Exotic plant species: drivers and impacts of plant invasions in an eastern deciduous forest community

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Abstract

Humans are moving plants around the globe, both intentionally and accidentally, which may lead to the range expansion of broadly tolerant exotic invasive species. When these exotic species invade and become naturalized in an ecosystem, they can threaten the biodiversity and alter the function of the system, causing economic losses through damages to crops or structures or the loss of native species. In addition to transportation of invasive plants, humans have also caused disturbances to ecosystems that may create opportunities for non-natives at the expense of native species who are unable to adapt to the disturbance. One example of such disturbance is the substantial increase in the range and population density of white-tailed deer (*Odocoileus virginianus*) over the last century. This overabundant deer population can then have both direct and indirect effects on forest plant species and facilitate the success of invasive species.

This dissertation intends to understand the effects of deer on the plant communities and the effects of exotic species on the litter-dwelling communities of a deciduous forest ecosystem in the Shenandoah Valley of Virginia. In Chapter 1 I used paired open and fenced plots to demonstrate that deer had indirect effects when they were present, increasing soil compaction, altering soil nutrient pools, and lowering nitrate fluxes. I found that deer had direct effects when they were present, causing greater herbivory damage resulting in reduced plant height and biomass, lowering the survival of all study species except for the exotic shrub *Lonicera maackii*, whose survival was not affected by deer herbivory. I found that invasive species became much more common in the herbaceous layer of the plant community when deer were present.

In Chapter 2 I used a 2×2 factorial experiment to examine the separate and interactive effects of herbivory pressure from deer and the alteration to the shade environment through deer browsing. I found that deer had little direct (herbivory) or indirect (shade) effects on two native forest herbs (*Arisaema triphyllum* and *Podophyllum peltatum*). I found that a common invasive grass (*Microstegium vimineum*) benefitted from both indirect and direct effects of deer presence, increasing in size, reproduction, and abundance. I found that the invasive herb *Alliaria petiolata* had a more complicated response, with greater recruitment when unshaded and in the presence of deer, but had lower survival, growth, and reproduction when deer were present.

To evaluate the role of deer as endozoochorous seed dispersers, I collected scat from three locations in the landscape of the Blue Ridge and Shenandoah Valley. I found that deer were transporting seeds from many different taxa via endozoochory, but very few of these seeds germinated. Of the seeds identified in the samples of deer scat, the typical invasive seeds were from plants with small, hard seeds and non-fleshy fruits, while the typical native seeds were from fleshy-fruited plants. The results of the germination trials suggest that, instead of being seed dispersers, deer are acting more as seed predators and are not benefitting many of the species whose seeds they consume.

Finally, I tested for the effects of leaf litter from three invasive plants (*Ailanthus altissima*, *Lonicera maackii*, and *Rhamnus davurica*) on the detrital-layer food web. I found that invasive litter was more nutritious, but decomposed much faster than native litter. I found that bacteria, fungi, and arthropods all preferred litter from the invasive species, suggesting that these exotic invasive species are beneficial, novel resources for the litter-dwelling community. However, due to the high rates of decomposition, this

resource is short-lived and native leaf litter may provide the most stable resource for this food web.

Taken together, the results of my dissertation indicate that deer are having significant impacts on this forest system. By altering the physical environment, deer are creating opportunities for invasive species to grow and reproduce. Native species are unable to take advantage of these disturbances. The effects of deer herbivory also appear to create advantages for invasive species that are either more tolerant of herbivory than native species or that benefit from the removal of preferred native competitors. Once invasive species are established, they may have strong effects on the food webs of this forest system. These invasive species may create a novel food web in this forest that is characterized by cycles of high abundance of litter-dwelling organisms in the spring that become very low in abundance in the summer. These cycles can then affect the higher trophic levels of the system, forcing them to find uninvaded habitats late in the growing season to sustain their populations.

Dedication

First and foremost, I dedicate this work to my wife Kara and our children. You have been my constant throughout my studies, encouraging me every step of the way to keep my focus and to maintain my spirits. You have remained steadfast in your support through times apart while working in the field and through my long nights working on my research and writing. Your confidence in my abilities has been integral to the completion of this work.

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INTRODUCTION

Humans are moving plants around the globe, intentionally introducing plants into new areas for horticultural or agricultural purposes, or accidentally transporting seeds (Sakai et al. 2001). The results are wide-spread changes to global plant distributions. This includes range expansion of widespread, broadly tolerant species that easily invade new regions and range contraction of endemic native species (Olden et al. 2004) as well as decline in the populations or localized extinctions of native species (Elton 1958; Davis 2003; Sax et al. 2007). The worldwide spread of commonly occurring, broadly tolerant species has created ecosystems that are more similar to each other in their composition, leading to the term 'biotic homogenization' (Olden et al. 2004; Rooney 2009). When these exotic invasive species (hereafter "invasive species") invade and become naturalized in an ecosystem, they can threaten the biodiversity and alter the function of the system, causing economic losses through damages to crops or structures or the loss of native species (Rodriguez 2006; Sax et al. 2007; Pyke et al. 2008; Lind and Parker 2010).

The introduction of a plant into a new region does not necessarily equal a successful invasion. For example, only 400 plant species have become naturalized in North America out of more than 4000 introductions (Davis 2003). Several hypotheses have been formulated to explain why some plant species are more successful at invading than others. Although these hypotheses are not mutually exclusive, understanding the potential drivers is of great importance to managing invasive species. The enemy release hypothesis (ERH) suggests that invasive plants are more successful in a new area because they do not have their native herbivores in their new location to suppress them (Elton 1958; Colautti et al. 2004; Eschtruth and Battles 2009; Huang et al. 2010; Lind and

Parker 2010; Roy et al. 2011). The novel weapons hypothesis (NWH) proposes that exotic plants have a competitive advantage over the natives because they produce defense compounds that the native herbivores are unable to process (Cappuccino and Arnason 2006; Lind and Parker 2010) or allelochemicals that limit the competitive ability of neighboring native plants that have not coevolved with the exotic (Rabotnov 1982; Mallik and Pelliser 2000; Callaway and Ridenour 2004). A third hypothesis is the evolution of increased competitive ability (EICA) that suggests that in the absence of enemies, natural selection favors invasive genotypes that allocate resources to increased growth and reproduction instead of defensive compounds (Blossey and Nötzold 1995; Lewis et al. 2006; Huang et al. 2010).

Another reason that invasive species may have advantages over native species is that the environment to which the native species were adapted has dramatically changed, creating opportunities for non-native species to become established. A significant change to the environments in eastern North America is the substantial increase in the range and population density of white-tailed deer (*Odocoileus virginianus*) over the last century (Rooney 2001; Eschtruth and Battles 2009; Pellerin et al. 2010), with current population estimates at approximately 30 million in the continental United States (Bagley 2017). In Virginia, recent estimates of deer populations are approximately 1.25-2.5 times that of estimates from early-colonial times (VDGIF 2015). Reasons for this expansion include protective laws and refuges that have allowed the deer population to recover from historic habitat loss and over-hunting (Adams and Hamilton 2011; Côté 2011), management strategies aimed at increasing herd numbers for hunters, and an increase in ideal habitat due to an increase in young growth forests following timber harvest and abandonment of

agricultural fields (Adams and Hamilton 2011). The overabundance of white-tailed deer can have both direct and indirect effects on forest plant species and facilitate the success of invasive species.

Direct effects of deer on native plant communities include altering the abundance of plants through herbivory and changing the composition of the plant community by selectively browsing more palatable plants. Invasive species often have chemical defenses that herbivores avoid, and selective browsing by deer could lead to an increase in their abundance (Côté et al. 2004; Averill et al. 2016). Selective browsing often has its greatest effect on seedlings and herbaceous plants, since they are easily reached by deer, lowering recruitment of canopy trees (Knight et al. 2009a; Knight et al. 2009b; Rooney 2009; Collard et al. 2010; Pellerin et al. 2010).

Deer can also directly affect the distribution of plants via endozoochory, the consumption and deposition of seeds. This can play an important role in long distance dispersal for both plants adapted for frugivory (i.e., fleshy fruits) and plants with small seeds that are accidentally consumed by herbivores (Janzen 1984; Willson 1993; Malo and Suarez 1995; Malo and Suarez 1996; Malo et al. 2000; Pakeman 2001; Vellend et al. 2003; Myers et al. 2004). Although deer are not commonly thought of as significant seed dispersers, their high abundances may result in an increasingly important role in the spread of plants, including invasive species, on local and regional scales.

White-tailed deer can also impact the plant composition and invasive species in forest ecosystems indirectly. Indirect effects occur when one organism affects a second organism through an impact on a third organism or by affecting the environment of the second organism. Indirect effects from ungulate herbivores and their impact on plant

species are not as well understood as the direct effects of herbivory. These indirect effects can include changes in resource availability and changes to the physical environment (Rooney and Waller 2003; Heckel et al. 2010; Kumbasli et al. 2010; Pellerin et al. 2010).

Nitrogen cycling may be changed in several ways when deer are overabundant. On a small, local scale, deposition of dung and urine from large herbivores, such as white-tailed deer, can return high amounts of nitrogen to the soil, leading to an increase in the heterogeneity of soil resources for plants (Bardgett and Wardle 2003; Côté et al. 2004). The direct effect of herbivory may alter the understory structure and may influence the nutrient inputs to the soil by changing the composition of the leaf litter. For example, white-tailed deer may decrease the overall contribution of shrub litter to nutrient cycling by over-browsing the shrub layer (Ritchie et al. 1998) or by allowing invasive species, with different nutrient contents of their leaves, to take over an area by preferentially browsing native plants (Shen et al. 2016).

A second resource that can be altered by an overabundant white-tailed deer population is available light in the understory. For example, sustained browsing of tree seedlings and avoidance of unpalatable ferns, grasses, and sedges, can cause tree regeneration to fail, resulting in sparse, savanna-like forests with little plant diversity in the herbaceous layer (Kolb et al. 1989; Rooney 2001; Horsley et al. 2003; Rooney and Waller 2003; Rooney 2009). This direct effect of herbivory opening up the understory may then indirectly favor light limited species by allowing more light to reach the forest floor, which can facilitate exotic species invasions by creating an environment with greater light resources for which light-adapted or light-generalist invasive species can

out-compete native species (Parendes and Jones 2000; Knight et al. 2009a). For example, Parendes and Jones (2000) found that 21 invasive species were most numerous in areas with high light produced by forest openings along roads compared to areas of lower light in a forest in the Pacific Northwest.

Soil compaction is another way that white-tailed deer can have indirect effects on the plant community. Heckel et al. (2010) indicated that browsing by an abundant deer population reduced leaf litter inputs, contributing to erosion of the upper layers of soil, and high traffic levels resulted in soil compaction. This overall increase in compaction resulted in a decline of both palatable and unpalatable native herb populations.

Once a forest community becomes heavily invaded by exotic species, changes may cascade through the ecosystem. For example, invaded areas have been shown to provide substantial increases in the primary productivity (Byers et al. 2012; Trammell et al. 2012), and in forest systems, approximately 90% of net primary production enters into detrital food webs (Cebrian 1999; Gessner et al. 2010). Trammell et al. (2012) found an increase of more than 20% of the total litterfall biomass in areas invaded by bush honeysuckle (*Lonicera maackii*). This detritus plays a critical role in ecosystems as it decomposes, serving as a major pathway for nutrient cycling (Handa et al. 2014). Decomposition is facilitated by bacteria, fungi, and invertebrates. Changes in the litter quantity and quality can impact the arthropod diversity and abundance in the detrital food webs (Uetz 1979; Bultman and Uetz 1984; Antvogel and Bonn 2001; Negrete-Yankelevich et al. 2008; Chen and Wise 1999). Understanding the changes in the dynamics of an ecosystem is therefore important for managing the problem of invasive species and restoring native species.

The purpose of my dissertation was to understand the effects of deer on the plant communities and the effects of invasive species on the litter-dwelling communities of a deciduous forest ecosystem. In Chapter 1, I used deer exclosures to test for indirect effects of deer on the physical environment, to test for direct effects of deer browsing, and to monitor changes in the plant community after deer exclusion. I found that deer had indirect effects, altering the physical environment by increasing soil compaction, altering the soil nutrient pools, and lowering nitrate fluxes (e.g. lower nitrate flux) when they were present. I also found that deer had direct effects. When deer were present, damage from herbivory was higher leading to reduced plant height. The effects of deer herbivory resulted in significantly lower biomass and survival for species that were planted in the study plots. The lone exception to the reduction in survival in the presence of deer was *Lonicera maackii* (Bush Honeysuckle). Deer browse on this invasive species is similar to most of the other species in the study, but its exceptional browse tolerance may be facilitating the invasion of this species. Invasive plant species also became much more common in the herbaceous layer of open plots compared to fenced plots, indicating that deer are clearly having a facilitative effect on the invasive species. The combination of these direct and indirect effects may result in a vastly different forest system due to the loss of native species unable to compete with invasive species that are avoided by deer in the deer-altered environment.

In Chapter 2, I looked at the indirect effect of deer herbivory increasing light in the understory by reintroducing shade and the direct effects of deer by excluding deer from study plots. This study examined two common native forest understory herbs, a common invasive herb, and a common invasive grass. I found that two native plants

(*Arisaema triphyllum* and *Podophyllum peltatum*) were largely unaffected by both the indirect effect of light availability and the direct effect of deer. I found that two invasive plants (*Alliaria petiolata* and *Microstegium vimineum*) did benefit from the indirect (shade) and direct (deer presence) effects of deer. Preferential browsing of competitors by deer appeared to provide an opportunity for *M. vimineum* to grow, reproduce, and spread. The indirect effect of deer increasing light availability also appeared to provide an opportunity for *M. vimineum* to grow and reproduce better than competitors. The invasive herb *Alliaria petiolata* had a more complicated response, recruiting more individuals when unshaded and when deer were present, but had lower survival, growth, and reproduction in the presence of deer. In the case of the natives, white-tailed deer are not playing a significant role in their abundance or reproductive outputs. However, the invasive species, especially *M. vimineum*, are affected by both the direct and indirect effects of deer herbivory and the indirect effect of light availability.

In Chapter 3, I examined the effects of deer as endozoochorous seed dispersers. I found that deer were transporting seeds from many different taxa via endozoochory. However, very few of the seeds identified from scat germinated, suggesting that deer are acting more as seed predators than seed dispersers and not benefitting many of the species whose seeds they consume. Of the seeds identified in deer scat, the typical invasive seeds were from plants with small, hard seeds from non-fleshy fruits. This fits with the "foliage is the fruit" hypothesis of Janzen (1984) which suggests that some plants rely on accidental ingestion of seeds by herbivores consuming the leaves and stems of the plant. On the other hand, native seeds identified in deer scat were typically from fleshy-fruited plants, indicating that deer are acting as frugivores for native species.

Using germination trials proved to be vital in understanding the role of seed dispersal by deer. Despite finding a wide-variety of seed taxa in deer scat, the low germination rates found in my study suggests that deer are playing only a minor role in seed dispersal in my study areas.

In Chapter 4, I tested for the effects of leaf litter from three invasive plants (*Ailanthus altissima*, *Lonicera maackii*, and *Rhamnus davurica*) on the detrital-layer food web. I found that invasive litter had higher nitrogen content and lower C:N ratios. I found that invasive litter was short-lived, decomposing much faster than native litter. In invaded habitats, the litter cover was much lower by early summer compared to native habitats. Using litter naturally found in the invaded and native habitats, as well as litter packets containing either invasive litter or native litter, I found that bacteria, fungi, and arthropods all preferred litter from the invasive species. This suggests that these invasive species are beneficial, novel resources for the litter-dwelling community. This positive response to invasive litter could be expected to propagate throughout the food web of this system. On the other hand, due to the short-lived nature of the invasive litter, the abundance of the litter-dwelling organisms crashed once the litter decomposed. This crash could also be expected to have negative effects on the food web that cascade to higher trophic levels. As a whole, despite lower overall abundance of litter-dwelling organisms, the native habitat supports a more stable litter-dwelling community over the course of a growing season because it has longer lasting litter that is both a nutritional and structural resource.

Taken together, the results of my study indicate that deer are having significant impacts on this forest system. By altering the physical environment, deer are creating

opportunities for invasive species to grow and reproduce that native species are unable to take advantage of. The effects of deer herbivory also appear to create advantages for invasive species that are either more tolerant of herbivory than native species or that benefit from the removal of preferred native competitors. Once invasive species are established, they may have strong effects on the food webs of this forest system. These invasive species may create a novel food web in this forest that is characterized by cycles of high abundance of litter-dwelling organisms in the spring that become very low in abundance in the summer. These cycles can then affect the higher trophic levels of the system, forcing them to find uninvaded habitats late in the growing season to sustain their populations.

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CHAPTER 1

The effects of excluding white-tailed deer (*Odocoileus virginianus*) on abiotic habitat components and the herbaceous-layer plant communities

Abstract

The expansion of white-tailed deer (*Odocoileus virginianus*) populations represents a disturbance to forest plant communities. Direct effects of deer herbivory may favor unpalatable and browse-tolerant plants, and indirect effects may increase soil compaction, altered nutrient cycling, and an increase in the openness of forest understories. These direct and indirect effects can favor the invasion of exotic plants by removing palatable native competitors and altering the understory habitat conditions for which native species are adapted. To understand the effects of deer and their interactions with native and invasive plants, I used paired exclosure and open plots in forest fragments at Blandy Experimental Farm in the northern Shenandoah Valley of Virginia. These paired plots allowed me to determine the changes in the physical properties of the abiotic environment, to measure the amount of herbivory damage and its effects on growth and survival, and to document changes in the plant community composition. I found that soil compaction increased and nitrogen flux decreased in the presence of deer. In control plots, the density of plants in the herbaceous layer increased, driven by the density of invasive plants, relative to exclosure plots. I found that the fence treatment led to differences in the plant community composition, but this did not change significantly over time. Herbivory damage from deer was significantly higher in open plots, and this led to significantly lower biomass and survival for most of the species measured. However, the survival of *Lonicera maackii* was not significantly different between treatments, despite lower biomass in open plots, indicating that this invasive species is especially tolerant of herbivory compared to the other invasive and native species in this study. The combined effects of deer on the abiotic environment and the direct effects of

deer herbivory may result in vast differences from the historical composition of this forest due to the loss of native species that are unable to compete with invasive species that are avoided by deer.

Introduction

Anthropogenic environmental disturbances can have compounding effects on exotic plant invasions. One example of human-related environmental disturbance is the overabundance of white-tailed deer (*Odocoileus virginianus*) throughout much of their range. Protective laws and refuges have allowed white-tailed deer to recover from historic habitat loss and over-hunting (Adams and Hamilton 2011; Côté 2011). The regeneration of young growth forests following timber harvest and abandonment of agricultural fields have created ideal habitat for deer, and management strategies aimed at increasing herd numbers for hunters have led to an overabundance of this species (Adams and Hamilton 2011). In Virginia, recent estimates of deer populations are approximately 1.25-2.5 times that of estimates from early-colonial times (VDGIF 2015). The overabundance of white tailed-deer can have both direct and indirect effects on forest plant communities and facilitate the success of invasive species (Rooney 2001; Côté et al. 2004; Eschtruth and Battles 2009; Pellerin et al. 2010).

Direct effects of deer include selective browsing of more palatable plants, which may benefit less palatable or better defended plants. Exotic invasive species (hereafter "invasive species") often have chemical defenses that herbivores avoid (Côté et al. 2004; Averill et al. 2016). Selective browsing commonly has its greatest effect on slow-growing, shade-tolerant trees and shrubs and small herbaceous species because they are unable to regrow quickly (Knight et al. 2009a; Knight et al. 2009b; Rooney 2009; Collard et al. 2010; Pellerin et al. 2010). Deer have also been shown to alter successional dynamics, slowing succession (DiTommasso et al. 2014) and accelerating exotic plant

invasion (Eschtruth and Battles 2009) through selective consumption of native species, favoring the persistence of introduced species.

Indirect effects occur when one organism affects a second organism by altering its biotic or abiotic environment. Indirect effects from ungulate herbivores and their impact on exotic plant invasions are not as well understood as the direct effects of herbivory. These indirect effects can include changes in resource availability and changes to the physical environment (Rooney and Waller 2003; Heckel et al. 2010; Kumbasli et al. 2010; Pellerin et al. 2010).

Nitrogen cycling may be changed in several ways when deer are overabundant. On a small, local scale, deposition of dung and urine from large herbivores, such as white-tailed deer, can return high amounts of nitrogen to the soil, leading to an increase in the heterogeneity of soil resources for plants (Bardgett and Wardle 2003; Côté et al. 2004). White-tailed deer may decrease the overall contribution of shrub litter to nutrient cycling by over-browsing the shrub layer (Ritchie et al. 1998) or by allowing invasive species, with different nutrient contents of their leaves, to take over an area by preferentially browsing native plants. For example, Ritchie et al. (1998) indicated that in a nitrogen-limited system, a shift from nutrient-rich plants to unpalatable or browse-tolerant plants that were nutrient-poor led to lower amounts of nitrogen being returned to the soil. On a long-term scale, the effects of deer browsing, by changing the plant composition, can either accelerate or decelerate nitrogen cycling by causing a shift in the canopy species (Frelich and Lorimer 1985; Ferrari 1999; Russell et al. 2001; Rooney and Waller 2003; Côté et al. 2004).

A second resource that can be altered by an overabundant white-tailed deer population is available light in the understory. For example, sustained browsing of tree seedlings can cause regeneration to fail, resulting in sparse, savanna-like forests with little plant diversity in the herbaceous layer (Kolb et al. 1989; Rooney 2001; Horsley et al. 2003; Rooney and Waller 2003; Rooney 2009). This direct effect of herbivory opening up the understory may then indirectly favor light limited species by allowing more light to reach the forest floor. This can facilitate exotic species invasions by creating an environment with greater light resources for which light-adapted or light-generalist invasive species can out-compete native species (Parendes and Jones 2000; Knight et al. 2009a). For example, Parendes and Jones (2000) found that 21 invasive species were most numerous in areas with high light produced by forest openings along roads compared to areas of lower light in a forest in the Pacific Northwest.

Soil compaction is another way that white-tailed deer can have indirect effects on the plant community. Heckel et al. (2010) indicated that browsing by an abundant deer population reduced leaf litter inputs, contributing to erosion of the upper layers of soil, and high traffic levels resulted in soil compaction. This overall increase in compaction resulted in a decline of both palatable and unpalatable native herb populations. For example, compacted soils can reduce the size and female:male sex ratio of *Arisaema triphyllum* (Heckel 2010), negatively affect the size and biomass of oak seedlings (Jourgholami et al. 2017), and limit root growth, length, and dry mass (Kormanek et al. 2015; Piche and Kelting 2015).

The goal of this study was to document the direct and indirect effects of an overabundant white-tailed deer population on eastern deciduous forest plant communities

and to understand the interactions among white-tailed deer and native and invasive plants. I set up pairs of exclosures and open plots that allowed me to test for broad effects of deer on the composition of the herbaceous layer, differentiating between exotic and native plants, as well as the specific composition of the herbaceous layer. I also used these plots to determine the amount of browse and physical damage to plants and to measure growth rates of plants when herbivory is limited for quantity selective group of native and invasive plants that were planted in the study plots. These plots also allowed me to measure the changes in the physical properties of the abiotic environment when deer traffic is prevented. I expected to see greater growth and survival of native species relative to invasive species inside exclosures due to preferential browsing of native species. I expected to see change over time in the composition of the herbaceous layer community in exclosures because the structuring force of deer had been removed. In open plots, I expected the plant community to show very little change, because the community is growing under the same high density deer conditions it has experienced for many years. I expected to see differences between exclosure and open plots to increase over time in abiotic properties. By reducing deer traffic, I expected that exclosures would have reduced soil compaction compared to open plots, and I expected that soil nutrient pools and fluxes would be altered due to a change in the leaf litter input and composition. I also expected that light intensity in exclosures would decrease over time compared to open plots, as the plants inside the exclosures would be able to grow taller and more densely in the absence of deer.

Methods

Site Description

This study was conducted at Blandy Experimental Farm, a 283 ha research facility in the northern Shenandoah Valley (Clarke County, VA) owned by the University of Virginia (39.061°N, 78.065°W). Annual mean precipitation is 97.6 cm. Mean January and July high/low temperatures are 6.2ºand -4.5ºC, and 31.4º and 17.5º C. The habitats at Blandy are similar to the surrounding rural environment, with forest fragments and open fields, including mowed lawns, managed native plant meadows, and fields in different stages of succession. Four forest fragments, ranging from 2.3 to 18.6 ha were used in this study. The canopy species of each of these forest fragments are composed primarily of red oak (*Quercus rubra*), white oak (*Q. alba*), mockernut hickory (*Carya tomentosa*), hackberry (*Celtis laevigata*), and black walnut (*Juglans nigra*). The smallest forest fragment (2.3 ha) differs from the other fragments in having an open understory, comprised primarily of grasses and scattered shrubs. The amount of understory cover in the next smallest fragment (4.9 ha) is similar to the cover in the two largest fragments, however it is primarily a mix of invasive Dahurian buckthorn (*Rhamnus davurica*) and bush honeysuckle (*Lonicera maackii*). The two largest fragments (5.8 and 18.6 ha) are similar in composition with a mix of primarily spicebush (*Lindera benzoin*) and bush honeysuckle.

Deer Exclosures

Within each of the four forest fragments, I established paired fenced/open plots (Supplemental Figure S1). I established four paired plots in winter 2010/2011, two pairs

in each of the two largest fragments. I set up three more pairs in spring 2011 in the two smallest fragments, one pair in the smallest and two pairs in the 4.9 ha fragment. Each study plot measured 25 m^2 (5 x 5 m), with the corners marked by survey flags. Around fenced plots, I erected a 2.3 m tall poly deer fence (Deerbusters, Frederick, MD) measuring 7 x 7 m, leaving a 1 m buffer around the plot.

Abiotic Variables

Each year, I took measurements of abiotic variables from each plot to test for the effects of deer exclusion or presence in the study sites. I measured soil compaction using penetrometers, one for surface penetration resistance (Pocket Penetrometer, Forestry Suppliers, Jackson, MS), and one for penetration resistance at 7.62 cm intervals down to 45.72 cm (Soil Compaction Tester, Dickey-John Corp., Auburn, IL). To record penetration resistance, the tip of the penetrometer is pushed into the soil, and the pressure needed to penetrate the soil was recorded in kg/cm^2. These measurements were made twice a year from five locations in each plot over the course of this study, once in late spring and once in late summer.

I also collected soil cores each summer to measure bulk density. Between 2011 and 2015, one 20 cm deep soil core was collected from each plot, and from 2013 to 2015 an additional 5 cm deep soil core was collected to better evaluate compaction at the surface. I collected these soil cores using a slide hammer attached to the corer, with the 20 cm cores measuring 273 mL in volume, and the 5 cm cores measuring 91 mL. To measure the bulk density of the soil cores, I oven-dried them at 105 ºC, placed the dried soil in a 2 mm sieve. I then poured the material remaining in the sieve into a tray and

crushed it with a rolling pin, and poured the crushed material back into the sieve. I repeated this process until only rock and plant material remained in the sieve. I recorded the mass of the dried soil, rocks, and plant material separately then, using a graduated cylinder partially filled with water, I measured the volume of gravel and plant material. I then calculated bulk density (g/mL) as

$$\text{Bulk Density} = \frac{\text{Mass dry soil } < 2\text{mm}}{\text{Volume of corer } - \text{ Volume of gravel} - \text{Volume of plant material}}$$

To measure the effect of deer exclusion on the soil nutrient pools in the study sites, I collected soil cores from each plot every spring. The cores were collected from four locations in each plot, to approximately 8 cm depth, and mixed together, then placed in a sample box to be sent to the Virginia Tech Soil Analysis lab. Variables returned from these analyses are listed in Appendix 2 and 3.

In addition to the soil nutrient pools, I also measured the soil nutrient flux each summer using soil PRS probes (Western-Ag Innovations, Inc., Saskatchewan, Canada), that mimic plant roots by absorbing cations and anions to a membrane surface. In late May of each year, I inserted four pairs of anion and cation probes in each plot. The pairs of probes remained in the ground for eight weeks, then collected, cleaned with deionized water, and returned to Western-Ag Innovations for analysis. Variables returned from these analyses were: Total Nitrogen, NO_3, NH_4, Calcium, Magnesium, Potassium, Phosphorous, Iron, Manganese, Copper, Zinc, Boron, Sulfur, Lead, Aluminum, and Cadmium (all $\mu g/10cm^2/8$ weeks).

The final abiotic variable I measured was the light intensity in the study plots. To do this, I used Hobo UA-002-08 data-logger pendants (Onset Computer Corp., Bourne,

MA) to record the light intensity (lux) at 0.0 m, 0.5 m, and 1.0 m from the forest floor from each plot in late summer 2013 and 2015. Light intensity readings were recorded on days with no cloud cover, between 10:00 AM and 3:00 PM. In each plot, four PVC stakes were placed approximately 1 m from each corner, toward the center of the plot. The data-loggers were attached at the appropriate heights and recorded the lux at 10 second intervals for five minutes. The light intensity at each height was then averaged over the five minute interval from the four data-loggers. Additionally, four data-loggers were placed in open locations at Blandy, so that the light intensity measured in the plots could be reported as the proportion of light in open areas (mean lux within plots/mean lux in open areas).

Plant community measurements

To document the effects of deer exclusion on the forest plant community, I performed quadrat surveys at least twice in each plot during the growing season (mid-spring and mid-summer) beginning in spring 2011. In each plot, five 1 m^2 quadrats were randomly assigned to each plot using a 5×5 m grid (Supplemental Figure S2). During each survey, I recorded the identity and abundance of all plant species in each quadrat, as well as their origin (native or invasive) and growth habit (herbaceous/grass, vine, or woody). At the end of each year, I totaled the maximum abundance of each species, the mean plant density (plants/m^2), and the density of plants by origin and growth habit for each plot.

Due to the heterogeneity of the study sites, comparing paired plots was unreasonable because each plot pair started with a moderate difference in composition.

Instead, the community composition of both fenced and open plots were compared using Sorensen's quantitative similarity index to measure the similarity in plants species of each plot between its first year of establishment and each successive year. This method of comparison yields lower values of similarity for plots that have greater change in plant composition over time, and higher similarity for plots with little change in plant composition. I expected that plots within deer exclosures would be less similar at the end of the study, due to the release from deer herbivory and traffic, and that control plots would be more similar at the end of the study, because the effects of deer were still present.

