Novel Weaponry in the Arsenal of an Invasive Shrub (Dahurian buckthorn)

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1.0 .0 Abstract

The introduction of novel, invasive plant species can pose ecological problems for native communities. The prevalence of these invasive species have increased over the last century due to ease of international movement. The northeastern U.S. contains large swathes of abandoned agricultural fields left behind as farming moved west. Blandy Experimental Farm (BEF) is a research station and the State Arboretum of Virginia, BEF includes abandoned agricultural fields at varying periods of succession. In 1939, Dahurian buckthorn was introduced to BEF as an ornamental planting. The shrub escaped it's original planting and has created dense thickets with little or no native woody regeneration in these abandoned fields. The mechanisms used by buckthorn to overtake the landscape are not well known but may include: changes in plant species composition, reduction of light resources, alteration of soil properties and processes, shifts in nutrient cycling, and the use of secondary compounds in allelopathy. My thesis focused on the following questions: 1.) how does Dahurian buckthorn influence plant species composition and light resource availability in secondary succession at BEF? 2.) How do Dahurian buckthorn thickets affect soil properties and nutrient cycling? 3.) Do Dahurian buckthorn leaves and berries exhibit allelopathic qualities that hinder native species germination? 4.) Has the presence of Dahurian buckthorn in dense thickets significantly altered the course of secondary succession at Blandy Experimental Farm? I used field measurements and a greenhouse study to determine the effects of the invasive shrub on the trajectory of secondary succession. In areas with high levels of buckthorn presence, soil temperature was significantly lower than areas with low levels of buckthorn (19.4 °C versus 20.5°C). Light resources at the ground level were lowest in areas with high buckthorn. Decomposition rates were significantly faster in buckthorn leaf litter than white oak litter, likely due to buckthorns higher N content (k =

0.0054 versus k = 0.0019). The greenhouse study found evidence of allelopathy in buckthorn litter and berries. Honey locust, white ash, white oak, and buckthorn had lower biomass and higher mortality when large quantities of buckthorn litter and berries were present in the soil mixture. This species is having an ecosystem level impact on the regeneration of native forests at BEF, buckthorn has these abandoned fields in arrested succession without forward movement in the plant community. Dahurian buckthorn is one of many invasive plants on the rise, they threaten our native communities and the ecosystem services we rely on. It is critical to study these invasive-native ecosystem interactions to understand their impacts and mitigate their lasting effects.

2.0.0 Introduction

Invasive plant species can cause widespread changes to native forests, especially in regenerating systems (Nyland et al., 2006; Schulte et al., 2011). After long-term agricultural disturbances, forest systems are highly susceptible to invasions from non-native early successional species (Waller et al., 2016), which can hinder native woody plant regeneration (Dey et al., 2018). Invasive species often share similar characteristics, such as increased plasticity to variable conditions, longer growing seasons, prolific seed viability with widespread dispersal, and increased growth rates; these aggressive traits increase the competitive advantage of invasive species, and can decrease the productivity and regeneration of native species (Mascaro and Schnitzner, 2007; Fridley, 2012; Smith, 2013; Jo et al., 2015).

Invaders can affect regenerating systems in a variety of ways, including changing soil properties (e.g. rapid nutrient mineralization, and release of secondary compounds), shading out young and slow growing seedlings, perturbing microbial communities, later fall senescence, and even altering bird and mammal browsing and grazing preferences and subsequent seed dispersal

(Gill and Marks, 1991; Heneghan, 2006; Rodrigues et al., 2015; Jo et al., 2015; Bartowitz and Orrock, 2016; Guiden and Orrock, 2017; Woodworth et al., 2019). These changes can impact ecosystem services, such as carbon sequestration and water purification (Mascaro and Schnitzner, 2011). High severity disturbances, such as agriculture (and subsequent abandonment) and clear-cutting, present these plants with a “get rich quick” scheme, as the high light and low competition of a disturbed area allows invasive species to explode in growth (Charles-Dominique, 2012, Dey et al., 2019). Invasive species then quickly shade out native species and alter aspects of the ecosystem to reduce regeneration (Harrington et al., 1989). Although some invaders can be found in late successional forests, they are rarely able to establish in the older forests to the same degree that they do in more recently disturbed sites (Kalkman et al., 2019).

