, and sprouting invasion. The heavy and light classifications were assigned based on the size and abundance of Amur honeysuckle individuals. We classified sprouting invasions as areas that had previously been naturally invaded by Amur honeysuckle, all shrubs were cut, but the site did not receive subsequent cutting treatments and individuals were allowed to sprout. All heavy invasions were at the Lugar Farm (minimum of 0.5 km apart) and all light invasions were at the Martell Forest (minimum of 0.9 km apart). Two sprouting invasions were at the Lugar Farm (located 0.4

km from nearest heavy invasion treatment and 0.2 km apart) and one was at the Martell Forest (0.7 km from nearest light invasion treatment). At eight out of nine sites, we haphazardly selected 20 individuals with basal diameters (measured at root collar) greater than 2 cm and with at least two branches displaying 20 or more floral buds. For each shrub, we recorded basal diameter and distance to the nearest other Amur honeysuckle individual displaying floral buds (Table 2.1). The sprouting invasion site at the Martell Forest only contained 11 individuals that met our criteria. To confirm our pervious subjective assessments of the sites, we performed an ANOVA ($\alpha = 0.05$) on the basal diameters ($F_{8,162} = 18.54$, $p < 0.001$) and distance to nearest conspecific neighbor ($F_{8,162} = 7.78$, $p < 0.001$) between types of invasions; both variables being log-transformed for the analyses. Groupings were determined using a TukeyHSD test ($\alpha = 0.05$; Table 2.1). Our tests generally confirmed our previous categorizations of the sites with heavy invasion sites consisting of larger individuals growing more closely together.

Table 2.1. Mean and standard deviation of basal diameter and distance to nearest mature individual for light, sprouting, and heavy-invasion sites at Martell Forest and Lugar Farm. Each site contained 20 Amur honeysuckle individuals, except for the sprouting invasion at Martell Forest, which only contained 11 mature individuals. Superscripts represent groupings between invasion types within each variable and are the results of a TukeyHSD test ($\alpha = 0.05$) on the log-transformed variables.

Invasion Type	Distance (m)	Basal Diameter (cm)
Heavy	0.95 (0.72)[a]	12.91 (7.81)[a]
Light	2.31 (1.45)[b]	7.98 (3.83)[b]
Sprouting	1.84 (1.89)[c]	6.57 (3.04)[b]

In early May of 2020, prior to anthesis, we fastened a pollination bag (PBS International; Bag Type PBS 10-1) to one of the pre-selected branches that displayed abundant numbers of floral buds (Figure 2.1A). Normally used in breeding trials, these bags are specifically designed to allow the transfer of air and sunlight but exclude pollen. This eliminated potential outcrossing by excluding both pollination from the wind and the primary mode of pollination: insects (McKinney and Goodell 2011). The open end of the bags was tightly wrapped with both a twist-tie and a zip-tie around a layer of non-absorbent cotton wrapped around the branch inside the bag, thus preventing damage to the stem and pollen entry.

We removed the pollination bags in early September of 2020. At this time, we counted the number berries on both the open- and closed-pollinated (bagged) branches for each Amur honeysuckle individual. Additionally, we counted the number of 'nodes' on each branch to standardize the extent of our measurements. A node was defined as the junction between two opposite leaves on a branch, where four flowers typically emerge. On closed-pollinated branches, we counted from the tip as far along the branch as the bag covered. For open-pollinated branches, we counted to an equivalent point on the branch. Both points were marked with a paint pen.

The bags were opened and counted prior to the berries being ready for collection due to a late summer heat wave that caused more bagged branches than expected to exhibit some level of chlorosis or necrosis (48%). Berries were left on the branches to fully develop. For closed-pollinated branches that produced berries, we placed thinner, mesh bags around the branch to prevent bird frugivory and collect any berries that dropped.

All berries were collected from closed-pollinated branches in late October, along with 10 berries from the paired open-pollinated branch on the same individual. If the corresponding open-pollinated branch did not contain 10 berries, then berries were collected from other open-pollinated branches on the same plant until we reached 10 berries, or none were left on the individual plant. We counted the number of seeds within each berry and prepared a germination trial in accordance with a published protocol for the species (Hidayati et al. 2000). Each branch's seeds were placed in their own petri dish, which was lined with filter paper and kept moist throughout the trial. The germination trial began on November 18th and lasted 20 weeks. We cold-stratified the seeds for 12 weeks at ~5-10 °C, with a12-h photoperiod. The germination period lasted 8 weeks with the same photoperiod and with daytime and nighttime temperatures of 20 and 10 °C, respectively. We treated seeds with a 10 mg l^{-1} solution of gibberellic acid (GA$_3$) at the beginning of both the

stratification period and the germination period to aid in the breaking of seed dormancy. To stem fungal growth within the petri dishes, we applied three treatments of a sulfur-based fungicide (Bonide®; Sulfur Plant Fungicide) as needed throughout the 20-week period. Each week after the seventh, we recorded the number of germinating seeds in each petri dish and removed the germinants. Germination was determined by the presence of a 'radical hook' extending from the seed coat (Figure 2.1B).

Figure 2.1. (A) A pollination bag (PBS International; Bag Type PBS 10-1) fastened to an Amur honeysuckle branch prior to anthesis. (B) Germinating Amur honeysuckle seeds with radical hooks extending from their seed coats.

2.3.1 Statistical Analyses

We constructed models for five dependent (response) variables using mixed-effects models in R ('lme4'): the number of berries resulting from open-pollination ("open-pollinated berries"), the number of berries from closed-pollination ("closed-pollinated berries"), bagged branch death, the number of seeds produced by a branch, and the proportion of seeds germinated in the germination trial. Negative binomial distributions were chosen for models containing the following count data (after checking over-dispersion, $\alpha = 0.05$): open-pollinated berries ($\hat{c} = 12.32$, $p < 0.001$), closed-pollinated berries ($\hat{c} = 2.47$, $p < 0.001$), and number of seeds ($\hat{c} = 2.14$, $p < 0.001$). Bagged branch death was a binomial metric and, thus, a logistic distribution was used (Appendix 2.A). The proportion of seeds germinated in the germination trial was also analyzed in a logistic distribution weighted by the number of seeds collected from the branch.

All models included the following biologically relevant explanatory independent variables: basal diameter, distance to nearest flowering conspecific neighbor, and invasion type. A relevant fixed-effect for the extent of each individual, which varied between dependent variables, was also included (e.g., number of berries collected was included to explain the number of seeds from a branch). Site was a random effect for the models explaining number of open-pollinated berries, closed-pollinated berries, and bagged branch death. Since we were only looking at individuals that displayed closed-pollination in the number of seeds and number of germinants models, we nested the Amur honeysuckle individual within site as a random effect to avoid pseudo-replication resulting from treating the open- and closed-pollinated branches separately. Branch type (closed- or open-pollinated) was included as a fixed effect in these models.

Model selection occurred by first creating a model with no interaction effects and then a model with all relevant interaction effects. Subsequent models with fewer interaction effects were included until we arrived at a model with the lowest Akaike information criterion (AIC). All final models were checked against a null model in log-likelihood test ($\alpha = 0.05$) for overall model significance, and all were significantly better predictors.

We performed a type II ANOVA ('car') on each model to determine which factors significantly predicted the response variable ($\alpha = 0.05$). Significant categorical variables were analyzed using pair-wise comparisons ($\alpha = 0.05$; 'emmeans'), while significant continuous variables had their predictions mapped ($\alpha = 0.05$, 'effects'). We displayed interaction effects using the 'interactions' package.

2.4   Results

Berries resulting from self-pollination developed on 48 of 171 (28%) closed-pollinated branches, despite 48% of bagged branches exhibiting at least some amount of chlorosis or necrosis. Open-pollinated branches generally produced more berries per node (open mean ± SD: 0.50 ± 0.43; closed mean ± SD: 0.01 ± 0.03) and more seeds per berry (open mean ± SD: 3.25 ± 1.54; closed mean ± SD: 0.01 ± 1.40), but both berry types germinated at similar rates (open: 41.7%; closed: 47.3%; Figure 2.2).
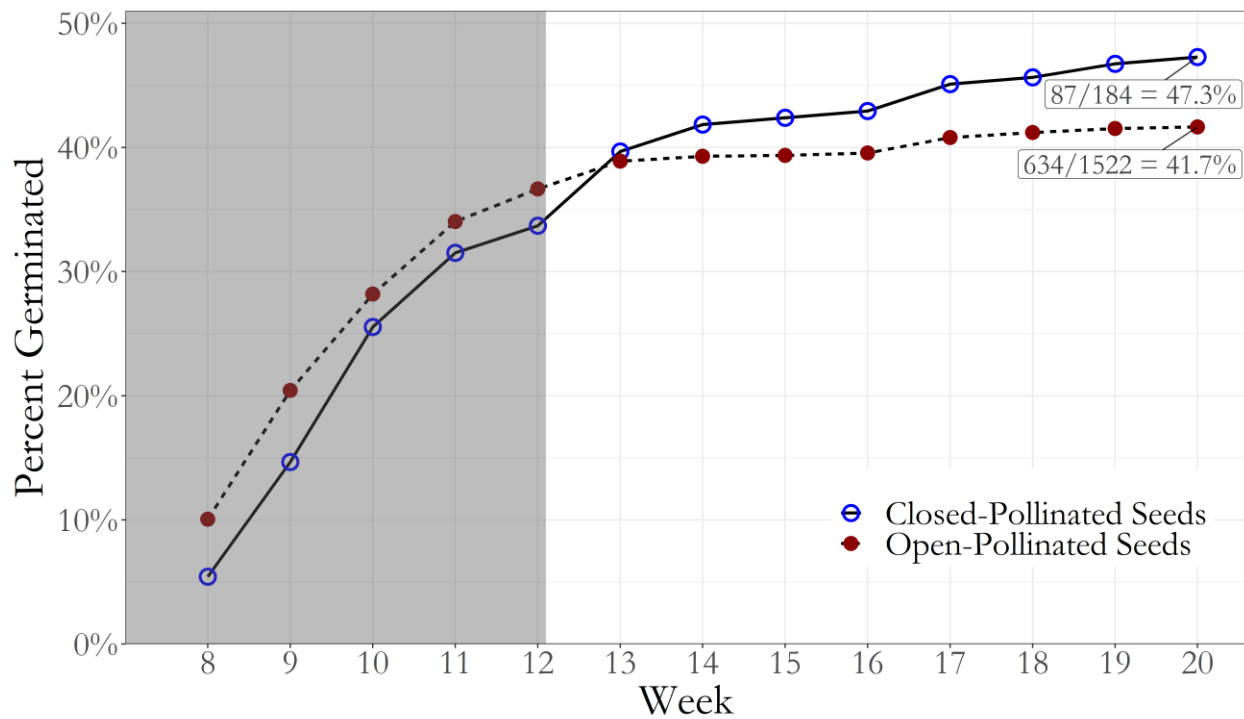
Figure 2.2. Percent germination of seeds from closed- and open-pollinated branches. The grey-shaded area represents germination during the stratification period.

2.4.1 Berries from Open-pollinated Branches

The model that best explained the number of berries from open-pollinated branches on an Amur honeysuckle individual consisted of eight fixed effects, four of which were interaction effects, in a negative binomial distribution (Table 2.2). We found the highest order, significant variable to be an interaction effect between an individual's basal diameter, distance to nearest conspecific individual, and invasion type (Figure 2.3).

Table 2.2. Variables that best explained the number of berries from open- and closed-pollinated branches on Amur honeysuckle individuals in a negative binomial model. Individual variable significance was determined by a type II ANOVA ($\alpha = 0.05$).

Variable	χ^2	df	p ($>\chi^2$)
Open-pollinated Berries			
Number of Open Nodes	2.367	1	0.124
Basal Diameter	0.018	1	0.894
Distance	1.258	1	0.262
Invasion Type	3.101	2	0.212
Basal Diameter : Distance	9.673	1	0.002*
Basal Diameter : Invasion Type	11.143	2	0.004*
Distance : Type	4.509	2	0.105
Basal Diameter : Distance : Invasion Type	10.867	2	0.004*
Closed-pollinated Berries			
Number of Closed Nodes	6.149	1	0.013*
Open-pollinated Berries per Node	7.677	1	0.006*
Bagged Branch Death	31.384	1	< 0.001*
Basal Diameter	5.711	1	0.017*
Distance	1.510	1	0.219
Invasion Type	8.304	2	0.016*
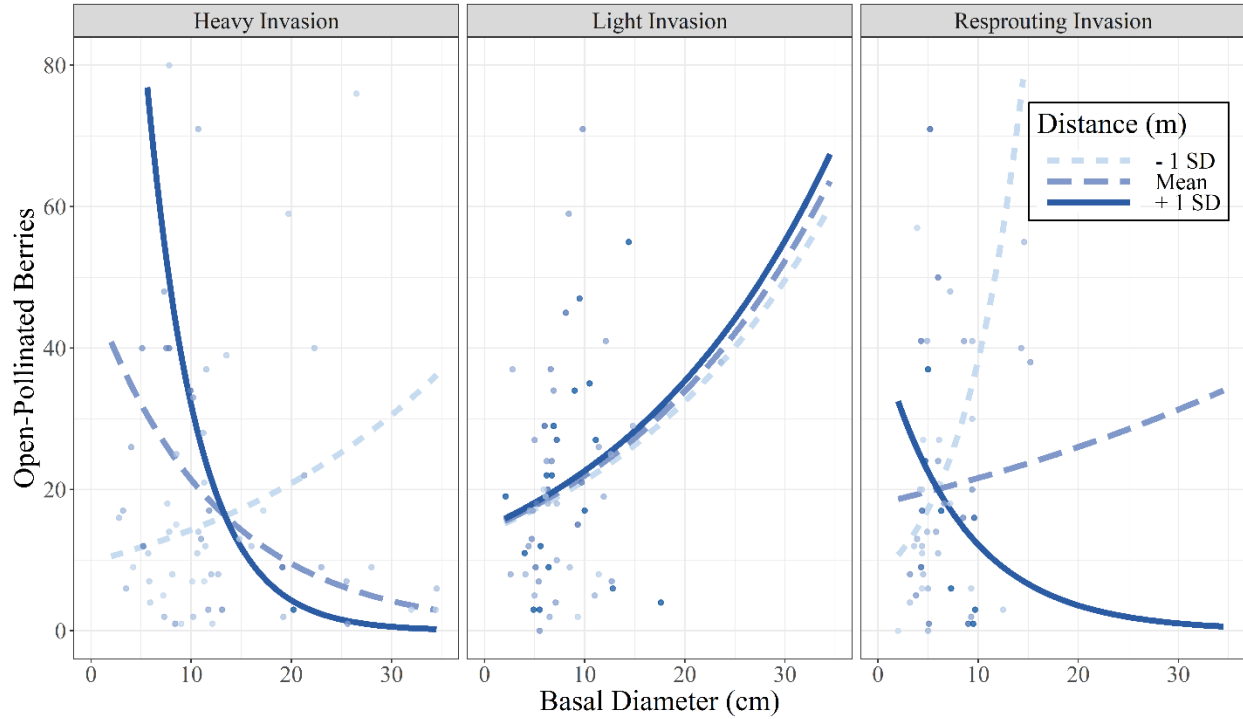
Figure 2.3. Relationships between basal diameter and the number of berries from open-pollinated branches across invasion sites. Different lines represent predictions from the best-fit model and how the relationships change across the distribution of the variable 'distance to nearest conspecific'. Points represent raw data.