In the final year of the study, used a LIDAR scanner to measure the plant structure in the study plots to test for differences between fenced and open plots. To do this, two ground-based LIDAR scans were taken at each plot from opposing directions. The point-return data from the two scans were then fitted together to form one point-cloud using SCENE 3D laser scanner software (FARO, Lake Mary, FL). Points returned from downed dead-wood and trees greater than 2 m were removed manually using CloudCompare processing software (CloudCompare.org). Using QuickTerrain (Applied Imagery, Chevy Chase, MD), I estimated the mean understory canopy height by first calculating the "Max Z" value for cells on a 0.1 m^2 grid, then taking the average of all the "Max Z" values for all the cells. I expected the mean understory canopy height to be higher for fenced plots and lower for open plots, as the plants inside the fence did not have browsing pressure from deer. I also used QuickTerrain to calculate the density of point-returns in 0.5 m intervals from 0 m to 2 m. The density of plants at each interval was then represented as the proportion of returns in a given interval to the total number of

point-returns for the plot. I expected to see greater density of returns in the fenced plots with increasing height compared to the open plots due to the absence of deer browsing.

Herbivory surveys

In order to measure the effects of white-tailed deer on plant growth and recruitment, I planted an assortment of 12 species, including native and invasive trees, shrubs, vines, and herbaceous species (Table 2) in each of the four plot pairs in the two largest forest fragments (established in 2011). Two additional species were planted but failed to survive to the end of the study, the invasive species *Alliaria petiolata* and *Microstegium vimineum*. I chose to plant species that were common to this region and that were readily available or easy to grow. Each plot, therefore, started with identical numbers and types of species. In each plot, 12 individuals of each species were randomly planted in a 12 x 12 grid. Beginning in winter 2011/2012, I documented the occurrence of damage (yes/no), number of stems damaged, and source of damage (deer/other) for all trees and shrubs planted in the plots. In spring 2013, I began recording if the plant had been previously damaged and the proportion of total stems damaged. In fall 2013, I began recording the number of apical buds (or main growing stems for bush honeysuckle), and the mean length of stems. These additional measurements give a clearer picture of the ability of these trees and shrubs to tolerate damage and continue growing despite the effects of herbivory.

In fall 2015, I collected all surviving plants from the study plots. I then recorded the aboveground biomass by drying the plants for 72 hours at 105 ºC and recording their

dry biomass. Finally, I recorded the number of surviving plants of each species out of the total planted for each plot.

Statistical analyses

Soil compaction and bulk density data was summarized with a principal components analysis (PCA) on the penetration and bulk density measurements. The resulting principal components were then tested using an ANOVA to test for the effects of deer exclusion on the soil compaction, with sites of the pairs as a block effect and fence or open as the treatment effect.

The effect of deer exclusion on the soil nutrient pools were tested similarly. First, the variables were separated into three categories: inorganic nutrients, acidity measurements, and soil organic matter. I then performed a PCA on the inorganic nutrients and acidity measurements, then tested for differences in the principal components of the inorganic nutrients, acidity, as well as the soil organic matter between fenced and open plots using an ANOVA with sites of the pairs as a block effect and fence or open as the treatment effect.

I tested for differences in soil nutrient flux between fenced and open plots using a repeated measure ANOVA for nitrate (NO_3) ammonia (NH_4) with sites of the pairs as a block effect, and fence, year, and fence×year as the treatment effects. Year was the repeated variable with sites(fence) as the subject. The inorganic nutrients were tested by first performing a PCA, then testing the principal components with a repeated measures ANOVA as with nitrate and ammonia.

The effect of deer exclusion on light intensity was tested using mixed model ANOVA to compare the lux at the forest floor, 0.5 m, and 1.0 m between fenced and open plots. The proportion of the lux in open areas was the response variable, with sites of pairs as a block effect, and fence, height, and fence×height as the treatment effects.

The effect of deer exclusion on the density of plants and the density of plants by origin and growth habit was tested using mixed model ANOVA. The density of plants in 2015 was the response variable, sites of pairs was a random block effect, and fence treatment was the fixed effect. The density measurements of plants from the preliminary surveys of each plot was not significant, but was included as a covariate in the model to account for change from the initial composition.

The effect of deer exclusion on the similarity of plant composition between plots in 2015 and the first year of establishment was tested using mixed model ANOVA. The similarity for each plot was the response variable, sites of pairs was a random block effect, and fence treatment was the fixed effect.

I used non-metric multidimensional scaling (NMDS) to represent the species composition and abundance of each plot in their initial year of establishment and 2015. I used the "autopilot" mode in PC-ORD (MjM Software, Gleneden Beach, Oregon, U.S.A.), with the Sorensen distance and a random starting configuration for the analysis. The procedure included 500 runs with real data and 500 runs with randomized data and use of a Monte Carlo test to help select final dimensionality. Dimensions that did not reduce stress by 5 or more were not considered useful and the highest dimensionality that met this criterion was used for the final ordination. A solution with two dimensions was optimal, and a total of 53 iterations was used for the final solution at which point the

reduction in stress had stabilized. NMDS represents sampling units in ordination space where sampling units closer to one another are more similar than units farther away. Using the NMDS coordinates of each plot in its initial year and in 2015 from the final ordination, I tested for the effect of deer exclusion using multivariate ANOVA (MANOVA) to compare the two dimensions between fenced and open plots, and between the initial year and 2015. The coordinates of the two dimensions for each plot were the response variables, fence, year, and fence×year were the fixed treatment effects, and sites of pairs was included as a block effect.

The effect of deer exclusion on the plant structure data from the LIDAR scans was tested using ANOVA to compare the understory height and the proportion of point-returns between fenced and open plots. The mean understory canopy height and the proportion of total returns for the 0.5 m intervals were the response variables, with sites of pairs as a block effect, and fence was the treatment effect.

The effect of deer exclusion on the planted species growth variables was tested using mixed model ANOVA. The proportion of stems damaged, number of apical buds, and stem length were the response variables, and fence, species, and fence×species were fixed treatment effects.

I tested for differences in the aboveground biomass of the species planted in the study plots using a mixed model ANOVA with sites of the pairs as a random block effect, and fence, species, and fence×species as the fixed treatment effects. Because no individuals of *Alliaria petiolata* or *Microstegium vimineum* survived to the end of the study, they were not included in the analysis. Individuals that were still present but were standing dead were also removed from the analysis.

The effect of deer exclusion on the survival of the planted species was tested using logistic regression to compare the probability of survival between fenced and open plots. Survival was the response variable and fence, species, and fence×species were the fixed treatment effects. Due to very few non-woody species surviving the duration of the study, only the woody trees and shrubs were included in the analysis.

Results

Soil Analyses

The principal component analysis on the soil compaction variables indicated a positive association with overall soil compaction for principal component 1 and a positive association with soil bulk density for principal component 2 (Appendix 1), together accounting for 66.7 percent of the variation in the data. Principal component 1 was significantly higher in open plots than fenced plots (Table 1; $F_{1,6}$=23.02, P=0.003), indicating higher soil compaction when deer were present. Principal component 2 was not different between treatments (Table 1; $F_{1,6}$=0.18, P=0.6823).

The principal component analysis on the inorganic soil nutrient pool variables indicated a positive association with potassium, calcium, zinc and boron for principal component 1, with a negative association with copper and iron (Appendix 2). Principal component 2 was positively associated with phosphorous, potassium, zinc, copper, and iron. The first two principal components accounted for 81.5 percent of the variation in the data. Principal component 1 was marginally higher in open than fenced plots (Table 1; $F_{1,6}$=4.94, P=0.0679), indicating greater availability of the positively associated nutrients in the presence of deer and greater availability of the negatively associated

nutrients when deer were absent. Principal component 2 was not different between treatments (Table 1; $F_{1,6}$=0.82, P=0.399).

The principal component analysis on the acidity variables from the soil nutrient pool measurements indicated a positive association with pH, buffer index, and cation exchange capacity and a negative association with acidity for principal component 1 (Appendix 3). Principal component 1 accounted for 93.2 percent of the variability in the data. There was no difference between treatments (Table 1; $F_{1,6}$=1.55, p=0.259).

The percent soil organic matter from the soil nutrient pool measurements was not different between fenced and open plots (Table 1; $F_{1,6}$=1.36, P=0.2963), indicating no impact from deer on the soil organic matter.

The repeated measure ANOVA on the nitrate flux indicated significantly greater nitrate flux over the 8-week burial period in fenced plots compared to open plots (Table 1; $F_{1,42}$=9.51, P=0.0036). The interaction of fence×year was not significant ($F_{3,42}$=0.54, P=0.6545). The repeated measure ANOVA on the ammonia flux indicated no difference between treatments (Table 1; $F_{1,42}$=0.02; P=0.8892), and there was no interaction of fence×year ($F_{3,42}$=1.39, P=0.2603).

The principal component analysis on the inorganic soil flux nutrients indicated a positive association with calcium, iron, copper, zinc, sulfur, and lead, and a negative association with potassium, and aluminum for principal component 1 (Appendix 4). Principal component 2 was positively associated with potassium, manganese, and aluminum, and negatively associated with calcium, and cadmium. The first two principal components accounted for 52.8 percent of the variation in the data. There was no difference in the inorganic soil nutrient flux between open or fenced plots for either of the

principal components (Table 1; Principal component 1: $F_{1,42}$=2.76, P=0.1038; Principal component 2: $F_{1,42}$=0.77, P=0.3867) and there were no interactions with year (Principal component 1: $F_{3,42}$=1.05, P=0.3805; Principal component 2: $F_{3,42}$=0.12, P=0.9463).

Light Analysis

There was no difference between open and fence treatments in the proportion of lux reaching the study plots relative to full sun (Table 1; $F_{1,30}$=0.1, P=0.7517), nor was there a difference in the proportion of lux at 0m, 0.5m, and 1.0m heights in the study plots ($F_{2,30}$=1.94, P=0.1618). There was also no fence×height interaction for the proportion of lux reaching the study plots ($F_{2,30}$=0.03, P=0.967).

Plant Community Analyses

Mean understory plant density was 1.9 times greater in open plots than fenced plots (Figure 1a; $F_{1,5}$=13.15, P=0.0151). The difference between treatments was seen almost exclusively in the herbaceous plants, which were 2.1 times more dense in open plots than in fenced plots (Figure 1b; $F_{1,5}$=12.41, P=0.0169). Woody plants and vines did not differ between open and fenced plots (Figure 1c; $F_{1,5}$=0.52, P=0.5041; Figure 1d; $F_{1,5}$=0, P=0.9547). The mean density of native plant species was only marginally higher in open relative to fenced plots (Figure 1e; $F_{1,5}$=5.38, P=0.0680), but the mean density of invasive plants in open plots was 2.5 times the density of invasive plants in fenced plots (Figure 1f; $F_{1,5}$=7.88, P=0.0377). Invasive plants accounted for 71 percent of the total plants in open plots, but only 52 percent of fenced plots. The invasive species that showed the biggest differences in open plots relative to fenced plots *Alliaria petiolata*,

Celastrus orbiculatus, *Microstegium vimineum*, *Perilla frutescens*, *Polygonum* sp., and *Rhamnus davurica* (all more abundant in open plots). Of these, the invasive species *A. petiolata*, *C. orbiculatus*, *M. vimineum*, and *Polygonum* sp. were found in the majority of the study sites, whereas the others were commonly found in only one or two of the woodlots. The native species that were most abundant inside the fences were *Cryptotaenia canadensis*, *Impatiens pallida*, *Phryma leptostachya*, and *Toxicodendron radicans*. Of these, only *T. radicans* was found in a majority of the study sites, with the others only found in one of the woodlots.

The similarity in plant composition of the plots in 2015 compared to their composition the first year they were surveyed was not different between open and fence treatments (Open mean: 0.5018 (SE=0.06); Fence mean: 0.5194 (SE=0.06); $F_{1,6}$=0.04, P=0.8407). The overall mean similarity index was 0.51, which indicates that species composition showed approximately a 50% turnover during the course of this study. Testing the NMDS ordination dimensions (Appendix 5) indicated significant differences in plant community composition between years ($F_{2,17}$=10.67, P=0.001) and fence treatments ($F_{2,17}$=4.46, P=0.0278). If deer were really having an effect on the species composition, we would have expected a fence×year interaction because the effects of deer accumulating over the course of the experiment should have led to increasingly divergent plant communities, but there was no year×fence interaction (Figure 2; $F_{2,17}$=1.38, P=0.2792), suggesting that the difference between fenced and open plots did not change over the course of the study

The mean understory canopy height based on the average max Z values of point returns from LIDAR scans was slightly higher in fenced plots than in open plots, but this

was not significant (Open mean: 0.36m (0.23-0.57m); Fence mean: 0.59m (0.37-0.95m); $F_{1,6}$=3.52, P=0.1097). The proportion of point returns at 0.5 m intervals (Figure 3; 0-0.5m: $F_{1,6}$=5.99, P=0.05; 0.5-1.0m: $F_{1,6}$=10.31, P=0.0183; 1.0-1.5m: $F_{1,6}$=5.49, P=0.0576; 1.5-2.0m: $F_{1,6}$=0, P=0.9967) was significantly different for the two lowest intervals, marginally significantly different for the 1.0-1.5 m interval, and not significant for the 1.5-2.0 m interval. Between 0-0.5 m, the proportion of total point returns in open plots was 1.6 times greater than that of the fenced plots, indicating greater biomass at this cross-section. Between 0.5-1.0 m the proportion of total point returns was 2.9 times greater in fenced plots than that of the open plots and between 1.0-1.5 m (marginal), the proportion of total point returns was 5.1 times greater in the fenced plots than the open plots, indicating greater biomass at this cross-section when deer were absent.

Herbivory Analyses

In open plots, proportion of stems damaged of the tree and shrub species planted in the study plots was 6.3 times greater than in the fenced plots (Open mean: 0.18 (SE=0.014); Fence mean: 0.03 (SE=0.017); $F_{1,332}$=47.19, P=<0.0001). Buckthorn individuals had the highest proportion of stems damaged, 3.8 times greater than dogwoods, which had the lowest proportion of stems damaged ($F_{5,332}$=3.19, P=0.0079). The fence×species interaction was significant, with buckthorn individuals having 10.4 times the proportion of stems damaged in open plots compared to individuals in fenced plots whereas white oak individuals in open plots only had 2.1 times the proportion of stems damaged compared to individuals in fenced plots (Table 2; $F_{5,332}$=3.33, P=0.006)

In fenced plots, individuals had 1.3 times the number of apical buds compared individuals in open plots and there was no fence×species interaction ($F_{1,332}$=6.69, P=0.0101; $F_{5,332}$=1.12, P=0.3501). Species in fenced plots grew significantly taller than species in open plots (Table 2; $F_{5,330}$=2.52, P=0.0295). Inside fences, buckthorn, bush honeysuckle, and red maple were approximately 40% taller than in open plots and northern red oak was 22% taller inside the fenced plots. Dogwood and white oak did not differ between the fenced and open plots.

At the end of the study, the above-ground biomass for individuals planted in the fenced plots was 2.6 times greater than in the open plots (Open mean: 3.01 g (SE=0.13); Fence mean: 1.15 g (SE=0.14); $F_{1,284}$=41.54, P<0.0001). Species responded differently to the fence treatment (Table 2; $F_{9,284}$=9.42, P<0.0001). The effect of the fence treatment had a significant effect on the biomass of all the woody species except for *Quercus alba*, but did not affect the biomass of the herbaceous species, grasses or vines. Inside the fence, *Rhamnus davurica* grew 5.0 times larger, *Lonicera maackii* grew 9.2 times larger, *Cornus florida* grew 8.1 times larger, *Quercus rubra* grew 5.6 times larger, and *Acer rubrum* grew 6.0 times larger compared to individuals growing in the open plots. Species also differed in the effect of the fence treatment on the likelihood of surviving to the end of the study (Table 2; χ^2=17.362, 5 df, P=0.0039). The biggest difference in probability of survival was for *Quercus rubra* which was 13.9 times more likely to survive to the end of the study in the fenced plots than in the open plots. At the other extreme, *L. maackii* and *C. florida* showed almost no benefit from the fence treatment. *L. maackii* had relatively high survival inside and outside the fences, but *C. florida* had uniformly low survival.

Discussion

Abiotic Variables

The presence of white-tailed deer in this forest community had clear effects on the abiotic properties. When deer were present, the overall soil compaction was much greater than inside the exclosures. Compacted soils have been shown to reduce the size and biomass of *Quercus castaneifolia* (Jourgholami et al. 2017), limit root growth in forests growing on abandoned agricultural land (Piche and Kelting 2015), lower root system length and dry mass in *Pinus sylvestris* and *Fagus sylvatica* (Kormanek et al. 2015), and reduce the size and female:male sex ratio of *Arisaema triphyllum* (Heckel et al. 2010). Loss of vegetation cover and reduced leaf litter deposition due to browsing effects of ungulates can increase surface water runoff and soil erosion (Cumming and Cumming 2003; Sharrow 2007), which may then lead to increased soil compaction resulting in decreased germination rates (Basset et al. 2005; Kyle et al. 2007), decreased nutrient availability (Cumming and Cumming 2003; Sharrow 2007; Heckel et al. 2010), and reduced absorption of mineral nutrients by plant roots (Kozlowski 1999). It is possible that soil compaction was responsible for some of the detected chemical differences between the fenced and open plots.

When deer were present, the pool of the inorganic nutrients potassium, calcium, zinc, and boron were marginally higher compared to fenced plots, and the pool of copper and iron was marginally higher inside exclosures compared to open plots. The flux of nitrate, the amount of nitrate absorbed by the soil Plant Root Simulator (PRS) probes, inside exclosures was 1.38 times that of the open plots, indicating that more nitrate is moving through the system and being absorbed by plant roots when deer are absent. This

suggests that plants are able to use more of the available nitrate, allowing them to grow bigger and allocate more to reproduction.

The consumption of nitrogen-fixing and woody plants has been shown to decrease the nitrogen content of aboveground plant tissue, litter, and belowground plant tissue, resulting in lower soil nitrate and total available nitrogen concentration in the presence of deer (Ritchie et al. 1998). Similarly, tree saplings of some species, when growing in an abundance of nitrogen, have been shown to increase the nitrogen content of their stem tissue which resulted in higher browse frequency by white-tailed deer (Tripler et al. 2002). This consumption of plant tissues results in an indirect effect on the nitrogen inputs to the ecosystem via reduced litter-fall, lowering the resource availability, which may lead to a reduction in understory and woody plant biomass (Ritchie et al. 1998, Tripler et al. 2002, Côté et al. 2004; St. John et al. 2012). The higher nitrate flux in the exclosure plots in this study may be the result of increased litter-fall from the unbrowsed vegetation as well as reduced soil compaction that may have allowed for greater root growth and uptake of nutrients (Kozlowski 1999). Conversely, the reduced nitrate flux in the open plots is best explained by a combination of reduced litter inputs and the effects of soil compaction on limiting the growth and extent of root systems described previously.

At the end of this study, I found no difference in the light intensity between open and fenced plots, and no difference in the light intensity at different heights within plots. This result was surprising given the contrast in the height of the understory plants between the treatments, as indicated by the higher point returns from 0.5-1.5 m in the exclosure plots. The proportion of open canopy lux that I found was similar to other

studies in deciduous forests (e.g., Canham and Burbank 1994; Tinya and Ódor 2016). It is possible that the difference in shade created by the taller plants inside the fences was not yet strong enough to separate them from the open plots, especially considering that the majority of the light is already attenuated by the canopy. I do expect that, given enough time, these treatments would result in differences in light intensity as the understory plants increase their height and shrubs and saplings are able to grow into the subcanopy, adding additional shade.

Plant Community

By the end of my study, the plant community showed clear changes from its initial composition, but the interaction of fence×year was not significant for the NMDS ordination, indicating that open and fenced plots showed the same degree of change. However the difference in the fence treatment, which was significant, was primarily from 2015, not 2011, suggesting that there was some accumulation of the effects of deer over the course of the experiment that I was unable to detect.

I did find effects from deer on the plant community in both the herbaceous layer quadrat surveys and the LIDAR scans. In the herbaceous layer, I found higher plant density in the presence of deer, primarily due to herbaceous invasive plants. The LIDAR scans also indicated greater density in the herbaceous layer, with significantly greater point returns from 0-0.5 m in the open plots, but greater density from 0.5-1.0 m in fenced plots. In open plots, the majority of plants are therefore limited to 0-0.5 m tall, beyond which they are damaged from deer browsing. For shrub and tree seedlings, this means that there is little chance of reaching the subcanopy when deer are present. On the other

hand, when deer are absent, the plants are able to grow taller, reaching more light resources, and have greater potential of reaching the subcanopy for shrubs and tree seedlings. Therefore, although the plant communities in this system are undergoing regular species turnover, when deer are present there will be a shift to an invasive-dominated system.

I had expected to see regeneration of tree seedlings inside the fences, but I did not observe this effect. After five years of exclusion, the density of woody plants did not differ between treatments. Previous studies have primarily reported positive effects of deer exclusion on woody vegetation including higher growth (Inouye et al. 1994; Horsley et al. 2003; Tripler et al. 2005; McGarvey et al. 2013), greater abundance or density (Horsley et al. 2003; Rossell et al. 2005; Rossell et al. 2007; Rooney 2009; McGarvey et al. 2013; DiTommasso et al. 2014), and greater survival (Tripler et al. 2005; Rossell et al. 2005). However, in some cases deer exclosure or reduced deer densities are not always enough for the community to recover. High mortality from small rodent herbivores (Inouye et al. 1994) or limited recruitment of deer-browse sensitive species (Tanentzap et al. 2011) may limit the beneficial effects of deer exclusion. It is possible, in the case of my study system, the relatively short time of exclosure (5 years) and the effects of other herbivores present in this forest are limiting the recovery of the woody vegetation. In fact, during the establishment and monitoring of the species used in the herbivory and survival analysis, I did observe damage and mortality, especially shortly after planting, from rodents.

As the invasive species became more prevalent in the herbaceous layer in open plots, native species did not become more prevalent when deer were excluded. Although

the density of invasive plants decreased inside fenced plots, the native species did not fill in the gaps, leading to overall lower plant density when deer were absent, as evidenced by the fewer point returns in the LIDAR scans. Several studies have found that when deer are excluded from an area, the legacy of chronic herbivory resulted in only limited recovery of plant communities. Native species recovery was limited only to those species that were able to persist under intense herbivory prior to deer exclusion and there was little to no recolonization of browse-sensitive species (Webster et al. 2005; Goetsch et al. 2011; Pendergast et al. 2016). This led to long time lags for recruitment from refugia due to low dispersal and reproductive rates for many understory species.

In addition to legacies of herbivory, it is also important to consider the legacy of the land-use history at Blandy Experimental Farm and the surrounding region. Historically, much of Virginia was converted to agriculture, resulting in a highly fragmented and disturbed landscape. As agricultural fields have been abandoned, forests have regrown, but the composition is heavily influenced by the surrounding vegetation. The forest fragments at Blandy used for this study, likewise were previously farmland with the largest (south-west) fragment only approximately 100 years old. As these forests at Blandy have regrown, the plant composition is limited to the most commonly occurring species and those species that are able to disperse from the surrounding fragmented and disturbed landscape. Although during the course of my study I did not observe the recovery of the native understory plant community, it is possible that, given enough time, the community in the deer exclosures would begin to restore itself.

The long-term effects of deer browsing can have significant impacts on the vegetation dynamics of forest systems. This can include delays in successional processes

by reducing plant biomass, recruitment of woody species, and selective browsing of native species that favors the persistence of short-lived, introduced species that recruit from an altered seed bank (DiTommaso et al. 2014). Deer browsing may also alter the trajectory of vegetation development favoring unpalatable or browse-tolerant species at the expense of native species that are preferentially browsed or browse-intolerant (Horsley et al. 2003; Rossell et al. 2007; Knight et al. 2009a; Knight et al. 2009b; Shen et al. 2016). Intense herbivory can lead to extirpation of browse sensitive species as well, such as *Trillium* sp., with only the most resilient species persisting in the presence of long-term deer browsing (Webster et al. 2005; Jenkins et al. 2007; Knight et al. 2009b). The result of chronic herbivory, therefore, can drastically alter the composition of the vegetative community, leaving behind a system dominated by invasive plants and species that are tolerant of herbivory or unpalatable to deer.

The impacts on the plant community from overabundant browsers is not only limited to white-tailed deer. At Yellowstone National Park, the absence of wolves for 70 years resulted in an overabundant elk (*Cervus elaphus*) population which significantly impacted woody plants, resulting in negative impacts to soils and wildlife habitat which was only recently reversed with the re-introduction of wolves (Ripple and Beschta 2012). In southeastern Australia, the reduction of red fox (*Vulpes vulpes*) populations, an introduced predator, and the lack of other predators has resulted in an extremely high population of native wallabies (*Wallabia bicolor*) (Dexter et al. 2013). The post-fire recruitment and growth of tree species was significantly lower and an understory fern (*Pteridium esculentum*) increased in abundance in open plots compared to fenced plots.

The long-term effect of this herbivore may result in a low diversity fern parkland (Dexter et al. 2013) similar to the effects of deer (Rooney and Waller 2003).

Herbivory Effects

Plants in open plots received 6.3 times more stem damage than plants inside fences, were significantly shorter, and had significantly fewer apical buds. The increase in the number of apical buds inside fences indicates that these plants have more opportunities for growth in height and breadth in the absence of deer. The relative amount of damage differed widely across species, however. The invasive species *R. davurica* and *L. maackii* grew approximately 40 percent taller inside fences than open plots, and the native species *A. rubrum* and *Q. rubra* grew 40 and 22 percent taller inside fences, respectively. There was, however little difference between treatments for the native species *C. florida* and *Q. alba*. Overall, plants growing in the presence of deer are at a vast disadvantage compared to plants growing in the absence of deer, regardless of if they are native or invasive species. In forests with high deer populations similar to Blandy, this can greatly alter the understory dynamics.

At the end of the study, the biomass and survival of the species planted in the plots followed a similar pattern as that for the herbivory surveys. Biomass inside fences was 2.6 times that of open plots, and there was a significant interaction of fence×species treatment. The native tree *Q. alba*, and the grasses, vines, and herbaceous species did not differ between treatments, but the invasive shrubs *R. davurica* and *L. maackii* and the native trees *C. florida*, *Q. rubra*, and *A. rubrum* all had significantly greater biomass inside fences (5-9.2 times that of open plots). The likelihood of survival to the end of the

study also differed between treatments, but in contrast to the biomass differences, this was primarily driven by the native tree *Q. rubra*. Overall, most of the woody species greatly benefitted from deer exclosure, growing larger and surviving longer in the absence of deer. Two species, the invasive shrub *L. maackii* and the native tree *C. florida*, however, showed almost no benefit from the fence treatment. The native tree had uniformly low survival regardless of the presence or absence of deer. The invasive shrub, on the other hand, had both the highest overall survival, and that survival was almost identical between treatments, despite the strong evidence of herbivory and much lower biomass in open plots. Because *L. maackii* is able to tolerate herbivory so well, it will have more opportunities for growth and spread through this forest compared to the other species that are unable to cope with damage from deer. Tolerance or resistance to herbivory can lead to changes in forest composition and successional dynamics. White-tailed deer have been shown to modify the forest structure on the Piedmont Plateau of Virginia (Rossell et al. 2005), which may lead to a shift from oak-hickory forests to stands with fewer species that are dominated by the more tolerant *Fraxinus* sp., *Prunus serotina*, and *Celtis occidentalis*. Successional processes on abandoned agricultural land has been shown to be dominated by tolerant and resistant species (Van Uytvanck et al. 2010). In the long-term, the cover of resistant and tolerant plants allowed non-resistant plants to grow beyond the browse line, promoting forest succession.

I have shown that white-tailed deer have both indirect and direct effects on this forest ecosystem that strongly favor the success of a number of invasive species. By increasing soil compaction and altering the nutrient dynamics deer may change the composition of the forest community to species that have lower nitrogen requirements

and that are able to persist in compacted soils. The direct effects of deer herbivory has resulted in a greater proportion of invasive species in the herbaceous layer, and significantly reduces the growth and survival of tree and shrub seedlings, with only the most tolerant or resistant species persisting in the presence of deer. Together, these effects may result in a vastly different forest due to the loss of native species that are unable to compete with invasive species that are avoided by deer.

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Table 1: Means (and 95% confidence intervals) for soil and physical variables for the exclosure and open plots. An asterisk (*) indicates a significant difference between open and exclosure plots (*P<0.05, **P<0.01, ***P<0.001).

	Open	Exclosure
Soil Compaction (PC1)	1.02 ** (0.28 - 1.76)	-1.02 (-1.76 - -0.28)
Bulk Density (PC2)	0.11 (-0.79 - 1.01)	-0.11 (-1.01 - 0.79)
Inorganic Soil Nutrient Pool (PC1)	0.422 (-0.24 - 1.08)	-0.422 (-1.08 - 0.24)
Inorganic Soil Nutrient Pool (PC2)	-0.12 (-0.60 - 0.35)	0.12 (-0.35 - 0.60)
Soil Acidity (PC1)	0.15 (-0.27 - 0.58)	-0.15 (-0.58 - 0.27)
Percent Soil Organic Matter	5.90 (5.42 - 6.38)	6.23 (5.68 - 6.79)
Soil Nitrate Flux ($mg/10cm^2/8$ weeks)	421.5 ** (346.4 - 496.6)	583.8 (508.7 - 658.9)
Soil Ammonia Flux ($mg/10cm^2/8$ weeks)	3.7 (3.1 - 4.4)	3.7 (3.1 - 4.3)
Inorganic Soil Nutrient Flux (PC1)	-0.37 (-1.01 - 0.27)	0.37 (-0.27 - 1.01)
Inorganic Soil Nutrient Flux (PC2)	-0.16 (-0.68 - 0.36)	0.16 (-0.36 - 0.68)
Proportion of Open Canopy Lux	6.83 (4.68 - 8.98)	6.58 (4.43 - 8.73)

Table 2. The damage levels of planted invasive and native species to deer exposure (open plots) and deer protection (fences plots) Reported are least square means (and 95% confidence intervals). Asterisks indicate that open means are significantly different from fences means (*P<0.05, **P<0.01, ***P<0.001).