2.1.0 Increasing Range of Rhamnus spp.

In 1939, both *Rhamnus cathartica* (common buckthorn), an invasive woody species from Europe, and *Rhamnus davurica* (Dahurian buckthorn), a shrub / small tree originating from Asia, were introduced to the State Arboretum of Virginia at the Blandy Experimental Farm (BEF) in Boyce, north-central Virginia (Curator of Blandy Experimental Farm). Dahurian buckthorn escaped its original planting at BEF and began to integrate into natural landscapes, it has been observed radiating out from BEF. Common buckthorn is a more well known invasive shrub in the Rhamnaceae family, but BEF was not “ground zero” for this species. As the northeastern United States has been in a widespread transition from abandoned agriculture to early successional forests, due to the westward movement of the farming industry (Compton and Boone, 2000), these areas are highly susceptible to the colonization of fast-growing invasive plant species. Over the past 80 years, buckthorn species have increased in dominance immensely.

In the Ohio/Michigan area, *Rhamnus* spp. cover increased by 39% from 690 ha in 2001-2006 to 940 ha in 2007-2011 (Becker et al., 2013). These species prefer alkaline soils and can be found as far north as Maine and Canada, and to the west their ranges are limited by the ecosystems of the Rocky Mountains (Barton, 2004; Muzzi and Fabbri, 2006; Delanoy-Archibold, 2007; Kurylo et al., 2007; Stewart et al., 2008). The spread of *Rhamnus* spp. in both Maine and Virginia began, or at least was supported, by intentional ornamental plantings. In Maine, *Rhamnus* spp. can be found most often in riparian zones, likely due to dispersers gathering seeds from lawn plantings and using the water as a corridor between habitat fragments (Barton, 2004; Rodrigues et al., 2015). These species seem to be limited by spodic, acidic soils and the longer winters in the North, but climate change could extend their invasive ranges further northward. As the ranges of these shrub species grow, so do their impacts on local plant-plant interactions and communities.

2*.2.0 Rhamnus Competition with Other Species*

Studies have shown that, due to the high growth rate and photosynthetic capacity of *Rhamnus spp*., native tree, shrub, and herbaceous species such as *Crataegus spp., Cornus racemosa, Geranium maculatum, Thalictrum dasycarpum, Symphyotrichum lateriflorum, Eurybia macrophylla,* and *Prunus serotina,* were outcompeted by *Rhamnus* spp. in all conditions (Harrington et al., 1989b; Stewart and Graves, 2004; Klionsky et al., 2011). However, *Crataegus spp*. had fared better in drier, shadier areas (Kalkman et al., 2019). At the forest edge with high light, buckthorn can use its high leaf area to production ratio and rapid growth rate to expand and shade out some of the native regenerating plants (Harringtion et al, 1989; Schulte et al., 2011; Charles – Dominique, 2012; Whitfeld et al., 2014; Kalkman et al., 2019).

With regard to indirect interactions, buckthorn can also affect ecosystem properties that, in turn, negatively impact surrounding plants. Native species in Syracuse, New York have shown greater nitrogen (N) resorption rates, which is when a plant moves N resources back into the stem and trunk before losing its leaves in the fall, than both of these *Rhamnus* spp., but *Rhamnus* spp. have greater N uptake from the soil (reducing plant-available soil N by 11%) and greater fine root biomass (Jo et al., 2015; Hinman and Fridley, 2018). Decomposition rates of buckthorn leaf litter over a 400-day litterbag experiment were greater than those of *P. serotina, P. deltoides*, and *Q. ellipoidalles*, likely through increased microbial N mineralization due to high foliar N content in buckthorn (Heneghan, 2002). *R. davurica* litter at Blandy Experimental Farm had a higher nitrogen content than native litter and *Lonicera mackii* litter, as well as a 2.9 times faster decomposition rate (Woodworth et al., 2019). The low C:N ratio of *R. davurica* leads to a faster depletion of organic matter and a quick turnaround for nitrogen availability, which reinforces their dense thickets and rapid growth rates. By the end of the growing season in September, buckthorn thickets have little to no organic matter left on the ground surface, which can cause a boom-bust scenario and the collapse of local arthropod communities (Heneghan, 2004).