2.4.2 Berries from Closed-pollinated Branches

The model we constructed which explained the number of berries from closed-pollinated branches on an Amur honeysuckle individual consisted of six fixed effects, none of which were interaction effects, in a negative binomial distribution (Table 2.2). The number of berries from open-pollinated branches per node predicted the number of berries on closed-pollinated branches in a positive relationship (Figure 2.4A). Interestingly, a larger basal diameter led to fewer berries on closed-pollinated branches in our model (Figure 2.4B). Heavy invasions produced significantly more berries from closed-pollination than light invasions, with sprouting invasions not differing from either (Figure 2.5A). Additionally, the number of closed-pollinated nodes, unsurprisingly, was positively correlated with the number of berries resulting from closed-pollination. As expected, branches exhibiting chlorosis or necrosis produced fewer berries than those that did not (Figure 2.5B).
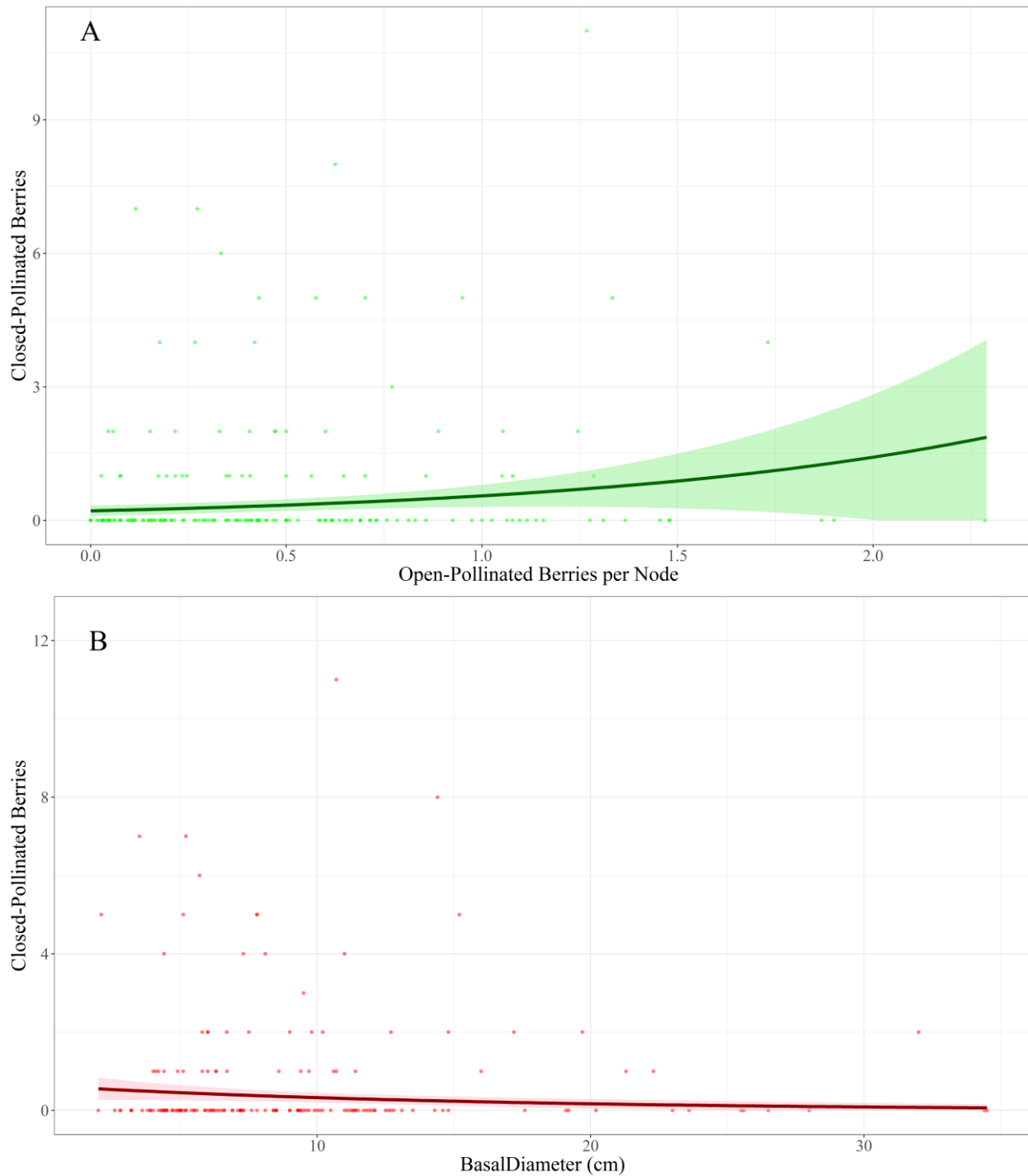
Figure 2.4. Predicted relationship of both the number of berries from open-pollinated branches per open node (A) and basal diameter (B) to the number of berries from closed-pollination, as determined by the best-fit negative binomial model. Points represent raw data.
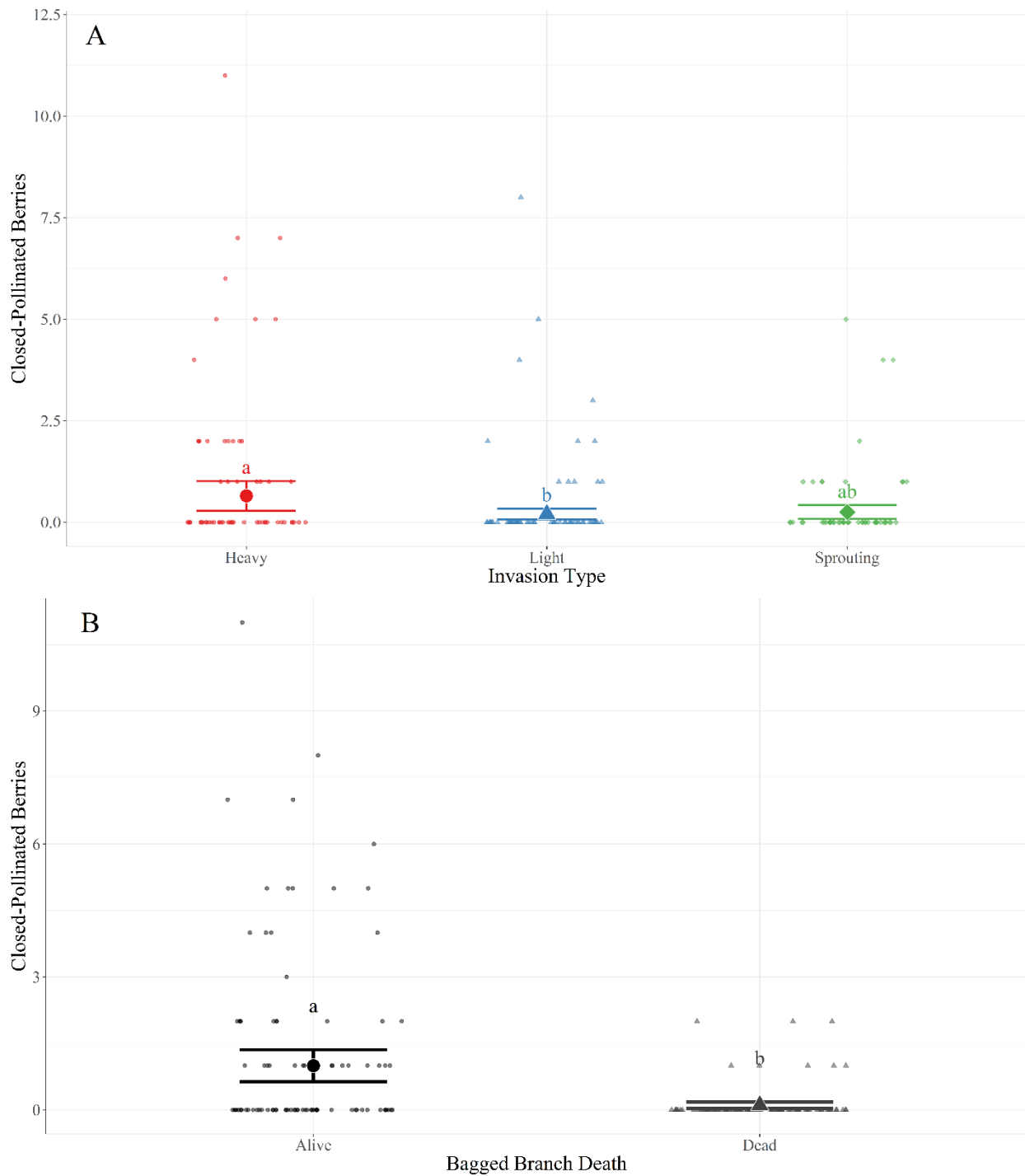
Figure 2.5. Predicted relationships type of invasion (A) and bagged branch death (B) to the number of berries resulting from closed-pollination, as determined by the best-fit negative binomial model. Significance was determined by a post-hoc pairwise comparison ($\alpha = 0.05$). Large points and error bars represent the predicted value and 95% confidence intervals. Smaller points represent raw data.

2.4.3   Number of Seeds

To predict the number of seeds from a closed-pollinated branch, we fit a negative binomial model with six fixed effects, one of which was an interaction effect (Table 2.3). Branch type was a significant variable within the model, where closed-pollinated branches produced much fewer seeds than open-pollinated branches (Figure 2.6A). Our model predicted that heavy invasions produced the most seed, followed by light invasions, and sprouting invasions produced the fewest seed (Figure 2.6B). The number of berries collected was a marginally significant variable and correlated positively with the number of seeds produced.

Table 2.3. The variables that best explained the number of seeds from a given Amur honeysuckle branch in a negative binomial model. Individual variable significance was determined by a type II ANOVA ($\alpha = 0.05$).

Variable	$\chi 2$	df	p ($>\chi 2$)
Number of Berries Collected	2.952	1	0.086
Branch Type	262.472	1	< 0.001*
Basal Diameter	0.146	1	0.702
Distance	0.007	1	0.935
Invasion Type	5.992	2	0.050*
Number of Berries Collected : Branch Type	2.195	1	0.138
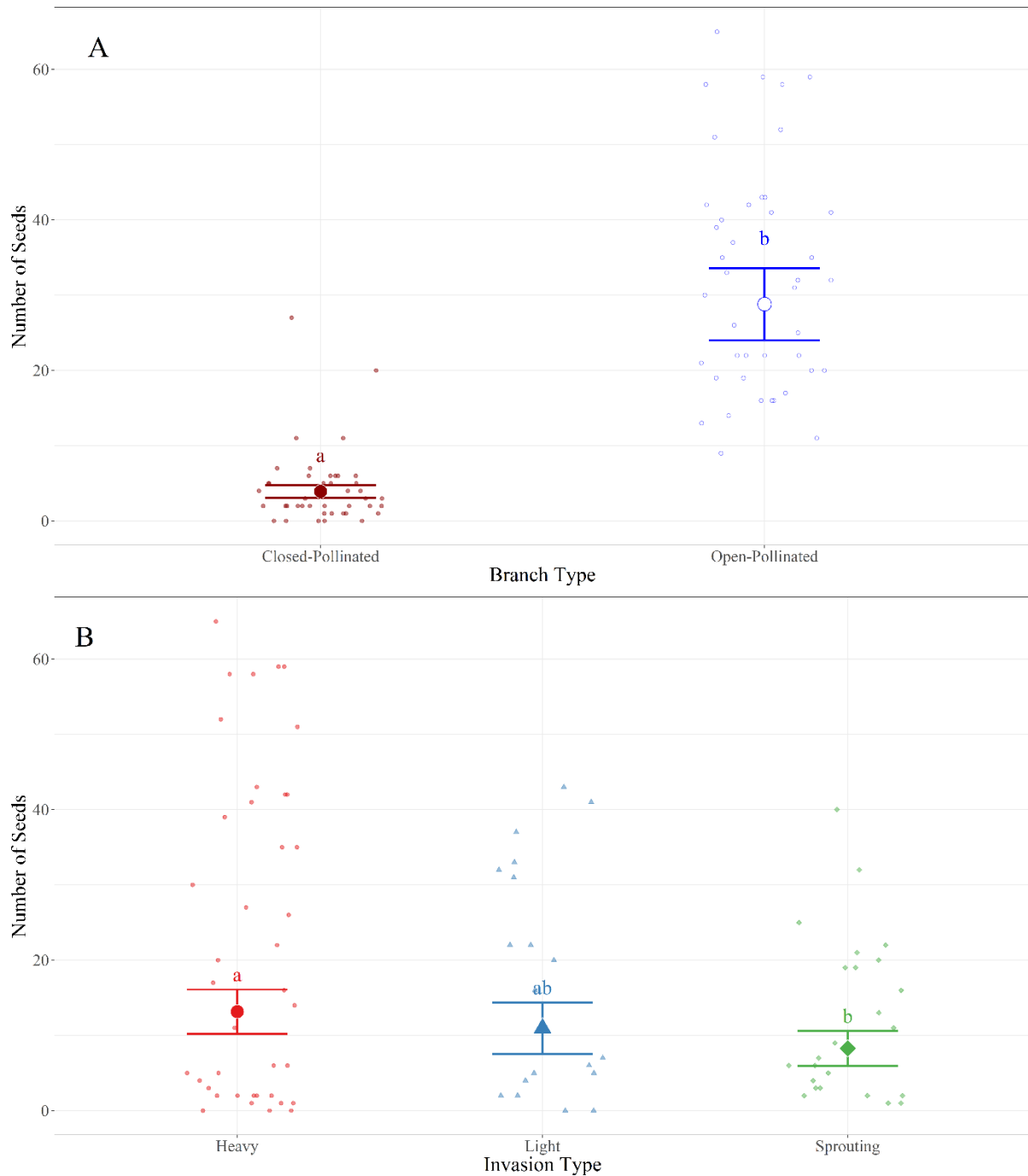
Figure 2.6. Predicted relationships branch type (A) and invasion type (B) to the number of seeds of both closed- and open- pollinated branches as determined by the best-fit negative binomial model. Significance was determined by a post-hoc pairwise comparison ($\alpha = 0.05$). Large points and error bars represent the predicted value and 95% confidence intervals. Smaller points represent raw data.

2.4.4 Proportion of Seeds Germinated

Our best-fit model to explain the number of germinants from each branch was a binomial distributed regression with seven variables, two of which were interaction effects (Table 2.4). The number of berries from each branch has a significant, positive relationship with the proportion of seeds germinated (Figure 2.7A). Branch type was significant alone and in an interaction effect with type of invasion (Figure 2.7B). Alone, basal diameter and distance to nearest conspecific individual were not significant. However, when the two variables were combined with the already significant variable branch type, the interaction effect was marginally significant, with greater distances displaying lower germination rates at smaller basal diameters, but greater germination rates at larger basal diameters.

Table 2.4. The variables that best explained the proportion of germinating seeds from a given Amur honeysuckle branch in a binomial model. Individual variable significance was determined by a type II ANOVA ($\alpha = 0.05$).

Variable	$\chi2$	df	p ($>\chi2$)
Number of Berries	3.860	1	0.049*
Branch Type	5.112	1	0.024*
Basal Diameter	1.536	1	0.215
Distance	2.025	1	0.155
Invasion Type	2.729	2	0.255
Branch Type : Invasion Type	44.758	2	< 0.001*
Basal Diameter : Distance	3.268	1	0.071
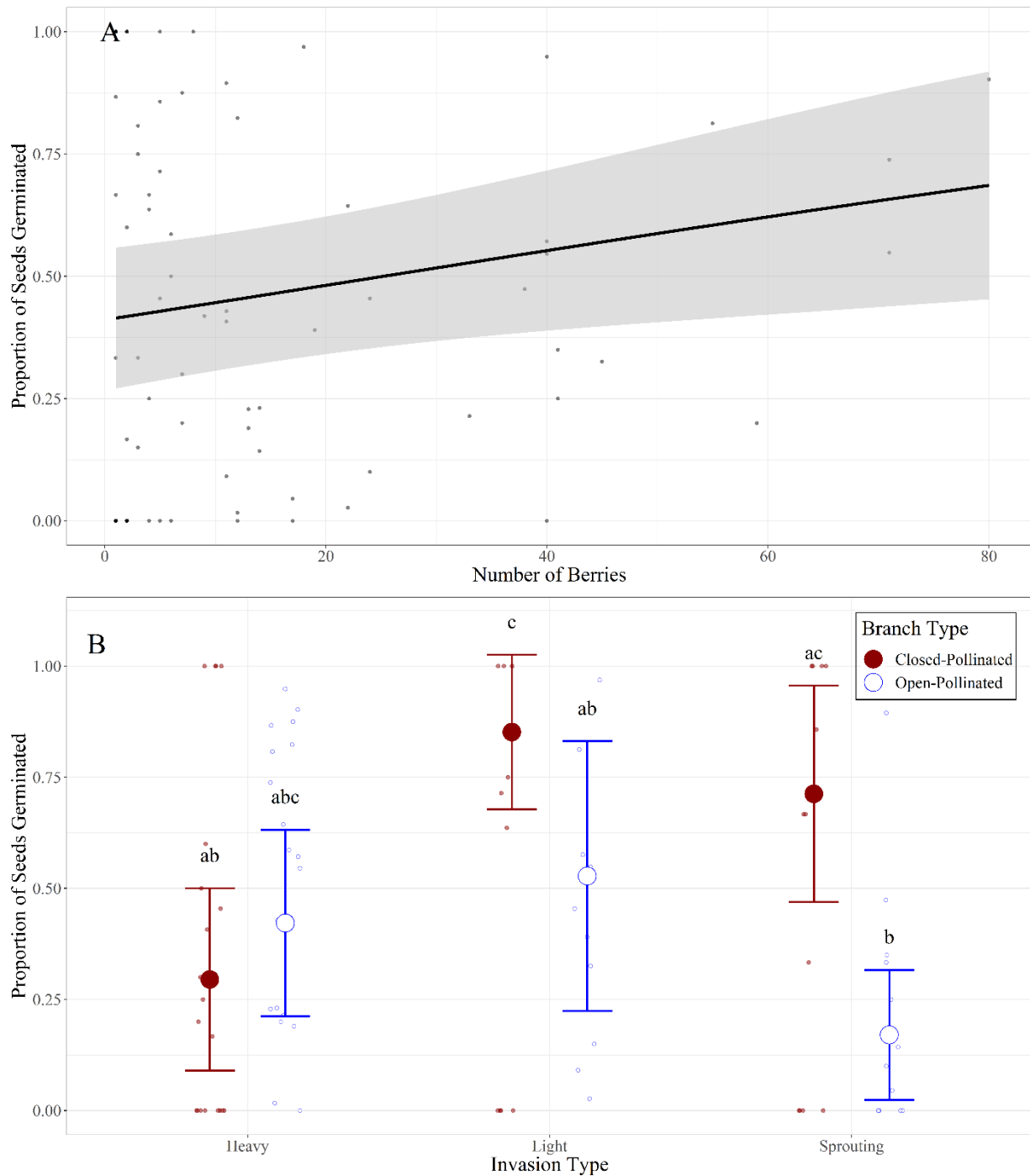
Figure 2.7. (A) Predicted interaction effect between branch type and total number of berries originally found on each branch, which significantly predicted the number of germinating seeds in the best-fit binomial model. Points represent raw data. (B) Predicted interaction effect between branch type and invasion type, which significantly predicted the number of germinating seeds in the best-fit binomial model. Significance was determined by a post-hoc pairwise comparison (α = 0.05). Large points and error bars represent the predicted value and 95% confidence intervals. Smaller points represent raw data.