Species	Status	Proportion Stems Damaged		Stem Height (cm)		Biomass (g)		Survival (%)	
		Open	Fence	Open	Fence	Open	Fence	Open	Fence
Lonicera maackii	Invasive	0.18*** (0.13 - 0.22)	0.06 (0.02 - 0.10)	43.19*** (36.65 - 49.74)	71.35 (65.26 - 77.44)	4.71*** (2.42 - 9.18)	43.44 (22.14 - 85.23)	66.7	68.1
Rhamnus davurica	Invasive	0.31*** (0.24 - 0.37)	0.03 (-0.02 - 0.08)	26.89** (16.54 - 37.23)	45.56 (38.04 - 53.09)	1.21*** (0.56 - 2.62)	6.05 (3.03 - 12.07)	29.2*	52.1
Celastrus orbiculatus	Invasive	N/A	N/A	N/A	N/A	1.24 (0.61 - 2.52)	0.90 (0.42 - 1.91)	N/A	N/A
Acer rubrum	Native	0.26*** (0.20 - 0.31)	0.03 (-0.01 - 0.08)	29.53** (20.92 - 38.14)	47.08 (40.22 - 53.93)	0.94*** (0.43 - 2.05)	5.64 (2.82 - 11.29)	27.7*	52.2
Cornus florida	Native	0.09 (-0.01 - 0.19)	0.00 (-0.17 - 0.17)	44.29 (29.65 - 58.92)	45.7 (20.35 - 71.05)	0.90*** (0.32 - 2.52)	7.32 (2.63 - 20.33)	10.4	10.4
Quercus rubra	Native	0.16*** (0.10 - 0.22)	0.00 (-0.04 - 0.04)	49.33* (40.18 - 58.49)	63.18 (56.97 - 69.39)	1.95* 0.46 - 8.36)	10.89 (5.53 - 21.43)	4.3***	59.2
Quercus alba	Native	0.11 (0.04 - 0.18)	0.05 (-0.01 - 0.11)	29.52 (18.54 - 40.49)	30.31 (21.53 - 39.09)	1.16 (0.38 - 3.53)	1.64 (0.75 - 3.59)	4.3*	27.7
Aquilegia canadensis	Native	N/A	N/A	N/A	N/A	0.18 (0.06 - 0.53)	0.33 (0.11 - 0.98)	N/A	N/A
Elymus hystrix	Native	N/A	N/A	N/A	N/A	2.33 (1.15 - 4.71)	1.89 (0.89 - 4.02)	N/A	N/A
Parthenocissus quinquefolia	Native	N/A	N/A	N/A	N/A	0.72 (0.34 - 1.52)	0.58 (0.27 - 1.24)	N/A	N/A

Figure Legend:

Figure 1. LS means of understory plant community variables. A) Density of understory plants/m^2. B) Density of herbaceous plants/m^2 in the understory. C) Density of woody plants/m^2 in the understory. D) Density of vines/m^2 in the understory. E) Density of understory native plants/m^2. F) Density of understory invasive plants/m^2. Error bars represent 95% confidence intervals.

Figure 2. Non-metric multidimensional scaling ordination of plant species composition. Ordination comparing the composition of open and fenced plots in 2011 and 2015. Centroids are the LS means of the two ordination dimensions for each treatment at the beginning and end of the study. Error bars represent 95% confidence intervals.

Figure 3. LS mean proportion of total LIDAR point returns at 0.5m intervals between open and fence treatments. Error bars represent 95% confidence intervals.

Figure 1:

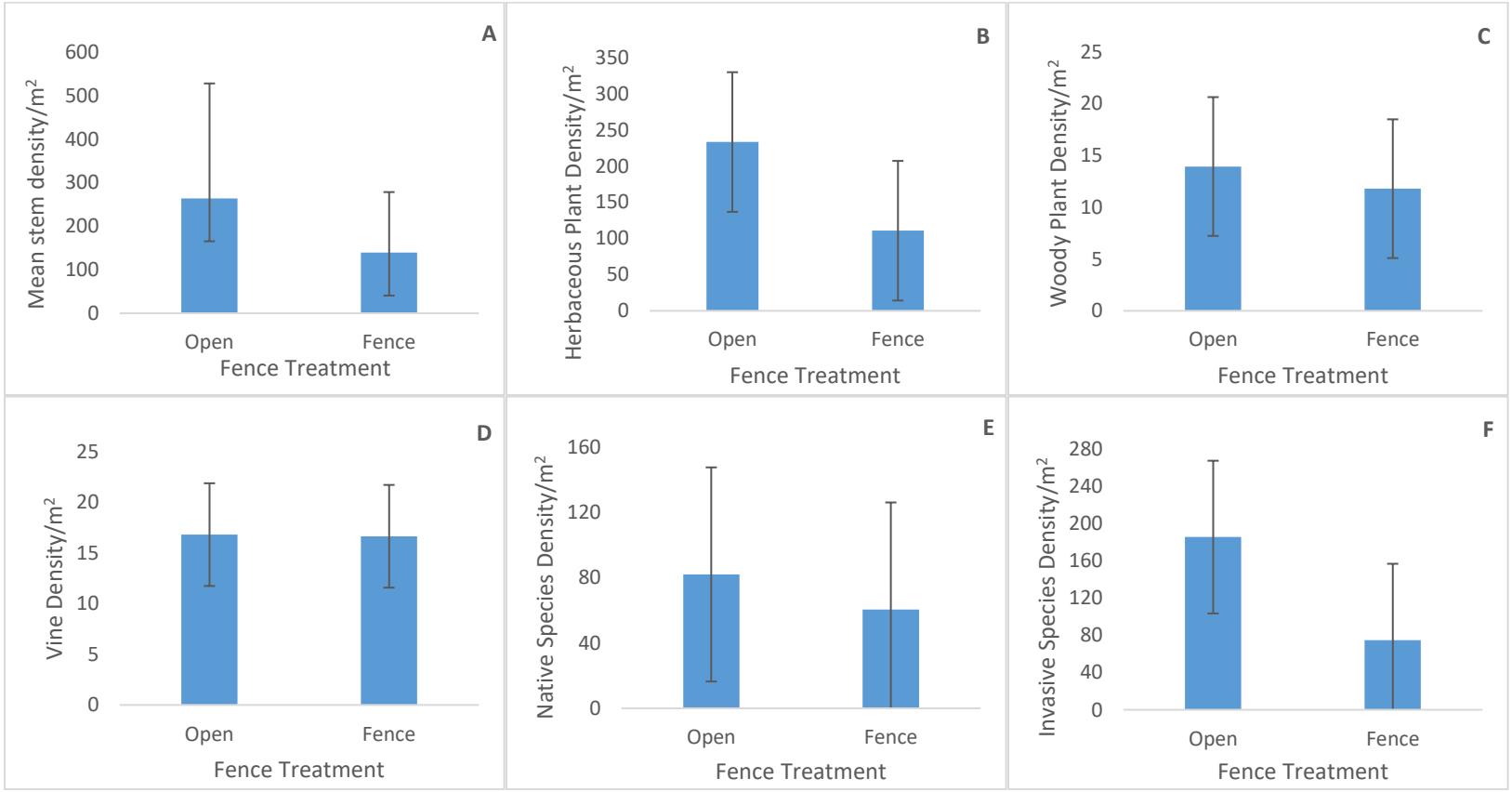

A
Mean stem density/m²
600
500
400
300
200
100
0
Open
Fence
Fence Treatment
B
Herbaceous Plant Density/m²
350
300
250
200
150
100
50
0
Open
Fence
Fence Treatment
C
Woody Plant Density/m²
25
20
15
10
5
0
Open
Fence
Fence Treatment
D
Vine Density/m²
25
20
15
10
5
0
Open
Fence
Fence Treatment
E
Native Species Density/m²
160
120
80
40
0
Open
Fence
Fence Treatment
F
Invasive Species Density/m²
280
240
200
160
120
80
40
0
Open
Fence
Fence Treatment

Figure 2:

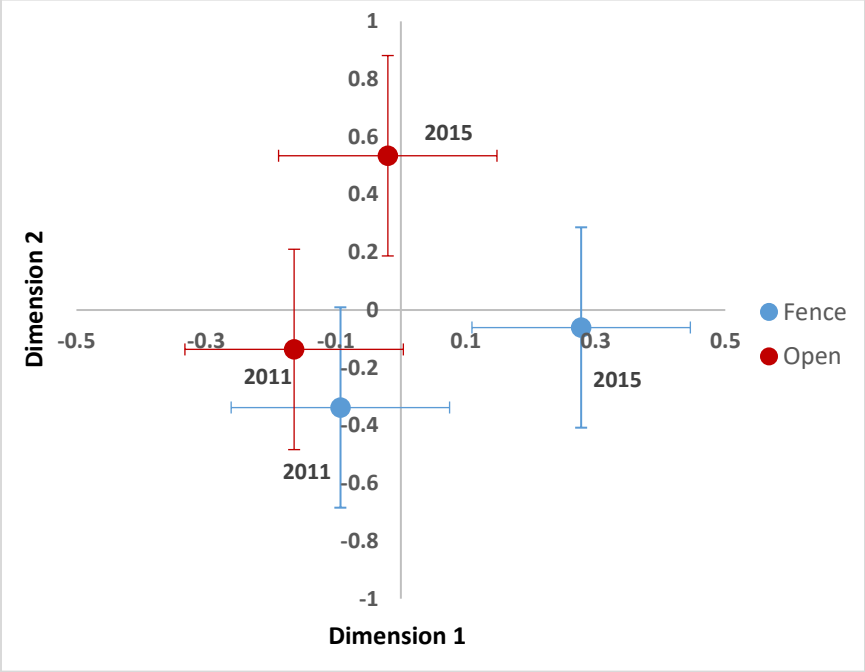
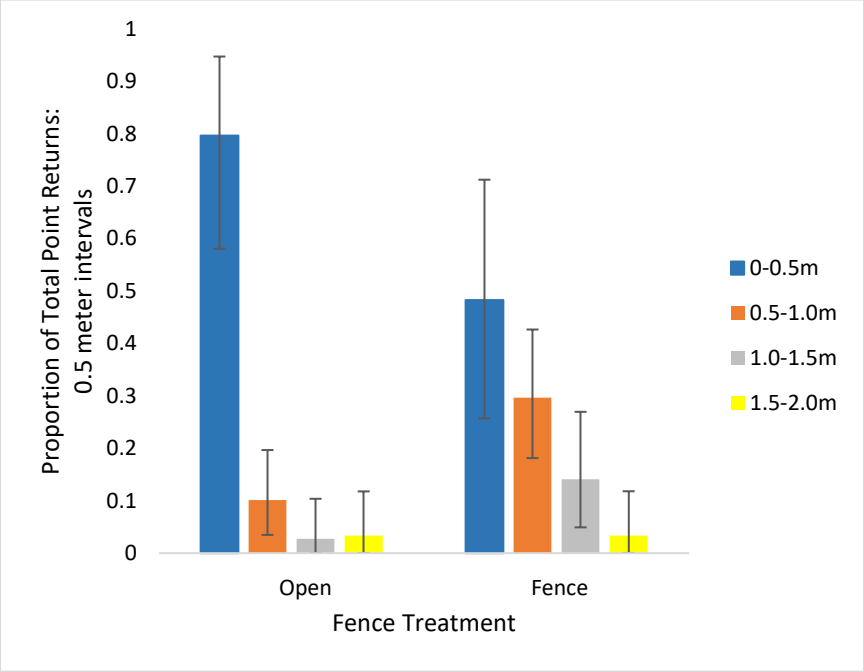
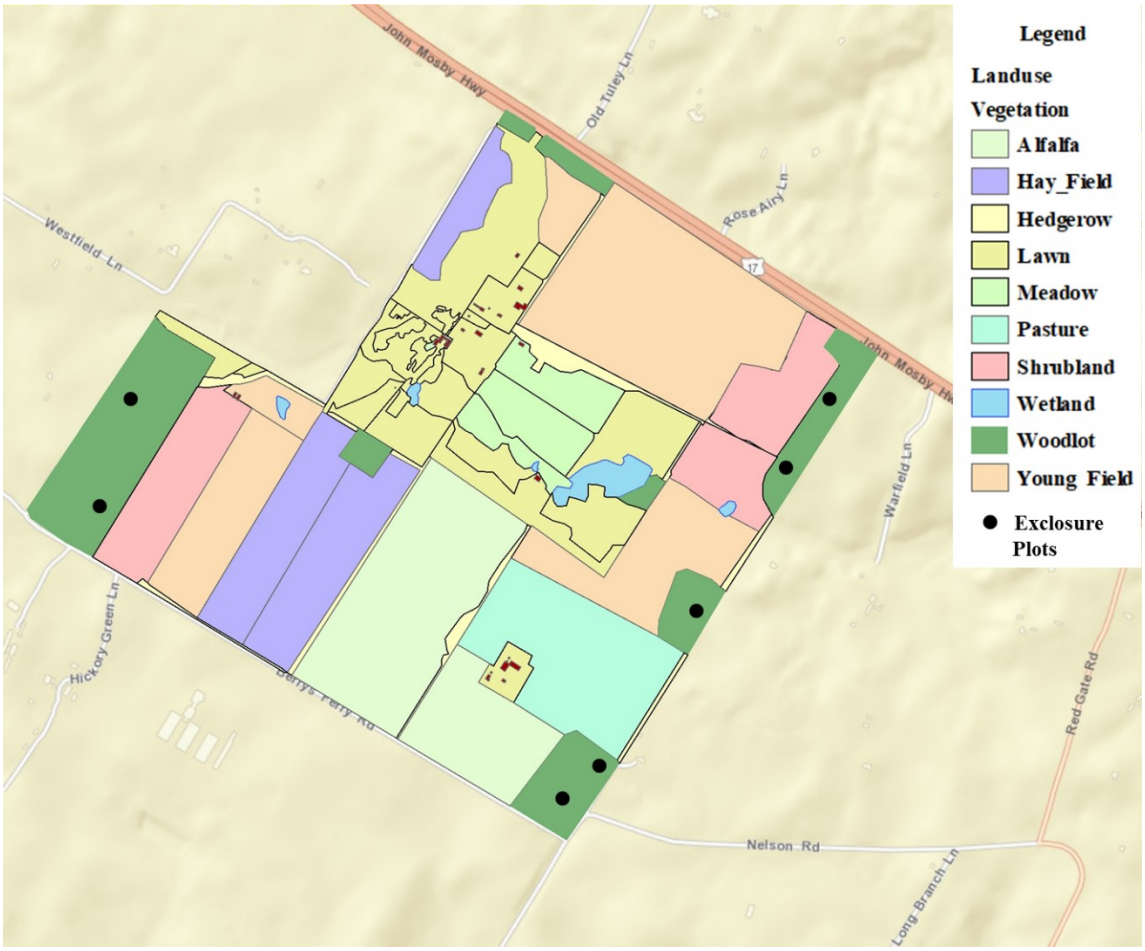

1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-0.5
-0.3
-0.1
0.1
0.3
0.5
Dimension 2
Dimension 1
2015
2011
2011
2015
Fence
Open

Figure 3:


Proportion of Total Point Returns:
0.5 meter intervals
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Open
Fence
Fence Treatment
0-0.5m
0.5-1.0m
1.0-1.5m
1.5-2.0m

Supplemental Figure S1. Map of paired exclosure/control plots at Blandy Experimental Farm.



Supplemental Figure S2. Diagram of the quadrats used for herbaceous layer surveys in each 5 × 5 m exclosure and control plot. A total of 5 quadrats were randomly selected in each plot, and repeatedly surveyed over the course of the study.

1	2	3	4	5
6	7	8	9	10
11	12	13	14	15
16	17	18	19	20
21	22	23	24	25

Appendices

Appendix 1: Factor loadings of principal components 1 and 2 for soil compaction variables. Measurements of soil compaction below the surface were at the indicated intervals as marked on the soil penetrometer.

	Prin1	Prin2
BD 10 cm	0.177	0.609
BD 5 cm	0.289	0.366
Surface	0.429	0.057
7.62 cm	0.485	0.042
15.24 cm	0.460	0.074
22.86 cm	0.443	-0.328
30.48 cm	0.240	-0.614

Appendix 2: Factor loadings of principal components 1 and 2 for soil inorganic nutrient pool variables.

	Prin1	Prin2
Phosphorous	-0.116	0.696
Potassium	0.333	0.298
Calcium	0.412	-0.001
Magnesium	0.397	0.181
Zinc	0.295	0.454
Manganese	-0.188	-0.048
Copper	-0.355	0.304
Iron	-0.374	0.304
Boron	0.400	-0.027

Appendix 3: Factor loadings of principal components 1 and 2 for soil pool acidity variables.

	Prin1	Prin2
pH	0.513	-0.231
Buffer	0.509	-0.324
CEC	0.464	0.883
Acidity	-0.512	0.247

Appendix 4: Factor loadings of principal components 1 and 2 for soil inorganic flux variables.

	Prin1	Prin2
Calcium	0.331	-0.306
Magnesium	0.175	-0.196
Potassium	-0.286	0.416
Phosphorous	0.101	-0.199
Iron	0.401	0.167
Manganese	-0.002	0.502
Copper	0.426	0.116
Zinc	0.355	0.204
Boron	0.190	0.041
Sulfur	0.340	0.152
Lead	0.368	0.046
Aluminum	-0.037	0.469
Cadmium	-0.102	-0.271

Appendix 5: Species associations with NMDS dimensions.

Species	Origin	Dim. 1	Dim. 2	Species	Origin	Dim. 1	Dim. 2
DUIN2	Invasive	-0.90393	-0.92738	GEUM	Native	-0.767	-0.0935
DAGL	Invasive	-0.87866	-0.89649	CYPERFAM	Native	-0.7643	0.42562
PEFR4	Invasive	-0.84512	0.08808	GECA7	Native	-0.7608	0.46048
ROMU	Invasive	-0.71971	0.47439	LEVI2	Native	-0.7345	0.58968
RHDA	Invasive	-0.60007	0.45395	CRATA	Native	-0.569	-0.1942
EUFO5	Invasive	-0.5553	-0.04359	PIPU2	Native	-0.5558	0.67015
LOMA6	Invasive	-0.54931	0.42053	ASTERFAM	Native	-0.4939	0.77861
AIAL	Invasive	-0.43226	0.35135	CARYA	Native	-0.4544	-0.1943
MIVI	Invasive	-0.37019	0.21286	CEOC	Native	-0.3863	0.04604
ALPE4	Invasive	-0.36305	-0.14799	CLVI3	Native	-0.3733	0.41687
LOJA	Invasive	-0.34341	0.22779	TORA2	Native	-0.2429	-0.2953
RUBUS	Invasive	-0.19648	0.50388	HAVI2	Native	-0.2259	0.24667
POLYG4	Invasive	-0.10488	0.75944	GALIU	Native	-0.1869	0.48684
STME2	Invasive	-0.10286	0.66089	POVI2	Native	0.01124	-0.0769
LISI	Invasive	-0.0342	0.43342	ARTR	Native	0.01322	0.17124
CEOR7	Invasive	0.21189	0.17586	PRSE2	Native	0.08269	0.24479
PHOTI	Invasive	0.30229	0.42779	VITIS	Native	0.10128	0.46282
PRAV	Invasive	0.42924	0.08562	PHAM4	Native	0.12909	0.13427
VERON	Invasive	0.46217	0.337	PAQU2	Native	0.29155	0.06379
HEHE	Invasive	0.46924	0.31273	CYPERFAM	Native	0.30794	0.48752
SOCA3	Native	-0.9161	-1.0228	POPE	Native	0.34092	0.36507
PIAV	Native	-0.895	-0.8396	CIL	Native	0.4355	0.11775
SPHE	Native	-0.8238	-0.2912	ULMUS	Native	0.47682	-0.4424
OXVI	Native	-0.8231	0.83628	MECA3	Native	0.49581	0.33306
OXALI	Native	-0.8116	0.40653	ELHY	Native	0.5088	0.11195
VIOLA	Native	-0.8084	0.56007	ACNE2	Native	0.5205	0.1863
CYPERFAM	Native	-0.7985	0.04926	PHLE5	Native	0.54121	-0.5371
CRCA9	Native	-0.7892	0.61067	CORYD	Native	0.67263	-0.1961
SOCA3	Native	-0.9161	-1.0228	ACRAR	Native	0.67332	-0.4559
PIAV	Native	-0.895	-0.8396	CACO26	Native	0.78982	0.12674

CHAPTER 2

The effect of reintroducing shade to an over-browsed understory on two native and two invasive plants using artificial shade structures

Abstract

The expansion of white-tailed deer (*Odocoileus virginianus*) populations represents a disturbance to forest plant communities through direct effects of herbivory that favor unpalatable and browse-tolerant plants and indirect effects including an increase in the openness of forest understories. These direct and indirect effects can favor the invasion of exotic plants by removing palatable native competitors and altering the understory habitat conditions for which native species are adapted. In this study I quantified the effects of reintroducing shade to the understory and preventing deer herbivory on the size, reproduction, and abundance of the native species *Podophyllum peltatum* and *Arisaema triphyllum* and the invasive species *Alliaria petiolata* and *Microstegium vimineum* in forest fragments at Blandy Experimental Farm in the northern Shenandoah Valley of Virginia. Neither native species showed a response to the shade treatment. *Alliaria petiolata* had greater per-capita recruitment in unshaded plots than shaded plots. *Microstegium vimineum* had greater abundance, had more fruiting plants, and had more spikelets/plant in unshaded plots than shaded plots. The native *A. triphyllum* did not show a response to deer exclusion, but the other native, *P. peltatum*, grew taller but was less likely to flower in fenced plots than open plots. The invasive *A. petiolata* had lower per-capita recruitment, but greater survival to adults, greater height, and more fruit/individual in fenced plots than unfenced plots. The invasive *M. vimineum* was less abundant and had fewer fruiting plants in fenced plots than unfenced plots. These treatments always acted additively but the effects of deer on plants were not always in the same direction for both shade and herbivory effects. It therefore seems important that

both direct and indirect effects of disturbances like deer overabundance be considered when evaluating the response of plant communities.

Introduction

Land-use change and the loss of predators have resulted in a substantial increase in the range and population density of white-tailed deer (*Odocoileus virginianus*) since European settlement over the last century (Rooney 2001; Eschtruth and Battles 2009; Pellerin et al. 2010). The current population is estimated at approximately 30 million in the continental United States (Bagley 2017). Reasons for the expanding deer population include protective laws and refuges that have allowed the deer population to recover from historic overhunting, management strategies aimed at increasing herd numbers for hunters, and an increase in ideal habitat due to an increase in young growth forests following timber harvest and abandonment of agricultural fields (Adams and Hamilton 2011).

White-tailed deer expansion represents a novel disturbance effect on forest plant communities. The overabundance of white-tailed deer may facilitate the success of exotic invasive plant species (hereafter "invasive species" through the direct effects of herbivory that may favor unpalatable (resistant) and browse-tolerant plants (Rooney 2001; Eschtruth and Battles 2009; Knight et al. 2009a; Knight et al. 2009b; Rooney 2009; Collard et al. 2010). Facilitation of invasion can be explained, in part, by the enemy release hypothesis, where specialist and generalist herbivores (via selective browsing) in an introduced range have a greater impact on native plant species, than on invasive species (Eschtruth and Battles 2009) that either resist or tolerate herbivory better than natives. For example, *Alliaria petiolata* (garlic mustard), *Microstegium vimineum* (Asian stiltgrass) (Eschtruth and Battles 2009; Knight et al. 2009a), and *Berberis thunbergii* (Japanese barberry) (Eschtruth and Battles 2009) were shown to successfully invade by

being avoided by deer while competing native plant species were preferentially consumed. In the case of Knight et al. (2009a), *A. petiolata* comprised 60% cover in unfenced plots while comprising less than 20% in fenced plots.

White-tailed deer may also indirectly affect forest communities through changes to the understory structure (Rooney and Waller 2003). The reduction or elimination of understory herbs, shrubs, and saplings, opens up the understory and may produce broad, open park- or savanna-like habitats with understories composed of plants (such as graminoids or ferns) that inhibit growth of tree seedlings (Kolb et al. 1989; Rooney 2001; Horsley et al. 2003; Rooney and Waller 2003; Rooney 2009). This decrease in diversity and increase in light availability may affect species in the herbaceous layer, favoring light limited species or light "generalists" over shade adapted species. For example, Parendes and Jones (2000) found that 21 invasive species were most numerous in areas with high light produced by forest openings along roads compared to areas of lower light in a forest in the Pacific Northwest. Both *A. petiolata* and *M. vimineum* grow well in shade (Cavers et al. 1979; Barden 1987; Cheplick 2005; Smith and Reynolds 2014), but both act as light "generalists", with *A. petiolata* most commonly found under partial shade, showing an increase in biomass with increasing light (Meekins and McCarthy 2000; Droste et al. 2010; Smith and Reynolds 2014; Stinson and Seidler 2014). *Microstegium vimineum* has been shown to exhibit phenotypic plasticity in its response to light availability, increasing biomass under high light and water conditions and increasing specific leaf area under low light conditions, allowing for greater use of the available light (Droste et al. 20010). Taken together with the direct effects of herbivory, the indirect effects of white-tailed deer opening up the understory and decreasing the abundance of native competitors can

often favor the invasion of exotic plants such as Asian stiltgrass (*Microstegium vimineum*) and garlic mustard (*Alliaria petiolata*) (Knight et al. 2009a).

Podophyllum peltatum (mayapple) and *Arisaema triphyllum* (jack-in-the-pulpit) are native herbaceous species commonly found in the eastern temperate deciduous forests where deer populations are overabundant. These species are considered unpalatable to deer (with a stronger avoidance of *A. triphyllum*) (Bierzychudek 1982; Heckel et al. 2010; Pendergast et al. 2016), but *P. peltatum* fruits are known to be eaten by deer (Niederhauser and Matlack 2015). *Podophyllum peltatum* is considered shade-tolerant (Niederhauser and Matlack 2015), but also shows plasticity in its response to openings in the canopy (Hull 2002; Cushman et al. 2005; Cushman et al. 2006). *Arisaema triphyllum* prefers interior forest habitats (Matlack 1994), but will tolerate partial-shade as well (Hull 2002). Due to their habitat preferences, the opening of the understory from over-browsing understory trees and shrubs by white-tailed deer could affect both of these native species.

Alliaria petiolata (garlic mustard) is an invasive herb commonly found throughout the eastern temperate deciduous forests. It is avoided by deer and can out-compete native plants in the presence of deer (Eschtruth and Battles 2009; Knight et al. 2009a). It grows under a wide range of light conditions from forest edges to the interior, especially in disturbed locations (Cavers et al. 1979; Meekins and McCarthy 2000; Lewis et al. 2006; Smith and Reynolds 2014; Stinson and Seidler 2014). *Microstegium vimineum* (Asian stilt grass) is an invasive grass also commonly found throughout the eastern temperate deciduous forests. Like *A. petiolata,* it is avoided by deer and can out-compete native plants in the presence of deer (Eschtruth and Battles 2009; Knight et al.

2009a). It will grow under a wide range of light conditions (Barden 1987; Claridge and Franklin 2002; Cheplick 2005; Droste et al. 2010) including open woods and upland fields, especially in heavily disturbed areas (Barden 1987; Manee et al. 2015) and has been shown to have reduced survival growing under shade from shrubs (Schramm and Ehrenfeld 2010).

The purpose of this experiment was to document simultaneously the indirect effects of shading and the direct and indirect effects of deer browsing in the understory through the use of combined shade and fence treatments on two native (*A. triphyllum* and *P. peltatum*) and two invasive (*A. petiolata* and *M. vimineum*) plants in the herbaceous layer. Though studies have shown that deer can create a more open understory by over-browsing saplings and shrubs, as well as facilitate invasions of exotic species by directly consuming natives species or indirectly lowering the abundance of native competitors, most of these are examining these effects separately or consider the effects of *increased* light availability combined with deer herbivory. This study, however, examines the effects of restoring understory shade in both the presence and absence of deer. This allowed me to test for the possibility of non-additive effects from the two treatments. By decreasing the available light in the understory, I expected to see reductions in the abundance, recruitment, and reproduction of all four species, especially on the light-adapted or light generalist species *A. petiolata* and *M. vimineum*. For the two invasive species, I expected to see the greatest positive response under both unshaded and unfenced treatments, as they are benefitting from both the higher light availability and the decrease in competition from species that are preferentially browsed. For the two native species, I expected to see the greatest positive response under unshaded and fenced

treatments, as they are benefitting from the higher light availability and are free from the direct effects of the deer.

Methods

Two native and two invasive species were the focus of this experiment. The native mayapple (*Podophyllum peltatum*; Berberidaceae) and jack-in-the-pulpit (*Arisaema triphyllum*; Araceae) are both common, perennial spring-flowering herbs in the understory of forests in the Shenandoah Valley. Both *P. peltatum* (Pendergast et al. 2016) and *A. triphyllum* (Heckel et al. 2010) are considered unpalatable to white-tailed deer. *Podophyllum peltatum* grows from a rhizome, often forming dense clonal populations, and also reproduces sexually from a single flower on mature individuals (Niederhauser and Matlack 2015). After flowering in mid- to late-spring, these flowers produce a fleshy green to yellow berry 2-5 cm wide containing many seeds. These berries mature in mid-summer and are known to be eaten and dispersed by white-tailed deer, raccoons (*Procyon lotor*), and Eastern box turtle (*Terrapene carolina*) (Niederhauser and Matlack 2015). *Arisaema triphyllum* grows from a corm, and can reproduce both clonally and sexually from a spadix that is either male or female. These plants exhibit a gender switching phenomenon where larger individuals tend to produce female flowers and smaller individuals tend to produce male flowers (Bierzychudek 1982). Some small *A. triphyllum* may skip sexual reproduction. After flowering in mid- to late-spring, female *A. triphyllum* produce an infructescence of berries that turn red when mature in late-summer and then either drop to the ground or are removed by fruit-eating birds or rodents (Bierzychudek 1982).

The invasive garlic mustard (*Alliaria petiolata*; Brassicaceae) and Asian stiltgrass (*Microstegium vimineum*; Poaceae) are native to the majority of Europe and eastern Asia respectively. *Alliaria petiolata* and *M. vimineum* are also unpalatable to white-tailed deer, and both species have been shown to out-compete native plants in the presence of deer (Eschtruth and Battles 2009; Knight et al. 2009a). *Alliaria petiolata* is a biennial species that germinates in early spring and grows as a basal rosette in the first year. In the second year, individuals then produce one to several flowering stalks (Biswas and Wagner 2015). After flowering in late-spring through early-summer, flowers produce siliques containing 10-20 seeds, which mature between June and September (Cavers et al. 1979; Biswas and Wagner 2015). *Microstegium vimineum* is an annual species. Seedlings emerge in early spring and when mature, they flower in late-summer to early-fall (Cheplick 2005), producing racemes of spikelets that can yield between 100 and 1000 seeds (Gibson et al. 2002). Dispersal of the seeds occurs by wind, water (flooding), animals (zoochory), and humans (machinery, zoochory), especially along rivers, ditches, and roads (Barden 1987; Anderson et al. 2013).