Another contributor to the invasion success of buckthorn is its extended growing season (earlier bud break and later senescence), which allows the shrub to retain its green leaves and continue to grow after other native species have ceased photosynthesis and dropped their leaves (Fridley, 2012; Jo et al., 2015). The open canopy gives buckthorn unrestricted access to light energy during the fall. Because this tactic is not used by many native species, due to possible frost or insect damage and resultant plant N loss, as climate change lengthens the growing season, invasive species can exploit this strategy with little risk (Schmidt and Whelan, 1999; Fridley, 2012).

Buckthorn was found to have highly allelopathic berries and leaves, which further alter the soils to the point where germination of native species decreases, as concentrations of buckthorn berries and leaves increase (Seltzner and Eddy, 2003; Warren et al., 2017; Pinzone et al., 2018). These secondary compounds are common in species that live in highly competitive environments. As invasive species increase in prevalence, the mutual beneficial relationship they seem to have with each other further highlights the importance of their role in successional ecosystems. For example, in I.C. King Park in Tennessee, soils previously occupied by other non-native or invasive species increased the aboveground biomass and height of *R. davurica* by factors of 1.8 and 1.5, respectively, creating a positive feedback for the invaders (Kuebbing et al., 2015). In places such as Blandy Experimental Farm, where there are high invasive species richness and densities, it seems this positive feedback can lead to the elevated success of invasive species establishment.

2.3.0 Buckthorn Effects on Ecosystems

The specific traits that make *Rhamnus* spp. successful invaders are still unknown; they could be related to phenology, growth rate, shade tolerance, nutrient cycling, and/or soil property alterations. Some studies have linked *Rhamnus* spp. directly to the loss of productivity and diversity in communities (Whitfeld et al., 2014). The main findings from the literature are: 1) soils that had dense buckthorn thickets (hereafter referred to as buckthorn soils) had higher turnover and uptake rates for inorganic N; 2) the shrub utilizes a longer growing season and higher photosynthetic capacity to grow faster and more prolifically than other species, and 3) *Rhamnus* spp. have possible allelopathic properties in their berries and leaves (Knight et al., 2007; Rodrigues et al., 2015; Jo et al., 2015). It is important to determine the defining characteristics of these and other invasive plant species to make future management and policy decisions.

2.4.0 Project Objectives

A large gap of knowledge remains with regard to buckthorn and the factors contributing to its dominance and success over native species across early secondary successional systems. My study focused on *Rhamnus davurica* at Blandy Experimental Farm due to its high abundance and successful invasion of the early successional forests (Photo 1). Specifically, I examined light levels, net N mineralization and nitrification, decomposition, soil properties and processes (temperature, moisture, and respiration), plant species composition, and possible allelopathic interactions of the shrub.

Photo 1.) A photograph of a chronosequence at Blandy Experimental Farm in the summer of 2019. The mid-level shrubs in light green are Dahurian buckthorn.

2.5.0 Thesis Goals

The main questions I answered:

1. How does Dahurian buckthorn influence plant species composition and light resource availability in secondary succession at Blandy Experimental Farm?
2. How do Dahurian buckthorn thickets affect soil properties and nutrient cycling?
3. Do Dahurian buckthorn leaves and berries exhibit allelopathic qualities that hinder native species germination?
4. Has the presence of Dahurian buckthorn in dense thickets significantly altered the course of secondary succession at Blandy Experimental Farm?

3.0.0 Dahurian buckthorn and its impacts on secondary successional ecosystems following abandoned agriculture (Chapter 1)

3.1.0 Introduction

The northeastern U.S. has been in a transition state from abandoned agriculture to regenerating forests, leaving wide swathes of land open to colonizing species (Waller et al., 2016; Dey et al., 2018). The typical trajectory of secondary succession in the eastern U.S. is a transition of vegetation over ~100 years starting with pioneer species such as weedy forbs and grasses, followed by early/mid-successional species such as tulip poplar, eastern red cedar, and cherries, and eventually late successional species, such as oak and black gum (Grime, 1979). This expected succession, however, is not always the path followed. Invasive species have become more and more prominent all over the globe, as ease of travel and transportation of goods became increasingly possible and affordable. Some invasive species are purposely planted in their non-native range as ornamentals or for biological control. These species can escape their original plantings and migrate into nearby landscapes. Invaders face little to no threats in their new range, described by the enemy release hypothesis, as they have essentially left all of their predators, parasites, and competitors behind; aggressive non-natives can compete unchecked with native species to dominate landscapes (Vila et al., 2004; Nyland et al., 2006; Schulte et al., 2011). These invaders can put a regenerating forest into arrested succession, where it is no longer changing in composition through time (Gill and Marks, 1991). If succession stalls in the shrub / small tree state, it may have less carbon sequestration potential and species diversity than a late successional hardwood forest (Mascaro and Schnitzner, 2011; Larkin et al., 2014; Kuebbing et al., 2015).