2.5   Discussion

Our study provides conclusive evidence that Amur honeysuckle not only produces berries via self-pollination, but that those berries produce viable seeds. The most immediate implication of these results comes in the context of long-distance dispersal during the establishment phase of the invasion of a forest patch (Shigesada and Kawasaki 1997). The first, lone individual to become established in a new area would require the establishment of at least one other individual in order for the establishment phase to continue, if it does not possess the ability produce viable, self-pollinated seeds. With the ability to self-pollinate, a single long-distance dispersal event could establish its own population giving the invasion a greater likelihood of establishing and persisting until conspecific individuals can disperse to the same area and prevent inbreeding depression (Duminil et al. 2009). While information on the establishment phase of woody invasions is limited, Amur honeysuckle has a much shorter lag period (10-15 years; Shields et al. 2014) in the establishment phase compared to the invasive tree *Acer plantoanoides* (34 years; Wangen and Webster 2006). Species in the genus *Acer* rarely have the ability to self-pollinate (Sullivan 1983), so self-compatibility may be one reason that Amur honeysuckle had a shorter lag period. Our results show that a given bagged branch can produce a self-pollinated berry on roughly one in 80 flowers for those that did not experience any chlorosis or necrosis, which is slightly lower than reported in other similar studies (~3 in 100 flowers in Goodell and Iler 2007, ~1 in 25 flowers in Barriball et al. 2014). Given that a mature Amur honeysuckle individual can produce thousands of flowers in a given year (Goodell et al. 2010), and those seeds resulting from self-pollination germinate at similar rates to seeds from open-pollinated plants, we can conclude that Amur honeysuckle's ability to self-pollinate likely contributes to the species' ability to invade new ecosystems.

Our study demonstrated ample self-pollinated berry production, which provides insight into the factors controlling their production. Contrary to our predictions, individual plants further away from the nearest source of conspecific pollen – as measured by the distance to the nearest flowering conspecific individual – did not produce more self-pollinated berries. If distance is a good proxy of conspecific pollen availability, this lack of relationship indicates that the rate of self-pollination was not influenced by this factor, at least at the range of distances measured in our study.

Based on the positive relationships we observed between the number of closed-pollinated berries and the number of berries per node on the open-pollinated branches, as well as basal diameter, our results indicate that general reproductive capacity, perhaps based on carbohydrate availability, is the best predictor of the number of berries on both closed-pollinated (bagged) and open-pollinated branches. Individuals in heavy invasions were larger and likely older (Shields et al. 2014), leading to greater photosynthetic capacity, contributing to overall greater production of seed. While we observed similar rates of germination between closed- and open pollinated seeds, we did not grow the germinants into plants to assess fitness. If plants resulting from closed-pollination are less fit, smaller shrubs in light invasion areas may allocate less of a limited supply of carbohydrates into producing self-pollinated berries. Our data show that the importance of basal diameter changes in seed production. Branches in sprouting invasions produced the fewest number seeds. Carbohydrate availability being the strongest predictor of reproductive capacity can also explain this trend, as individuals in sprouting invasions are likely depleted after regenerating vegetative tissues following treatment (Richburg 2005).

However, unlike other studies examining self-compatibility in woody plant species (Duminil et al. 2009, Rodger and Johnson 2013), we found no evidence of inbreeding depression in the germination rates of Amur honeysuckle seeds. Overall, seeds resulting from self-pollination germinated at higher rates than open-pollinated seeds with the highest rates occurring in light and sprouting invasions (Figure 2.7A). However, inbreeding depression may occur at a later life stage such as the progeny due to self-fertilization displaying slower growth three months after sowing compared to the progeny which resulted from outcrossing even when germination rates between the groups were similar (Rodger and Johnson 2013).

When conspecific pollen is excluded, Amur honeysuckle individuals only produced self-pollinated seed on one out of every 80 flowers, but a single Amur honeysuckle individual is capable of producing thousands of flowers in a single year (McNeish and McEwan 2016). Our results indicate not only that Amur honeysuckle possesses the ability to produce viable, self-pollinated seeds, but that it occurs at rates that may augment the ability of the species to invade new, isolated forest patches. Birds are a major vector of Amur honeysuckle seeds (Ingold and Craycraft 1983), and this method of dispersal may result in a limited number of seeds reaching an isolated forest patch (Gosper et al. 2005). More research is needed to determine how plants produced from self-pollinated propagules perform over time, with respect to their physiology,

growth, and survival (Rambuda and Johnson 2004, Rodger and Johnson 2013), but it is possible that propagules resulting from self-pollination can reduce the lag time in the establishment phase of a species invasion (Shigesada and Kawasaki 1997). Moreover, if self-pollinated plants have established in a forest patch, when more distantly related individuals do eventually disperse to the area, there will be more flowers ready to receive and provide conspecific pollen. Thus, the species would be able to more rapidly reproduce, compared to scenarios where viable self-pollination is not possible. This trait has been consistently downplayed in the invasion biology of Amur honeysuckle (Goodell and Iler 2007, Barriball et al. 2014). However, our results suggest that self-pollination is an important trait that facilitates the early establishment of Amur honeysuckle and may reduce the duration of the lag period during early population growth. If this is the case, self-pollination should be more closely studied in other problematic invasive plants.

2.6   Appendix Bagged Branch Death

We fit a binomial model to determine which variables would predict a bagged branch exhibiting chlorosis or necrosis (Table 2.A.1). Larger basal diameters (Figure 2.A.1) and greater distances between conspecific individuals (Figure 2.A.2) both correlated with decreasing likelihoods that a bagged branch would exhibit this damage. Amur honeysuckle individuals in heavy invasions showed a lower likelihood of branch death than those in light invasions, with sprouting invasions again not differing significantly from either (Figure 13.A.3). Interestingly, the number of nodes in the bagged branches did not significantly predict bagged branch death, which was contrary to our prediction.

The true rate at which conspecific pollen-excluded Amur honeysuckle produces berries via self-pollination is likely higher than we observed in our study. Given the high number of bagged branches exhibiting chlorosis or necrosis and that those branches produced fewer berries (Figure 2.6A), suggest that the pollinator bags were having a negative impact on the health of the branches that they enveloped. Overcrowding within the pollinator bags, which was our initial assumption, does not seem to have been the driver of branch death, as the number of nodes within the bags did not predict branch death (Table 2.A.1). Small-stemmed individuals close to another conspecific in light or sprouting invasions were the most likely to show bagged-branch death. This indicates that overall plant stress is likely the driver behind bagged-branch death, with plants under those conditions having less tolerance and, thus, being unable support branches experiencing hotter, moister conditions inside the bags. In future similar studies, we recommend removing the pollination bags as soon as pollen shed ends.

Table 2.A.1 The variables that best explained bagged-branch death on Amur honeysuckle individuals in a binomial model. Individual variable significance was determined by a type II ANOVA (α = 0.05).

Variable	χ2	df	p (>χ2)
Number of Closed Nodes	2.664	1	0.103
Basal Diameter	6.324	1	0.012*
Distance	5.605	1	0.018*
Invasion Type	6.762	2	0.034*
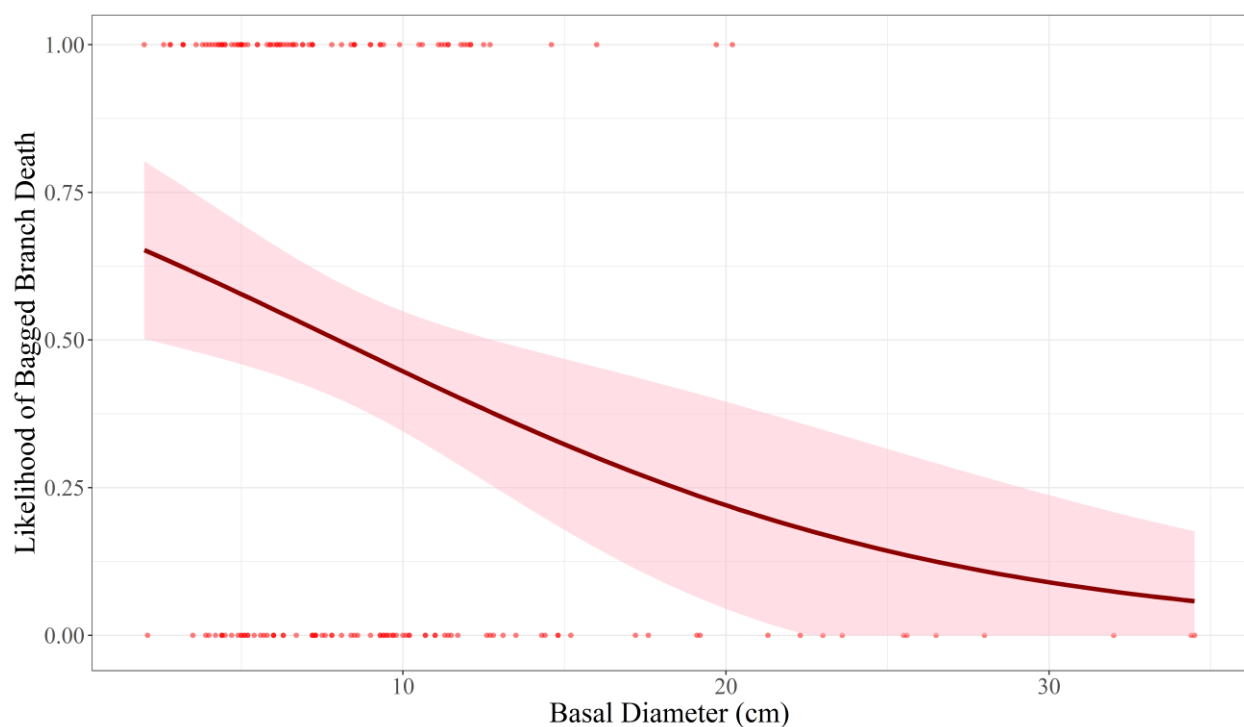
Figure 2.A.1. The predicted relationship between basal diameter of an Amur honeysuckle stem and the likelihood that a bagged branch exhibited chlorosis or necrosis as determined by the best-fit binomial model. Points represent raw data.
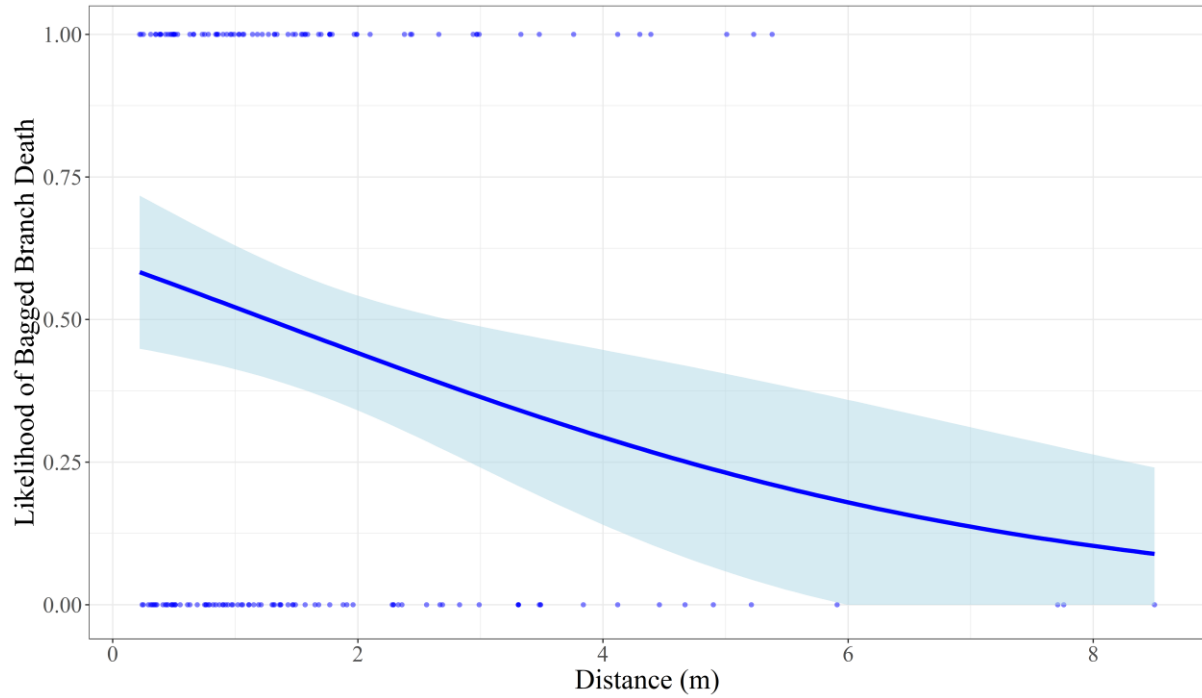
Figure 2.A.2. The predicted relationship between distance to nearest conspecific and the likelihood that a bagged branch exhibited chlorosis or necrosis as determined by the best-fit binomial model. Points represent raw data.
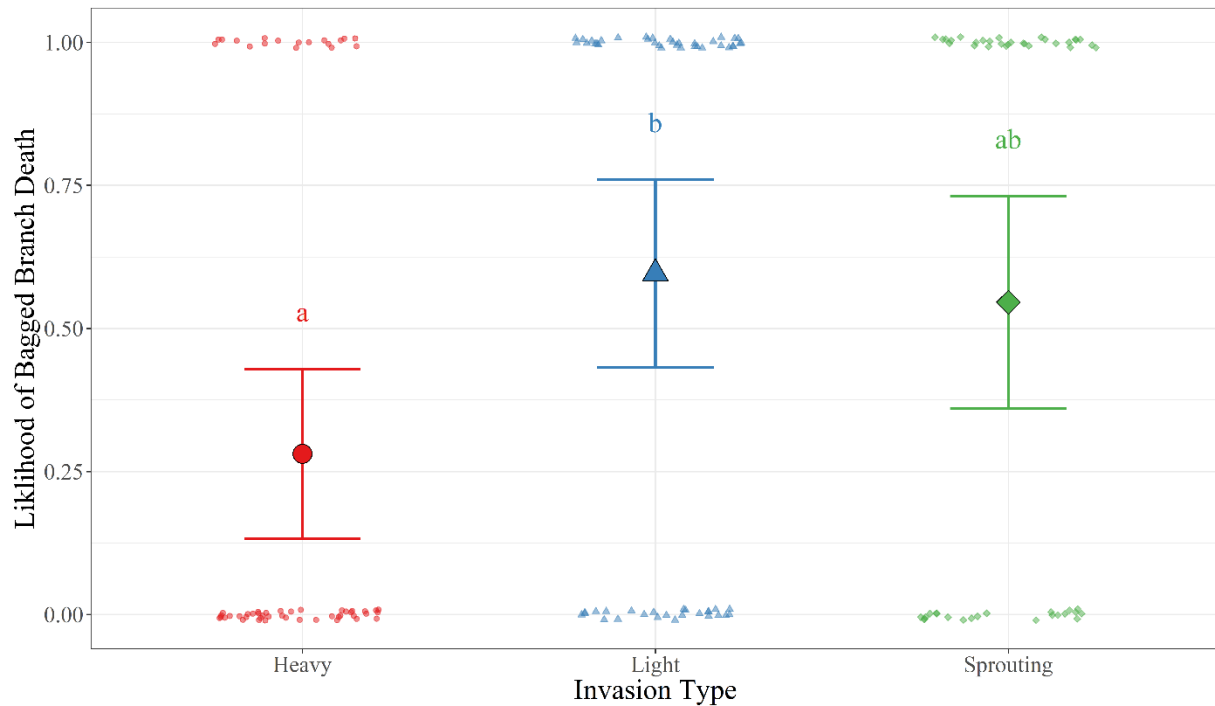
Figure 2.A.3. The predicted relationship between invasion type and the likelihood that a bagged branch exhibited chlorosis or necrosis as determined by the best-fit binomial model. Significance was determined by a post-hoc pairwise comparison ($\alpha = 0.05$). Large points and error bars represent the predicted value and 95% confidence intervals. Smaller points represent raw data.