Site Description

This experiment took place at Blandy Experimental Farm in the northern Shenandoah Valley in Clarke County, VA (39.061º N, 78.065º W). Annual mean total precipitation is 97.6 cm. Mean January and July high/low temperatures are 6.2º/-4.5ºC, and 31.4º/17.5º C, respectively. Nighttime spotlight censuses conducted in October 2016 at Blandy estimated white-tailed deer population at approximately 103.7 deer/km^2 (W.

McShea, pers. comm.) which is well above the estimated carrying capacity of 15.4 deer/km^2 for the nearby Virginia Piedmont (Whittington 1984).

Five forest fragments were used in this study, ranging from 1.2 to 18.6 ha (Appendix 1). The canopy species of each of these forest fragments were primarily red oak (*Quercus rubra*), white oak (*Q. alba*), mockernut hickory (*Carya tomentosa*), hackberry (*Celtis laevigata*), and black walnut (*Juglans nigra*). The understory of these fragments is open, with scattered clusters of primarily bush honeysuckle (*Lonicera maackii*), spicebush (*Lindera benzoin*), or Dahurian buckthorn (*Rhamnus davurica*) shrubs. One fragment (2.3 ha) had the majority of invasive shrubs removed in 2009 (primarily bush honeysuckle). The lack of a dense understory in these fragments due to heavy browsing by white-tailed deer results in greater light intensity in the herbaceous layer which can have differential effects on the species of interest.

Shade structures

In May 2013, I selected and marked with flags ten sites for each of the four study species, with four plots at each site (Appendix 2). Sites containing either the two native plants or *A. petiolata* were selected based on the presence of individuals such that each of the four plots would contain at least one plant. However, the majority of plots contained at least five or more individuals. *Microstegium vimineum* sites were selected based on the presence of at least several clusters of grass, with most plots having at least 25% coverage. Each plot was randomly assigned one of four treatment combinations: shade, fence, both shade and fence, or no shade and no fence (control plots) (Appendix 3). All plots other than controls had a PVC structure erected above them, approximately 1.5 m

tall and 2 × 2 m^2. The corners of the control plots were marked with survey flags. For shaded plots, I attached a sheet of charcoal-colored fiberglass window screen (mesh size 18 x 14/$in.^2$) to the top of each structure. I then placed nine additional strips of screen on top of the sheet in a 3 x 3 cross-hatch pattern to add variation to the shade pattern. This design resulted in a reduction in light intensity of 50-55% for late-morning sunlight. For fenced plots, I attached 1.8 m tall poly deer fence (Deerbusters, Frederick, MD) to the exterior of the structure.

Shade structures were in place beginning in July 2013. In October 2013 the shade cloths were removed to simulate a deciduous understory canopy. Shade cloths were put back in place in March 2014 and removed again in October 2014. This was repeated in March and October 2015. The plants in each plot, therefore, experienced the experimental conditions for two-and-a-half growing seasons.

Experimental Surveys

I used several methods for measuring the performance of each study species. Due to their differences in growth and reproduction, the variables measured on each species varied, but all variables encompassed three types of responses: size of individuals, reproduction, and abundance.

For *P. peltatum*, I counted the number of stems in each plot, measured stem height and leaf width, and I recorded whether each plant flowered and if they produced a fruit in late-May-June 2015. A preliminary multiple regression analysis of the length and width of 54 *P. peltatum* fruits suggested that the width alone was the best predictor of seed number (R^2=0.70). I therefore measured fruit width on all *P. peltatum* fruit in my

study plots in July 2015, when they were near maturity. As *P. peltatum* has a perennial growing pattern, analyzing data from 2015 would represent the cumulative effects of treatments on this species over the two-and-a-half growing seasons under experimental conditions.

For *A. triphyllum*, I counted the number of stems in each plot, measured the height of the tallest stem and leaf width, and recorded whether plants flowered, and recorded if the flowers were male or female in late-May-June 2015. In August 2015, I counted the number of mature berries on the infructescence for all individuals in a plot that produced fruit. As *A. triphyllum* has a perennial growing pattern, analyzing data from 2015 would represent the cumulative effects on this species over the two-and-a-half growing season of experimental conditions.

For *A. petiolata*, I measured first-year (non-flowering rosettes) and second-year (flowering adults) individuals separately in July-August 2014 and 2015. With rosettes, I counted the number of individuals in each plot in 2014 and 2015 and measured the number of leaves per rosette. With flowering adults, I counted the number of individuals in each plot in 2014 and 2015 and measured the number of stems per individual, and the stem height. I then counted the total number of fruits, and the length of ten randomly selected fruits for all adult *A. petiolata* individuals in a plot. Based on a sample of 100 siliques collected in summer 2014, fruit length is a good predictor of seed number (R^2=0.64). I first measured per-capita recruitment of this biennial under experimental conditions by measuring the ratio of the number of rosettes in 2015 to the number of flowering adults in the previous year (2014). I also measured the survival rate of 2014

rosettes to flowering adults in 2015 by calculating the ratio of the number of 2014 rosettes to the number of 2015 adults.

For *M. vimineum*, due to the high density of individuals in the plots I subsampled plots to estimate abundance in each plot in late August and September 2015. I randomly placed a 1 m^2 quadrat, divided into 100 10 x 10 cm grids, and then, using five randomly selected grid squares, I counted the total number of individuals in each grid and the number of individuals that produced spikelets. To estimate individual growth and reproduction, I randomly selected five plants by tossing a marker into the plot and measured the height and number of spikelets on the closest plant to the marker. As *M. vimineum* is an annual, any effects on the measured variables, whether positive or negative, would accumulate over time, so measurements occurred in 2015 to account for the cumulative effects of treatment.

Statistical Analyses

Each of the four species was analyzed separately. The experimental design was treated as a 2×2 factorial analysis with location as a random block effect. Shade and fence treatments and the shade×fence interaction were treated as fixed effects. Plot was treated as the experimental unit (40 plots per species). For variables such as plant height or leaf width (where multiple individuals were measured per plot), plot means were calculated for use as dependent variables to avoid pseudoreplication. All analyses were performed using SAS version 9.4.

The effects of shade and deer exclusion on *P. peltatum* abundance, stem height, and leaf width were tested using mixed model factorial ANOVAs. The total abundance

of *P. peltatum* was log-transformed to meet ANOVA assumptions. As the abundance of *P. peltatum* in 2015 was strongly correlated with the abundance in 2014, one year after plot establishment, I used the abundance in 2014 as a covariate in the analysis. Stem height and leaf width did not require transformation. I used a generalized linear mixed model factorial ANOVA with a binomial distribution and a logit link to test for differences in the proportion of flowering individuals between treatments.

The effects of shade and deer exclusion on *A. triphyllum* abundance, stem height, leaf width, and number of fruits were tested using mixed model factorial ANOVAs. The abundance of *A. triphyllum* was log-transformed to meet ANOVA assumptions. As the abundance of *A. triphyllum* in 2015 was strongly correlated with the abundance in 2013 when the plots were established, I used the abundance in 2013 as a covariate in the analysis. Stem height, leaf width, and fruit number did not require transformation. I used generalized linear mixed model factorial ANOVAs with a binomial distribution and a logit link to test for differences in the proportion of flowering individuals to total individuals in a plot and to test for differences in the proportion of females to total individuals in a plot.

I used a mixed model factorial ANOVA to compare the per-capita recruitment of *A. petiolata* rosettes. I log-transformed the ratio of the number of first-year rosettes in 2015 to the number of flowering adults in 2014. I used a generalized linear mixed model factorial ANOVA to test for the effects of shade and deer exclusion on the survival of first-year rosettes in 2014 to flowering adults in 2015. Using flowering adults from 2014 the effect of shade and deer exclusion on the number of stems per individual, stem height, fruit number, and fruit length was tested using a mixed model factorial ANOVA. Using

first-year rosettes from 2014 I analyzed the effects of shade and deer exclusion on the number of leaves per rosette using the same mixed model factorial ANOVA. All variables were log-transformed to meet ANOVA assumptions.

The effects of shade and deer exclusion on *M. vimineum* total abundance/m^2, mean height, total number of fruiting individuals/m^2, and mean number of spikelets were tested using mixed model factorial ANOVAs. Total abundance/m^2, total number of fruiting individuals/m^2, and mean height were log-transformed to meet ANOVA assumptions. The mean number of spikelets did not require transformation.

Results

Mayapple

The responses of *P. peltatum* to shade and deer exclusion are found in Table 1a. There was no difference in the total number of *P. peltatum* individuals between shade ($F_{1,35}$=0.88, P=0.354) or fence ($F_{1,35}$=2.08, P=0.1578) treatments and there was no shade×fence interaction ($F_{1,35}$=0.22, P=0.6407). Individuals of *P. peltatum* did not differ in mean stem height between open and shaded treatments ($F_{1,26}$=1.19, P=0.2851), but in fenced plots, individual *P. peltatum* stems were 9.5% taller on average than individuals in unfenced plots ($F_{1,26}$=9.12, P=0.0056). There was no shade×fence interaction for stem height ($F_{1,26}$=0.7, P=0.4108). The mean leaf width of *P. peltatum* individuals did not differ between shade ($F_{1,26}$=0.03 P=0.8718) or fence ($F_{1,26}$=1.53, P=0.2272) treatments, and there was no shade×fence interaction ($F_{1,26}$=0.4, P=0.5336).

The probability of flowering for *P. peltatum* individuals did not differ between open and shaded treatments ($F_{1,27}$=1.05, P=0.3147), but in unfenced plots, individual *P.*

peltatum were twice as likely to flower as individuals in fenced plots ($F_{1,27}$=13.65, P=0.001). There was no shade×fence interaction for probability of flowering ($F_{1,27}$=1.63, P=0.2125). Despite a modest number of flowering individuals (148 of 850 total ramets) very few set fruit that survived to maturity (24 of 148 flowering ramets, much less than one per plot). This small number precluded analysis of fruit production and fruit size.

Jack-in-the-Pulpit

The responses of *A. triphyllum* to shade and deer exclusion are found in Table 1b. Deer protection and shade did not affect *A. triphyllum* in any measurable way. Total abundance did not differ between shade or fence treatments and there was no shade×fence interaction. Stem height and leaf width of *A. triphyllum* did not differ between treatments and there was no shade×fence interaction. Neither the proportion of flowering individuals nor the proportion of females differed between treatments and there was no shade×fence. Too few individuals produced fruits (0.8%) to allow a test for any differences between treatments. All p-values were >0.0587.

Garlic Mustard

The responses of *A. petiolata* to shade and deer exclusion are found in Table 1c. The per-capita recruitment rate of first-year rosettes in 2015 from the flowering adults in 2014 was 2.7 times greater in unshaded plots than shaded ($F_{1,23}$=11.6, P=0.0024). The per-capita recruitment rate of first-year rosettes in 2015 was 3.0 times greater in unfenced plots than fenced ($F_{1,23}$=14.03, P=0.0011). However, rosettes in fenced plots were 3.1 times more likely to survive from 2014 to 2015 than in unfenced plots ($F_{1,23}$=13.9,

P=0.0011). Rosette survival was not affected by the shade treatment ($F_{1,23}$=0.49, P=0.4896) nor was there an interaction between fence and shade treatments ($F_{1,23}$=0.03, P=0.8547). The mean number of leaflets per rosette did not differ between shade ($F_{1,23}$=0.9, P=0.3529) or fence ($F_{1,23}$=1.94, P=0.1775) treatments and there was no shade×fence interaction ($F_{1,23}$=0.78, P=0.3859). The mean number of stems per adult also did not differ between shade ($F_{1,23}$=0.39, P=0.5388) or fence ($F_{1,23}$=2.69, P=0.1145) treatments and there was no shade×fence interaction ($F_{1,23}$=0.33, P=0.5724). In fenced plots, flowering adult *A. petiolata* individuals were 1.3 times taller than individuals in open plots ($F_{1,23}$=7.97, P=0.0096), but height was not affected by the shade treatment ($F_{1,23}$=0.06, P=0.8097), and there was no shade×fence interaction ($F_{1,23}$=0.04, P=0.8527).

In fenced plots, flowering individuals produced 1.9 times more fruits than individuals in open plots ($F_{1,23}$=6.61, P=0.0171). The mean number of fruits produced by flowering individuals did not differ between shade treatments ($F_{1,23}$=0.1, P=0.7539) and there was no shade×fence interaction ($F_{1,23}$=0.12, P=0.7299). The mean length of fruits did not differ between shade ($F_{1,23}$=0, P=0.9457) or fence ($F_{1,23}$=1.29, P=0.2671) treatments and there was no shade×fence interaction ($F_{1,23}$=0.27, P=0.6108).

Asian Stiltgrass

The responses of *M. vimineum* to shade and deer exclusion are found in Table 1d. The mean abundance of *M. vimineum* individuals/m^2 in unshaded plots was 2.3 times that of individuals in shaded plots ($F_{1,27}$=18.26, P=0.0002). The mean abundance of individuals/m^2 in unfenced plots was twice that of individuals in fenced plots

($F_{1,27}$=13.02, P=0.0012). There was no shade×fence interaction on the abundance of individuals/m^2 ($F_{1,27}$=0.01, P=0.9436).

The mean height of randomly selected individuals of *M. vimineum* was not different between shade ($F_{1,27}$=2.25, P=0.1448) or fence ($F_{1,27}$=3.44, P=0.0745) treatments and there was no shade×fence interaction ($F_{1,27}$=0.31, P=0.5852).

The mean number of fruiting *M. vimineum* individuals/m^2 in unshaded plots was 2.4 times that of individuals in shaded plots ($F_{1,27}$=21.14, P<0.0001). The mean number of fruiting individuals/m^2 in unfenced plots was 2.2 times that of individuals in fenced plots ($F_{1,27}$=16.97, P=0.0003). There was no shade×fence interaction for the number of fruiting individuals/m^2 ($F_{1,27}$=0.11, P=0.7373). The mean number of spikelets on randomly selected individuals of *M. vimineum* was 33% greater in unshaded plots than shaded plots ($F_{1,27}$=7.26, P=0.012). There was no difference in the mean number of spikelets on randomly selected individuals between fence treatments ($F_{1,27}$=0.91, P=0.3488) and there was no shade×fence interaction ($F_{1,27}$=1.22, P=0.2786).

Discussion

Shade

Reducing light in the understory did not have any effect on the abundance, size, or reproduction, of the native species *P. peltatum* or *A. triphyllum*. However, both of the invasive species were negatively affected by adding shade to the environment. The per-capita recruitment of *A. petiolata* in unshaded plots was 2.7 times greater than for the shaded plots. *Microstegium vimineum* was 1.3 times more abundant, had 1.4 times more individuals/m^2 with spikelets, and 1.3 times more spikelets/individual in unshaded plots

compared to shaded plots. These results suggest that the removal of the forest understory by deer does not have a particularly negative effect for shade-adapted native species, but has a very positive effect for the invasive species.

The lack of response to shade treatment by the native *P. peltatum* indicates that it does not necessarily require shade for optimal growth. Cushman et al. (2005) found that when shade was increased *P. peltatum* shoots took longer to senesce, had greater leaf area per plant, and greater shoot, but shade did not affect the number of emerging shoots, total leaf area, or leaf dry mass. Plants growing in full sun contained greater podophyllotoxin (a toxic lignin compound) and total lignin, indicating that these plants are able to commit more resources to defense than plants growing in shade (Cushman et al. 2005). While I did not observe differences in leaf size or stem height, a key difference between my study and Cushman et al. (2005) was that I focused on differences between shaded and unshaded plants growing under a forest canopy and did not include a full sun treatment compared to the garden setting used by Cushman et al. (2005). In a study on the photosynthetic response to sunflecks, Hull (2002) found that *P. peltatum* was intermediate between sun- and shade-plants, with a plastic response to lighting conditions, which is consistent with the lack of differences between my shade treatments. The removal of the shrub layer by deer should increase the frequency of sunflecks hitting the herbaceous layer, but it is not clear if the short-term increase in photosynthesis is enough to affect the growth or reproduction of *P. peltatum*, neither of which I observed in my study.

The lack of response to shade treatment by the native *A. triphyllum* also indicates that it does not necessarily require shade for optimal growth. In his study on the

photosynthetic response to sunflecks, Hull (2002) found that the response of *A. triphyllum* to sunflecks was more typical of a shade-plant, maintaining photosynthetic induction at lower irradiance compared to sun-plants. As with *P. peltatum*, it is not clear if the photosynthetic response of *A. triphyllum* to short-term direct irradiance will affect the growth or reproduction, neither of which I observed. Although it is considered a shade plant, Levine and Feller (2004) found that populations of *A. triphyllum* growing in 100-year old forests had a higher female:male sex ratio in gaps compared to closed canopy forests. They did not find any differences in clonal reproduction, concluding that *A. triphyllum* maintains its populations by tolerating shade until light availability increases, allowing for sexual reproduction and the potential for dispersal. My data suggest that the changes in light intensity by deer browsing in the shrub layer is not enough to produce the changes in sex expression reported by Levine and Feller (2004) in forest gaps.

Alliaria petiolata is highly competitive in shaded environments due to its highly plastic response to shade (Meekins and McCarthy 2000) and its winter-green habit (Engelhardt and Anderson 2011; Smith and Reynolds 2014; Stinson and Seidler 2014; Smith and Reynolds 2015; Heckman and Carr 2016). However, I observed a decrease in recruitment of *A. petiolata* in shaded plots compared to unshaded plots. This is consistent with other studies on *A. petiolata* that have found negative effects of shade, including reduced growth or biomass (Engelhardt and Anderson 2011; Smith and Reynolds 2014; Stinson and Seidler 2014), reproduction (Stinson and Seidler 2014), and reduced survival (Smith and Reynolds 2015). This suggests that deer removal of the shrub layer facilitates the invasion of the herbaceous layer by *A. petiolata* rosettes.

The negative effect of shade on the abundance and reproduction of *M. vimineum* is consistent with other studies that have examined this invasive species. Increased shade has been shown to decrease reproductive output (Cheplick 2005; Schramm and Ehrenfeld 2010), cover (Abrams and Johnson 2012), and lower survival (Schramm and Ehrenfeld 2010). *Microstegium vimineum* can persist in shaded environments however. This species is able to maximize its photosynthetic capability in the shade by increasing leaf biomass or area (Cheplick 2005; Droste et al. 2010) and allocating a greater proportion of biomass allocated to shoots (Claridge and Franklin 2002). By reintroducing shade to the understory, I have shown that deer appear to be increasing light levels in the herbaceous layer enough to promote the abundance and reproduction of *M. vimineum* and thereby facilitating its ability to invade forest habitats.

On a broader scale, the impact of increased light availability on the survival of natives or the successful invasion of exotic species varies. The increased shade under a dense canopy of *Lonicera maackii*, an invasive shrub with extended leaf phenology, has been shown to negatively affect native species height, recruitment, and reproduction (*Impatiens capensis*: Cipollini et al. 2009), survival and fecundity (*Galium aparine*, *Impatiens pallida*, and *Pilea pumila*: Gould and Gorchov 2000), reduced seed production and pollinator services (*Geranium maculatum*: McKinney and Goodell 2010), and reduced native species richness (Cipollini et al. 2009). As was seen in *A. petiolata* and *M. vimineum* in this study, when light availability increases, invasive species are often better able to invade, showing increased cover and biomass in unshaded plots (*Hesperis matronalis* and *Rhamnus cathartica*: Tanentzap and Bazely 2009). However, even in deep shade of intact forests some invasive species are still able to persist, with only slight

reductions in survival and growth at just 2% of full sun (*Celastrus orbiculatus*: Ellsworth et al. 2004). Overall however, increasing light in the understory is often beneficial for invasive species, and it is rarely harmful.

Deer Exclusion

Surprisingly, the effects of deer herbivory on these four species was often positive, with the plants in open plots outperforming the plants in fenced plots. When deer were present, the native *P. peltatum* was slightly but significantly shorter (10%) but was twice as likely to flower as in fenced plots (6% vs. 12.4% flowering). Deer presence did not have any effect on the abundance, size, or reproduction of the native species *A. triphyllum*. When deer were present, the invasive species *A. petiolata* had three times greater per-capita recruitment, but 68% lower survival, 25% shorter adult height, and 47% fewer fruits per individual compared to fenced plots. The invasive species *M. vimineum* had 1.3 times greater abundance, 1.3 times more individuals/m^2 with spikelets in the presence of deer than when deer were excluded.

Because they are considered unpalatable to deer (Bierzychudek 1982; Heckel et al. 2010; Pendergast 2016), I expected an overall negligible effect of the fence treatment on the two native species. Although this was observed for *A. triphyllum*, the response of *P. peltatum* did not follow my expectation. Unpalatable species are known to benefit from deer herbivory on competitor species, including both native graminoids (Rooney 2009) and invasive herbs (*A. petiolata* and *M. vimineum*: Knight et al. 2009a). However, it is also possible for nonconsumptive effects of highly abundant deer populations to negatively affect unpalatable herbs, for example through reductions in soil quality (*A.*

triphyllum: Heckel et al. 2010). Therefore, *P. peltatum* may be both benefitting from reduced competition, increasing its rate of flowering, and suffering in the presence of deer, by reductions in growth.

Alliaria petiolata is also regarded as unpalatable (Knight et al. 2009a; Averill et al. 2016). My results showed that *A. petiolata* recruitment increased in the presence of deer. This may be due to the indirect effect of deer creating greater opportunities for establishment by consuming more palatable species (Eschtruth and Battles 2009; Rooney 2009; Goetsch et al. 2011; Holmes and Webster 2011) and preferentially avoiding *A. petiolata* rosettes, similar to Knight et al. (2009a) and Averill et al. (2016). However, deer had a direct, negative effect on the second life-history stage of *A. petiolata*, reducing the survival to adulthood, adult height, and the number of fruits per individual. Unlike Knight et al. (2009a), these results suggest that *A. petiolata* at Blandy are not actually avoiding damage from deer throughout their life cycle. It is possible that the damage is not due to herbivory but from other physical damage, such as trampling, that weakens individuals, limiting their survival, growth, and reproduction. The much higher density of deer at Blandy compared to Knight et al (2009a), 25-40 versus 104 deer/km^2, could lead to deer browsing simply through the lack of preferred browse.

Like the other species in this study, *M. vimineum* is also considered unpalatable to deer (Knight et al. 2009a; Averill et al. 2016). My results showed that the presence of deer benefitted *M. vimineum* abundance and the number of plants producing spikelets. I am inferring that this is likely due to the indirect effect of both avoidance of herbivory that I expected and to the removal of preferentially browsed plants that would otherwise compete with *M. vimineum*, allowing greater germination success, survival, and

reproduction. These results are consistent with other studies that have implicated deer browsing in the successful invasion of *M. vimineum*. The abundance of *M. vimineum* was promoted in the presence of deer (Shen et al. 2016) and the cover of *M. vimineum* was reduced when deer were absent (Abrams and Johnson 2012), primarily due to high shrub cover inside fenced plots.

The lack of a strong negative response to deer herbivory by the two native species in this study might be an exceptional example compared to the majority of native herbs. Both *P. peltatum* and *A. triphyllum* are highly unpalatable, but many native species are highly susceptible to deer herbivory, including both herbaceous species (Webster et al. 2005; Knight et al. 2009a; Collard et al. 2010), and many tree and shrub species (Rooney 2009; Goetsch et al. 2011; Holmes and Webster 2011; Nuttle et al. 2013). I intentionally examined unpalatable species in this study so that my results would not be solely about browsing preference, but for those species that do experience significant effects of browsing, deer herbivory can lead to collapse of biodiversity (Rooney 2009; Goetsch et al. 2011) and can have long-term effects that impact forest communities for many years after deer have been removed (Webster et al. 2005; Pendergast et al. 2016). The native species in this study exhibit a perennial life history, whereas the invasive species have much shorter annual or biennial life histories. Numerical responses on the part of the natives may have been more difficult to detect. However, the effects of reduced light or changes in the competitive environment could have manifested in a number of ways in both native species (e.g., increased clonal spread, increased size, or increased reproductive output). The general lack of response in these species suggest either that

deer do not have a strong influence on their ecological success or that the effects of deer exclusion and the restoration of shade has a much longer lag time.

While previous studies have found that deer may promote invasive species by increasing light in the understory, I examined the effects of re-introducing shade on native and invasive species, combined with a deer fencing treatment. It is important to note that the interaction between these factors was never significant in any of my experiments. These treatments always acted additively. However, the effects of deer on plants were not always in the same direction for both shade and herbivory effects or the same for different life history stages. *Alliaria petiolata* appears to benefit from both shade removal by deer and removal of competing species by increasing its recruitment of rosettes, but browsing pressure seems to have a negative effect on survival from rosette to flowering adult. On the other hand, the effects of a more open understory and deer presence for *M. vimineum* were always positive. There are examples of interactive effects of shade and deer herbivory. *Impatiens capensis* showed a positive effect of shrub removal when protected from deer, but this effect disappeared in plots where deer were present, due to the effects of herbivory (Cipollini et al. 2009). It therefore seems important that both direct and indirect effects, and their interactions, of disturbances like deer overabundance be considered when evaluating the response of plant communities.

In conclusion, my results indicate that for the two focal native species *P. peltatum* and *A. triphyllum*, white-tailed deer are not playing a significant role in their abundance or reproductive output. Any negative effects these two species are experiencing are more likely due to competitive effects from other species that deer avoid. For the invasive species *M. vimineum*, my results clearly show that this species benefits from both the

direct and indirect effects of deer herbivory and the indirect effect of increased understory light availability. However, for the invasive species *A. petiolata*, at deer densities similar to Blandy, the results are less clear. This species does benefit from deer presence by recruiting more individuals into the population, but once established, it does appear to suffer negative effects from deer. It is important to note, however, that deer densities are extremely high at Blandy (104 deer/km^2), and at lower densities *A. petiolata* may be able to escape the negative effects of deer, with the result of a strongly successful invasion.

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Table 1. Means (and 95% confidence intervals) for native (*Podophyllum peltatum* and *Arisaema triphyllum*) and invasive (*Alliaria petiolata* and *Microstegium vimineum*) species responses to shade and fence treatments. Response means marked with asterisks (*) indicate significant differences between means (* P<0.05, ** P<0.01, ***P<0.001).

a) **Mayapple** (*Podophyllum peltatum*)

	Shade Treatment		Fence Treatment	
Response	**No Shade Mean**	**Shade Mean**	**Open Mean**	**Fence Mean**
Abundance	15.65 (12.64-19.33)	13.61 (10.96-16.83)	16.23 (13.13-20.02)	13.11 (10.57-16.21)
Stem Height (cm)	24.43 (21.30-27.55)	23.64 (20.51-26.77)	22.95** (19.82-26.07)	25.12** (21.99-28.26)
Leaf Width (cm)	19.99 (17.18-22.81)	19.87 (17.04-22.70)	19.45 (16.64-22.26)	20.41 (17.58-23.24)
Proportion Flowering	0.096 (0.028-0.287)	0.080 (0.022-0.246)	0.124*** (0.036-0.346)	0.061*** (0.017-0.199)

b) **Jack-in-the-pulpit** (*Arisaema triphyllum*)

	Shade Treatment		Fence Treatment	
Response	**No Shade Mean**	**Shade Mean**	**Open Mean**	**Fence Mean**
Abundance	13.12 (8.80-19.57)	11.13 (7.47-16..6)	13.73 (9.18-20.54)	10.64 (7.11-15.91)
Stem Height (cm)	13.91 (11.77-16.05)	13.81 (11.72-15.91)	12.96 (10.87-15.06)	14.76 (12.62-16.90)
Leaf Width (cm)	6.05 (5.01-7.10)	6.00 (4.98-7.03)	5.91 (4.89-6.94)	6.14 (5.10-7.19)
Proportion Flowering	0.067 (0.045-0.100)	0.058 (0.035-0.096)	0.065 (0.043-0.098)	0.060 (0.036-0.098)
Proportion Female	0.008 (0.002-0.026)	0.011 (0.003-0.037)	0.015 (0.006-0.041)	0.006 (0.001-0.023)

Table 1, continued

c) **Garlic Mustard** (*Alliaria petiolata*)

Response	Shade Treatment		Fence Treatment	
	No Shade Mean	**Shade Mean**	**Open Mean**	**Fence Mean**
Per-capita recruitment	3.69** (1.95-6.45)	1.36** (0.47-2.80)	3.85** (2.02-6.81)	1.28** (0.44-2.63)
Proportion Rosette Survival	7.16 (3.83-10.69)	5.78 (2.93-9.53)	3.60** (1.80-6.25)	11.24** (6.20-15.23)
Leaflets/Rosette	1.63 (1.45-1.83)	1.72 (1.52-1.93)	1.61 (1.43-1.81)	1.74 (1.54-1.95)
Stems/Adult	1.36 (1.13-1.63)	1.45 (1.20-1.74)	1.30 (1.06-1.56)	1.53 (1.27-1.81)
Adult Height (cm)	49.11 (41.07-58.69)	47.93 (39.79-57.69)	42.12** (34.95-50.72)	55.87** (46.74-66.73)
Fruit Number	13.13 (8.75-19.49)	12.16 (7.92-18.41)	9.20* (5.91-14.05)	17.23* (11.57-25.43)
Fruit Length (cm)	4.14 (3.86-4.42)	4.13 (3.84-4.43)	4.04 (3.76-4.34)	4.23 (3.95-4.52)

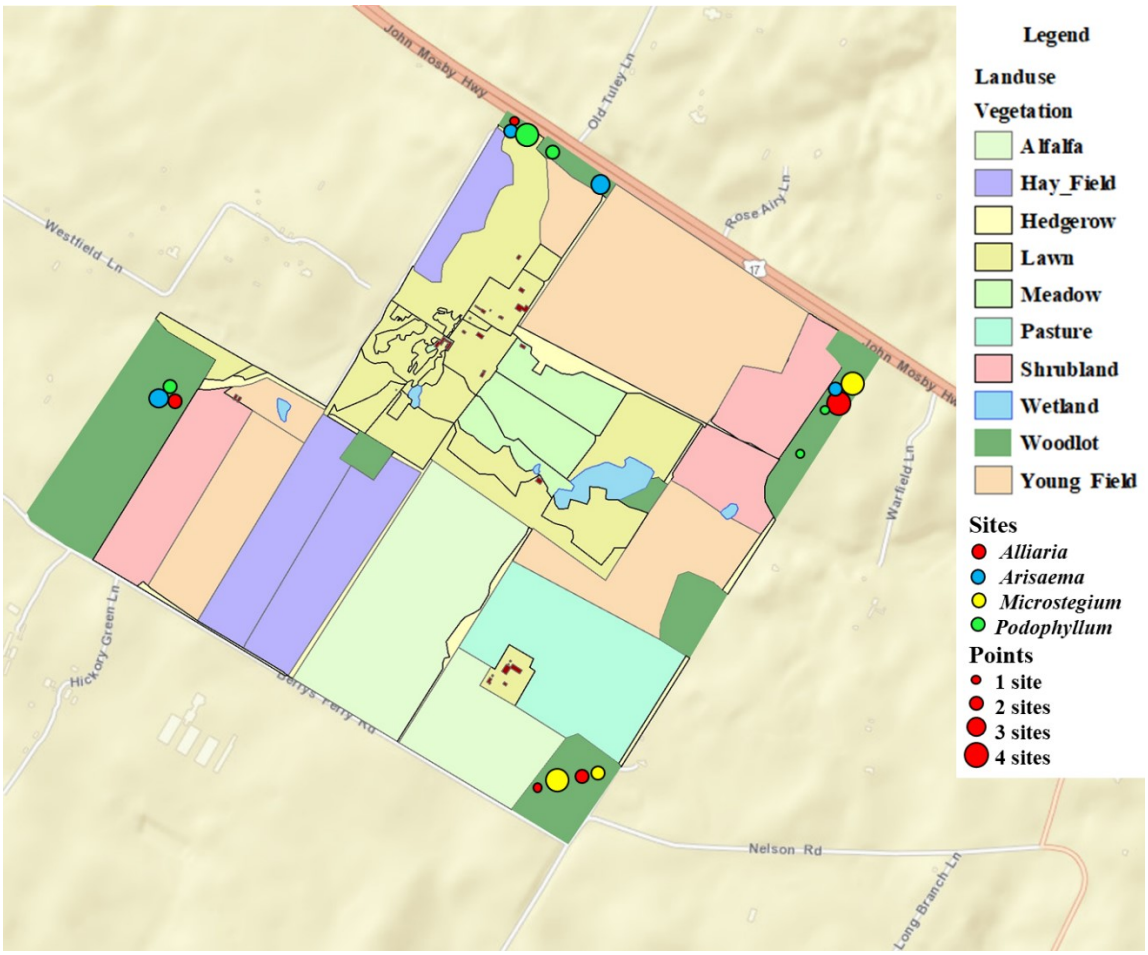
d) **Asian Stiltgrass** (*Microstegium vimineum*)—the height and spikelets/plant represent measurements of randomly selected individuals in each plot.