The high light, low competition conditions of abandoned agricultural fields are perfect areas for invaders to become established (Charles-Dominique, 2012, Dey et al., 2019). A positive feedback exists with invasive species through plant-soil interactions; as one invader changes aspects of the soil environment, it allows for further invasions (Kuebbing et al., 2015). Invaders grow rapidly and reproduce prolifically to spread and prevent the colonization of native species (Mascaro and Schnitzner, 2007; Fridley, 2012; Smith, 2013; Jo et al., 2015). Once these invaders are prominent in an ecosystem, they have been observed to change ecosystem processes and characteristics, such as nutrient cycling, resource availability, and species composition (Heneghan, 2006; Rodrigues et al., 2015, Woodworth et al., 2019).

Buckthorn species (*Rhamnus*) are examples of invasive shrubs that have become prevalent in landscapes across North America. Over the past 80 years, buckthorn species have increased in dominance immensely, and their range has spread as far west as the Rocky Mountains and as far north as Canada (Barton, 2004; Muzzi and Fabbri, 2006; Delanoy-Archibold, 2007; Kurylo et al., 2007; Stewart et al., 2008; Becker et al., 2013). Buckthorn species are known for their dense, monospecific thickets, which heavily reduce light resources to the understory. Stands of buckthorn may have dark, bare ground under a dense canopy of leaves, with buckthorn seedlings growing on the edges of the stand, as the species spreads (Whitfield et al., 2014). Invasive *Rhamnus* spp. create dense canopies, as they have a high photosynthetic capacity and grow rapidly to shade out the competition (Harrington et al., 1989b; Stewart and Graves, 2004; Klionsky et al., 2011; Kalkman et al., 2019). In areas with dense forest cover, buckthorn will act more like a small tree reaching towards the canopy, whereas in open areas the plant assumes the structure of a shrub, demonstrating its range of phenotypic plasticity (Charles-Dominque, 2012). So much shade is generated by the canopies of these shrubs that there may not be enough light for seedlings to grow directly under their parent shrubs (Gourley, 1985). Even within forest gaps, competition for light and belowground resources with glossy buckthorn (*Rhamnus frangula*) negatively impacted the growth and survival of red maple and white pine (Fagan and Peart, 2004). Although *Rhamnus* spp. are tolerant to a wide range of conditions, native species, such as hawthorn (*Crataegus* spp.), have the best chance to compete in low soil moisture and low light conditions (Kalkan et al., 2019). Fully mature, late successional forests are one of the few environments for which buckthorn is not suited to dominate; the competitive success of the shrub occurs predominantly at forest edges or in open fields (Gourley, 1985; Schulte et al., 2011; Kalkman et al., 2019).

Once buckthorn invades an ecosystem, it can alter ecosystem carbon budgets. The shrub has a high photosynthetic capacity due to its dense, active canopy with high foliar nitrogen (N) (Metcalfe et al., 2011). Buckthorn canopies often reduce surface and soil evaporation, yielding greater soil moisture than under other vegetation. Soil moisture was 59% greater under buckthorn shrubs than in areas without buckthorn (Heneghan et al., 2004). In higher moisture environments, decomposition can occur more rapidly which increases ecosystem respiration, especially when the high quality buckthorn leaf litter is present (Heneghan et al., 2002; Heneghan et al., 2004; Heneghan et al., 2006).