CHAPTER 3.   THE EFFECT OF A NOVEL HERBICIDE ADJUVANT IN TREATING AMUR HONEYSUCKLE (*LONICERA MAACKII*)

3.1   Abstract

Invasive woody perennials pose a serious threat to the diversity and function of forests in the eastern United States. While herbicide treatments have proven effective in controlling invasions, there is concern about non-target environmental effects and potential health risks associated with widespread herbicide use. We evaluated '2XL' – a cellulase enzyme derived from fungi – as a potential herbicide adjuvant in order to increase the efficacy of glyphosate, allowing for the use of lower concentrations of herbicides in control efforts. To test whether the addition of 2XL would increase the efficacy of glyphosate on treated woody stumps, we conducted a controlled, field-based experiment using the 'cut-stump' method of treatment on a particularly problematic invasive shrub, Amur honeysuckle (*Lonicera maackii* [Rupr.] Herder). Given its ability to degrade a critical cell-wall component, we hypothesized that 2XL would affect glyphosate uptake by vascular tissue(s). In our experiment, we tested combinations of three concentrations of 2XL with five concentrations of glyphosate and predicted that low concentrations of glyphosate combined with 2XL would be as effective in limiting the sprouting of *L. maackii* as higher concentrations of glyphosate without the enzyme. A negative binomial mixed-effects model on the number of sprouting stems revealed that 2XL decreased the effectiveness of glyphosate. The number of sprouting stems predicted by this model showed a consistent pattern related to 2XL addition, leading to one to five more sprouting stems in all concentrations of glyphosate treatment. Moreover, a type III ANOVA revealed that neither the interaction between 2XL and glyphosate nor 2XL alone had a significant effect on the height of the tallest sprouting stem after treatment. All of these patterns were consistent within the same year as application of the treatment, as well as in the following year. These results suggest that the degradation of cell walls may have led to less herbicide being absorbed into the plant's vascular tissue. While 2XL may not be effective as an adjuvant, our study shows that intact cell walls may be important for herbicide adjuvants in controlling invasive shrubs. Our results also showed effective control of *L. maackii* at the lowest concentration of glyphosate tested, suggesting that concentrations lower than those typically applied can be used to control *L. maackii* with the cut-stump method.

3.2   Introduction

Invasive species are causing wide-spread degradation of various ecosystems and are threatening the survival of many native plant species (Richardson et al. 2000, Sakai et al. 2001, Brown et al. 2002, Oswalt et al. 2015). The increased abundance and distribution of invasive species is closely tied to a decrease in global biodiversity and reduced ecosystem function (Butchart et al. 2010). The estimated costs of controlling invasive species and the ecological damage they cause may be as high as $120 billion annually and will likely continue to rise (Pimentel et al. 2005). Invasive woody-plant species are particularly pernicious and difficult to control. For example, between 1994 and 2005, over 17,000 worker hours were spent controlling invasive woody plants in Great Smoky Mountains National Park (Webster et al. 2006).

Woody-plant species, specifically shrubs originating from East Asia, often possess traits that make them particularly well-suited to invade eastern North American forests (Ricklefs et al. 2007, Iannone et al. 2015, 2016). East Asian shrub species have an extended leaf phenology that results in a longer growing season than native plant species, allowing them to fix additional carbon and outcompete native competitors (Fridley 2012). Thus, East Asian shrubs are aggressive invaders of eastern North American forests and controlling their establishment and spread in these areas has become difficult and costly for land managers (Rathfon and Ruble 2007). Developing safer, more effective ways to control invasive shrubs is critical to the maintenance of biodiversity and ecosystem function (Webster et al. 2006).

One particularly problematic invasive shrub is Amur honeysuckle (*Lonicera maackii* [Rupr.] Herder). Its vigorous root growth, woody character, and prolific sprouting make the species particularly difficult to control (McNeish and McEwan 2016). *Lonicera maackii* was introduced intentionally to the United States for the first time in 1897 (Luken and Thieret 1996). After multiple subsequent importations, it quickly spread across the eastern hardwood forest due to rapid maturation (Deering and Vankat 1999), high fecundity (Lieurance 2004), and widely dispersed seeds (Gorchov et al. 2014). *Lonicera maackii* is known to exclude native species by limiting access to light, due to its dense canopy and the extended leaf phenology (McNeish and McEwan 2016). *Lonicera maackii* leaves emerge earlier in the spring and are retained longer into the fall than most native plants, which negatively affects the establishment and growth of herbaceous and woody competitors (Schulte et al. 2011). The species also has indirect effects on forest ecosystems

through altered nutrient cycling (Schuster and Dukes 2017), as well as the production of potentially allelopathic chemicals (Bauer et al. 2012).

A variety of treatment techniques are used to control invasions of *L. maackii* and other woody shrubs, but all are included in at least one of the following four categories: physical removal, biological control, fire, and chemical treatment. Physical removal includes manual techniques such as: root wrenching, lopping, or root severing (Oneto et al. 2010), as well as mechanical removal in the form of brush-cutting and mulching-head treatments (Frank et al. 2018b). These methods are environmentally safe and can be relatively inexpensive to apply, but may require more work hours than other treatments, and some manual techniques require access to specialized equipment (Oneto et al. 2010, Bailey et al. 2011, Frank et al. 2018b). Biological control is the least common form of woody-species control but is gaining acceptance as an alternative treatment as concerns about chemical treatments become more prevalent (Rosa García et al. 2012). Goat grazing has been examined as a means of biological control for invasive shrubs, and while concerns over specificity and access exist, this method shows potential for future use (Luginbuhl et al. 1999, Elias and Tischew 2016, Rathfon et al. 2021). Fire is a widely used method for controlling some invasive woody plant species (Mandle et al. 2011). While efficient (Ward and Williams 2011, Ward et al. 2013), this method has varying effectiveness across shrub species and may promote the establishment of some invasive plants (Ward and Williams 2011, Mandle et al. 2011, Rebbeck 2012, Ward et al. 2013, Guthrie et al. 2016).

Chemical application, often in combination with mechanical methods, is one of the most commonly applied treatments for invasive woody shrubs (Webster et al. 2006). Foliar sprays are quick and efficient but can lead to non-target effects on native species (Howle et al. 2010, Bailey et al. 2011, Ma et al. 2018). Basal applications, such as 'hack and squirt', girdling, basal bark applications, and stem injections are also commonly used for an array of species (Hartman and McCarthy 2004, Rathfon and Ruble 2007, Loh and Daehler 2008, Oneto et al. 2010). The 'cut-stump' method typically involves the use of clearing saws to cut a woody stem horizontally at its base, followed by an application of a systemic herbicide to the exposed surface (Luken and Mattimiro 1991). This technique combines the specificity of manual removal with the efficiency of chemical treatment. Although the cut-stump method is generally preferred for invasive shrub control, post-treatment sprouting and the ability of seeds to remain viable in the soil for many years

typically require follow-up treatments, which can be costly and time-consuming (Bailey et al. 2011).

Glyphosate is the active ingredient in many herbicide formulations used in invasive shrub control and is the most commonly used herbicide in the world (Tarazona et al. 2017). It is generally regarded as safe, when compared to other herbicides (Baylis 2000). Despite its popularity, the use of glyphosate is not without its concerns. The application of glyphosate and herbicide formulations containing it have been shown to unintentionally harm soil microbes (Busse et al. 2001) and poses an increased risk of non-target effects in aquatic ecosystems (Tsui and Chu 2003), as well as to wildlife (Gill et al. 2018). Additionally, the International Agency for Research on Cancer (IARC) recently classified glyphosate as a probable carcinogen to humans (International Agency for Research on Cancer 2015). While the EPA has disagreed with this position, the scope of the EPA's review was limited to dietary ingestion of pure glyphosate and did not include mixtures with surfactants, which seem to influence glyphosate's mobility into the human body (Benbrook 2020). Additional studies have shown glyphosate may linger in ecosystems longer than previously reported and that its major metabolite (aminomethylphosphonic acid) may also pose toxicological risks to humans (Bai and Ogbourne 2016). Whether glyphosate poses an actual risk to the health of ecosystems or humans, the public has become increasingly wary of the use of herbicides in general. For example, since the IARC's designation of glyphosate as a probable carcinogen, three lawsuits have been filed in the United States concerning the connection between glyphosate and Non-Hodgkin Lymphoma with an average award of $47.6 million (Benbrook 2020). Additionally, landowners in the United States have become increasingly concerned about the non-target effects of herbicides (Ma et al. 2018), specifically glyphosate (Howle et al. 2010). Similar concerns have led to bans on glyphosate in many parts of Europe (Kudsk and Mathiassen 2020).

Finding ways to get the public, specifically landowners, to understand the importance of controlling invasive species is critical to protecting native species. One approach to reduce concerns about the use of herbicides is to develop ways to effectively treat invasive species with lower concentrations of herbicides. The use of chemical adjuvants may provide an approach that maintains efficacy with a lower concentration of herbicide. One potential adjuvant, '2XL' (1,4-[1,3:1,4]-β-D-Glucan 4-glucanohydrolase), is a commercially available cocktail of cellulases that were originally derived from the fungus *Aspergillus niger*. While similar enzymes have been suggested for use in the degradation of woody plants for bioethanol productions (Scharf and

Boucias 2010), cellulases may have a wide range of applications. Because these enzymes digests cellulose, which is a key structural component of the plant cell wall, we suggest that 2XL may increase herbicide efficacy in cut-stump treatments of invasive shrubs. It is assumed that when applied to a cut stump as part of an herbicide/2XL mixture, the enzyme would weaken the cell walls of woody plant's vascular tissue. This, in turn, may increase the rate at which the herbicide diffuses into the vascular tissue – specifically, the phloem – by degrading one barrier to herbicide uptake. With a larger proportion of the herbicide being absorbed, lower concentrations of herbicide could be as efficacious. Conversely, the damage to cell walls could function as a cumulative stress on the plant, which may have an additive effect when combined with the effects of the herbicide.

To test whether the addition of 2XL on a treated woody stump would increase the efficacy of glyphosate treatment, we conducted a controlled, field-based experiment using the 'cut-stump' method of treatment on a particularly problematic invasive shrub, *L. maackii*. In our experiment, we tested combinations of three concentrations of 2XL with five concentrations of glyphosate and predicted that low concentrations of glyphosate combined with 2XL would be as effective in limiting the sprouting of *L. maackii* as higher concentrations of glyphosate without the enzyme.

3.3   Methods
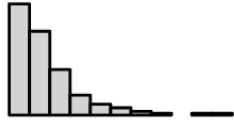
3.3.1   Study Sites

We established four replicate sites within mature, secondary, mixed-hardwood forests at the Richard G. Lugar Forestry Farm (henceforth referred to as 'Lugar Farm') in northwest Indiana (Figure 3.1). Overstories were dominated by oak species (*Quercus* spp. L.), honey locust (*Gleditsia triacanthos* L.), sugar maple (*Acer saccharum* Marshall), and black cherry (*Prunus serotina* Ehrh.). Forests at the Lugar Farm are heavily invaded with dense thickets of *L. maackii* (Shields et al. 2015a) and the sites have received little or no treatment for any invasive plant species. Topographically, the sites were generally flat with slopes of less than 5%. While the abundance of *L. maackii* stems was similar across replicates, an ANOVA ($F_{3,628}$ = 10.20, p < 0.001) and Tukey HSD test revealed that there were significant differences (p < 0.05) in the total basal area between replicates (Table 3.1). The total basal area of individual shrubs was calculated as the summed basal area of all individual stems comprising the individual.
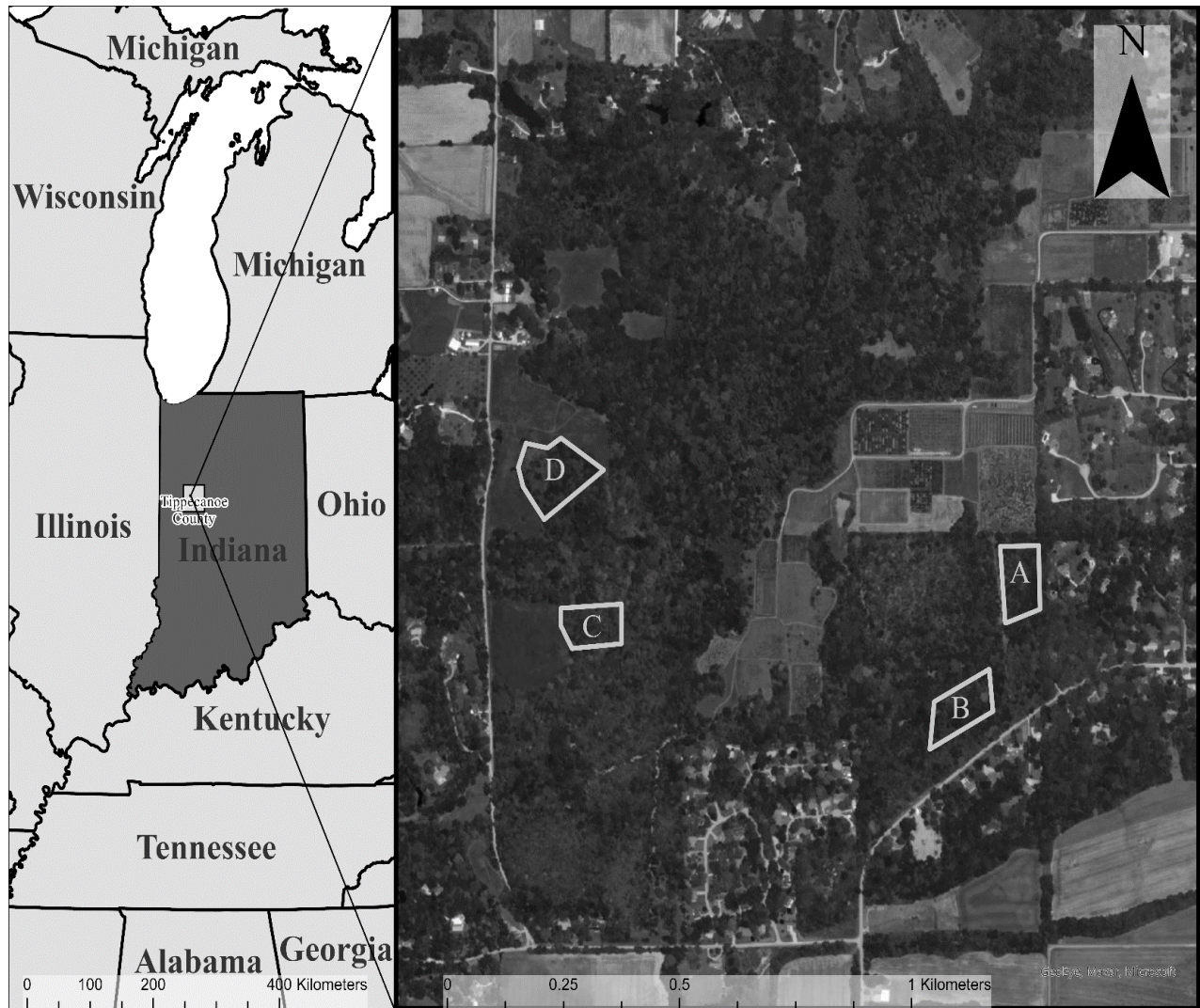
Figure 3.1. Location of study replicates. The four replicate sites were located within Richard G. Lugar Farm (86.9525993°W, 40.4296062°N) in west-central Indiana.

Table 3.1. Median and quartiles of total site basal area (calculated for 150 individuals at each site), and distribution of individual *Lonicera maackii* basal area across study sites at the Lugar Farm. Total basal diameter for each shrub was calculated by summing the basal area of each individual's stem or stems, which were calculated from their basal diameters. All units are in cm^2. Histogram X axis ranged from 3.1 cm^2 to 656.0 cm^2, bars represent 30-cm intervals. Sites with different letters differed significantly based on a Tukey HSD test ($p < 0.05$).

Block	Median	Quartile 1	Quartile 3	Stem Size Distribution	
A	21.24	12.41	41.85		*a*
B	40.72	22.27	73.14		*bc*
C	32.17	14.14	65.04		*ab*
D	38.48	19.63	81.71		*c*

3.3.2 Treatment

At each site, we haphazardly selected 150 *L. maackii* individuals. Criteria for selection included a basal diameter of >2 cm and a minimum distance of 2 m from the forest edge, in order to ensure adequate surface area for herbicide application and to avoid any potential edge effects, respectively. We measured the basal stem diameters of all selected individuals and used these measurements to calculate total basal area. Individuals were then randomly assigned to one of 15 treatment combinations. These combinations consisted of a water only control and various concentrations of herbicide and adjuvant, including five concentrations of glyphosate (between 0 and 240 g/L), each of which was paired with three levels of 2XL (Sigma-Aldrich, St. Louis MO, USA): 0, 25, and 50% (v/v; Table 3.2).

Table 3.2. Summary of the concentration combinations of glyphosate and 2XL, and the number of individual plants to which the treatments were applied.

Glyphosate (g/L)	2XL (%, v/v) 0	25	50	Totals
0				
30		10 plants per treatment		150 plants per replicate
60		x		x
120		15 treatments		4 replicates
240				
				600 treated plants

Each treatment was pre-mixed in spray bottles and applied on July 30, 2019, which was eight days after and 13 days prior to a rain event. Temperatures ranged between 11 and 32 °C during this period. Each individual was cut horizontally ~10 cm above ground level with a chainsaw. Application of the assigned mixture occurred immediately after the cut was made. The vascular zone was completely treated in order to ensure full coverage of the herbicide mixture and to achieve maximal entry into the phloem and, thus, transport to the root system. The application rate of the treatment was 3.5-4.5 mL of solution per ~6.5 cm of basal diameter per stem. This methodology closely replicates the application technique commonly used by land managers (Frank et al. 2018b). We did not add any surfactant to our mixtures.