Response	Shade Treatment		Fence Treatment	
	No Shade Mean	**Shade Mean**	**Open Mean**	**Fence Mean**
Abundance/m^2	3.24*** (2.88-3.60)	2.46*** (2.10-2.82)	3.18** (2.82-3.54)	2.52** (2.16-2.88)
Height (cm)	2.99 (2.79-3.20)	2.84 (2.63-3.04)	3.01 (2.80-3.22)	2.81 (2.61-3.02)
Fruiting Plants/m^2	2.99*** (2.64-3.33)	2.19*** (1.85-2.54)	2.95*** (2.60-3.29)	2.23*** (1.89-2.58)
Spikelets/Plant	4.99* (4.24-5.73)	3.75* (3.01-4.49)	4.59 (3.84-5.33)	4.15 (3.41-4.89)

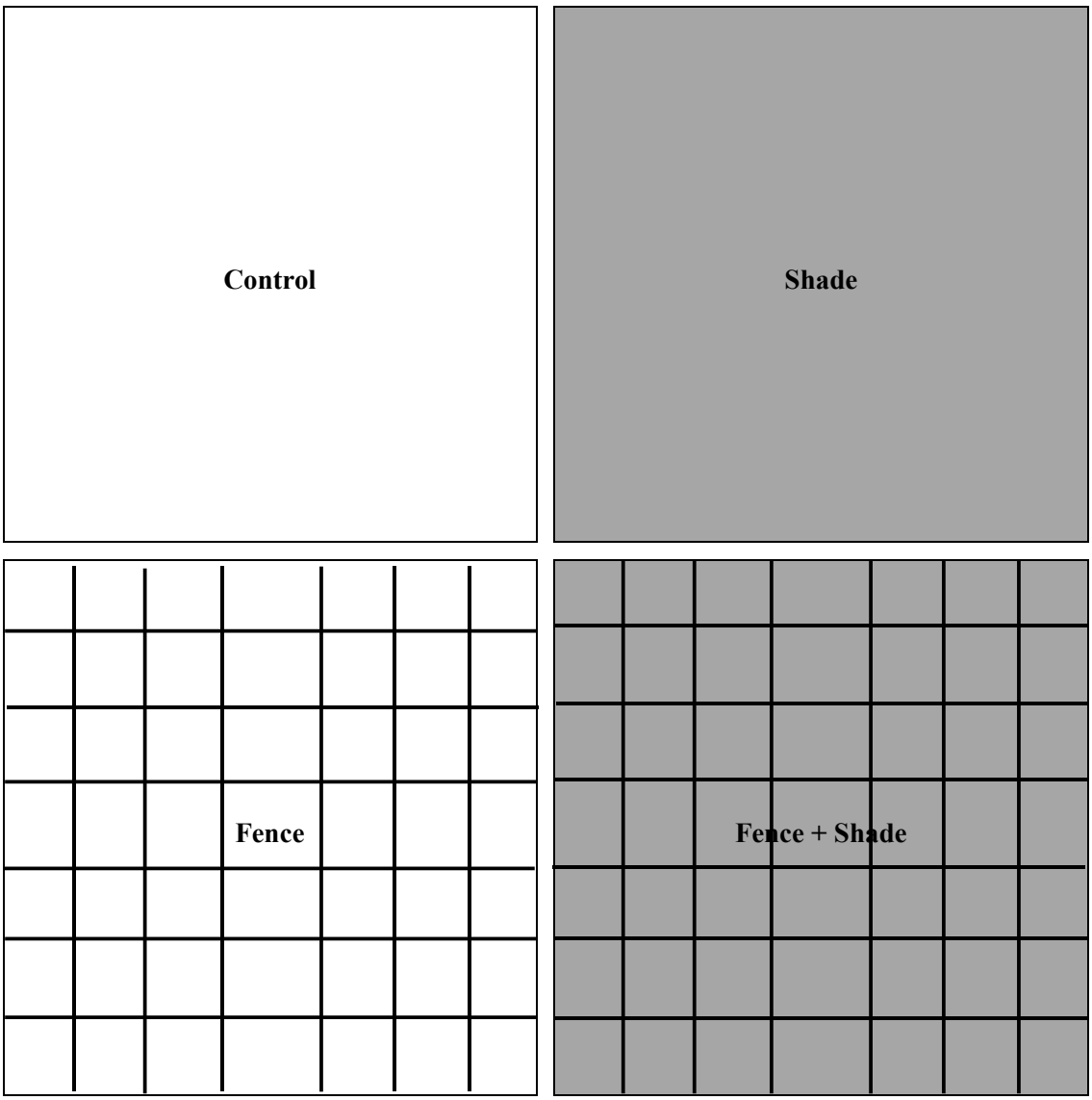
Appendix 1: Forest fragment sizes and the number and species identity of shade plots they contained

Forest fragment (size)	Shade plot species	Number of plots
North (1.2 ha)	*P. peltatum*	2
	A. triphyllum	3
Northwest (2.3 ha)	*P. peltatum*	4
	A. triphyllum	2
	A. petiolata	1
Northeast (4.9 ha)	*P. peltatum*	2
	A. triphyllum	2
	A. petiolata	4
	M. vimineum	4
Southeast (5.8 ha)	*A. petiolata*	3
	M. vimineum	6
Southwest (18.6 ha)	*P. peltatum*	2
	A. triphyllum	3
	A. petiolata	2

Appendix 2. Map of the location of study sites at Blandy Experimental Farm. Size of icons indicates the number of sites for each species at a given location. Where more than one site occurred in a location, sites were separated by at least 10 m.



Appendix 3. Diagram of study sites indicating treatment for each plot within a site.



CHAPTER 3

The dispersal of seeds by white-tailed deer (*Odocoileus virginianus*) through endozoochory

Abstract

Understanding the processes by which species invasions may be promoted is vital to combat species invasions and protect biodiversity. Seed dispersal is an integral component to colonizing new locations, and plants have adapted several ways to facilitate this process. White-tailed deer (*Odocoileus virginianus*) are not often thought of as important frugivores, but they have been recognized as having a potentially important role in long distance dispersal. In this study I collected scat from three locations across the landscape of the Blue Ridge and Shenandoah Valley. I identified the total abundance of seeds and the abundance of seeds from invasive species found in sub-samples of deer scat and tested for viability of seeds by stratifying sub-samples and allowing seeds to germinate. I compared the abundance of seeds between field and forest habitats, spring and fall seasons, and among locations. Although relatively few seeds were found in the scat samples (mean = 29.1 seeds per pellet group), I identified a diverse group of 41 different seed taxa, over one-third of which were exotic in origin. The seeds dispersed in the deer scat was both seasonally and habitat dependent and varied across locations. Of the 41 taxa identified, only 17 germinated, over half of which were invasive. Of the native taxa identified, the most abundant taxa were from fleshy-fruited taxa. In contrast, the most abundant invasive taxa did not have fleshy fruits, but they tended to have small, hard seeds. This indicates that deer may be playing the role of frugivores for some of these taxa, although the low germination rates suggest that deer may act more as seed predators than seed dispersers for many species.

Introduction

The ecological and economic costs of exotic invasive species (hereafter "invasive species") are well-known, including displacement of native species and loss of biodiversity, altering nutrient cycles, increasing the risk of fire, reducing recreational use of invaded areas, as much as $24 billion in lost crop production annually, the loss of pasture forage, and as much as $500 million spent annually to control invasions residential lawns and gardens (Pimental et al. 2005). It is therefore vital to understand the processes by which species invasions may be promoted. Seed dispersal is critical to the spread of invasive species. The majority of plants do not disperse their seeds very far, typically from zero to a few meters away (Howe and Smallwood 1982; Cain et al. 2000; Vellend et al. 2003). However, in order to colonize new habitats and exchange genes between metapopulations, plants must be able to disperse their seeds across hundreds of meters (Vickery et al. 1986; Cain et al. 2000; Vellend et al. 2003; Myers et al. 2004). In order to accomplish this, plants have adapted several ways to facilitate seed dispersal. Some have developed morphological adaptations which aid dispersion, such as achenes and samaras which facilitate wind dispersal, while other plants have seeds which are dispersed through the process of epizoochory, by sticking to animal fur using barbs, hooks, or spines (Willson 1993; Cain et al. 2000; Pakeman 2001; Myers et al. 2004).

Endozoochory, the consumption and deposition of seeds, is a common mode of dispersal for seeds contained in fleshy fruits, but it may also be especially important for seed dispersal in plants with no clear physical adaptations for dispersal (Willson 1993; Malo and Suarez 1996; Malo et al. 2000). This might be especially true for herbaceous plants with dry fruits and small seeds Janzen (1984). Although viable large seeds are

occasionally found in animal dung, they are more commonly destroyed by chewing and digestion, but small seeds are able to pass undamaged through the gut of the animal (Vellend et al. 2003; Myers et al. 2004). Though it has been established that endozoochory is an important mechanism for long distance dispersal, it is only recently that "non-typical" frugivores such as white-tailed deer (*Odocoileus virginianus*) and other ungulate herbivores have been recognized as having an important role in long distance dispersal (Malo and Suarez 1995; Malo and Suarez 1996; Malo et al. 2000; Pakeman 2001; Vellend et al. 2003; Myers et al. 2004)

The expanded range and population size of white-tailed deer (Rooney 2001; Eschtruth and Battles 2009; Pellerin et al. 2010; Adams and Hamilton 2011) could have a great impact on the dispersal of seeds. White-tailed deer are generalist feeders of both herbaceous and woody plants (Bagley 2017). With a mean gut passage time of 23 hours (Mautz and Petrides 1971) and home range of 23-31 hectares (Labisky and Fritzen 1998), white-tailed deer may travel long distances before they deposit any seeds consumed (Pakeman 2001). The broad diet and large range of deer, therefore, can play a significant role in the seed dispersal of the plants they consume across a vast landscape of varying habitats.

Recent studies on seed dispersal by white-tailed deer have indicated that deer can be significant dispersal vectors for seeds. In a late summer study in New York, Vellend (2002) identified seeds of invasive *Lonicera* sp. shrubs in 66/72 scat samples. Although deer were not commonly recognized as dispersal vectors, estimates indicated up to 5.2 *Lonicera* sp. seeds/m^2 could be deposited in scat samples compared to 0.7 seeds/m^2 for birds, which were the commonly recognized dispersal vectors. Depending on the season

and migration patterns, deer could potentially transport these seeds up to 10 km (Vellend 2002). In the same part of New York, Myers et al. (2004) collected scat samples over one year and identified 72 germinating species, 46 of which were not native to the area. Out of the 72 species, the majority (58) were from open habitats and 45 did not have any special dispersal adaptations. Based on the abundance of seeds in the scat samples, up to 10 seeds/m^2 could be deposited in the landscape each year (Myers et al. 2004). A similar study in Connecticut (Williams and Ward 2006) collected scat samples over 10 months and identified germinating 57 species, 32 of which were not native to the area. Based on the abundance of seeds in the scat samples, deer could deposit 390-1046 exotic seeds/day/km^2 (Williams and Ward 2006) depending on the time of year. For both Myers et al. (2004) and Williams and Ward (2006) a large proportion of seeds were small, hard seeds that fit Janzen's (1984) "foliage is the fruit" hypothesis. The number of species that germinated in these studies, including both native and invasive, indicate that white-tailed deer play an important role in long distance seed dispersal, both for plants with no dispersal adaptations and those that do have dispersal adaptations (Cain et al. 2000; Pakeman 2001; Myers et al. 2004).

With regard to seed dispersal by white-tailed deer, it is important to identify the numbers of seeds dispersed by deer, the number of species dispersed by deer, the proportions of seeds dispersed that are invasive species, and the number of seeds that may germinate from deer scat (Pakeman 2001; Vellend et al. 2003). Due to the wide range and broad diet of white-tailed deer in North America, these identifications need to cover large, community scales, in different locations and habitat types (Malo and Suarez 1995; Malo et al. 2000; Vellend 2002). In addition to dispersing small, hard seeds as

proposed by Janzen (1984), white-tailed deer may also act as frugivores, a role not commonly applied to deer. For example, several studies have shown that deer act in this manner for some species including *Lonicera maackii*, *Panax quinquefolius*, *Podophyllum peltatum*, *Rhus aromatica*, and *Solanum carolinense* and *S. nigrum* (Li et al. 1999; Furedi and McGraw 2004; Myers et al. 2004; Williams and Ward 2006; Blyth et al. 2013; Castellano and Gorchov 2013; Niederhauser and Matlack 2015). However, these seeds being consumed does not always lead to the deposition of viable seeds (*P. quinquefolius*, Furedi and McGraw 2004; *P. peltatum*, Niederhauser and Matlack 2015) or a reduction in the viability of seeds (*L. maackii*; Castellano and Gorchov 2013), in which case the consumption of the fruits may be considered to be a seed predation event rather than a seed dispersal event.

The purpose of this study was to identify what seed taxa, their abundance, and the origins (native or invasive) and fruit type (fleshy or non-fleshy) of these taxa that deer are moving across the landscape. Previous studies have shown that many taxa germinate from deer scat, including both native and invasive taxa, and these taxa often germinate at rates that could strongly benefit the species being dispersed. In my study, I identified and counted the abundance of taxa contained in deer scat, in addition to germination trials. I examined both the abundance of seeds as well as the abundance of invasive species in scat samples from three locations across the landscape of the Blue Ridge and Shenandoah Valley of Virginia to get a better regional description of what taxa deer are dispersing. I examined differences between field and forested habitats and the variation between seasons. In addition to identifying the seeds in the scat samples, I attempted to germinate seeds to establish whether deer were competently dispersing viable seeds. I expected the

majority of seeds to be either adapted for endozoochory (e.g., seeds from fleshy fruited species) or to have small, hard seeds as observed by Myers et al. (2004) and according to the hypothesis of Janzen (1984).

Methods

Site Description

This project took place at three locations in northwest Virginia: Blandy Experimental Farm (39.061°N, 78.065°W) in the Shenandoah Valley (Clarke County), and Sky Meadows State Park (38.989°N, 77.966°W) and the private Wheeler property (38.938°N, 77.969°W), both near Delaplane, Virginia in the Blue Ridge Mountains (Facquier County). Blandy is approximately 13 km northwest of Sky Meadows and 16 km northwest of the Wheeler property, and Sky Meadows is approximately 4.5 km north of the Wheeler property. All three locations contained a mixture of forest fragments and field habitats. The forests at all three locations were primarily a mixture of oaks and hickories, with native spicebush (*Lindera benzoin*) and invasive bush honeysuckle (*Lonicera maackii*) commonly found in the understory. The field habitats at Blandy are a mixture of mowed lawn areas, a native plant meadow, and early successional fields. At both Sky Meadows and the Wheeler property, field areas were primarily hay fields and pastures (absent of cattle during the project), as well as abandoned fields and a bottomland meadow near a stream at Sky Meadows.

Sample Collections

In June 2012, I established 20 100m transects at Blandy, ten transects each in forest and field habitats. In July 2012, because fewer samples of scat were found relative to forest transects, three additional field transects were established, bringing the total number of transects at Blandy to 23. In late September 2012 to get a better regional description of deer endozoochory, additional transects were established at Sky Meadows State Park and the Wheeler property, including nine forest and nine field transects at each location for a total of 59 transects in the study. Initially, the transects at Blandy were surveyed weekly for deer scat during the summer. With the addition of the transects at Sky Meadows and the Wheeler property, surveys were conducted approximately monthly from September through November. Sampling resumed again in early March 2013 and continued through April.

To collect deer scat, I walked the length of the transects, and when I encountered intact, recently deposited pellet groups, I collected the samples in plastic bags. Every 10 meters along the transect I performed intensive searches in a 1 m^2 quadrat in order to find samples that may have been covered by leaf litter or vegetation and to allow me to estimate the density of deer pellet groups at each location. This intensive searching proved to be especially important in field habitats. Pellet samples were stored in a refrigerator at approximately 7 °C until further analysis.

Seed identification and germination

In the lab, I divided each pellet group into four subsamples of equal weight. To count and identify seeds, I placed one subsample in a 0.5 mm sieve to remove excess

plant and fecal material (Myers et al. 2004), and then searched the remaining material under a dissecting scope for seeds. I then used pictorial and dichotomous seed keys (Delorit 1970; Martin and Barkley 2000) to identify all intact, potentially germinable seeds from these subsamples, recording the total number of seeds and the number of each seed taxon (primarily genus or species). I then collected information from the literature (Digital Atlas of the Virginia Flora; USDA Plants Database) to document characteristics of each seed taxon, including if the seeds were borne in fleshy or non-fleshy fruits and if the plant was native or invasive.

I used two more subsamples to determine which plants would germinate from the deer scat. To best encompass the variety of germination conditions required by different plant species, I sieved one of these subsamples in the same manner as the seed identification, placing the remaining material from the sieve in a petri dish on wet filter paper. The other subsample was air dried at room temperature and then placed in a petri dish. These subsamples underwent cold, wet or cold, dry stratification, respectively, for at least three months at 7 °C, then spread on potting mix and kept moist under greenhouse conditions. I then monitored these subsamples for germination of seeds from the pellet groups, allowing them to grow, transplanting if necessary, until the necessary characters for identification were evident.

Statistical analyses

Due to a large proportion of samples containing no viable seeds and a low proportion of germinations, sample periods were combined by season. To test for habitat and seasonal effects and differences across locations, I used the seed collection data from

Blandy (fall and spring only), Sky Meadows, and the Wheeler property. At all three sites I combined samples to represent a fall (September and November 2012) and a spring collection (March and April 2013). Using generalized linear models with an exponential distribution and a log link, total seed abundance, total abundance of invasive species seeds, and total abundance of native species seeds were response variables. Season, location, and habitats were the independent variables and were treated as fixed effects. The interaction effects of season×location, season×habitat, location×habitat, and season×location×habitat were also included in the model and were the primary variables of interest.

Results

A total of 252 pellet groups were collected over the course of the study. At Blandy, over a period of eight weeks in the summer of 2012 when transects were sampled bi-weekly, I found 0.16 pellet groups/m^2. The density of pellet groups at Blandy and Sky Meadows was greater in the spring (0.21/m^2 at Blandy and 0.14/m^2 at Sky Meadows) than in the fall (0.13 pellet groups/m^2 for Blandy and 0.09 pellet groups/m^2 at Sky Meadows). The Wheeler property showed almost no seasonal effect (0.16 and 0.18 pellet groups/m^2 for spring and fall, respectively).

From these 252 samples I was able to extract 1543 seeds. This comes to a mean of 7.3 seeds identified per sub-sample (SD = 37.3; Range: 0-450). It is important to note that the scat samples were divided into four sub-samples, therefore this represents approximately one-quarter of the total seed abundance per pellet group. The seeds represented 41 taxa (Table 1), 32 of which were identified to species (Table 2). The other

9 taxa were identified to either genus or family (Table 2). The greatest richness was at Blandy (35, including spring, summer, and fall samples), followed by Sky Meadows (15), and the Wheeler property (10) (Table 1). Seeds from 15 invasive taxa were identified, 13 of which were found at Blandy, while Sky Meadows and the Wheeler property each had 5 invasive taxa seeds identified (Table 2).

The most commonly identified taxa of native seeds were variable between locations and habitats (Table 2). Seeds from wild raspberries (*Rubus* spp.) were the most abundant overall (39.3 % of all seeds), but these were found only at Blandy and primarily from field habitats and primarily from the summer when no collections were made at the other two sites. However, due to the similarity of seeds, it was impossible to differentiate between native and invasive *Rubus* species. Seeds of the genus *Galium* were treated in the same manner, but these represented only 1.6% of all seeds identified. The next most abundant taxon from the seed identifications was wild grape, *Vitis* spp. (20.9% of all seeds), which was found only in forest habitats at both Sky Meadows and the Wheeler property. The most commonly identified seeds of invasive taxa (Table 2) were less variable across locations, with three of the six taxa found at all three locations. The thistle *Cirsium vulgare* was the most abundant of the invasive taxa and was found at all three sites (68.5% of all invasive species across all sites, not including *Rubus* sp. seeds).

There were no consistent differences in germination between wet and dry stratification treatments. Among the taxa that germinated, eight had higher germination in wet stratification and seven had higher germination in dry stratification, with two taxa having equal germination. I am reporting the pooled data for total germinations. Despite the high abundance and diversity of seeds identified from scat samples, the diversity of

germinating taxa was much lower (Table 1 and 2). A total of 17 taxa were found among the 134 seedlings that germinated from the scat samples (Table 1). The greatest richness of germinating seeds was at Blandy (12, including spring, summer, and fall samples), followed by Sky Meadows and the Wheeler property (both 8). Nine invasive species germinated, seven of which were found at Blandy, while the Wheeler property had four, and Sky Meadows had three invasive species that germinated. A native grass (*Muhlenbergia schreberi*) was the most common native taxon among the germinating seedlings, and accounting for 14.9% of all germinating seeds and being represented at all three locations (Table 2). The native horsenettle (*Solanum carolinense*) was the next most common native taxon, representing 11.5% of all seedlings and being found at all three locations. Lambsquarters (*Chenopodium album*) was the most common invasive taxon among the germinating seedlings (27.7% of all seedlings, and 60.3% of invasive seedlings, not including *Rubus* sp.) and was found at all three locations. Only ten *Rubus* spp. germinated, compared to the 619 seeds identified from scat collected at Blandy. No *Cirsium vulgare* seeds germinated, compared to the 231 seeds identified.

For native seeds, 9 of the 24 identified taxa came from fleshy fruits (Table 2), and seeds from native fleshy fruits accounted for 29.9% of all seeds (Table 2). Of the native taxa that germinated, 5 of the 6 were from fleshy fruits, accounting for 26.4% of all germinating seeds. For invasive seeds, only 2 of the 14 identified taxa came from fleshy fruits, accounting for 2.4% of all invasive seeds and only 0.5% of all seeds. Of the invasive taxa that germinated, 2 of 7 came from fleshy fruits, accounting for 13.2% of germinating invasive seeds and 6.1% of all seeds that germinated. *Rubus*, which has fleshy fruits, accounted for 39.3% of all seeds identified, but only 6.8% of germinating

seeds. Both native and invasive *Rubus* are common at Blandy, but I was unable to identify seeds of this genus down to species level.

Differences in total seed abundance from deer scat samples between forest and field habitats depended on the season (Figure 1a; Habitat×Season interaction, $F_{1,169}$=18.89, P<0.0001). Collections from the fall contained the greatest abundance of seeds, but the field and forest habitats did not differ significantly. In the spring, however, seeds from forest collections were 5.1 times greater than from the field habitats. When *Rubus* sp. were omitted from the analysis (because their native/invasive status was ambiguous) there was a significant 3-way interaction of native seed abundance, suggesting that seasonal effects and habitat effects were site-specific (Figure 1b; $F_{2,169}$=5.74, P=0.0039). This interaction was primarily due to the high abundance of native seeds (mostly Vitis sp.) identified from forest habitats at Sky meadows in the fall, which had 17.8 times more seeds per sample of scat than the next closest fall forest habitat (Blandy). For invasive seeds, differences between habitats depended on the season of collection (Figure 1c; Habitat×Season interaction, $F_{1,169}$=4648.31, P<0.0001). In the spring, invasive seeds from forest habitats were 5.7 times more abundant than in field habitats, but almost no invasive seeds were found in forest samples in the fall.

Discussion

The mean of 7.3 seeds/sub-sample found in this study translates to approximately 29 seeds identified/pellet group. However, only 134 seedlings germinated from the sub-samples, and this would translate into only 1.1 seedlings per entire pellet group. This is a far smaller number than the 5.3 – 38 seedlings per pellet group reported by Myers et al.

2004. Both Blyth et al. (2013) and Williams et al. (2008) found that the number of seedlings germinating from scat samples showed strong seasonal differences with no seedlings being found in some months but with peaks over 160 seedlings in July (Williams et al. 2008).

Relatively few seeds were found in the deer scat samples, but overall, a diverse group of seed taxa were identified, over one-third of which were exotic in origin. A few taxa dominated, however. The fleshy-fruited taxon, *Rubus* sp., which included both invasive and native species, was the most abundant seed, followed by the fleshy-fruited native taxon *Vitis* sp. and the non-fleshy-fruited invasive taxon *Cirsium vulgare*. Of all the taxa identified from the scat samples, 69.8% produce fleshy fruit.

Of the 41 taxa of seeds found in the scat samples, only 17 germinated. invasive taxa represented over half of these. Of the native taxa to germinate, the majority were from fleshy fruits, but the most abundant native to germinate was *M. schreberi*, a grass that produces seeds that are not specially adapted for endozoochory. Of the invasive taxa to germinate, the majority did not have fleshy fruits, the lone exceptions being *D. indica* and *L. maackii*.

Although white-tailed deer are not commonly thought of as frugivores, it is clear that they are performing this role as seed dispersers for many species. Seeds of additional fleshy-fruited species that have been found in deer scat include *Podophyllum peltatum*, *Rhus aromatica*, and *Solanum nigrum* (Li et al. 1999; Myers et al. 2004; Williams and Ward 2006; Blyth et al. 2013; Niederhauser and Matlack 2015), and a number of taxa found in scat in my study were reported by other authors. However, despite the high abundance of seeds from fleshy fruited taxa, relatively few of these actually germinated.

For example, seeds from *Rubus* sp., *Vitis* sp., and *Viburnum prunifolium* totaled 1024 seeds, but only 10 *Rubus* sp. and 4 *Vitis* sp. germinated, and there were no germinations for *V. prunifolium*. When looking at dispersal of *L. maackii* by white-tailed deer Castellano and Gorchov (2013) found that 68% of seeds found in deer scat were viable after stratification using tetrazolium tests. This was significantly lower than the viability of stratified (87%) and non-stratified (84%) seeds collected directly from *L. maackii* fruit. By comparison, the viability of *L. maackii* seeds consumed by birds tended to be much higher, varying between 94-100% in *Turdus migratorius* (American Robin) and *Sturnus vulgaris* (European Starling) to as low as 75-83% in *Mimus polyglottos* (Northern Mockingbird) and *Bombycilla cedorum* (Cedar Waxwing) (Bartuszevige and Gorchov 2006). In a study on *P. peltatum*, Niederhauser and Matlack (2015) found that 82% of fruits that survived beyond 2 weeks were consumed unripe, primarily by deer. Seeds from unripe fruits were shown to have significantly lower proportion germinating than seeds from ripe fruits. In addition, only 1% of seeds from ripe fruits were passed intact after ingestion by deer compared to 28% of seeds ingested by raccoons (*Procyon lotor*) (Niederhauser and Matlack 2015). Seeds that were passed by raccoons resulted in 100% germination rates (Niderhauser and Matlack 2015). In this case, consumption by deer primarily results in the loss of the seed instead of dispersal. While deer seem to be capable of dispersing seeds from fleshy-fruited species, in many cases they seem to act more as seed predators than seed dispersers. Even when they are capable of dispersing viable seeds they seem much less efficient in comparison to other common frugivores, such as birds (Bartuszevige and Gorchov 2006) or raccoons (Niederhauser and Matlack 2015).

In addition to acting as seed dispersers for fleshy fruited taxa, deer are also consuming a large number of seeds that fit the "foliage is the fruit" hypothesis of Janzen (1984). These are typically small, hard seeds that are consumed incidentally along with the foliage of these plants. Many of these taxa were of exotic origin in this study. The two most abundant invasive species germinating from the deer scat were members of the Amaranthaceae, *C. album* and *D. ambrosioides*. These taxa certainly fit within the hypothesis of Janzen (1984), having small seeds that are consumed along with the foliage of these plants. Seeds of *C. album* are known to be dispersed in the manure of ungulates (Aper et al. 2013), and they were the most common seeds dispersed by the *Cervus elaphus*, *Dama dama*, and *Capreolus capreolus* in a study of cervids in England (Eycott 2007). However the other Amaranthaceae, *D. ambrosioides*, is generally considered noxious and avoided by herbivores (Georgia 1914), so its presence in the deer scat was somewhat surprising. In contrast to these successfully germinating species, *C. vulgare* was the most abundant invasive taxa identified in the scat samples, yet none of these wind-dispersed seeds germinated from the scat, indicating that deer are acting more as seed predators than dispersers for this species. The most abundant germinating native taxon was the turf-grass *Muhlenbergia schreberi*. This species also fits with the "foliage is the fruit" hypothesis (Janzen 1984), having small seeds (1-1.5 mm length) and lacking fleshy fruits. Other studies have also indicated that size (primarily small, round seeds) is a strong predictor of the ability of ungulates to disperse these types of grasses (Pakeman et al. 2002; Rosas et al. 2008).