Sprouting stems were measured once in early November of the same year as the initial treatment (2019) and again in mid-June of the following year (2020). The number of sprouts and length of the longest sprout, measured from tip to the base of the sprout on the cut stump, were recorded. Additionally, we recorded the occurrence of deer browse. Browse rates were low both years (same year: 9%, following year: 5%) and similar between sites.

3.3.3   Statistical Analyses

Due to the abundance of zero values (no sprouting), a negative binomial regression model predicting number of sprouts was constructed for each year (R package: "lme4"), which included total basal area of all 150 stems at a replicate site as a fixed-effect and glyphosate dosage, 2XL dosage, and the interaction between glyphosate and 2XL, as well as Block (site) as random effects. For all models, $\alpha = 0.05$. We checked for an over-dispersion assumption using a log-likelihood comparison between the full negative model and a Poisson model, with the same variables (same year: df = 18, p < 0.001; following year: df = 18, p < 0.001) and a chi-squared test on the over-dispersion ratio (same year: $\hat{c} = 3.44$, p < 0.001; following year: $\hat{c} = 3.46$, p <0.001). Results confirmed that the data were over-dispersed, and that the negative binomial distribution was appropriate.

The overall significance of the full negative binomial models was checked through a log-likelihood test, comparing it to a null model. The significance of interaction effects was also tested through a log-likelihood test by comparing the full model to a negative binomial regression that

included the same variables aside from the interaction effect between glyphosate and 2XL dosages. The full model was significantly better than the reduced model, indicating that the interaction between glyphosate and 2XL is important in predicting sprouting. The previous two checks were confirmed through comparisons of Akaike information criterion (AIC) for each model. Using the full negative binomial regression model, we performed pairwise comparisons between all combinations of glyphosate and 2XL for each year (R packages: "emmeans" and "multcomp"). Response estimates of the number of sprouting stems and 95% confidence intervals were generated and displayed for these comparisons. For combinations of glyphosate and 2XL that the model predicts zero sprouting stems, confidence intervals cannot be created because there is no variance in the data. Thus, these estimates did not appear statistically different from other estimates, despite a clear biological and visually apparent difference. For sprouting stems, we ran a type III ANOVA ($\alpha = 0.05$) comparing the heights of the tallest stem across glyphosate concentrations, 2XL volumes, the interaction effect between glyphosate and 2XL dosages, with total basal area of *L. maackii* as a fixed effect.

3.4   Results

The number of sprouts on individual *L. maackii* stumps varied with treatment type, where those receiving glyphosate treatment exhibited few sprouts, regardless of concentration. The addition of 2XL exhibited no effect on sprouting, irrespective of the concentration of either chemical. This trend was consistent for measurements in the same year as treatment and in the following year and can be seen in the predicted values from each year's full negative binomial model (Figures 3.2A and B; Table 3.3).

Both the same-year negative binomial model ($df = 18$, $p < 0.001$) and the model for the following year ($df = 18$, $p < 0.001$) were significant predictors of the number of sprouts, when compared to null models. We also found the interaction effect between glyphosate and 2XL significantly contributed to the model's predictive ability in both the same year ($df = 18$, $p < 0.001$) and following year ($df = 18$, $p < 0.001$). The AIC was lowest for the full negative binomial models (same year: 1,051.1; following year: 1,247.8) compared to both the null (same year: 1376.8; following year: 5095.8), reduced models (same year: 1,057.9; following year: 1,285.2), and

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Poisson models (same year: 1,175.2; following year: 1,416.5). This confirmed that the full negative binomial models were the strongest models in both years. The coefficient estimates from negative binomial mixed effects model revealed that 2XL only had non-significant or positive effects on the predicted number of sprouts in both years. Specifically, we observed a small but statistically significant increase in the number of sprouts in the following year after treatment when 30 g\L of glyphosate was mixed with a 25% volume of 2XL compared to no 2XL (Figure 3.2B).
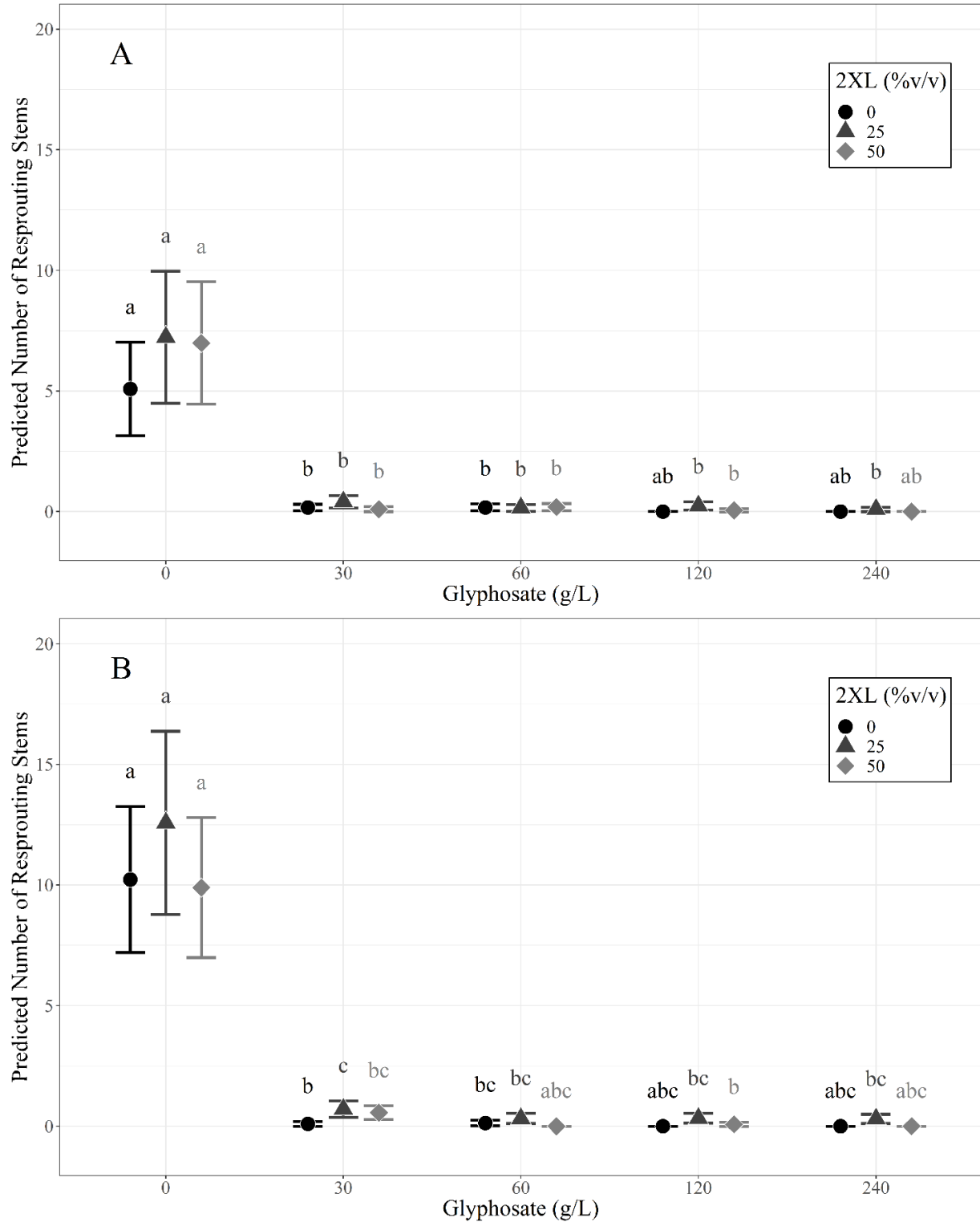
Figure 3.2. Predicted number of sprouting *Lonicera maackii* stems and 95% confidence intervals, based on the full negative binomial regression model for (A) the same year as treatment and (B) the following year. Superscripts indicate significant differences ($\alpha = 0.05$) between concentration combinations within each year respectively.

Table 3.3. Summary of the negative binomial models explaining the number of sprouting *Lonicera maackii* stems in both the same and the following years after treatment. Regression coefficients and p-values (in parentheses) are included for each variable.

Variable	Same Year	Following Year
(Intercept)	1.562 (0.000)	2.254 (0.000)
Total Basal Area	0.001 (0.240)	0.001 (0.223)
Glyphosate-30	-3.395 (0.000)	-4.636 (0.000)
Glyphosate-60	-3.378 (0.000)	-4.350 (0.000)
Glyphosate-120	-22.605 (0.571)	-22.950 (0.590)
Glyphosate-240	-21.897 (0.730)	-23.043 (0.669)
2XL-25	0.353 (0.117)	0.207 (0.326)
2XL-50	0.318 (0.143)	-0.033 (0.872)
Glyphosate-30:2XL-25	0.523 (0.338)	1.763 (0.004)
Glyphosate-60:2XL-25	-0.433 (0.495)	0.690 (0.256)
Glyphosate-120:2XL-25	19.197 (0.631)	19.303 (0.651)
Glyphosate-240:2XL-25	17.537 (0.782)	19.328 (0.720)
Glyphosate-30:2XL-50	-0.838 (0.225)	1.766 (0.004)
Glyphosate-60:2XL-50	-0.229 (0.701)	-18.638 (0.810)
Glyphosate-120:2XL-50	17.672 (0.658)	18.097 (0.671)
Glyphosate-240:2XL-50	3.190 (0.971)	0.100 (0.999)
Num.Obs.	588	588
AIC	1051.1	1247.8
BIC	1129.9	1326.6

A Type III ANOVA revealed no significant differences among any 2XL-glyphosate combinations in both the same-year sprout lengths (Figure 3.3, Table 3.4) or in those for the following year (Figure 3.4, Table 3.5), although the low number of sprouting stems in combinations containing glyphosate may have hidden any potential relationship. This provides additional evidence that 2XL does not have a negative impact on sprouting vigor post-treatment.
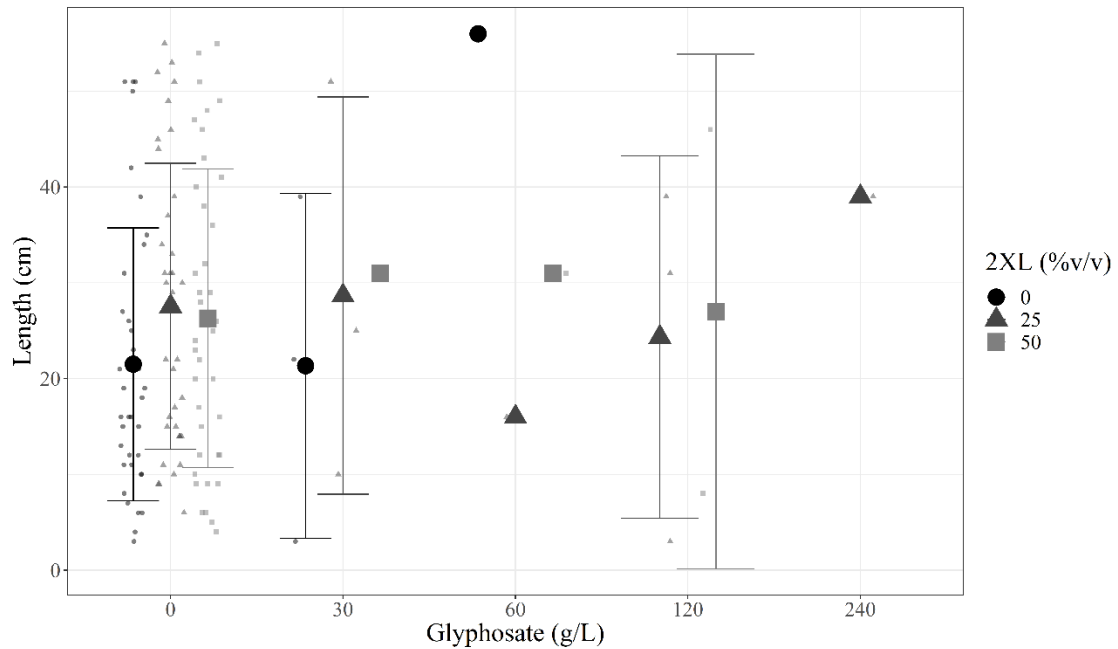
Figure 3.3. Length (mean ± 1 standard deviation) of tallest sprouting *Lonicera maackii* stem (cm) of individuals that sprouted the same year as treatment across treatment types. Large symbols represent means, individual data points are displayed as smaller symbols of the same shape.

Table 3.4. Type III ANOVA results from comparisons of tallest sprouting *Lonicera maackii* stem in the same year as treatment. Glyphosate level did have a marginally significant impact on the length of the longest sprouting stem.

Same Year Variable	Sum Sq	df	F values	P (>F)
Total Area	39.683	1	0.167	0.683
Glyphosate	1,161.292	2	2.448	0.091
2XL	645.311	2	1.36	0.261
Glyphosate:2XL	1,080.406	5	0.911	0.477
Residuals	26,567.819	112		
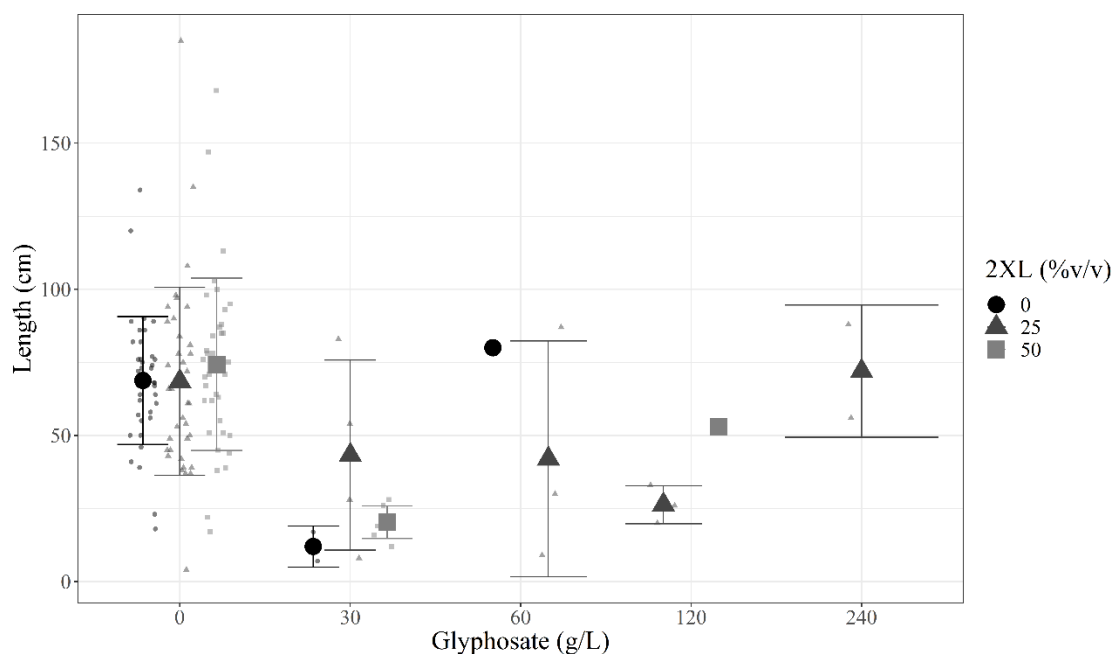
Figure 3.4. Length (mean ± 1 standard deviation) of tallest sprouting *Lonicera maackii* stem (cm) of individuals that sprouted the year following treatment across treatment combinations. Large symbols represent means, individual data points are displayed as smaller symbols of the same shape.

Table 3.5. Type III ANOVA results from comparisons of tallest sprouting *Lonicera maackii* stem in the year following treatment.

Following Year Variable	Sum Sq	df	F values	P (>F)
Total Area	7.465	1	0.019	0.890
Glyphosate	705.316	2	0.906	0.407
2XL	189.778	2	0.244	0.784
Glyphosate:2XL	2,601.737	4	1.67	0.161
Residuals	48,282.271	124		

3.5 Discussion

The primary goal of our study was to determine if lower concentrations of glyphosate can effectively control an aggressive, non-native shrub. Interest in reducing the concentration of glyphosate used to control invasive plants reflects studies of environmental (Busse et al. 2001, Tsui and Chu 2003, Gill et al. 2018) and human health (International Agency for Research on Cancer 2015, Bai and Ogbourne 2016) concerns, as well as reservations expressed by private landowners (Howle et al. 2010, Ma et al. 2018). We found that all tested concentrations of glyphosate were highly effective at preventing the sprouting of *L. maackii* from cut stumps; lower concentrations of glyphosate were as inhibitory as higher concentrations.

Similar studies using the cut-stump method of control applied higher concentrations of glyphosate on *L. maackii*. Hartman and McCarthy (2004) used a 50% glyphosate solution, which is roughly equivalent to 244 g/L of glyphosate. While they saw similarly high levels of control–94% mortality–our study achieved equivalent levels of control with 30 and 60 g/L (Hartman and McCarthy 2004). Other studies have shown 90-100% efficacy in preventing sprouting within a year of treatment using 88 g/L glyphosate (18%) in the cut-stump method for controlling *L. maackii*, which is similar to what we achieved with our lower concentrations (McDonnell et al. 2005, Schulz et al. 2012). The cut-stump method, with a 20-25% concentration of glyphosate (~98 g/L–122 g/L), is widely recommended as a control technique to private landowners (Smith and Smith 2010, Miller et al. 2013). However, our results suggest that a lower concentration could achieve the desired level of control.

Similar studies on other invasive shrubs provide some support for lower glyphosate concentrations. For example, lower concentrations of glyphosate have been used to effectively control a closely related invasive species, Morrow honeysuckle (*Lonicera morrowii* A. Gray; Mervosh and Gumbart 2015). In this study, 41 g/L was as effective as 205 g/L in completely preventing sprouting in the cut-stump method of treatment. However, another study had less success in controlling *L. morrowii* with a moderate concentration of glyphosate (~98 g/L), particularly when treatments were applied in autumn (Love and Anderson 2009). A concentration of 120 g/L has been successfully used to control another problematic invasive shrub: Chinese privet (*Ligustrum sinense* Lour.; Enloe et al. 2018).

For the control of invasive shrub species, glyphosate concentrations of 20-25% (~98 g/L – 122 g/L) applied in the late summer or early autumn are typically recommended to land managers

and private landowners (Smith and Smith 2010, Miller et al. 2013). However, the results of our study, and those of similar studies that examined control techniques for invasive shrub species, suggest that reevaluating these recommendations may be warranted, given that effective control can be achieved with lower concentrations of glyphosate. Based on our results, concentrations as low as 30-60 g/L can be effective, which could reduce the expense of treatments, while also alleviating concerns about non-target effects of the herbicide.

Our study was the first to evaluate the effectiveness of cellulase as an herbicide adjuvant. Contrary to our predictions, we found no evidence that 2XL is an effective adjuvant when used in conjunction with glyphosate. Our review of the literature found no published reports describing the mechanism by which glyphosate enters the vascular tissue(s) when applied to the stumps of woody plants. However, it has been suggested that phosphate transporters play a role in the uptake of glyphosate when applied as a foliar spray to herbaceous plants (Misson et al. 2005, Gu et al. 2016). In the foliar application of glyphosate on woody plants, Pereira et al. (2019) described how phosphate transporters embedded in the cell membranes of rose gum (*Eucalyptus grandis* W. Hill ex. Maid.) may facilitate glyphosate absorption into the tree's vascular tissue. While glyphosate absorption through the leaves likely differs from absorption through a stump, it is possible that phosphate transporters are involved in glyphosate uptake in stump applications. This is consistent with our results, which suggest that 2XL actually slightly reduces glyphosate efficiency (Figure 3.2b). In this framework, cellulase activity would compromise uptake by damaging cell walls or interfering with phosphate transporters, thus reducing the efficacy of glyphosate. While passive diffusion may still allow glyphosate to gain access to vascular tissue(s), our results suggest that glyphosate uptake was not enhanced when combined with 2XL. The reduced uptake of glyphosate likely cancelled out any additive effect 2XL may have through its degradation of plant cell walls. Clearly, glyphosate uptake is still not fully understood, and recent research suggests that an ATP-binding cassette (ABC) transporter is the primary means by which glyphosate enters into the cytoplasm of a cell (Pan et al. 2021). In either case, protein mitigated transportation seems pivotal to glyphosate uptake into the plant.

In order to devise methods to increase uptake efficiency, more research is needed to identify the precise mechanism of glyphosate uptake. Evaluating the role of phosphate transporters may be a first step in understanding glyphosate uptake into the vascular tissues of woody plants through either foliar spray and/or cut-stump applications. Ideally, this will ultimately reduce the

concentrations of glyphosate needed to control invasive woody plants, particularly if a suitable adjuvant that works in conjunction with glyphosate uptake can be identified.

CHAPTER 4. HIGHER INTENSITIES OF MULCHING-HEAD TREATMENTS LIMIT THE RESPONSE OF A TARGET INVASIVE SHRUB (*LONICERA MAACKII*) AND PROMOTE HERBACEOUS-LAYER SPECIES DIVERSITY

4.1 Abstract

Amur honeysuckle (*Lonicera maackii* [Rupr.] Herder) and other invasive shrubs represent serious threats to the biodiversity and ecosystem services of forests in the eastern United States. Controlling invasive shrubs can be costly and time consuming, highlighting the need for efficient techniques to control heavy invasions across large areas of forest. Mulching-heads have shown promise in efficiently controlling multiple species of invasive shrubs, including Amur honeysuckle, potentially allowing the restoration of suppressed herbaceous-layer plant communities. In this study, we examine how mulching intensity (i.e., treatment depth below the soil surface) affects Amur honeysuckle sprouting response, as well as the post-treatment response of the plant community. We found that deeper applications of a mulching-head inhibited the post-mulching treatment regrowth of Amur honeysuckle stumps. According to NMDS analysis, post-treatment species composition became more similar across plots and shifts in post-treatment axis values were associated with increased species diversity and floristic quality: a measure of species conservation value. Additionally, a canonical correspondence analysis revealed that deeper mulching-head intensities were correlated with increases of herbaceous-layer plant species diversity and floristic quality. We conclude that mulching-head treatments are an effective technique for controlling invasive shrubs without degrading native plant communities, and deeper treatment reduce the rate of post-treatment sprouting.

4.2 Introduction

Invasive shrubs are ubiquitous and a threat to the health of forest ecosystems in the eastern United States, particularly shrub species originating from East Asia (Webster et al. 2006, Ricklefs et al. 2007, Iannone et al. 2015, 2016). These invasive shrubs threaten native plant species in a myriad of ways. Most notably, their extended leaf phenology allows additional carbon fixation earlier and later in the year, resulting in a longer growing seasons and greater potential to shade

out competitors in the understory (Fridley 2012). Not only are invasive shrubs directly harmful to forest ecosystems, but they are also extremely difficult to control across landscapes (Webster et al. 2006). The vigorous root growth and a propensity to sprout new stems after treatment make invasive shrubs a costly and persistent challenge for control efforts by land managers (Rathfon and Ruble 2007, McNeish and McEwan 2016). For example, between 1994 and 2005, Great Smoky Mountains Nation Park alone spent over 17,000 worker hours controlling woody invasive plant species (Webster et al. 2006). Developing efficient and cost-effective invasive shrub control techniques is critical to restoring and protecting the forest ecosystems of the eastern United States.

Amur honeysuckle (*Lonicera maackii* [Rupr.] Herder) is an archetypal invasive shrub that was deliberately introduced in the United States multiple times for many reasons, including soil stabilization, horticultural value, and wildlife habitat, and has become nearly ubiquitous in eastern forest (Luken and Thieret 1996). Similar to other invasive shrubs from East Asia, Amur honeysuckle is noted for having an extended leaf phenology (McNeish and McEwan 2016), the ability to alter nutrient cycling (Schuster and Dukes 2017), and early developing and prolific reproductive capabilities (Luken and Thieret 1996, Deering and Vankat 1999, Lieurance 2004, Gorchov et al. 2014). Additionally, there is evidence that Amur honeysuckle may be allelopathic (Bauer et al. 2012).

Control techniques for Amur honeysuckle and other invasive shrubs tend to employ one or more of four treatment categories: physical removal of individuals, biological control, fire, and herbicide treatments. Root wrenching, lopping, root severing, and brush-cutting are all common examples of physical removal (Oneto et al. 2010, Frank et al. 2018b). Goat grazing has recently been suggested as a potential means to biologically control shrub invasions (Luginbuhl et al. 1999, Elias and Tischew 2016, Rathfon et al. 2021). Prescribed fires are also commonly used to treat invasive shrubs (Mandle et al. 2011). Studies have shown that direct fire treatment from propane torches can be effective in controlling Japanese barberry (*Berberis thunbergii* DC.) in forests of the northeastern United States when applied multiple times (Ward and Williams 2011) or used with herbicides (Ward et al. 2009). Herbicide applications, such as foliar spraying, hack and squirt, girdling, stem injections, and cut-stump applications, are the most common means of invasive shrub control and often also involve a method of physical removal (Hartman and McCarthy 2004, Webster et al. 2006, Rathfon and Ruble 2007, Loh and Daehler 2008, Howle et al. 2010, Oneto et al. 2010, Bailey et al. 2011).

While all of these methods can be effective in controlling invasive shrubs, they also all have major drawbacks. Physical removal techniques often require specialized equipment and are one of the most inefficient control methods (Bailey et al. 2011). While goats and other forms of biological control show potential (Luginbuhl et al. 1999, Elias and Tischew 2016, Rathfon et al. 2021), the techniques are still relatively untested and potential non-target effects are not fully understood. Prescribed burns are very efficient in terms of time and cost (Ward and Williams 2011, Ward et al. 2013), but the technique may have limited effectiveness for some invasive shrubs and may even favor some invasive plant species (Ward and Williams 2011, Mandle et al. 2011, Rebbeck 2012, Ward et al. 2013, Guthrie et al. 2016).

Herbicide application is a highly effective control technique for invasive shrubs. However, the cost of application can be limiting (Bailey et al. 2011) and landowners are often cautious about the potential dangers of herbicide application (Ma et al. 2018). Glyphosate, which is the most heavily used herbicide in the world and known to relatively benign compared to other herbicides (Baylis 2000, Tarazona et al. 2017), has been the subject of multiple lawsuits awarding an average of $47.6 million (Benbrook 2020), and research has found evidence of non-target environmental effects (Busse et al. 2001, Tsui and Chu 2003, Bai and Ogbourne 2016, Gill et al. 2018). While herbicides will continue to be widely used in the control of invasive plants, control techniques that reduce the amount of herbicide applied can reduce the cost of control, while also helping to assuage public concerns.

Mechanical control of invasive shrubs offers an efficient control technique that can be applied to heavy invasions across large areas. Recently, mulching-head treatments (also known as rotary mulching-heads, forestry heads, wood shredders, or mastication heads) have gained attention as an efficient method of controlling dense thickets of invasive shrubs (Hanula et al. 2009, Bailey et al. 2011, Ward et al. 2013, Frank et al. 2018b). Conceptually, the treatment should be able to shatter the root collar of the shrub, inhibiting the post-treatment sprouting of stumps (Frank et al. 2018b). The effectiveness of mulching-head treatments seems to vary by species. A study comparing mulching-head treatments to stem cutting and removal without herbicide for the treatment of Japanese barberry found the two treatments to be equally effective (Ward et al. 2013). Hanula et al. (2009) found both of these methods failed to significantly reduce the herbaceous-layer cover of Chinese privet (*Ligustrum sinense* Lour.) but did significantly increase cover of non-privet plant species within a year after treatment. Foliar application a year after either

treatment nearly eliminated Chinese privet from the treated sites (Hanula et al. 2009). While the effects of mulching-head treatment on the herbaceous-layer plant community have received little attention, Hudson et al. (2014) found that species richness was higher in following treatment of Chinese privet with mulching-heads compared to cut-stump treatment.

Frank et al. (2018b) found that mulching-head treatments were nearly as effective as cut-stump in controlling Amur honeysuckle invasions. Frank et al. (2018b) observed increased herbaceous-layer plant species diversity and conservation value in mulching treatments, compared to the cut-stump method. However, the authors noted that Amur honeysuckle sprouted prolifically, highlighting the need to examine how the intensity of mulching-head treatments affects post-treatment sprouting. Frank et al. (2018b) identified a negative relationship between depth of mulching-head treatment and the volume of sprouts on treated Amur honeysuckle stumps in the spring following a winter treatment. However, whether this relationship would persist across multiple years was not evaluated. Additionally, increased intensity may damage belowground plant structures and seeds, which could affect the response of post-treatment herbaceous-layer species composition and diversity (Frank et al. 2018b).

In this study, we used plots previously established by Frank et al. (2018b) to examine the multi-year response to mulching-head intensity – as measured by treatment depth below the soil surface. Specifically, we examined how intensity affects the multi-year sprouting of treated Amur honeysuckle stumps, as well as the species composition and diversity of the herbaceous layer. We predicted that more intense treatment would reduce Amur honeysuckle sprouting vigor by more thoroughly damaging vegetative buds around the root collar. However, we also predicted that more intense treatments would shift herbaceous layer species composition toward greater importance of early seral and non-native species, such as garlic mustard (*Alliaria petiolata* [M. Bieb.] Cavara & Grande), which often increases in cover after Amur honeysuckle control (Shields et al. 2015b).

4.3   Methods

In the autumn of 2016, three replicates of a field experiment to examine the effects of mulching-head intensity were established at two sites in west-central Indiana, near West Lafayette. One replicate was established at the Purdue Wildlife Area (PWA; 40.45621492702777, -87.05916902428363) and two other replicates were established at the Richard G. Lugar Forestry Farm (40.42886251864756, -86.9533685827635; henceforth referred to as "Lugar Farm"). At

each site, we also sampled a single, untreated reference area for comparison to the replicates at that site. Prior to treatment, understories of the replicates and reference areas were dominated by Amur honeysuckle and had received no prior invasive species treatments (Frank et al. 2018b).

At each treatment and reference area, eight plots were established with four plots in two parallel rows: plot centers 8 m apart. Prior to any treatment, the percent cover of each herbaceous-layer species was visually estimated in four 1-m^2 quadrats positioned one meter away from plot center in all cardinal directions (north, east, south, and west). Aside from grass species, which were combined into a single taxonomic group, all plants were identified to at least the genus level, and most were identified to the species level (USDA Plants Database 2021). The percent ground-cover type (leaf litter, fine woody debris, coarse woody debris, bare ground, and bole) was also estimated in these quadrats.

Mulching-head treatments of the two replicates at Lugar Farm and one at PWA occurred in the winter of 2017. To measure mulching-head treatment intensity, a 9 x 5 grid of pre-marked wooden stakes was established by placing stakes four meters apart in between the centers of each plot (Frank et al. 2018b). Stakes were hammered into the soil with 10 cm left above ground to measure the depth of treatment. After treatment, the number of centimeters below the soil surface where breakage occurred was recorded for each stake. While the mulching-head completely pulled some stakes out of the ground, at least three out of the nine stakes nearest each plot center remained measurable after treatment (median 4.5). We calculated the mean depth of the remaining stakes closest to the center of each plot and used this as the measurement of treatment intensity.

In the autumn of 2019 (two years post-treatment), we remeasured herbaceous-layer cover during the same time of year and using the same methodology as Frank et al. (2018b), except that a rock cover type was estimated, and bole cover was not estimated following treatments. In the fall of 2020 (three years post-treatment), we recorded the percent of midstory Amur honeysuckle cover in 2 x 2 m quadrats randomly placed at each plot. We also measured the number of stems sprouting from treated Amur honeysuckle stumps in 40-m^2 circular plots extending from each plot center. The height, crown length, and crown width of each sprout was measured and used to calculate the ellipsoidal volume of the individual shrub. We summed all these volumes as a measure of Amur honeysuckle response to mulching treatment. Total shrub volume is considered a more informative metric for stump response, as it combines stem density and shrub cover into a single measurement (Frank et al. 2018b).

4.4   Statistical analyses

4.4.1 Amur Honeysuckle Response

Similar to an analysis of short-term response to mulching-head treatments (Frank et al. 2018b), we evaluated the correlation between treatment intensity and the volume of shrubs found at each plot using linear regression. A Shapiro-Wilk normality test revealed that shrub volume was not distributed normally (W = 0.84407, p = 0.002). A natural log transformation was applied, and the log-transformed distribution was normal (W = 0.9577, p = 0.394); thus, we fit a linear regression and plotted the results in R (version 3.6.3).

4.4.2 Herbaceous Community Response to Treatment

Based on the percent herbaceous-layer cover, we calculated taxonomic species richness (richness), Shannon's Diversity Index (diversity), and Pielou's Evenness Index (evenness) for each plot both before and after mulching-head treatment. We also determined the average coefficient of conservation (C value) and floristic quality index (FQI), according to Indiana state guidelines (Rothrock 2004). To make these metrics comparable to a previous study, we also excluded grasses (Poaceae), sedges (*Carex* spp.), and unidentified taxa from C value calculations, assigned exotic species a C value of 0, and averaged possible C values for other taxa only identified to genus (Frank et al. 2018b).

To understand how the herbaceous layer responds to mulching-head treatment across the full range of treatment intensities, we used non-metric multidimensional scaling (NMDS) with a Bray-Curis dissimilarity matrix to fit herbaceous-layer community metrics (richness, diversity, evenness, average C value, and FQI) and the ground-cover types (bare ground, coarse woody debris, fine woody debris, and litter) from the treated plots both before and after treatment within a single ordination. Analysis was conducted using the 'vegan' package in R; specifically, the "metaNMDS" command. The data were square-root and Wisconsin transformed within and constrained to two axes. A maximum of 100 attempts were allowed to find a convergent solution to the ordination. The linear R^2 and stress of the ordinations were recorded as measures of how much of the Bray-Curtis dissimilarity matrix was preserved in the ordination, with a stress of less than 0.2 being considered adequate for analysis, according to the guidance for the 'vegan' package in R.