The dispersal of seeds by deer was also seasonally and habitat dependent and varied from across the region. This is not surprising, and these patterns were probably

driven by differences in species composition across locations and seasons. As mentioned earlier, other studies also found strong seasonal patterns (Myers et al. 2004; Williams et al. 2008; Blyth et al. 2013), but peak seasons varied from study to study. Generally in my study, seeds were more abundant in scat samples collected in the fall, and this likely corresponds to when seeds and fruit, especially fleshy fruit, are maturing. For example, in the fall, seeds from *Vitis* sp. were abundant in scat from Sky Meadows, while at Blandy seeds from *Rubus* sp. were most abundant. For invasive species, *C. vulgare* accounted for most of the seeds in spring samples, none of which germinated, however. *Chenopodium album* was found in both spring and fall, but primarily in field habitats. No invasive species were found in samples from forest habitats in the fall.

This study indicated that deer are not moving many seeds, but they are moving many different taxa. Many of these are invasive species. Although deer appear to be attracted to fleshy fruits, the most commonly dispersed invasive taxa were not fleshy-fruited. While I did identify a wide range of taxa in the deer scat, a much smaller proportion of these taxa actually germinated. Although most of these were from fleshy fruited plants for native taxa, this was not true for invasive plants. In my study areas, white-tailed deer are playing only a minor role in dispersing the seeds of invasive species with the possible exceptions of *C. album* and *D. ambrosioides*. However, due to low germination rates, my study suggests that in future evaluations of the role of white-tailed deer, it is important to consider both the abundance of seeds being dispersed as well as the viability of those seeds. Simply identifying the seeds contained in scat samples may overestimate the role of deer endozoochory and relying only on germination trials may not give a clear picture of the impact of deer on the plants they consume. By evaluating

the viability of seeds with germination trials relative to the total abundance of seeds dispersed by deer, one may get a better idea the impacts of deer and whether they are acting more as seed predators or seed dispersers

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Table 1: Richness of seeds counted in white-tailed deer scat samples.

	Total	Blandy	Sky Meadows	Wheeler
Seed Count Richness	41	35	15	10
Invasive Seed Count Richness	15	13	5	5
Germination Richness	17	12	8	8
Invasive Germination Richness	9	7	3	4

Table 2: Total abundance, abundance by habitat, total germinations, germinations by habitat, locations found, and fruit type for species identified from scat samples.

Species	Origin	Fruit Type	Location(s)	Abundance			Germinations		
				Total	Field	Forest	Total	Field	Forest
Vitis sp.	N	Fleshy	SM, WH	**329**	0	329	**4**	0	4
Viburnum prunifolium	N	Fleshy	SM	**76**	0	76	**0**	0	0
Phytolacca americana	N	Fleshy	BL, SM	**20**	6	14	**7**	1	6
Solanum carolinense	N	Fleshy	BL, SM, WH	**19**	12	7	**17**	10	7
Symphoricarpos orbiculatus	N	Fleshy	BL, SM, WH	**10**	2	8	**1**	1	0
Physalis sp.	N	Fleshy	BL, SM	**9**	5	4	**10**	7	3
Morus rubra	N	Fleshy	BL	**5**	0	5	**0**	0	0
Asimina triloba	N	Fleshy	SM	**2**	0	2	**0**	0	0
Toxicodendron radicans	N	Fleshy	BL	**1**	0	1	**0**	0	0
Sporobolus vaginiflorus	N	Other	BL, SM	**27**	17	10	**0**	0	0
Muhlenbergia schreberi	N	Other	BL, SM, WH	**21**	16	5	**22**	16	6
Silene sp.	N	Other	BL	**20**	1	19	**0**	0	0
Potentilla pensylvanica	N	Other	BL	**15**	1	14	**0**	0	0
Poa palustris	N	Other	BL	**7**	4	3	**0**	0	0
Bouteloua curtipendula	N	Other	BL	**6**	3	3	**0**	0	0
obtusa	N	Other	BL	**6**	6	0	**0**	0	0
Ludwigia alternifolia	N	Other	BL	**5**	0	5	**0**	0	0
Polygonum lapathifolium	N	Other	BL	**2**	0	2	**0**	0	0
Oxalis stricta	N	Other	BL	**2**	0	2	**0**	0	0
Andropogon gerardii	N	Other	BL	**1**	0	1	**0**	0	0
Cenchrus longispinus	N	Other	BL	**1**	1	0	**0**	0	0
Geum canadense	N	Other	BL	**1**	0	1	**0**	0	0

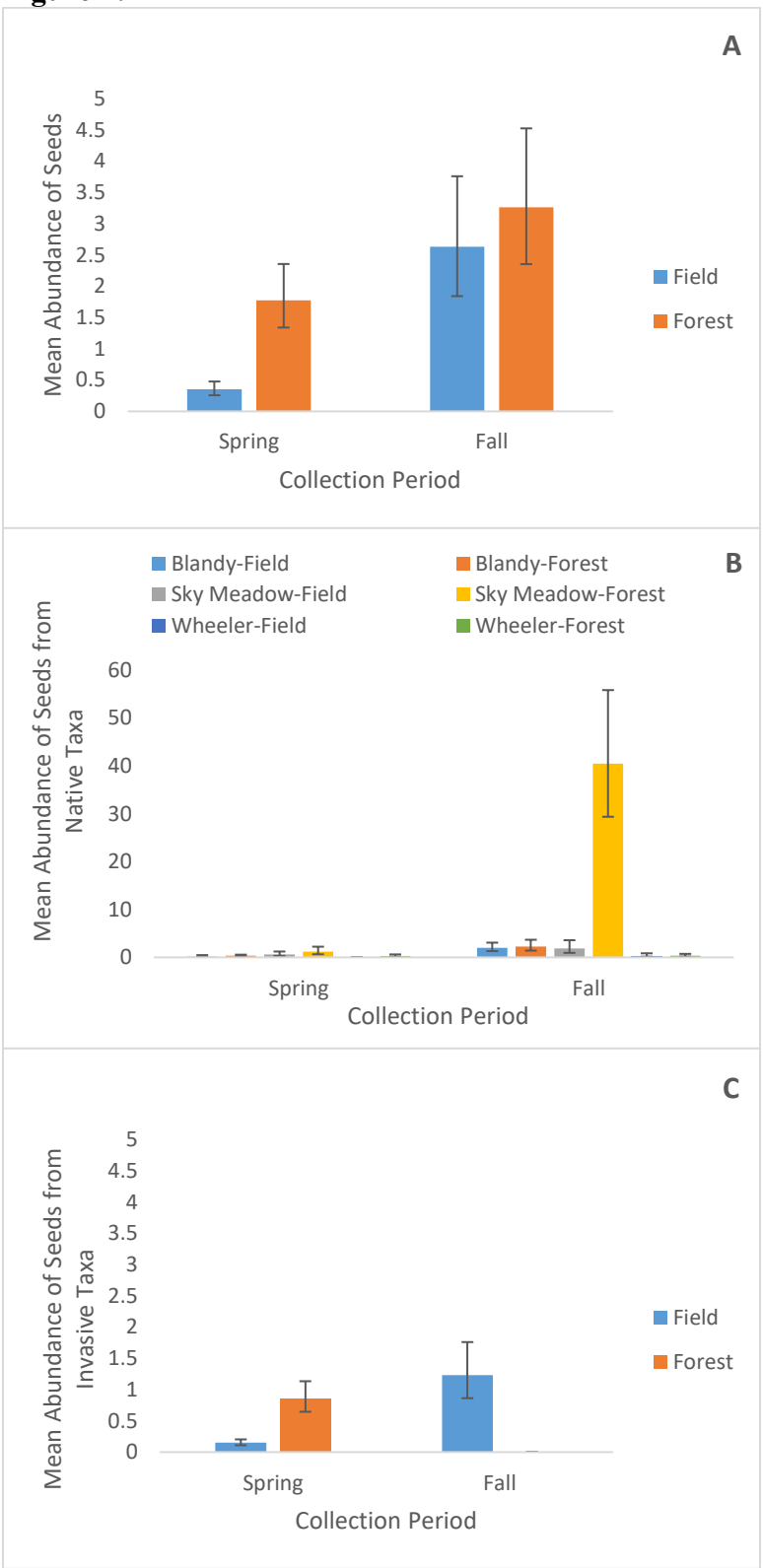
Table 2, continued:

Species	Origin	Fruit Type	Location(s)	Abundance			Germinations		
				Total	Field	Forest	Total	Field	Forest
Lycopus americanus	N	Other	BL	**1**	0	1	**0**	0	0
Potentilla sp.	N	Other	BL	**1**	0	1	**0**	0	0
Rubus sp.	I/N	Fleshy	BL	**619**	514	105	**10**	10	0
Solanaceae	I/N	Fleshy	SM, WH	**6**	1	5	**6**	1	5
Galium sp.	I/N	Other	BL, SM	**25**	17	8	**1**	1	0
Poaceae	I/N	Other	BL	**0**	0	0	**2**	1	1
Duchesnea indica	I	Fleshy	BL	**6**	1	5	**7**	3	4
Lonicera maackii	I	Fleshy	BL	**2**	2	0	**2**	2	0
Cirsium vulgare	I	Other	BL, SM, WH	**231**	92	139	**0**	0	0
Chenopodium album	I	Other	BL, SM, WH	**63**	52	11	**41**	20	21
Dysphania ambrosioides	I	Other	BL, SM, WH	**12**	5	7	**14**	9	5
Veronica sp.	I	Other	BL	**6**	1	5	**0**	0	0
Melilotus officinalis	I	Other	BL	**6**	6	0	**0**	0	0
Cynodon dactylon	I	Other	BL	**2**	2	0	**0**	0	0
Fallopia convulvulus	I	Other	BL	**2**	0	2	**0**	0	0
Persicaria longiseta	I	Other	BL, WH	**2**	1	1	**1**	1	0
media	I	Other	BL	**2**	0	2	**0**	0	0
Trifolium campestre	I	Other	BL, WH	**1**	1	0	**2**	2	0
Microstegium vimineum	I	Other	SM	**1**	0	0	**1**	0	1
Portulaca oleracea	I	Other	BL	**1**	0	1	**0**	0	0

Figure Legend:

Figure 1: LS Mean abundance of seeds counted in samples of White-tailed Deer (*Odocoileus virginianus*) scat for the autumn 2012 and spring 2013, and field and forest habitats located at Blandy Experimental Farm, Sky Meadows State Park, and the Wheeler property. A) Interaction of season×habitat on the total abundance of seeds identified. B) Interaction of season×habitat×location on the abundance of seeds identified from native taxa. C) Interaction of season×habitat on the abundance of seeds identified from invasive taxa. Error bars represent 95% confidence intervals.

Figure 1:


A
Mean Abundance of Seeds
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Spring
Fall
Collection Period
Field
Forest
B
Blandy-Field
Blandy-Forest
Sky Meadow-Field
Sky Meadow-Forest
Wheeler-Field
Wheeler-Forest
Mean Abundance of Seeds from Native Taxa
60
50
40
30
20
10
0
Spring
Fall
Collection Period
C
Mean Abundance of Seeds from Invasive Taxa
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Spring
Fall
Collection Period
Field
Forest

CHAPTER 4

How exotic tree and shrub invasions alter the leaf-litter-dwelling communities in a northern Virginia deciduous forest

Abstract

Studies of exotic species invasions have primarily focused on the negative effects of invasive species. However, they may also be treated as large-scale 'accidental experiments', through which we can gain valuable information on the ecological processes regulating biodiversity. In this study, I examine how the leaf litter inputs from three invasive species may be impacting the litter-dwelling community of an eastern deciduous forest ecosystem. I tested leaf litter from two invasive shrubs, *Lonicera maackii* and *Rhamnus davurica*, and the invasive tree *Ailanthus altissima* and litter from native oak-hickory forest for differences in decomposition rates and nutritional quality. I then tested for the effect of litter source (native vs. invasive litter) and habitat (native vs. invasive canopy) on the abundance and composition of the litter-dwelling community. Litter from all three invasive species decomposed much more rapidly, disappearing from the forest floor much earlier than native litter. Invasive litter had significantly higher nitrogen content and significantly lower C:N ratios relative to native litter. I found clear preferences for bags containing invasive litter by bacteria, fungi, and arthropods. My results indicate that these invasive species are a beneficial, novel resource for the litter-dwelling community. However, the short-lived nature of this resource results in a crash in the abundance of the litter-dwelling organisms once the litter is decomposed. As a whole, despite lower overall abundance, the native habitat supports a more stable litter-dwelling community over the course of a growing season.

Introduction

Studies of species invasions have primarily focused on the negative effects of exotic invasive species (hereafter "invasive species") on native species and the mechanisms that explain why exotics become invasive (Elton 1958; Rabotnov 1982; Blossey and Nötzold 1995; Mallik and Pelliser 2000; Brown and Sax 2004; Callaway and Ridenour 2004; Colautti et al. 2004; Cappuccino and Arnason 2006; Lewis et al. 2006; Rodriguez 2006; Eschtruth and Battles 2009; Huang et al. 2010; Lind and Parker 2010; Roy et al. 2011). Although the study of invasive species is often motivated by efforts to control their effects, another way to view species introductions is as large-scale 'accidental experiments' because we can gain valuable information on the ecological and evolutionary processes regulating biodiversity (Parker et al. 1999; Wardle 2002; Brown and Sax 2004; Sax et al. 2007). When plant invasions occur, they may alter the leaf litter resources of an ecosystem by adding to and changing the availability of the litter resources. In this study, I examine how the invasion of three invasive species may be impacting the litter-dwelling community of an eastern deciduous forest ecosystem.

By studying heavily invaded communities, we can gain an understanding of how invasive species impact the multi-trophic interactions of an ecosystem. For example, invaded areas have been shown to provide substantial increases in the primary productivity in ecosystems (Byers et al. 2012; Trammell et al. 2012). Trammell et al. (2012) found an increase of more than 20% in the total litterfall biomass in areas invaded by bush honeysuckle (*Lonicera maackii*). Previous studies have shown that approximately 90% of net primary production of forests and shrublands enters into detrital food webs (Cebrian 1999; Gessner et al. 2010), and this detritus plays a critical

role in ecosystems as it decomposes, serving as a major pathway for nutrient cycling (Handa et al. 2014). Decomposition is facilitated by bacteria, fungi, and invertebrates. Changes in the litter quantity and quality can impact the detritivore food webs in the litter layer. This can include changes in arthropod diversity and abundance due to alterations in litter quality, structure, and depth (Uetz 1979; Bultman and Uetz 1984; Antvogel and Bonn 2001). Litter quality has been found to be a major factor in determining the arthropods found under a tree (Negrete-Yankelevich et al. 2008), and changes in the quality of the resource base have been shown to affect the abundance of arthropods at all trophic levels in a detrital food web (Chen and Wise 1999).

Alterations to the leaf litter in areas invaded by exotic species may have either positive or negative effects on the consumer populations inhabiting the litter. For example, *Ailanthus altissima* was found to increase the abundance of some litter-dwelling organisms and decrease the abundance of others relative to uninvaded areas (Gutiérrez-López et al. 2014; Motard et al. 2015). Comparing invaded and uninvaded habitats, litter from *Lonicera maackii* was found to increase the abundance of litter organisms (Poulette and Arthur 2012) or support a different community of organisms from uninvaded habitats (Arthur et al. 2012). Positive effects on the litter-dwelling communities have also been noted for *Rhamnus davurica*, leading to an increase in the relative abundance of bacteria responsible for nitrogen cycling in the presence of *Rhamnus* litter (Rodrigues et al. 2015). Therefore, through the inhibition or promotion of growth of consumer populations invasive species have the potential to cause cascading effects through the food webs of the invaded community. Understanding these changes in the dynamics of an ecosystem is important for managing the problem of invasive species and restoring native species.

The goal of this study was to determine what effects invasive species may have, once they have heavily invaded a habitat, on the leaf litter-dwelling communities of a forest ecosystem. Previous studies have shown both positive and negative effects of invasive species on the litter-dwelling community, but these have primarily focused on the response of one to few species, trophic groups, or levels of the food web to either an invaded habitat or the leaves of the invasive species. In my study, I used leaf litter from two invasive shrubs, *Lonicera maackii* and *Rhamnus davurica,* and the invasive tree *Ailanthus altissima* (hereafter referred to as *Lonicera*, *Rhamnus*, and *Ailanthus*) to investigate the effects of these species on the bacteria, fungi, and arthropods in the litter in both uninvaded hardwood forest habitat and habitats already invaded by these species. Specifically, I looked at the effect of litter source (native vs. invasive litter) and habitat (native vs. invasive canopy), using both litter naturally on the ground in each habitat and litter packets of native or invasive litter, on the abundance and composition of the litter-dwelling community. I expected that litter sources with high nutrient content, and that are easily digested, will promote higher numbers of litter-dwelling organisms (bacteria, fungi, and arthropods) with a more diverse composition of trophic groups. I also expected that habitat type (invaded vs. native) may differentially affect the abundance of organisms or the types of trophic groups that are present in the habitat. This will lead to differences in the litter-dwelling communities that colonize novel litter sources that are introduced to the habitats.

Methods

Site Description

This experiment took place at Blandy Experimental Farm in the northern Shenandoah Valley in Clarke County, VA (39.061º N, 78.065º W). Annual mean precipitation is 97.6 cm, 61.2 cm of which falls as snow. Mean January and July high/low temperatures are 6.2ºand -4.5ºC, and 31.4º and 17.5º C, respectively. Variation from the mean monthly high/low temperatures and precipitation can be found in Appendix 1.

Invaded and Native Communities

This study took place in four types of communities: forests dominated by native trees and shrubs, and habitats heavily invaded by one of three non-native species: Dahurian buckthorn (*Rhamnus davurica*), bush honeysuckle (*Lonicera maackii*), and tree of heaven (*Ailanthus altissima*). Native habitats were characterized primarily by canopy species of red oak (*Quercus rubra*), white oak (*Q. alba*), mockernut hickory (*Carya tomentosa*), hackberry (*Celtis laevigata*), and black walnut (*Juglans nigra*). Two study sites were located in a large forest fragment (18.6 ha), and two sites were located in a 5.8 ha fragment (Appendix 2 and 3 for locations). A fifth native site (0.5 ha) was located in an area that had previously undergone removal of invasive tree and shrub species (primarily bush honeysuckle) in 2009. Areas invaded by the Dahurian buckthorn (*Rhamnus davurica*) include a 5.0 ha monoculture (two sites), as well as portions of a 14-year old successional area (134.7 ha, last disturbed in 2000), which contained three sites. Locations used that are invaded by bush honeysuckle include one site in the 14-year old

successional area, two sites in a 4.9 ha forest fragment, one site in the 5.8 ha fragment, and one site in the 18.6 ha fragment. Although all of these sites were located underneath a dense canopy of bush honeysuckle, sites located in the forest fragments did have a native hardwood canopy over the honeysuckle canopy. For tree of heaven (*Ailanthus altissima*), study sites were placed in areas with clusters of canopy-sized trees. This included two sites each in the 4.9 ha forest fragment, one site in the 14-year-old successional area, and one site in a 26-year old successional area (102.8 ha, last disturbed in 1986) in between the 18.6 ha forest fragment and the 14-year old successional area.

Leaf Litter Density, Physical and Chemical Properties

To measure the density of litter cover, I collected leaves from each of the five sites in all four habitats from 22 March-20 June 2014. I randomly placed a 0.25 m^2 square at each location and collected all litter within the square. Once all the litter was collected, I weighed each sample to get "fresh" mass. All samples were then dried for at least 48 hours at 60º C and re-weighed to get dry mass. The dry mass/0.25 m^2 was then multiplied by 4 so that litter cover was represented as g dry litter/m^2 for each site.

To determine the decomposition rate and nutrient content of the various litter types, I collected fresh fallen leaves from each habitat at Blandy Experimental Farm in October 2013. To collect litter, leaves were raked from under native mixed hardwoods in uninvaded forest fragments, from monospecific stands of *Rhamnus* and tree of heaven, and shaken from bush honeysuckle onto a tarp underneath several individuals of this species.

To test for differences in decomposition rates, I used mesh fruit bags to create litter packets containing 45-50 g "fresh" mass of either native litter or one of the respective invasive species litter. I also dried ten representative samples of each litter type (45-50 g) of "fresh" litter for 48 hours at 60º C to obtain a conversion factor for the dry weight of litter placed in each packet. This conversion factor was the ratio of the dry mass/fresh mass, averaged for all ten samples of each litter type. I was then able to convert the "fresh" mass to Dry $Mass_{initial}$ of each packet at the beginning of the experiment.

To compare the decomposition rates of each type of litter and to test whether decomposition rates varied among habitats, I placed packets at each experimental site on 29 November 2013. Sites in invaded habitats contained six packets each of native and the respective invasive litter, while native habitats contained six packets of each invasive litter type and six packets of native litter. Beginning 1 February 2014, and the first of each following month, I collected one packet of each litter type from all experimental sites. Samples were then dried for 48 hours at 60º C and then weighed to get Dry $Mass_{final}$. Mean proportion of litter lost was then calculated by

$$\text{Proportion of Litter Lost} = (\text{Dry Mass}_{initial} - \text{Dry Mass}_{final}) / \text{Dry Mass}_{initial}$$

To measure the leaf litter nutrient composition, samples were taken from the October 2013 collection and were allowed to air dry for one week. Dried samples were ground and 2-5 mg was measured into tin capsules for analysis of carbon and nitrogen. C:N ratios were also calculated as a ratio of the masses of carbon and nitrogen in the

tinned samples. These analyses were performed using a Thermo Fisher Flash 2000 Organic Elemental Analyzer.

Statistical Analyses

Differences in decomposition rates of the litter placed in native habitats was tested using mixed model repeated measures analysis of variance (ANOVA), with proportion of initial mass remaining the dependent variable. Litter type was the fixed independent variable, the time variable was repeated on each of the native sites, and litter type×time was the fixed interaction variable. Based on the lowest Akaike's Information Criterion (AIC) score, I used the compound symmetric approach for the variance-covariance matrix.

Differences in litter cover (g/m^2) was tested using mixed model ANOVA, with the mass of litter (g/m^2) naturally present in the study sites the dependent variable. Habitat, sampling date, and their interaction were treated as fixed effects.

The effect of litter source on nutrient content was also tested using mixed model ANOVA. Carbon (g C/g leaf), nitrogen (g N/g leaf), and the C:N ratios were the dependent variables, and the litter source was the independent variable.

Arthropod Communities in Native and Invaded Habitats

In order to determine the abundance of arthropods in the leaf litter present in each habitat, I collected monthly samples of litter naturally present at each site beginning 22 March through 20 June 2014. These samples were collected by raking 60-100 g "fresh" mass of litter that had naturally fallen in each of the five sites from all habitats into plastic

bags, which were immediately transferred to the laboratory for arthropod collection. The range in litter mass that was collected was due to the varying environmental conditions at the time of each collection, with the goal of approximately 50 g of dry litter. Arthropod collections were made by placing these samples of the naturally present litter into Berlese-Tullgren funnels for 10 days. At the end of 10 days, the litter was removed from the funnels, and placed in drying ovens at 60º C for an additional 24 hours, to obtain the dry mass of the litter. All arthropods were preserved in ethanol and identified to the lowest taxon necessary to categorize a trophic function, most commonly family or genus, under a dissecting microscope. The primary trophic categories were predators, detritivores, herbivores, fungivores, omnivores, and scavengers. Rare categories included parasitoids and hematophagous arthropods (ticks, mosquitoes, and biting flies). For each sample, the total abundance and richness, and the abundance and richness of each trophic category were measured. Finally, because each site in each habitat varied in the amount of litter cover/m^2, and because I collected 60-100g "fresh" mass of litter regardless of how much litter cover there was, I calculated a multiplier for each site, for each collection date:

$$\frac{Abundance}{m^2} = \frac{\left(dry\ mass\ litter\frac{g}{0.25m^2}\right)*4}{dry\ mass_{funnel}} \times abundance$$

allowing me to estimate the abundance variables/m^2 of litter at each site.

Statistical Analyses:

I tested for differences in the abundance/m^2 and richness of arthropods found in the litter naturally present in the four study habitats using generalized linear mixed model ANOVA. I selected the model with the best fit using pseudo-AICC scores. For total

abundance/m^2, the best fit was using an exponential distribution and a log link, and for richness the best fit was using a Poisson distribution with a log link. In both models the dependent variable was either abundance/m^2 or richness, and habitat, sampling date, and their interaction time were fixed effects. Sampling date was treated as a repeated variable for sites(habitat).

Leaf Litter Colonization Experiments

To test for differential microorganism and arthropod colonization of different types of leaf, I placed leaf litter packets in each of the four habitats and characterized colonization throughout the spring. Leaf litter was collected in November of 2012 and October 2013 from a variety of sources and locations at Blandy Experimental Farm. Leaves were raked from under native mixed hardwoods in uninvaded forest fragments, from monospecific stands of buckthorn and tree of heaven, and shaken from bush honeysuckle onto a tarp underneath several individuals of this species.

To test for the effect of litter mixture on arthropod and microorganism colonization, I created litter packets containing three "doses" of litter: 100% invasive, 50% invasive-native mix, or 100% native. Each packet contained 90-100 g of litter. To test for the effect of habitat on colonization, packets were placed in native and in each of the three invaded habitats. In the invaded habitats, I placed three different litter packets at each site: one of the respective invasive litter, one native, and one mixed. In the native habitats, I placed seven packets at each site, one each of all possible litter mixtures: 100% invasive x 3 invasive species, 50% mix x 3 invasive species, and 100% native (see Appendix 4 for diagram of litter packet placement). Litter packets were

collected from only one site of each habitat per collection date for a total of 16 packets (3 packets × 3 invaded habitats + 7 packets uninvaded habitat). The total number of litter packets was 80 each year (3 packets × 5 sites × 3 invaded habitats + 7 packets × 5 native sites). Packets were placed in their respective sites on 24 November 2012 for year one, and 29 November 2013 for year two. In year one, samples were collected beginning 27 April 2013 and then retrieved at approximately 10-day intervals until 12 June 2013. In year two, samples were collected beginning 1 March 2014 and retrieved monthly until 4 July 2014.

In year one of the experiment, bacterial and fungal abundance estimates were determined using Acridine Orange Direct Counting (AODC), a microscopic enumeration technique (Hobbie et al. 1977). Two 1 g samples of litter were taken from each packet on their collection date and stored in 4% formalin. Within one week of storing the samples in formalin, each sample was then homogenized in 100 mL of a 2% formaldehyde solution in preparation for staining. For the staining procedure, 10 mL of deionized water was placed in a filter column, followed by an aliquot of sample, and then 1.0 mL Acridine Orange was added to the column. If necessary, ten-fold sample dilutions were made to give between 20-200 bacterial cells per microscope field. Prepared slides were viewed through a fluorescence microscope (bacteria: oil immersion lens 100x, fungi: non-oil immersion 40x; ocular magnification 10x), and counted in accordance with general AODC guidelines. Bacterial abundance is reported as the number of cells/g dry weight of litter material. Fungal abundance estimates are reported as hyphal length/g dry litter material using the hyphal intersection approach of Jones and Mollison (1948).

To estimate biovolume of the microbiota, digital images were taken of five random fields per sample for both bacteria and fungi. Using ImageJ 1.49, the length and width of 20 cells were measured from each field for bacteria, and the mean diameter of hypha was measured from each field for fungi. Bacterial cell volume was estimated using the formula:

$$V = (\pi/4) \times w^2 \times (l\text{-}w/3)$$

where V = biovolume; w = width of the bacterial cell; and l = length of the cell (Krambeck et al. 1981). Fungal hypha volume was determined by treating hyphae as cylinders for biovolume conversion.

Arthropod collections were made with Berlese-Tullgren funnels as described previously. One aspect of the litter packet measurements that differed from the measurements of abundance in the innate litter, ants were rarely encountered, and in only one sample were they a large proportion of the abundance. In the litter packets it was common to find ants nesting in packets that still contained relatively high amounts of litter. Because their presence in the litter seemed to be more an artifact of the litter providing structural cover, instead of the ants actually colonizing the litter, the abundance of ants was subtracted from the abundance measurements. Tests on the abundance of ants separately from the rest of the arthropods indicated no differences between litter mixtures or habitats.

Statistical Analyses

When analyzing the communities that colonized the litter packets, each of the three invasive species were analyzed separately. Included in the analysis of each invasive

species were the three packets of litter (100% invasive, 50% invasive, and 100% native) collected from that habitat and the corresponding litter packets collected from the native habitat.

The effects of leaf litter type (invasive or native) and habitat (invaded or native) on the abundance (g^{-1} dry litter) of bacterial cells, length of fungal hyphae, and the biovolume (μm^3/g dry litter) of bacterial cells and fungal hyphae was analyzed by a factorial ANOVA with litter type, habitat, and their interaction as fixed effects. All response variables were log-transformed to meet normality and homogeneity of variance assumptions. Sampling date was also included in the model as a fixed effect, but because only one sample of each litter type was collected from each habitat at each sampling date, 2-way and 3-way interactions involving time were not included in the model. Separate analyses were run for each invaded habitat (*Ailanthus*, *Lonicera*, and *Rhamnus*).

The effects of litter type and habitat on the total abundance and richness was analyzed by a generalized linear mixed model ANOVA with litter type, habitat, and their interactions as fixed effects. The year of collection (2013 or 2014) and day of year were included as random effects, and interactions with these variables were not included. Separate analyses were run for each invaded habitat (*Ailanthus*, *Lonicera*, and *Rhamnus*). I selected the model with the best fit using pseudo-AICC scores. The best fit for all models used a Poisson distribution with a log link except for *Rhamnus* abundance, which had a best fit using an exponential distribution with a log link.

The effects of litter type and habitat on the trophic group abundance and trophic group richness was analyzed using a multivariate ANOVA (MANOVA) with litter type, habitat, and their interactions as fixed effects. Year of collection (2013 or 2014) was

included as a fixed block effect. Site nested within habitat was also included as a fixed effect, and was used as the error term for habitat in the MANOVA. The dependent variables in the MANOVA were the abundances of predators, detritivores, herbivores, fungivores, omnivores, and "other" (a combination of the rarer scavengers and hematophagous arthropods). The dependent variables in the MANOVA for richness did not include the "other" category. Separate analyses were run for each invaded habitat (*Ailanthus*, *Lonicera*, and *Rhamnus*).