After a stable NMDS ordination was derived, we plotted the ordination with arrows drawn connecting each plot's pre-treatment location within the ordination to its location post- treatment, to visualize changes in herbaceous-layer species composition after treatment. The weights of each variable (referred to as 'species' within the ordination) were also plotted to visualize which factors shifted after treatment. To statistically determine if there were differences in pre- and post-treatment plots, we used the "adonis" analysis (also referred to as a PERMANOVA) from the 'vegan' package in R ($\alpha = 0.05$). The adonis analysis provides an analysis of variance, which examines differences between groups within distance matrices. We tested if measured metrics of diversity and ground-cover types varied between pre- and post- treatment plots within a Bray-Curtis dissimilarity matrix using 999 permutations. To determine what factors contributed most to the dissimilarity of the two groups, we used a similarity percentage analysis ("simper" command in 'vegan'; also known as a SIMPER analysis). This analysis revealed which variables (community metrics or ground-cover types) contributed significantly to the compositional divergence of groups (pre- and post-treatment plots) in a Bray-Curtis dissimilarity matrix ($\alpha = 0.05$).

4.4.3 Effect of Intensity on Community Response

After examining how herbaceous-layer composition shifted in treated compared to reference plots, we performed a canonical correspondence analysis (CCA) in order to understand how mulching-head intensity and other factors drove changes in the herbaceous species response. A CCA combines regression and ordination techniques where a matrix of explanatory (independent) variables are constrained by a matrix of response (dependent) variables (Borcard et al. 2011). Although CCA typically handles unimodal or linear relationships within the data matrix, this technique allowed us to directly examine the influence of both pre- and post-treatment variables on species composition. To determine how the post-treatment herbaceous community was related to the pre-treatment herbaceous-layer community (richness, diversity, evenness, average C value, and FQI), site, ground-cover type (bare ground, bole, coarse woody debris, and fine woody debris), and intensity of mulching-head treatment, we utilized an explanatory matrix consisting of these variables. Our response matrix consisted of post-treatment measures of the same metrics of herbaceous-layer communities (richness, diversity, evenness, average C value, and FQI), along with measures of Amur honeysuckle sprouting (Amur honeysuckle volume, number of sprouting stems on treated Amur honeysuckle stumps, and Amur honeysuckle midstory

cover). To determine if our matrix of explanatory variables significantly predicted our matrix of response variables, we used the "anova.cca" command from 'vegan' to derive overall model significance ($\alpha = 0.05$) and also determine the amount of variance the model explained (pseudo-R^2). In addition, "anova.cca" was used to examine the significance of individual explanatory variables ($\alpha = 0.05$) in contributing to the constrained ordination. A triplot of the CCA was created to demonstrate which explanatory variables correlated with which response variable.

4.4.4 Effect of Intensity on Species Composition

To understand how the composition of plant species varied with intensity of treatment, we first used NMDS to ordinate all post-treatment plots with a Bray-Curtis dissimilarity matrix of the percent cover of all plant species and ground cover types (litter, fine woody debris, coarse woody debris, bare ground, and rock) found in post-treatment plots. The parameters of this ordination were similar to the previous NMDS ordinations we created: two axes, all data were Wisconsin and square-root-transformed, 100 attempts were allowed to find a convergent solution, and a stress above 0.2 being considered inadequate for analysis. After a stable ordination of herbaceous-layer species percent cover was created, we assigned all plots to one of three intensity classes based on the values for depth of treatment: above soil surface (less intense), below soil surface (more intense), and reference (untreated). We plotted the different intensity classes within the ordination to display differences. To statistically determine if there were differences in species composition between intensity classes, we again used the "adonis" analysis (also referred to as a PERMANOVA) from the 'vegan' package in R ($\alpha = 0.05$). We tested if herbaceous-layer cover varied between intensity classes within a Bray-Curtis dissimilarity matrix using 999 permutations. Following this, we were able to examine pairwise differences between intensity classes using the command "pairwise.adonis" from the package 'pairwiseAdonis' with 999 permutations within a Bray-Curtis dissimilarity matrix ($\alpha = 0.05$). Finally, to determine which plant species or ground cover types varied between each intensity class, we performed a similarity percentage analysis ("simper" command in 'vegan'). This analysis revealed which variables (plant species or ground cover types) contributed significantly to the compositional divergence of groups (intensity classes) in a Bray-Curtis dissimilarity matrix ($\alpha = 0.05$).

4.5 Results

4.5.1 Amur Honeysuckle Response

The relationship between the intensity of mulching-head treatment and the natural log of resprouting Amur honeysuckle volume was significant in our study ($F_{1,22} = 16.69$, $R^2 = 0.406$, p < 0.001), displaying a negative relationship between intensity and volume (Figure 4.6). This is congruent with the previous study, which found a similar short-term trend (Frank et al. 2018b). Differences in intensity between forests also became apparent, with Lugar Farm having a mean intensity of 0.2 cm above the soil surface and PWA having a less intense average treatment intensity of 1.4 cm above the soil surface.
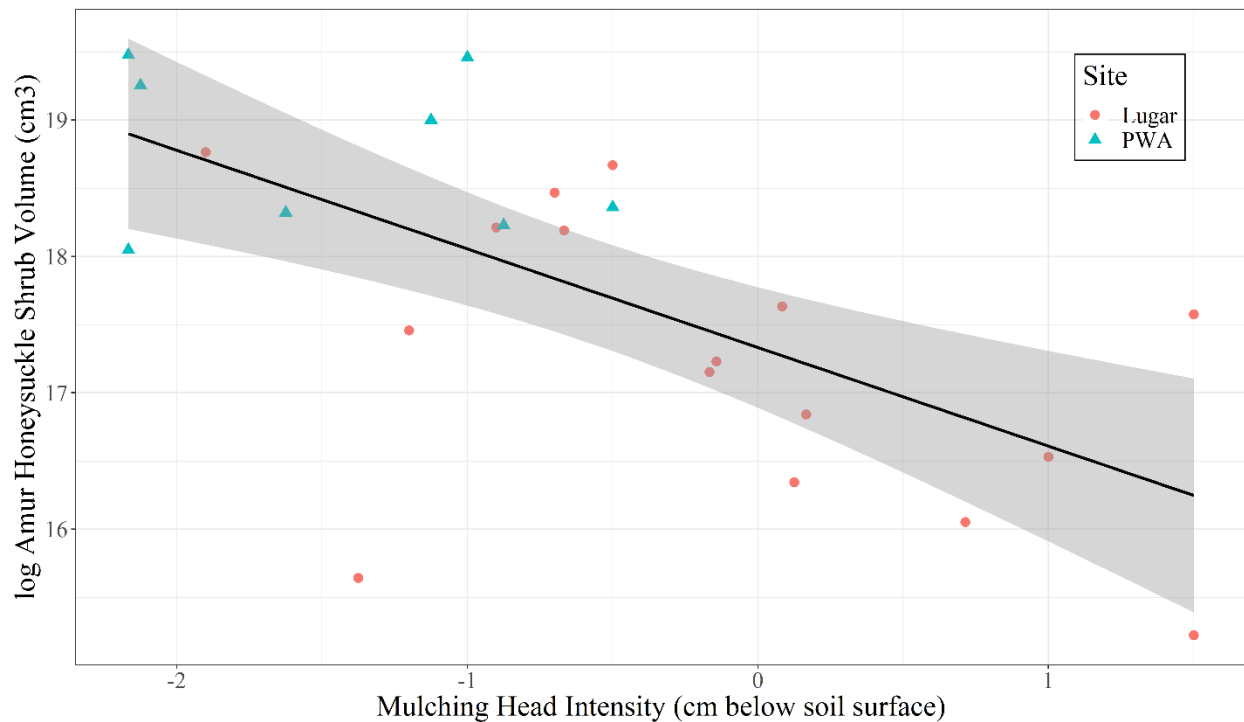

Figure 4.1. The negative relationship between mulching-head intensity and volume of sprouting Amur honeysuckle stems (F1,22 = 16.69, R2 = 0.406, p < 0.001). Negative x-axis values indicate height above soil level.

4.5.2 Herbaceous Community Response to Treatment

The NMDS, including measures of plant diversity and ground-cover types in both pre- and post-treatment plots, found a convergent, two-axis solution in 20 attempts with a stress of 0.11 and a linear R^2 of 0.973, which was acceptable for this analysis. The pre- and post-treatment plots were significantly separate from one another in the adonis analysis ($F_{1,47} = 23.881$, p = 0.001) and the post-treatment sites were clustered within the ordination (Figure 4.2). Plots converged post-treatment, displaying largely unidirectional movement in ordination space. Overall, the dispersion of plots decreased post-treatment. The weights of species richness, diversity, average C value, and FQI all trended in the same direction, as the cluster of post-treatment plots with all other factors trending away from the cluster (Figure 4.2). The SIMPER analysis confirmed this trend, with richness, diversity, evenness (which weight the ordination was in the opposite direction within Figure 4.2), average C value, and FQI all increased in post-treatment plots and significantly contributed to the Bray-Curtis dissimilarity between groups (Table 4.1). Leaf litter was the most strongly related to the dissimilarity and decreased in post-treatment plots. Bare ground and coarse woody debris also significantly related to dissimilarity and decreased after treatment, but to a lesser extent. Fine woody debris, however, did not relate significantly to the dissimilarity between pre- and post-treatment plots.
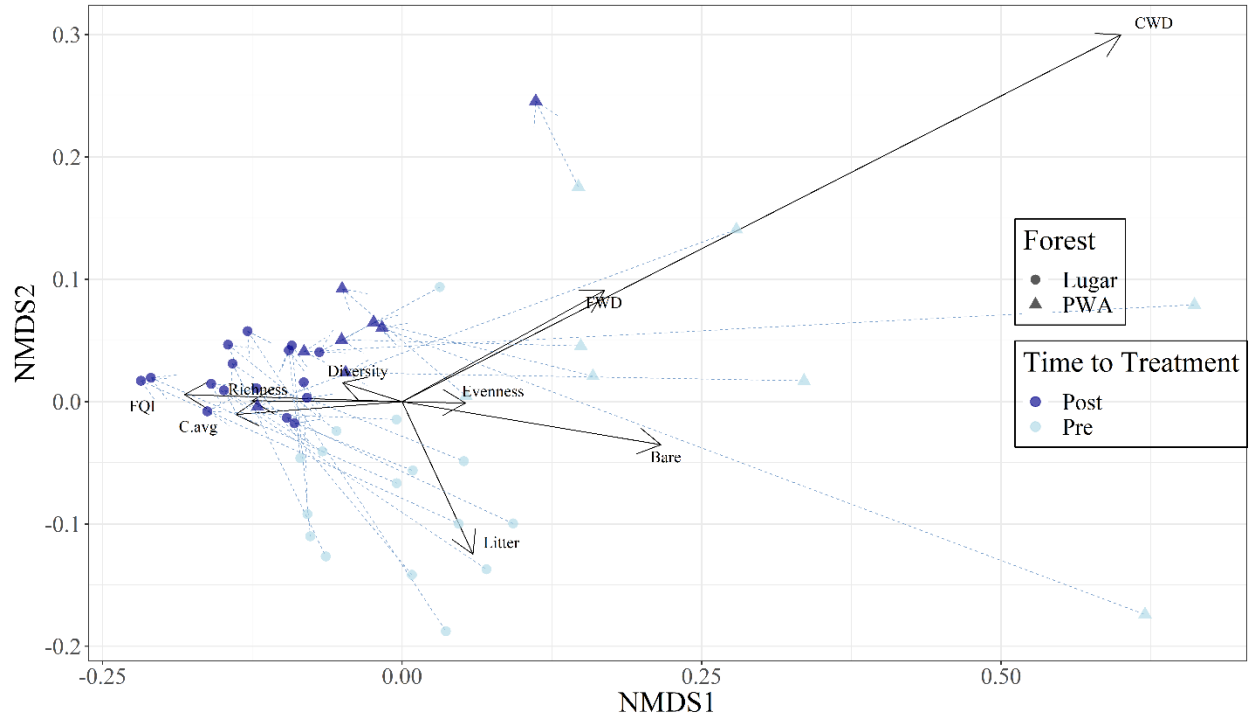
Figure 4.2. A non-metric multidimensional scaling comparing the plots pre- and post-mulching-head treatment. Dashed lines connect measurements of plots before treatment to measurements after treatment. Solid lines represent the weights variables within the ordination.

Table 4.1. Results of a SIMPER analysis ($\alpha = 0.05$) comparing the variables which contributed to the Bray-Curtis dissimilarity of pre- and post-treatment plots. Mean difference compares the post-treatment plot means of each variable to those of pre-treatment plots. Asterisks denotes significance of the variable.

Contributing Species	Mean Difference	% Contribution	p-value
Litter	-26.17	13.47%	0.001*
Fine Woody Debris	7.08	6.23%	0.357
Bare Ground	-10.38	5.67%	0.001*
Richness	11.33	5.37%	0.001*
FQI	5.91	2.78%	0.001*
Coarse Woody Debris	-0.67	0.48%	0.031*
Diversity	0.96	0.45%	0.001*
Average C Value	0.68	0.39%	0.001*
Evenness	0.15	0.09%	0.001*

4.5.3 Effect of Intensity on Community Response

The CCA we fit to examine response to mulching-head intensity explained a significant amount of variation in post-treatment plots ($F_{12,11} = 4.232$, $R^2 = 0.822$, p = 0.001). Our dependent variables (post-treatment diversity, richness, evenness, FQI, C average, number of sprouts on treated Amur honeysuckle stumps, Amur honeysuckle volume, and Amur honeysuckle mid-story cover) were significantly predicted by our pre-treatment explanatory variables (Table 4.2). Specifically, site, C average, and intensity significantly influenced the constrained ordination (Table 4.2). Graphing the CCA reveals that increasing intensity is tightly correlated with increasing diversity, richness, evenness, average C value, and FQI in post- treatment plots (Figure 4.3). The Lugar Farm and PWA sites were ordinated in opposite directions from one another. While the number of sprouts on treated Amur honeysuckle stumps were related to Lugar Farm sites, Amur honeysuckle volume and mid-story cover were higher at the PWA sites. The average C value of pre-treatment plots was not clearly related to the trend of other variables but was tightly related to the second axis of the CCA. The CCA axis 2 explained 21.3% of the variation in the model, while the first axis explained 69.8% of the variation. Although pre-treatment average C value significantly contributed to explaining overall model variation, it did not correlate closely with any post-treatment dependent variables, even to post-treatment average C value.

Table 4.2. Pre- treatment explanatory variables and the contribution made to a canonical correspondence analysis explaining post-treatment variables at each plot ($\alpha = 0.05$). Asterisks denote significance of the variable.

Explanatory Variable	Df	χ^2	F	p-value
Site	1	0.113	26.641	0.001*
Intensity	1	0.031	7.396	0.004*
Bare Ground	1	0.008	1.784	0.149
Tree bole	1	0.004	0.935	0.352
Coarse Woody Debris	1	0.007	1.653	0.162
Fine Woody Debris	1	0.006	1.456	0.203
Litter	1	0.005	1.231	0.272
Richness	1	0.006	1.332	0.221
Diversity	1	0.006	1.350	0.250
Evenness	1	0.004	0.910	0.380
C Average	1	0.016	3.813	0.025*
FQI	1	0.010	2.276	0.106
Residual	11	0.046		
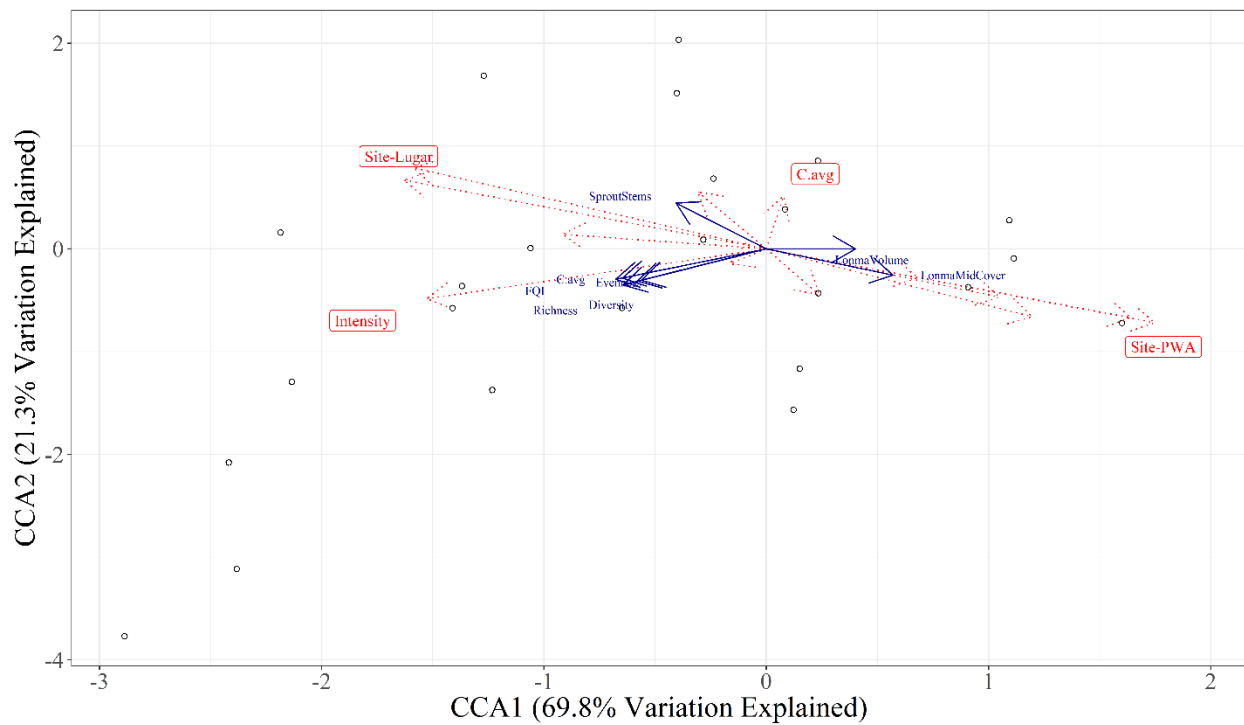
**Figure 4.3.** Triplot of canonical correspondence analysis (CCA) explaining post-treatment plot measurements with pre-treatment plot measurements, forest, and intensity. Open circles represent post-treatment plots within the ordination. Full, blue arrows show response (post-treatment) variables. Dotted red arrows represent explanatory (pre-treatment) variables with significant variables being labeled ($\alpha = 0.05$).

4.5.4 Effect of Intensity on Species Composition

The NMDS we fit based on post-treatment herbaceous-layer species composition compiled after 28 attempts in two axes and exhibited low stress (0.109), as well as a linear R^2 of 0.951 showing that the ordination was acceptable for use in this analysis. An adonis analysis revealed significant differences between the three intensity groups ($F_{2,39}$ = 15.362, p = 0.001). Within the ordination, the untreated reference sites are clearly dissimilar from both treatment intensities, but the above- and below-ground intensity groups have more overlap in the ordination (Figure 4.4). The pairwise adonis tests revealed significant differences in species composition between all intensity classes (Table 4.3). The SIMPER analysis revealed that the cover variables most responsible for the differences between intensity classes consist of variables with relatively low importance to conservation, such as Amur honeysuckle, ground-cover types, or plants with relatively low C values (Table 4.3). The mean C value of the plants that contributed most to the dissimilarity between all intensity classes was 3.5 out of a possible 10. This indicates that while species composition does differ between intensity classes, the differences are not necessarily driven by the establishment of plant species of particularly high value in conservation efforts.
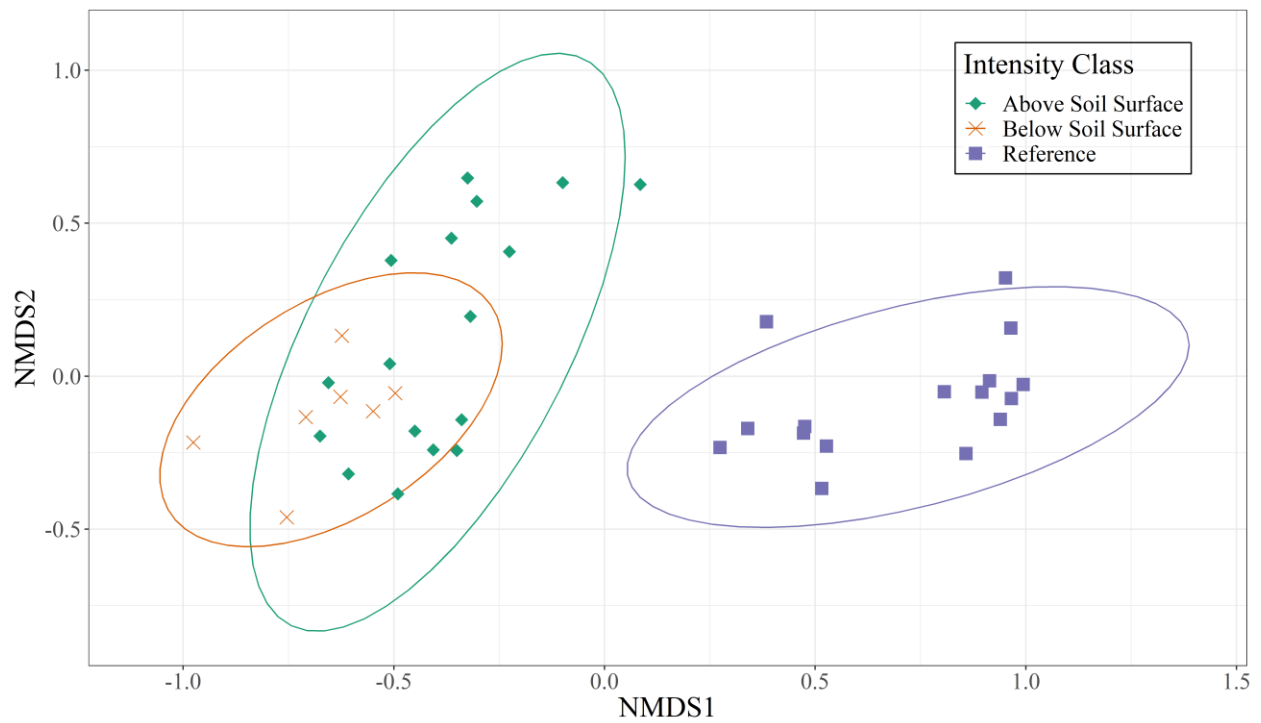
Figure 4.4. A non-metric multidimensional scaling ordination comprised of percent cover of herbaceous-layer plant species and ground-cover types. Intensity classes of mulching-head treatment with 95% confidence interval ellipses surrounding them are displayed.

Table 4.3. Results from a pairwise adonis comparison of species composition between intensity classes (**in bold**) and SIMPER analyses comparing the five herbaceous-layer species or ground-cover types which most related to Bray-Curtis dissimilarity between intensity classes. Species which contributed the most to the Bray-Curtis dissimilarity between two intensity classes as determined by the SIMPER analysis were listed in descending order of % Contribution underneath the pairwise adonis comparisons of the same groups. All tests of significance were set at $\alpha = 0.05$.

Pairs	Contributing Species	Mean Difference in Percent Cover	% Contribution	Df	Sums of Squares	F	R^2	p value
Below vs Above	-	-	-	**1**	**0.457**	**5.104**	**0.188**	**0.006**
	Pilea pumila	18.56	5.889%	-	-	-	-	0.013
	Symphyotrichum spp.	1.69	0.711%	-	-	-	-	0.019
	Viola striata	0.66	0.004%	-	-	-	-	0.034
	Polygonum virginianum	0.71	0.002%	-	-	-	-	0.001
	Populus tremuloides	0.45	0.001%	-	-	-	-	0.022
Below vs Reference	-	-	-	**1**	**1.700**	**29.746**	**0.586**	**0.003**
	Fine Woody Debris	-34.33	12.348%	-	-	-	-	0.002
	Grass species	29.00	10.308%	-	-	-	-	0.001
	Pilea pumila	26.82	9.368%	-	-	-	-	0.001
	Carex spp.	17.22	6.049%	-	-	-	-	0.001
	Bare ground	-16.07	5.8644%	-	-	-	-	0.001

Table 4.3. Continued

Pairs	Contributing Species	Mean Difference in Percent Cover	% Contribution	Df	Sums of Squares	F	R^2	p value
Above vs Reference	-	-	-	**1**	**1.299**	**16.607**	**0.349**	**0.006**
	Grass species	21.21	7.063%	-	-	-	-	0.002
	Lonicera maackii	15.15	5.544%	-	-	-	-	0.002
	Bare ground	-11.23	4.058%	-	-	-	-	0.010
	Ageratina altissima	4.90	1.688%	-	-	-	-	0.001
	Vitis spp.	3.878	1.272%	-	-	-	-	0.001

4.6 Discussion

Our study confirmed the short-term results of Frank et al. (2018b) in showing a strong association between the intensity of mulching-head treatment and decreasing volumes of sprout-origin Amur honeysuckle. This trend was confirmed immediately post-treatment (Frank et al. 2018b), but differences persisted three years after treatment (Figure 4.1). Treatments deeper into the ground have the ability to inhibit the sprouting of treated stumps, most likely because deeper treatments resulted in greater damage to the root collar. Amur honeysuckle does not possess the ability to generate new sprouts from distal, horizontal roots (i.e., 'root sucker'; B. Rivera, personal observation), so damaging the root collar likely reduces the number of dormant, preformed buds available for sprouting.

However, with soil disturbance – like those from mulching treatments – generally comes an increased risk of invasive or non-desirable species establishing in the disturbed seedbed resulting treated area (Kulmatiski et al. 2006). Our results indicate that the number of species present in post-treatment plots was higher than in pre-treatment plots, regardless of intensity (Figure 4.2, Table 4.1). However, increases in average C value and FQI also were associated with post- treatment plots, indicating that species composition following mulching-head treatment did not include increased proportions of invasive plant species or weedy species of lower conservation value.

Not only did increasing intensities of mulching-head treatment correlate to decreasing volumes of sprout-origin Amur honeysuckle, but our results also indicate that more intense mulching-head treatments correlated with increasing herbaceous-layer species diversity, richness, evenness, FQI, and C value average (Figure 4.3). This trend, however, may not be absolute. Our study avoided maximizing soil disturbance and damage to native saplings (Frank et al. 2018b), which could have led to a lower intensity of treatment, compared to control efforts where those precautions were not taken. The maximum mean intensity for a plot was 1.5 cm below the soil surface. It is unclear if even deeper treatments would increase the susceptibility of a plot to invasive species establishment and may warrant future study. Increases in plant species diversity, richness, evenness, C value average, and FQI are likely being driven by decreasing dominance of treated Amur honeysuckle stump sprouts at sites with more intense treatments. Less competition from Amur honeysuckle sprouts when stumps are treated with herbicide can lead to a more vigorous short-term response from the herbaceous layer (Shields et al. 2015b) and our study shows this

trend also occurs with increased intensity of mulching-head treatments. Amur honeysuckle is known to inhibit understory plant growth through shading (McNeish and McEwan 2016), and is potentially allelopathic (Bauer et al. 2012), so with less Amur honeysuckle present in more intensely treated areas, the more native plants have the opportunity to establish.

While the conservation value of these communities increased with treatment and was correlated with more intense mulching-head treatments, neither pre- or post-treatment plots exhibited particularly high mean C values (means of 2.05 and 2.73, respectively). According to Rothrock and Homoya (2005), forested natural areas occurring in the same state natural region as our study sites exhibited mean C values ranging between 3.8 and 4.1. Both of these sites (Lugar Farm and PWA) are in the Central Hardwood Region which is considered to be historically disturbed forests (Jenkins 2013). Agriculture, livestock grazing, high-grade logging, surface fires, and the removal of Indigenous peoples have long altered the species composition of this region, and, thus, the existing species pool in the seed bank or through dispersal from the local area may not include plant species targeted for conservation. While *Pilea pumila* may be considered an early seral species, the plant species responsible for most of the increases in diversity following treatment, such as *Ageratina altissima* (Jenkins and Parker 1999), are often associated with these historically disturbed forests rather than recent disturbances (Table 4.3). The species driving changes between the different intensity classes (above soil surface, below soil surface, and untreated reference sites) were also of low conservation value in Indiana (Table 4.3). More intense mulching treatments do, however, create opportunities for the establishment of more plant species, which could include plant species targeted for conservation by random chance or by active post-treatment seeding by land managers after long-term treatments.

Our results show that positive effects observed in the short-term (<1 year) by Frank et al. (2018b) persisted after three years. Mulching-head treatments appear to be an effective and cost-efficient tool to remove heavy invasions of Amur honeysuckle, especially when applied with deeper intensity. Not only did more intense mulching-head treatment reduce Amur honeysuckle sprouting (Figure 4.1), but it also led to increased plant diversity and conservation value in the herbaceous layer. A common relationship exists between the treatment of Amur honeysuckle and the emergence of the invasive garlic mustard after treatment (Shields et al. 2015b), indicating that the control of one invasive plant can lead to the establishment of other invasive plants after treatment. However, no such relationship was found in our data, with no clear increase in any

particular invasive plant species after treatment. Future studies should examine the effects of deeper treatment intensities in less historically disturbed forest. Additionally, determining the relationship with post-treatment invasive species establishment in forests with less historical disturbed than in this study will help show how broadly this control technique can be applied in other forest systems. Sensitive plant species not common at our sites may be more vulnerable to non-target effects of mulching-head treatments.

Finally, land managers should strongly consider adding intense mulching-head treatments to their suite of techniques to control heavy shrub invasions in historically disturbed forests. Amur honeysuckle is an archetypal invasive woody shrub (Luken and Thieret 1996) which suggests that the treatment technique will be effective against an array of other East Asian shrub species. While the cost of mulching rental may be prohibitive in some cases (Bailey et al. 2011), it is a fixed cost, and rapid treatment means that scaling up to large invasions may be cost-effective. Reduced sprouting of treated stumps in more intense mulching-head treatments allows for less herbicide usage in initial and follow-up treatments. Foliar applications a year after mulching treatment have been effective in greatly reducing Chinese privet densities and may be a recommendable strategy for controlling other invasive shrubs following mulching treatments (Hanula et al. 2009). In summary, mulching-head treatment is a promising technique for controlling invasive shrubs.

CHAPTER 5.   CONCLUSION

My thesis has provided both practical and conceptual insights into Amur honeysuckle invasions. Practically, this work has examined two ways to reduce herbicide usage in the control of Amur honeysuckle invasions. While 2XL (cellulases derived from fungi) did not prove to be an effective adjuvant for increasing the efficacy of glyphosate in the cut-stump method of control, the experiment did show that concentrations of less than half the recommended treatment were equally effective at preventing post-treatment sprouting of Amur honeysuckle (Figure 3.2). Glyphosate is the most commonly used herbicide in the world (Tarazona et al. 2017), and while it is known to be relatively benign compared to other herbicides (Baylis 2000), reducing the concentration of glyphosate needed to achieve effective control can save money (Bailey et al. 2011) and assuage public concerns (Howle et al. 2010). Examining if lower concentrations of glyphosate and other herbicides are effective for the control of other invasive shrubs should be a focus of future research.

The second most immediate impact of my thesis is the potential expanded use of more intense mulching-head treatments. Mulching-head treatments can also be an alternative to chemical application and have previously been shown to inhibit the short-term sprouting of Amur honeysuckle stumps (Frank et al. 2018b). However, I found that the inhibition of stump sprouting through more intense application of the mulching-head did not negatively affect herbaceous-layer composition, diversity, or conservation value. However, this study took place in historically disturbed forests so the positive relationships we identified between deeper intensities and increased diversity and conservation value may not translate to all forest types. However, much of the forests in the eastern United States are similar to the forests we studied and thus our results have broad applications. Deeper mulching-head treatments in historically disturbed and heavily invaded forests give native flora a chance to establish in the absence of dense thickets of Amur honeysuckle invasions (Schulte et al. 2011). While the cost of renting mulching-head equipment may be prohibitive when treating small areas, scaling to large invasions can not only make this treatment effective at controlling Amur honeysuckle, but cost-effective as well (Bailey et al. 2011), particularly when less effort and expense are incurred to chemically treat sprouts following mulching heard treatments.

My thesis has also provided conceptual input into the invasion biology of invasive shrubs. While 2XL did not prove to be an effective adjuvant for glyphosate, the potential reason behind

its ineffectiveness is, in of itself, important. We hypothesized that cellulases would break down cell walls to the vascular tissue, allowing for easier diffusion of the herbicide into the plant. However, we observed no positive effect of the addition of 2XL. This implies that protein mitigated transportation across cell membranes may be critical to the uptake of glyphosate into the plants vascular tissue which is supported by recent literature (Pereira et al. 2019, Pan et al. 2021).

The second conceptual insight into the invasion biology is establishing that Amur honeysuckle possess the ability to self-pollinate and produce viable seed sets from these self-pollination events may, in part, account for the relatively rapid establishment of the species in isolated forest patches (Shigesada and Kawasaki 1997, Wangen and Webster 2006, Shields et al. 2015a). Not only does this result confirm the existence of this trait in Amur honeysuckle, but it also sheds light on the importance of examining self-pollination in other woody invasive plant species. Self-pollination is considered to be rare in woody plants (Duminil et al. 2009), and the trait is more associated with short-lived plants such as annuals of biennials (Sutherland 2004). However, our results provide support for "Baker's Law" (Stebbins 1957, Baker 1974) which imparts that self-compatibility is an important trait for invasive plant species. While this insight may have limited impacts on current land-management efforts, it should be considered when screening and prioritizing potential invasive plant species going forward.

Providing both conceptual and pragmatic contributions to the understanding of plant invasions was the goal of my thesis and multiple contributions were made to each category. Amur honeysuckle remains a threat to forests in the eastern United States, but with increased understanding of how this and other invasive shrubs spread, and how they can be effectively and safely controlled, the more the negative impacts of invasive species can be mitigated.

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