Results

Leaf Litter Density, Physical and Chemical Properties

Leaf litter density was significantly different among habitats over the course of the collection period (Figure 1; $F_{3,64}$=15.10, P<0.0001). Native and *Lonicera* habitats began with 1.8 times more litter than both *Ailanthus* and *Rhamnus* in March. By June native habitats had 5.7 times more litter than *Ailanthus* habitats, 3.0 times more litter than *Rhamnus*, and 1.9 times more litter than *Lonicera*. There was a significant interaction between litter decomposition and time (Figure 2; $F_{15,60}$=3.31, P=0.0005), with the three invasive species losing mass at a higher rate than the native litter, which lost very little mass until late in the season. By July 78-87% of the invasive litter had decomposed, but only 36% of the native litter had decomposed.

Leaf litter nutrient content was significantly different between litter sources for both nitrogen (Figure 3a; $F_{3,12}$=116.57, P<0.0001), carbon (Figure 3b; $F_{3,12}$=47.81, P<0.0001), and C:N ratios (Figure 3c; $F_{3,12}$=428.49, P<0.0001). *Ailanthus* (1.6x, 2.4x) and *Lonicera* (1.4x, 2.1x) had higher nitrogen with respect to both *Rhamnus* and native

litter, and *Rhamnus* was 1.5 times higher than native. Native litter had the greatest carbon content (1.1x more than *Ailanthus* and *Lonicera*, 1.2x than *Rhamnus*), followed by *Lonicera*, *Ailanthus*, and *Rhamnus*. All litter sources differed from each other for C:N ratio, with native litter 1.9 times higher than *Rhamnus*, followed by *Lonicera*, and *Ailanthus* respectively. These results indicate that the nutrient content of invasive litter is much more readily available to decomposers than that of native litter.

Arthropod Communities in Native and Invaded Habitats

The mean arthropod abundance/m^2 in litter naturally present in each habitat was not different between habitats (Figure 4a; $F_{3,36}$=2.42, P=0.1035). However, there was a significant interaction between habitat and collection time (Figure 4b; $F_{3,36}$=3.38, P=0.0028). For both *Ailanthus* and *Lonicera* habitats, there were extreme decreases in arthropod abundance as collection date progressed into the summer. Native habitat did show a slight increase before decreasing slightly, while *Rhamnus* habitat had consistently low abundance of arthropods. Richness of arthropods varied between habitats (11.2-14.6 taxa) and between habitats over time (8.4-19.2 taxa) but did not differ significantly ($F_{3,16}$=0.9, P=0.4648; $F_{9,48}$=0.45, P=0.8988).

Leaf Litter Colonization Experiments

Habitat type (native or invasive) had no effect on the mean bacterial cell abundance/g dry leaf (all P>0.18) nor the mean bacterial cell biovolume (μm^3/g dry leaf) (all P>0.31). There were significant differences in cell abundance/g dry leaf between litter source for all three invasive species compared to native (Table 1; *Ailanthus*:

$F_{1,12}$=24.81, P=0.0003, *Lonicera*: $F_{1,12}$=48.92, P<0.0001; *Rhamnus*: $F_{1,12}$=9.6, P=0.0092). For all three, the invasive litter contained the greater abundance of bacteria (2x, 3.8x, and 2.5x respectively) compared to the native litter. There was no significant difference in cell biovolume (μm^3/g dry leaf) between *Ailanthus* and native litter packets (Table 1; $F_{1,9}$=2.28, P=0.1651), but bacterial cell biovolume on *Lonicera* and *Rhamnus* litter was significantly greater than on native litter (Table 1; *Lonicera*: $F_{1,9}$=9.89, P=0.0016; *Rhamnus*: $F_{1,9}$=6.98, P=0.0268). In both *Lonicera* and *Rhamnus* litter packets, the invasive litter (8.9x and 3.4x respectively) had greater cell biovolume than native litter.

There was no difference in mean fungal hyphae length between habitats (all P>0.14). However, there was 1.9 times greater fungal biovolume (μm^3/g dry leaf) in the litter placed in native habitats compared to litter placed in *Ailanthus* habitats ($F_{1,9}$=6.83, P=0.0281). There was no difference in the fungal biovolume for litter placed in *Lonicera* or *Rhamnus* habitats (all P>0.10). Comparing the mean hyphae length between litter packets indicated that *Lonicera* litter had 1.9 times greater fungal hyphae length than native litter, with a similar pattern to that found with bacteria. There was a similar trend, though not significant, between *Rhamnus* and native litter, but not for *Ailanthus* litter (Table 1; *Ailanthus*: $F_{1,12}$=2.35, P=0.1513; *Lonicera*: $F_{1,12}$=5.54, P=0.0365; *Rhamnus*: $F_{1,12}$=4.07, P=0.0667). There was no difference in the mean fungal biovolume (μm^3/g dry leaf) between *Ailanthus* (Table 1; *Ailanthus*: $F_{1,9}$=0.01, P=0.9335; *Lonicera*: $F_{1,9}$=1.24, P=0.2936; *Rhamnus*: $F_{1,9}$=3.04, P=0.1153) litter packets and native litter.

Total arthropod abundance was significantly different between habitats for both *Ailanthus* and *Lonicera* habitats compared to native habitat (Figure 5; *Ailanthus*:

$F_{1,40}$=484.2, P<0.0001; *Lonicera*: $F_{1,40}$=620.34, P<0.0001; *Rhamnus*: $F_{1,40}$=0.83, P=0.3688), but not between *Rhamnus* and native habitat. Litter packets placed in native habitat contained 1.6 times more arthropods than the corresponding litter packets placed in *Ailanthus* habitat. Litter packets placed in native habitat contained 1.6 times more arthropods than the corresponding litter packets placed in *Lonicera* habitat. Contrastingly, the total arthropod abundance in litter mixtures was significant for all three invasive species mixtures (Figure 5; *Ailanthus*: $F_{2,40}$=1654.98, P<0.0001; *Lonicera*: $F_{2,40}$=2863.81, P<0.0001; *Rhamnus*: $F_{2,40}$=3.76, P=0.0318), with the invasive litter mixtures containing 4.3, 5.1, and 2.5 times greater abundances respectively. I detected significant interactions between litter mixture and habitat for the *Ailanthus* and *Lonicera* analyses, but not for *Rhamnus* (Figure 5; *Ailanthus*: $F_{2,40}$=7.3, P=0.002; *Lonicera*: $F_{2,40}$=248.51, P<0.0001; *Rhamnus*: $F_{2,40}$=0.24, P=0.7915). More arthropods colonized *Ailanthus* (1.6x) and mixed-*Ailanthus* (1.8x) litter packets in native habitat than in the *Ailanthus* habitat. In native habitat, more arthropods colonized *Lonicera* (1.9x) and mixed-*Lonicera* (2.4x) litter packets than in *Lonicera* habitat.

Total arthropod richness was significantly greater in native (1.2x) habitat than *Rhamnus* habitat, but not between *Ailanthus* or *Lonicera* and native habitat (Supplemental Table S1; *Ailanthus*: $F_{1,40}$=2.87, P=0.0981; *Lonicera*: $F_{1,40}$=2.95, P=0.0934; *Rhamnus*: $F_{1,40}$=4.71, P=0.036). Total arthropod richness was significantly greater in *Ailanthus* litter (1.2x) than native litter, but not for any other litter mixture compared to native litter (Supplemental Table S1; *Ailanthus*: $F_{2,40}$=3.94, P=0.0274; *Lonicera*: $F_{2,40}$=2.8, P=0.0725; *Rhamnus*: $F_{2,40}$=1.82, P=0.1748). There was no interaction between litter mixtures and invaded or uninvaded habitats for arthropod

richness (*Ailanthus*: $F_{2,40}$=0.23, P=0.7918; *Lonicera*: $F_{2,40}$=0.04, P=0.9597; *Rhamnus*: $F_{2,40}$=0.88, P=0.424).

Trophic abundance of arthropods did not differ significantly between habitats (Supplemental Figure S1; *Ailanthus*: $F_{6,3}$=2.66, P=0.226; *Lonicera*: $F_{6,3}$=0.59, P=0.7323; *Rhamnus*: $F_{6,3}$=0.88, P=0.5945) but was significantly different between litter mixtures (Figure 6; *Ailanthus*: $F_{12,68}$=3.02, P=0.0019; *Lonicera*: $F_{12,68}$=3.16, P=0.0013; *Rhamnus*: $F_{12,68}$=2.45, P=0.0103). For all three comparisons with the invasive species, the abundance of trophic groups was uniformly lower in native litter relative to invasive litter. Trophic richness, as with trophic abundance, did not differ significantly between habitats (Supplemental Figure S2; *Ailanthus*: $F_{5,4}$=0.94, P=0.5409; *Lonicera*: $F_{5,4}$=0.44, P=0.8065; *Rhamnus*: $F_{5,4}$=0.78, P=0.6113). Trophic group richness in native leaf litter was uniformly lower relative to *Ailanthus* litter but did not differ between *Lonicera* or *Rhamnus* litter (Supplemental Figure S3; *Ailanthus*: $F_{10,70}$=2.99, P=0.0034; *Lonicera*: $F_{10,70}$=1.69, P=0.1010; *Rhamnus*: $F_{10,70}$=1.92, P=0.0568).

Discussion

There were clear differences in both the quantity and quality of the litter types in this study. Native habitats had the greatest biomass of litter per square meter, followed by the *Lonicera* habitats both of which were significantly greater than the *Ailanthus* and *Rhamnus* habitats. The majority of *Lonicera* habitats were under a native canopy, so there is some additional input of native leaves in these habitats that adds to the litter cover, explaining why the *Lonicera* habitat was more similar to the native habitat. Invasive litter placed in decomposition bags lost mass at a much higher rate than native

litter. The longer lasting litter in native habitats represents a more reliable resource for nutrition and shelter for the litter-dwelling community compared to litter in invaded habitats. Increasingly complex structure in the litter layer (via biomass and/or litter depth) has been shown to increase the abundance of arthropods and affect predator/prey interactions across trophic levels (Bulman and Uetz 1984; Langellotto and Denno 2004; Castro and Wise 2010; Sayer et al. 2010; Morice et al. 2013).

There were also clear differences in the physical and chemical properties of the litter. Nitrogen content was significantly greater in *Ailanthus* and *Lonicera* litter, followed by *Rhamnus*, all of which were greater than native litter. Native litter also had a significantly higher C:N ratio than all of the invasive litter. Although native habitats have more and longer lasting litter than invaded habitats, it is less nutritious, and the nitrogen is less available than the invasive litter.

The invasive litter supported much greater growth of bacteria and fungi than native litter, and *Lonicera* and *Rhamnus* also had a higher biovolume of bacteria than native litter. These patterns may be due to the greater available nitrogen seen in invasive litter, and the higher rates of decomposition of the invasive litter are likely due in part to the rapid growth of these microbial communities. Litter from *Lonicera* has been shown to support a distinct microbial community from native litter, likely present prior to senescence, which appears to drive the higher rate of decomposition compared to native litter (Arthur et al. 2012). In the case of *Rhamnus*, Rodrigues et al. (2015) showed that the relative abundance of nitrogen-cycling bacteria increased in the presence of *Rhamnus* litter compared to native litter. The abundance of bacteria hosted by *Ailanthus* was similar to bacterial abundance in the other two invasive species, but *Ailanthus* hosted

lower biovolume of fungus and bacteria. This may be due to antimicrobial secondary chemicals in *Ailanthus* leaves that have been observed to lower soil microbial activity in another study (Motard et al. 2015). The short-lived nature of the invasive litter, due to its high rate of decomposition, means that the microbial community will not be able to sustain these high numbers over the course of a growing season.

The arthropod communities colonizing invasive litter packets followed the same pattern as seen with the microbial community. Arthropod colonization was higher in all three invasive litter types relative to native litter. The abundance of most trophic groups was also higher in the invasive litter than native litter. Packets containing a mix of native and invasive litter were almost always intermediate, suggesting a "dosage" response by the arthropods to the litter quality. As with the microbial community, these results suggest that the invasive litter is a much better resource for the arthropods than native litter. In addition to the litter itself being a better resource for the arthropods, the greater abundance of bacteria and fungi on the invasive litter may also be promoting the abundance of arthropods. For example, isopods feeding on plant material with biofilm formed from microbes gained significantly more biomass than isopods feeding on plant material without biofilm (Horváthová et al. 2015).

The arthropod communities found in the litter sampled from each habitat showed a very different pattern from what was observed in the litter bag study. Arthropod abundance in *Lonicera* habitats peaked in the spring but crashed by early summer. The arthropods in *Rhamnus* were always very low in abundance. The arthropods in the native habitat and *Ailanthus* habitat still had substantial populations of arthropods by June. Litter bags placed in native habitats generally supported greater arthropod abundance

than the litter bags in invaded habitats. This suggests that the native habitat is supporting more arthropods overall, due to the low decomposition rate of native litter, than habitats invaded by *Ailanthus* and *Lonicera*, despite clear preference for the litter from these species. Similarly, Poulette and Arthur (2012) found that the density of microarthropods trended higher in litter bags placed under native oaks (*Q. muehlenbergii*) and hickories (*C. ovata*) than in invasive *Lonicera* and mixed litter bags.

By inhibiting or promoting the growth of the consumer populations in the presence of invasive litter, exotic species invasions have the potential to cause cascading effects through the food webs of the invaded community. For example, Chen and Wise (1999) showed that when a resource limited detrital food web received food enhancement treatments (in the form of chopped mushrooms and potatoes and fruit fly growth medium), the detritivore and fungivore taxa responded positively. This positive response propagated through the food web, with omnivorous and predaceous taxa both increasing their abundance in food enhancement treatments as well (Chen and Wise 1999). In my study, the response to the invasive litter changed the composition of the trophic groups of arthropods in the detritus food web of this forest ecosystem, favoring detritivores, fungivores, herbivores, and, to a lesser extent, predators. Omnivores, however, tended to be more common in native and mixed litter packets. Similar changes to the diversity and abundance of arthropods at all trophic levels in the detrital food web have been seen in other studies that have examined the effects of increased nutrient inputs through the leaf litter (Chen and Wise 1999; Negrete-Yankelevich et al. 2008; Poulette and Arthur 2012). In their study comparing the litter from *Lonicera maackii*, *Cary ovata*, *Fraxinus quadrangulata*, and *Quercus muehlenbergii*, Poulette and Arthur (2012) found that

Oribatid mites (primarily detritivores, fungivores, and herbivores) were more abundant in litter mixes of *Lonicera* and *Q. muehlenbergii* or *C. ovata* compared to native litter. Increased nutrients (Chen and Wise 1999) and higher quality litter (primarily lower lignin content; Negrete-Yankelevich et al. 2008) were found to increase the abundance of the decomposers in the litter layer, leading to a bottom-up cascade that increased the abundance of higher trophic levels in the litter community.

Approximately 90% of net primary production of forests enters into detrital food webs (Cebrian 1999; Gessner et al. 2010), and invaded areas can provide substantial increases in the primary productivity in some ecosystems (Trammell et al. 2012). My results indicate that invasions by these exotic species represent a novel resource for the litter-dwelling communities, and one that is more nutritious than native litter, leading to an increase in the abundance of a wide range of organisms at multiple trophic levels in this community. However, this resource is short-lived, which leads to a crash in the abundance once the litter is decomposed. On the other hand, native litter decomposes at a much slower rate than the invasive litter. As a whole, the native habitat supports a more stable litter-dwelling community over the course of a growing season. Compared to the invaded habitats, which lost most of their litter by early summer, the native habitats have a more consistent nutrient resource and structural habitat for the litter-dwelling community. This allows the litter-dwelling organisms to maintain their populations which, in turn, supports the higher trophic levels of this forest food web.

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Table 1: Means (and 95% confidence intervals) of bacterial and fungal response to invasive and native litter. Paired columns represent comparisons of invasive and native litter bags placed in invaded and uninvaded habitats. Means marked with asterisks (*) indicate significant differences between means of the paired columns (* P<0.05, ** P<0.01, ***P<0.001).

	Litter Mixture					
Response	*Ailanthus*	Native	*Lonicera*	Native	*Rhamnus*	Native
Bacterial cell abundance (Abundance/g dry leaf)	1.29^{10} *** (1.09^{10}- 1.49^{10})	6.34^{9} (4.31^{9} - 8.37^{9})	1.77^{10} *** (1.48^{10} - 2.06^{10})	4.64^{9} (1.77^{9} - 7.52^{9})	1.27^{10} ** (8.94^{9} - 1.64^{10})	5.16^{9} (1.41^{9} - 8.90^{9})
Bacterial biovolume (μm^3/g dry leaf)	2.43^{8} (9.80^{7} - 4.87^{8})	1.14^{8} (5.11^{7} - 2.54^{8})	6.06^{8} *** (2.77^{8} - 1.33^{9})	6.79^{7} (3.10^{7} - 1.49^{8})	2.28^{8} * (1.10^{8} - 4.74^{8})	6.80^{7} (3.27^{7} - 1.41^{8})
Fungal hyphae length (m/g dry leaf)	1.77^{8} (1.08^{8} - 2.46^{8})	2.46^{8} (1.77^{8} - 3.15^{8})	4.71^{8} * (3.24^{8} - $6.18^{8)}$	2.46^{8} (9.90^{7} - 3.94^{8})	3.60^{8} (2.51^{8} - 4.69^{8})	2.17^{8} (1.07^{8} - 3.26^{8})
Fungal biovolume (μm^3/g dry leaf)	8.378 (5.69 - 1.23^{9})	8.20^{8} (5.58^{8} - 1.21^{9})	1.11^{9} (4.52^{8} - 2.73^{8})	5.93^{8} (2.41^{8} - 1.46^{9})	1.80^{9} (7.76^{8} - 4.19^{9})	7.20^{8} (6.27^{8} - 3.38^{9})

Figure Legend:

Figure 1. LS mean litter (g/m^2) cover in *Ailanthus altissima*, *Lonicera maackii*, *Rhamnus davurica*, and native habitats. Error bars represent 95% confidence intervals.

Figure 2. LS Mean proportion litter mass lost over six months for packets of *Ailanthus altissima*, *Lonicera maackii*, *Rhamnus davurica*, and native leaf litter placed in native habitat. Mean proportion of litter lost between each sample period. Error bars represent 95% confidence intervals.

Figure 3. LS means of leaf nutrient variables. A) Nitrogen content, B) Carbon content, C) C:N ratio of litter from *Ailanthus altissima*, *Lonicera maackii*, *Rhamnus davurica*, and native leaves. Error bars represent 95% confidence intervals.

Figure 4. LS mean abundance of arthropods/m^2 of litter naturally present in each habitat. A) Combined mean abundance/m^2 between habitats. B) Mean abundance/m^2 for each collection date for each habitat. Error bars represent 95% confidence intervals.

Figure 5. LS mean abundance of arthropods showing the interaction of habitat and litter mixtures placed in invaded and uninvaded habitats. Blue columns are the means of litter mixtures placed in *Ailanthus*, *Lonicera*, or *Rhamnus* habitats, red columns are the means of litter mixtures placed in native habitat. Error bars represent 95% confidence intervals.

Figure 6. LS mean trophic group abundance of arthropods between litter mixtures placed in their respective invaded habitats or uninvaded habitats. Error bars represent 95% confidence intervals.

Figure 1:

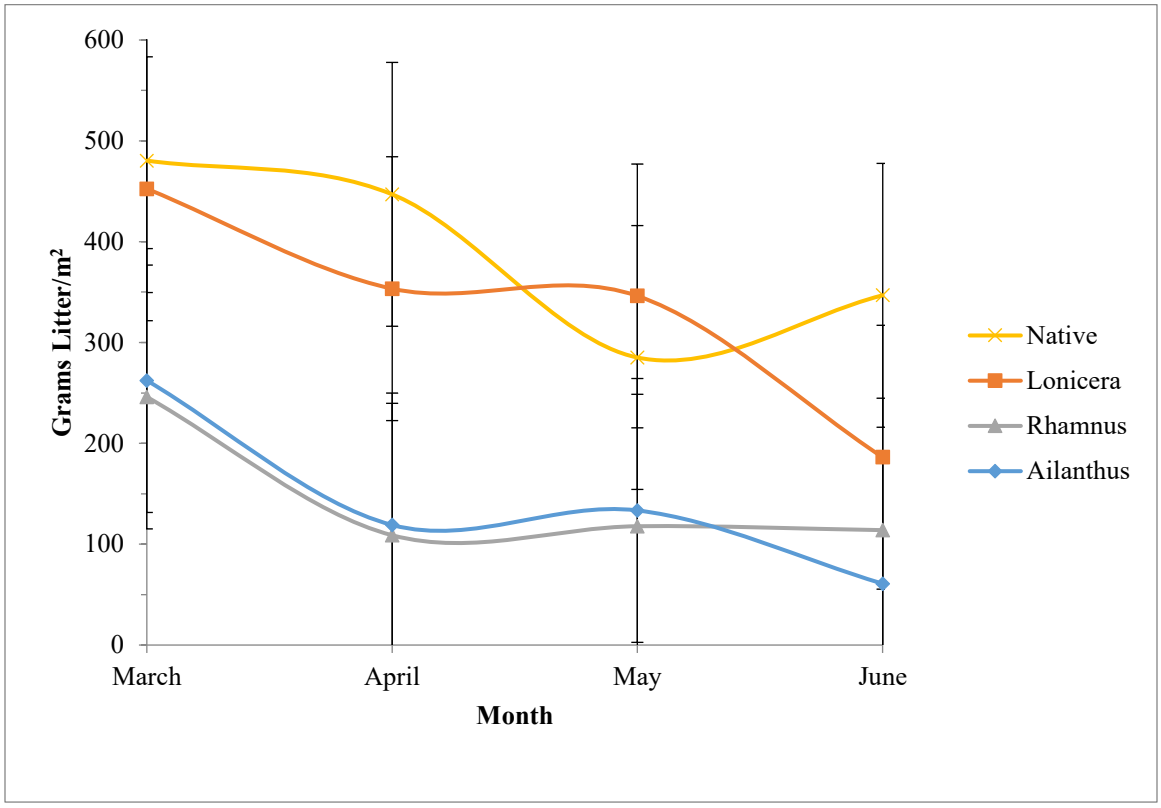

600
500
400
300
200
100
0
Grams Litter/m²
March
April
May
June
Month
Native
Lonicera
Rhamnus
Ailanthus

Figure 2:

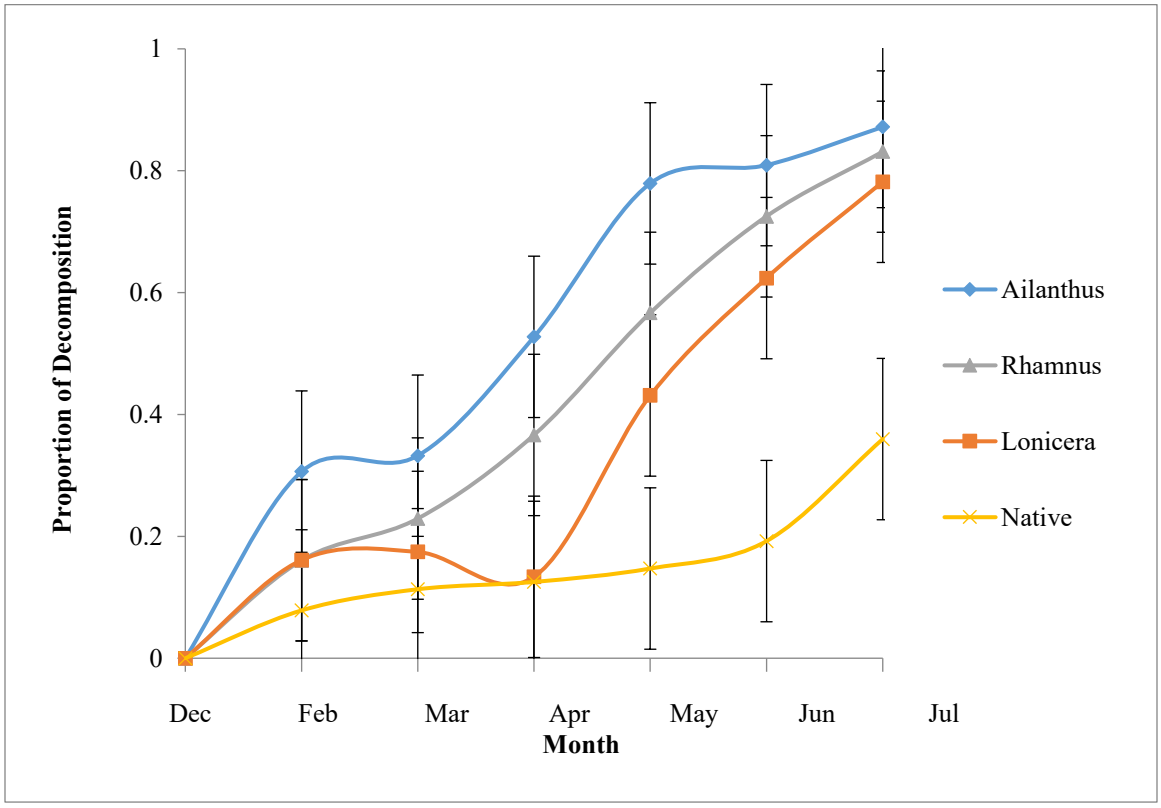

Proportion of Decomposition
1
0.8
0.6
0.4
0.2
0
Dec
Feb
Mar
Apr
May
Jun
Jul
Month
Ailanthus
Rhamnus
Lonicera
Native

Figure 3:

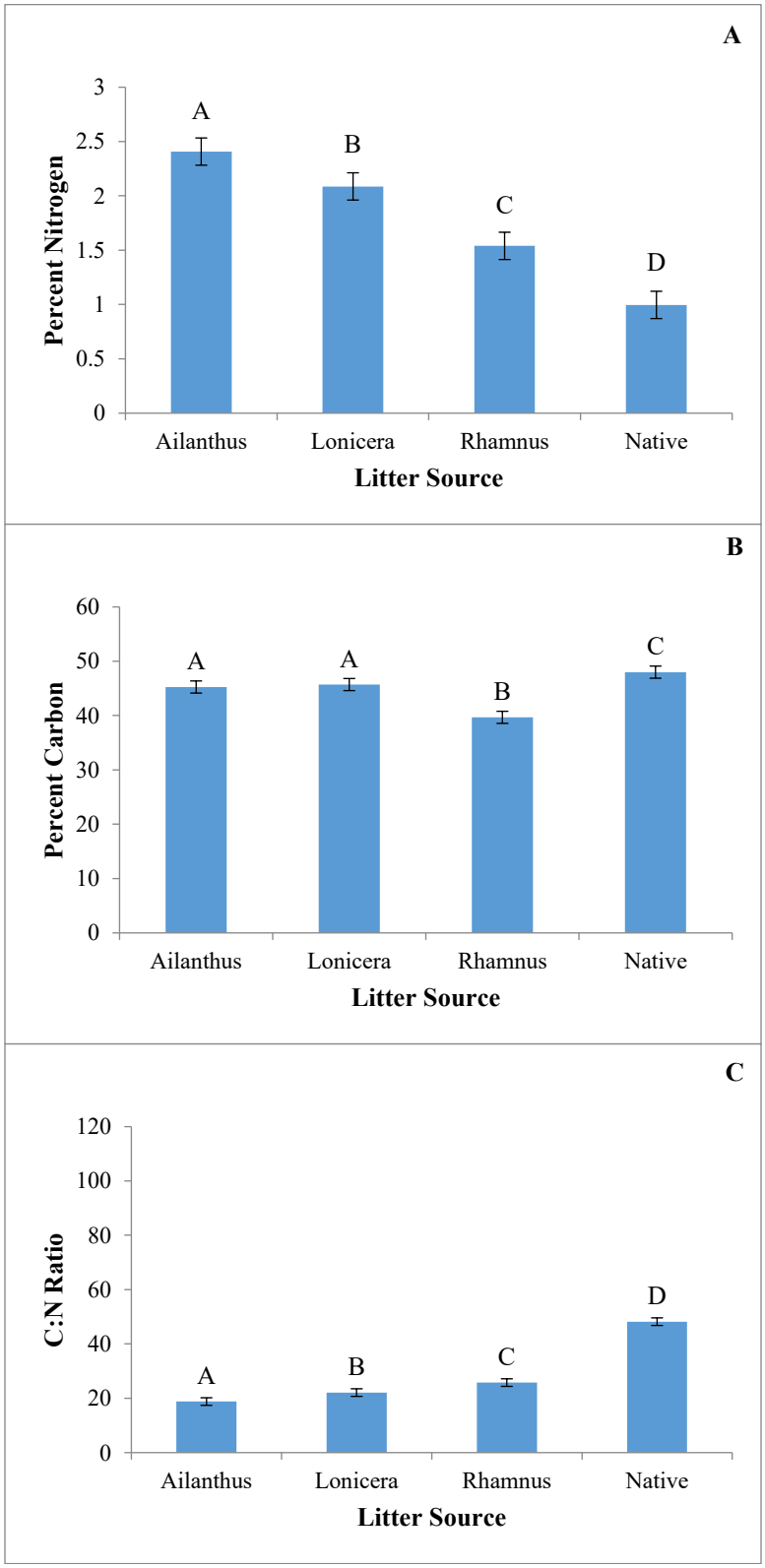

A
Percent Nitrogen
3
2.5
2
1.5
1
0.5
0
A
B
C
D
Ailanthus
Lonicera
Rhamnus
Native
Litter Source
B
Percent Carbon
60
50
40
30
20
10
0
A
A
B
C
Ailanthus
Lonicera
Rhamnus
Native
Litter Source
C
C:N Ratio
120
100
80
60
40
20
0
A
B
C
D
Ailanthus
Lonicera
Rhamnus
Native
Litter Source

Figure 4:

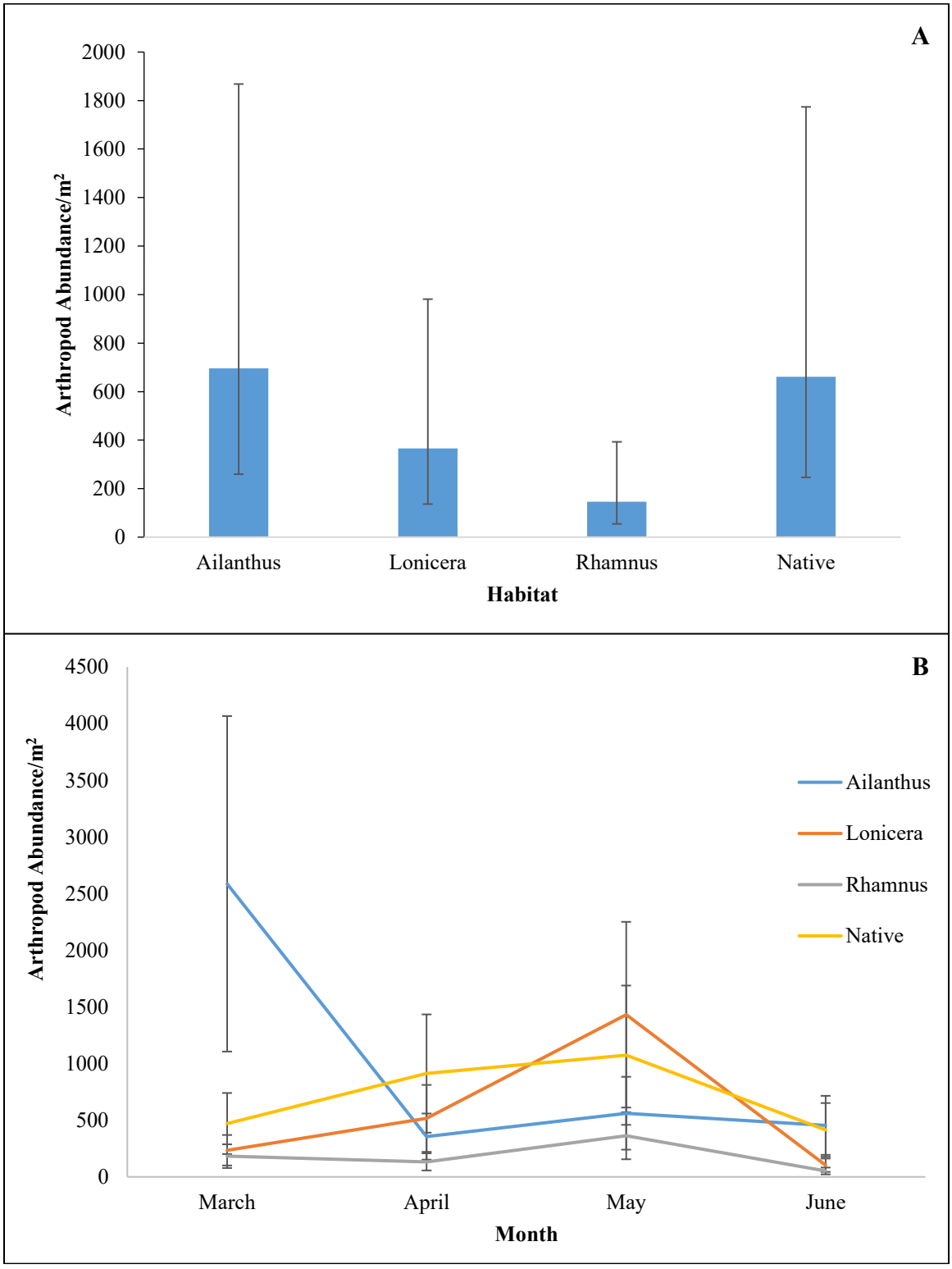

A
Arthropod Abundance/m²
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Ailanthus
Lonicera
Rhamnus
Native
Habitat
B
Arthropod Abundance/m²
4500
4000
3500
3000
2500
2000
1500
1000
500
0
March
April
May
June
Month
Ailanthus
Lonicera
Rhamnus
Native

Figure 5:

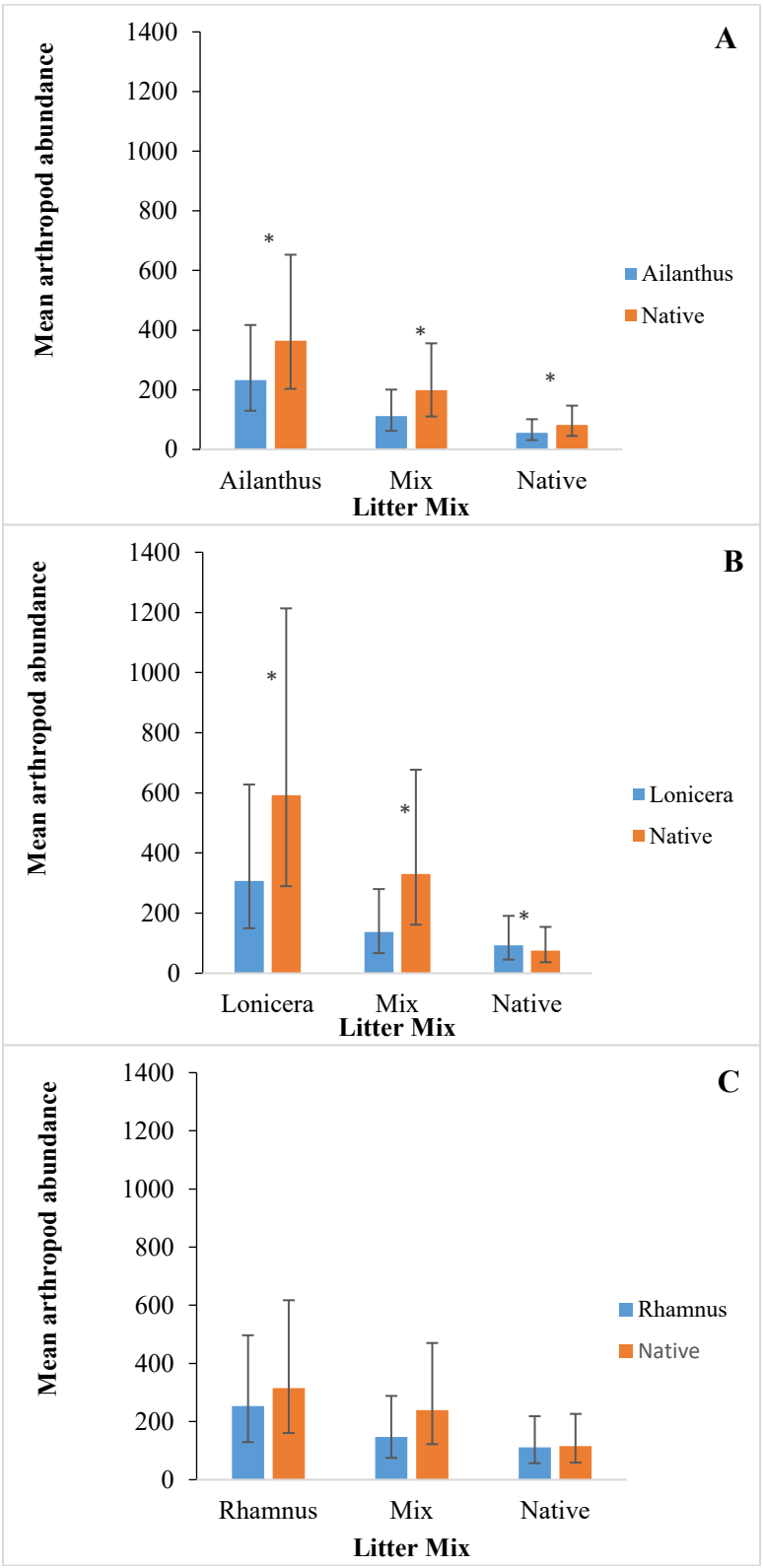
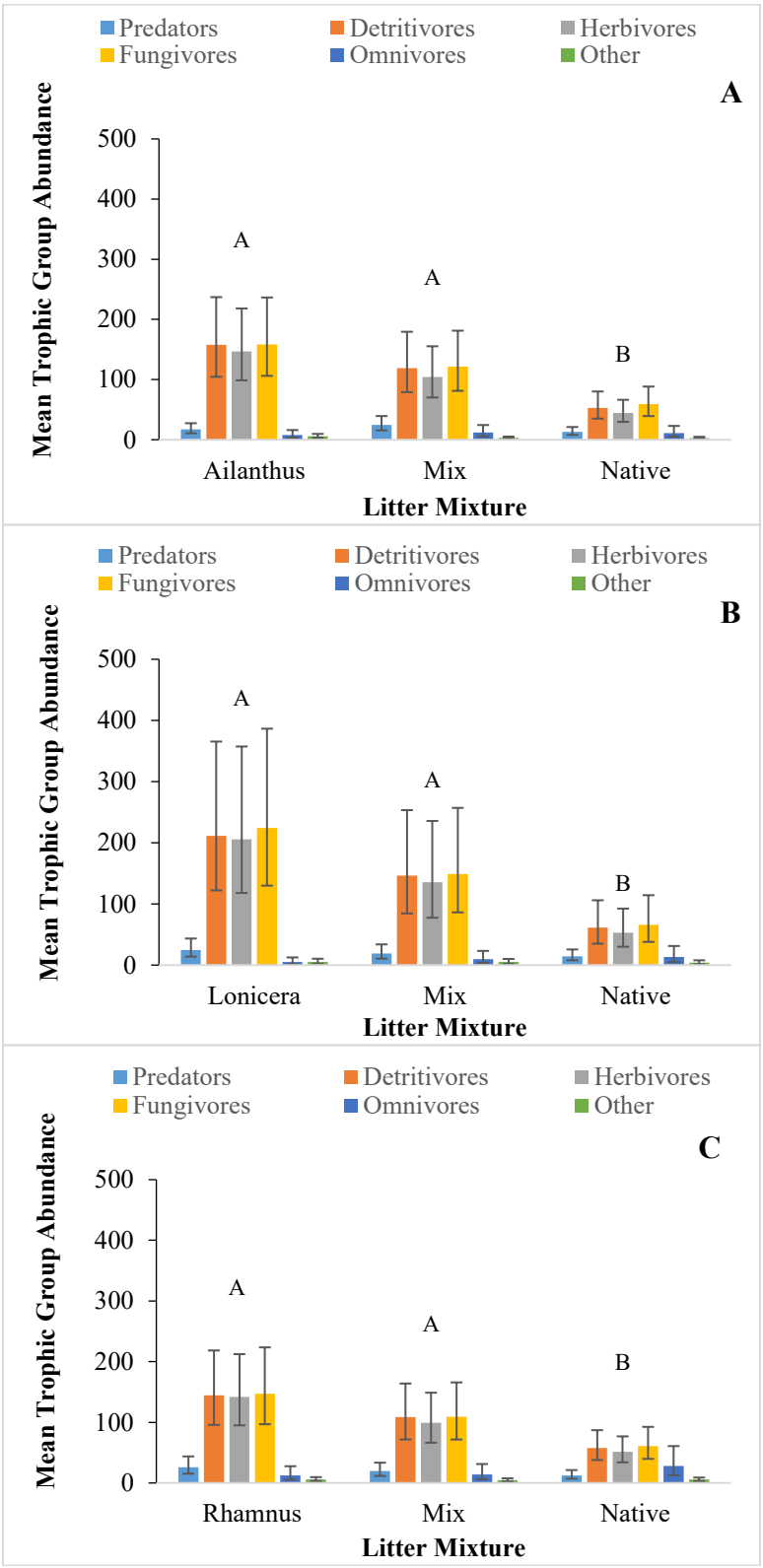

A
Mean arthropod abundance
1400
1200
1000
800
600
400
200
0
*
*
*
Ailanthus
Native
Ailanthus
Mix
Native
Litter Mix
B
Mean arthropod abundance
1400
1200
1000
800
600
400
200
0
*
*
*
Lonicera
Native
Lonicera
Mix
Native
Litter Mix
C
Mean arthropod abundance
1400
1200
1000
800
600
400
200
0
Rhamnus
Native
Rhamnus
Mix
Native
Litter Mix

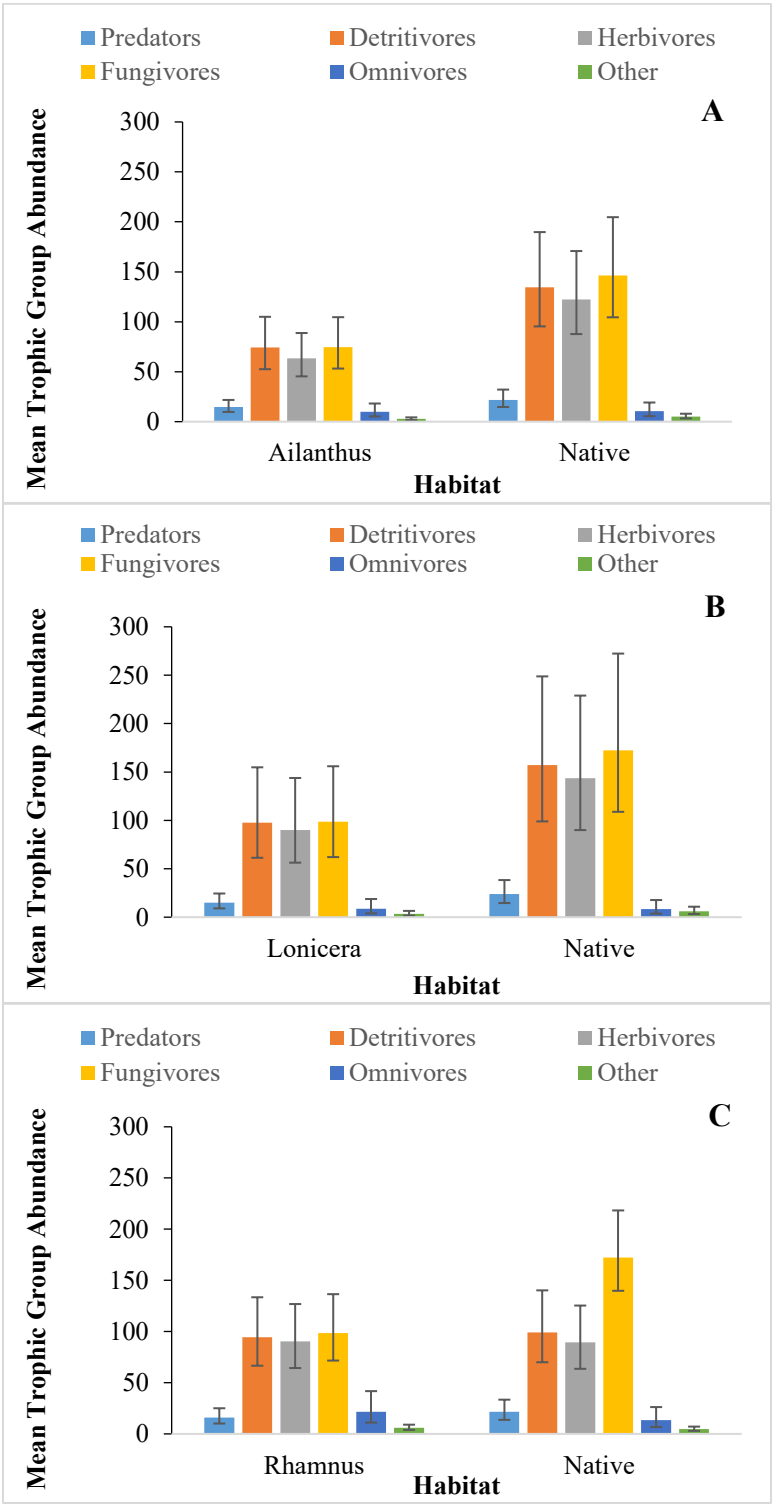
Figure 6:


Predators
Detritivores
Herbivores
Fungivores
Omnivores
Other
A
Mean Trophic Group Abundance
500
400
300
200
100
0
A
A
B
Ailanthus
Mix
Native
Litter Mixture
B
Lonicera
C
Rhamnus

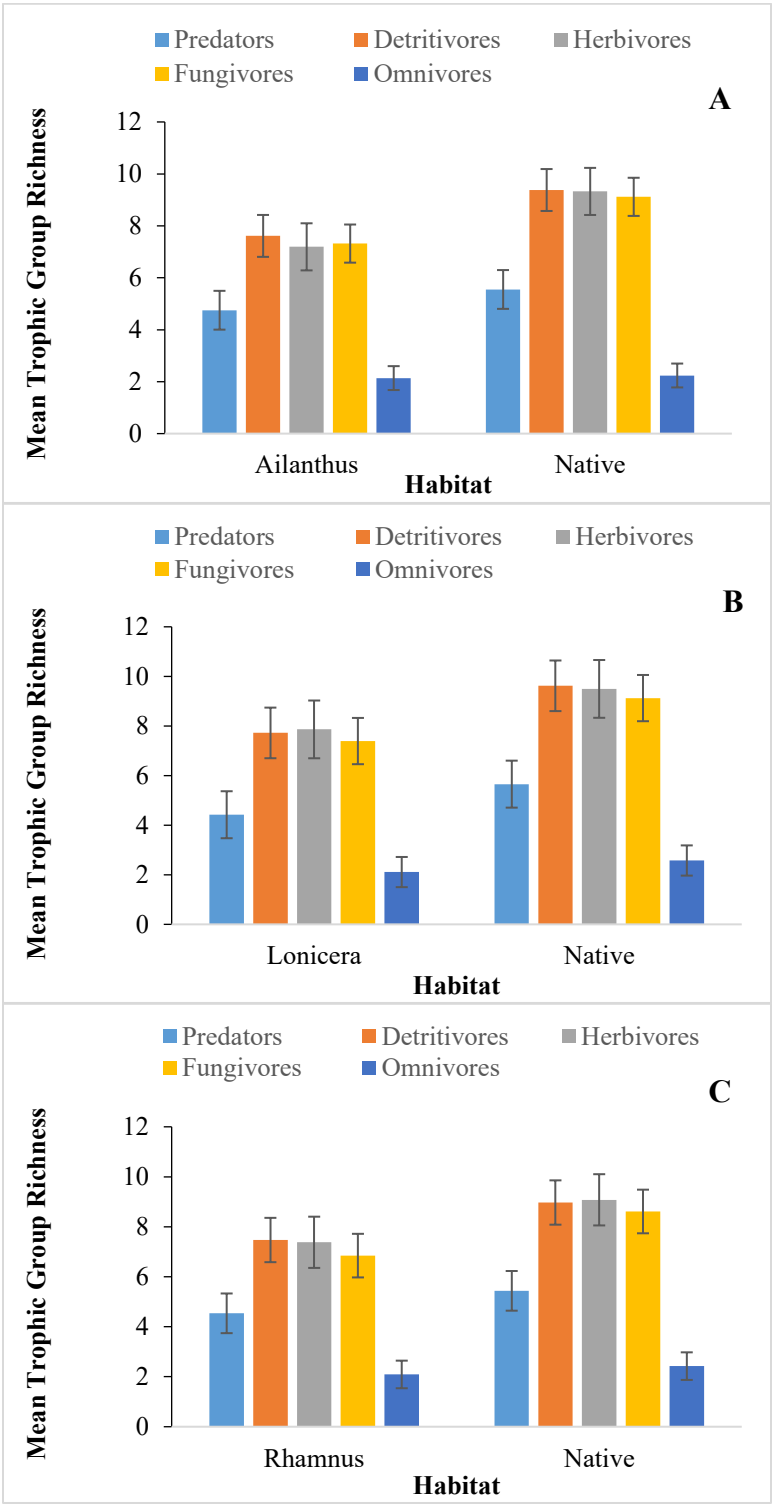
Supplemental Table S1. Mean richness (and 95% confidence intervals) of arthropods. A) Mean richness between litter mixtures placed in invaded habitats and litter mixtures placed in uninvaded habitats. Means marked with asterisks (*) indicate significant differences between means of the litter mixtures being compared (*P<0.05, **P<0.01, ***P<0.001). B) Mean richness between litter mixtures placed in their respective invaded habitats or uninvaded habitats. Means marked with asterisks (*) indicate significant differences between means of the litter mixtures being compared (*P<0.05, **P<0.01, ***P<0.001) and letters indicate significant differences between specific litter mixtures.

A)	**Habitats**								
	Ailanthus	Native	*Lonicera*	Native	*Rhamnus*	Native			
Richness	16.0 (13.4 - 19.0)	17.9 (15.1 - 21.1)	16.8 (13.4 - 21.1)	18.7 (15.0 - 23.5)	15.2 * (12.8 - 18.0)	17.6 (14.8 - 20.8)			
B)	**Litter Mixture**								
	Ailanthus	Mixed-*Ailanthus*	Native	*Lonicera*	Mixed-*Lonicera*	Native	*Rhamnus*	Mixed-*Rhamnus*	Native
Richness	19.1 *A (16.0 - 22.9)	16.2 AB (13.5 - 19.5)	15.5 B (12.9 - 18.7)	18.6 (14.7 - 23.5)	18.9 (15.0 - 23.9)	15.9 (12.5 - 20.2)	17.0 (14.2 - 20.4)	17.2 (14.3 - 20.6)	14.9 (12.3 - 18.0)

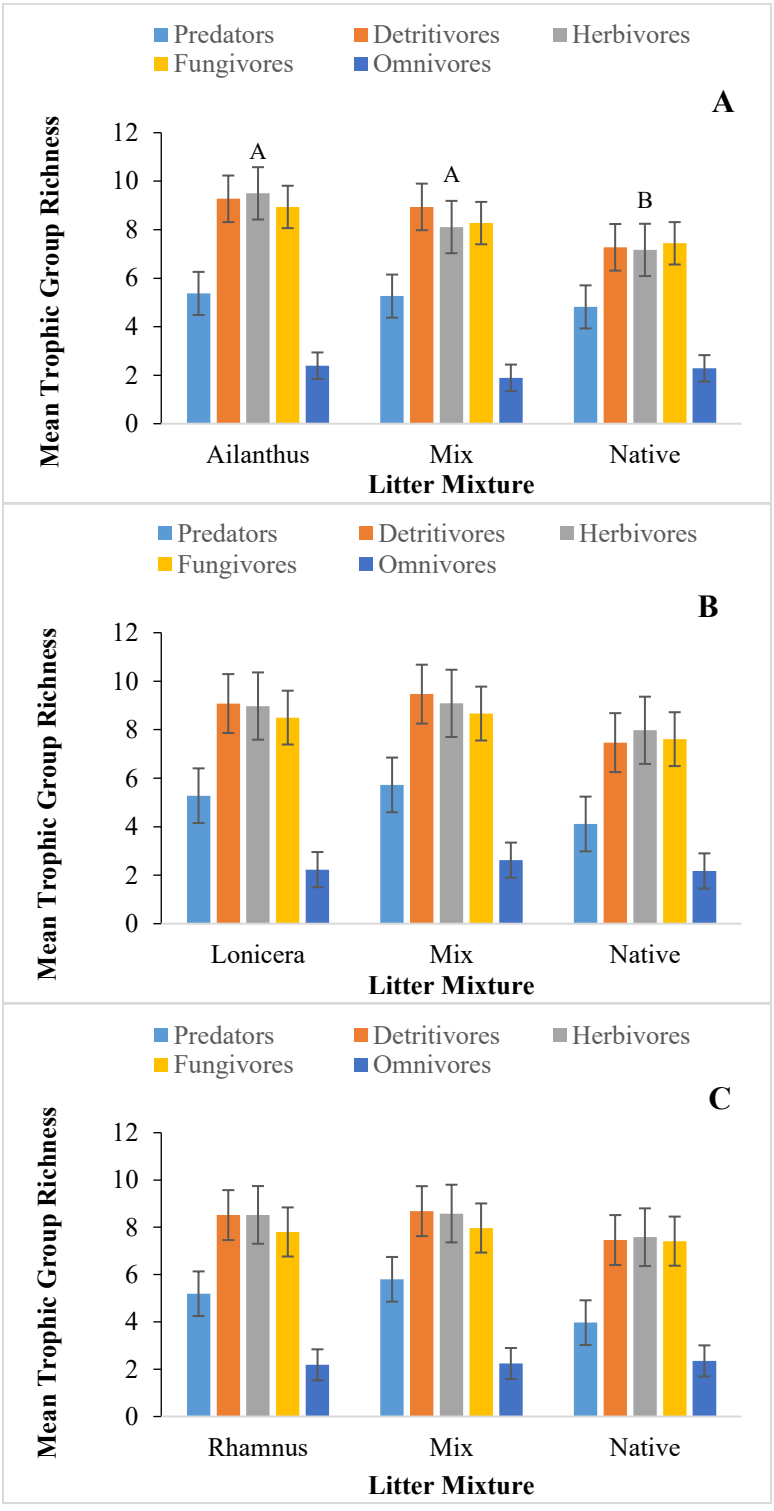
Supplemental Figure S1: LS means for trophic group abundance between litter mixtures placed in invaded habitats and litter mixtures placed in uninvaded habitats. Error bars represent 95% confidence intervals.



Supplemental Figure S2: LS mean trophic group richness of arthropods between litter mixtures placed in their respective invaded habitats or uninvaded habitats. Error bars represent 95% confidence intervals.



Supplemental Figure S3: LS mean trophic group richness of arthropods between litter mixtures placed in their respective invaded habitats or uninvaded habitats. Error bars represent 95% confidence intervals.



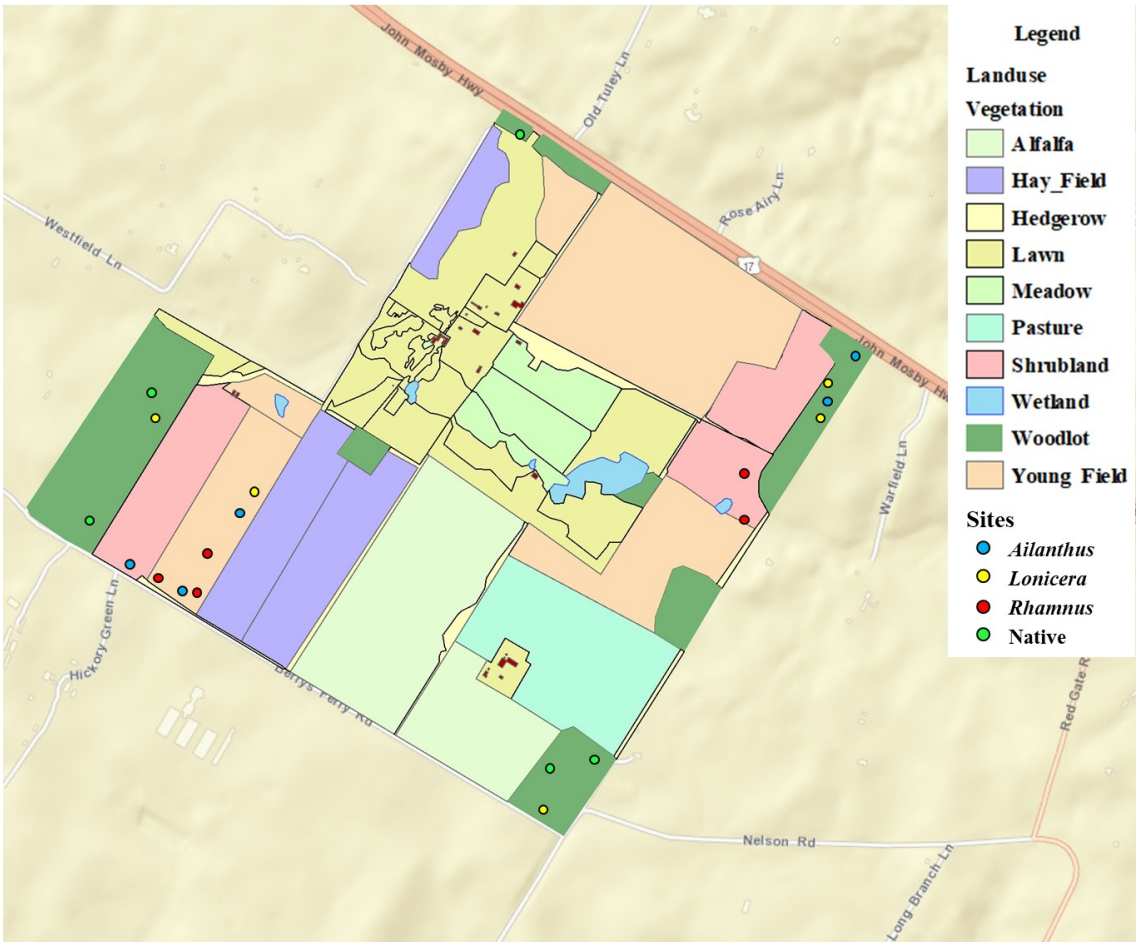
Appendix 1

Month	Mean Precipitation (cm)	2013 Precipitation (cm)	2014 Precipitation (cm)	Mean Temp. (ºC)	2013 Temp. (ºC)	2014 Temp. (ºC)
January	6.3	11.07	4.42	6.2/-4.5	7.3/-1.5	3.2/-8.4
February	5.8	4.45	9.09	8.1/-3.8	6.7/-4.4	5.2/-5.2
March	8.4	8.46	8.33	13.3/-0.3	9.2/-0.9	9.3/-3.1
April	7.9	4.17	6.53	19.7/4.8	18.1/4.4	18.8/4.8
May	10.2	12.34	10.49	24.7/10.0	24.2/9.5	25.7/10.1
June	9.8	16.94	11.28	29.4/15.1	28.9/16.8	29.2/16.3
July	9.5	11.99	11.10	31.4/17.5	30.9/19.6	30.2/16.4

Appendix 2

Habitat	Site ID	Latitude (º N)	Longitude (º W)
Ailanthus	Ail-1	78.05128	39.06266
Ailanthus	Ail-2	78.05083	39.06322
Ailanthus	Ail-3	78.07602	39.05762
Ailanthus	Ail-4	78.07231	39.05891
Ailanthus	Ail-5	78.07664	39.05775
Lonicera	Lon-1	78.05137	39.06237
Lonicera	Lon-2	78.05160	39.06294
Lonicera	Lon-3	78.07206	39.05932
Lonicera	Lon-4	78.07514	39.06244
Lonicera	Lon-5	78.06245	39.05111
Rhamnus	Rha-1	78.05476	39.05939
Rhamnus	Rha-2	78.05518	39.05886
Rhamnus	Rha-3	78.07452	39.05687
Rhamnus	Rha-4	78.07346	39.05730
Rhamnus	Rha-5	78.07558	39.05731
Native	Nat-1	78.07526	39.06254
Native	Nat-2	78.07731	39.05891
Native	Nat-3	78.06112	39.05208
Native	Nat-4	78.06212	39.05194
Native	Nat-5	78.06267	39.06944

Appendix 3. Map of the location of study sites at Blandy Experimental Farm.



Appendix 4. Diagram for litter bag placement at each site for invaded and native habitats. Litter bags were placed randomly, approximately 2 meters apart.