Often in temperate forests, the limiting factor for plant growth and litter decomposition is nitrogen availability (Reich et al., 1997; LeBauer and Treseder, 2008). When invasive species enter a system, they can affect nutrient cycling through changes in litter quality and quantity, altering the composition of the microbial community, which collectively may change rates of nitrogen mineralization and nitrification (Heneghan et al., 2004; Corbin and D’Antonio, 2004; Vila et al., 2011; Jo et al., 2015; Kuebbing et al., 2015; Hinman and Fridley, 2018; Pinzone et al., 2018). Invaders not only change nitrogen cycling while they are prevalent in an area, they may also have lasting effects on soil nitrogen pools that affect ecosystem functions and services for years following removal (Milchunas and Lauenroth, 1995; Elgersma et al., 2011).

Some invasive species, such as *Rhamnus*, have high N content in their leaves (~2.2% for *Rhamnus* compared to ~1.4% for cottonwood and oak), and low C:N ratios, which increase decomposition rates (Heneghan et al., 2002). Buckthorn litter decomposed up to 5x faster than native litter, and the buckthorn soils had N turnover rates 40% faster than non-buckthorn invaded soils (Heneghan et al., 2002; Heneghan et al., 2004; Rodrigues et al., 2015; Woodworth et al., 2020). Even the berries of buckthorn have higher N contents than the berries of 11 other invasive species (Sherburne, 1972). The conditions of the forest floor as described above influence decomposition rates; buckthorn can intensely shade the forest floor, which increases soil moisture and maintains an optimal temperature for decomposition during hot growing seasons (Heneghan et al., 2004; Reth et al., 2005). This causes an interesting shift in soil-dwelling invertebrate communities. It initially gives arthropods and microbes a large input of carbon and nitrogen, which they quickly break down; but the litter is decomposed so rapidly that the soil organisms have limited substrate/food for the remainder of the year, which can lead to the collapse of these communities (Heneghan et al., 2002; Schuh and Larsen, 2015). Once the litter has been broken down, buckthorn uses its large mass of fine roots to take up high amounts of the recycled nitrogen back into the plant; it can decrease soil N content by 11% compared to non-invaded areas (Jo et al., 2015). Buckthorn should be increasing fertility in the soil, as N is released from senesced leaves, but because the shrub is able to take up that nitrogen so fast, it causes a deficit in N availability for other plants (Heneghan et al., 2004).

In 1939, both *Rhamnus cathartica* (common buckthorn), an invasive woody species from Europe, and *Rhamnus davurica* (Dahurian buckthorn), a shrub/small tree originating from Asia, were intentionally introduced to the State Arboretum of Virginia and Blandy Experimental Farm (BEF) in Boyce, north-central Virginia. These shrubs escaped their original plantings and began to integrate into the communities around them; eventually Dahurian buckthorn became rampant and well established (Rodrigues et al., 2015). Dahurian buckthorn dominates certain landscapes at BEF with a few other invasive shrubs, mainly autumn olive and bush honeysuckle. In previous studies at BEF, there has been evidence that buckthorn is affecting nitrogen cycling, decomposition rates, and soil pH in heavily invaded areas (Rodrigues et al., 2015).

The goal of my study was to look further into the impacts of Dahurian buckthorn on early secondary succession. My main objective was to determine if the presence of *Rhamnus davurica* in dense thickets has significantly altered the course of secondary succession. I had two main research questions for this chapter: 1) How does Dahurian buckthorn influence light availability and species composition in secondary succession, and 2) How does Dahurian buckthorn affect soil properties and nutrient cycling during secondary succession?

3.2.0 Methods

3.2.1 Site Description

This study took place at the Blandy Experimental Farm (BEF) in Boyce, VA located within the Valley and Ridge region (39.064007, -78.065211). BEF comprises ~700 acres of forest and meadowland used for research and education; it is also home to the State Arboretum of Virginia. The mean annual precipitation is 1008 mm, and the mean annual temperature is 12.7°C. Blandy was widely used for agriculture until 1976, which was when most of the commercial farming ended, and extensive research began. Two fields were used in my study; one was left to regenerate in 1997, and the other was mechanically managed by mowing, discing, and herbicide (Round Up) until 2003, and then it was abandoned and allowed to naturally regenerate. Common species found within these areas include shrubs; *Lonicera spp., Rhamnus davurica, Rubus spp., Elaeagnus umbellata* and trees; such as *Ailanthus altissima, Gleditsia triacanthos,* and *Juglans nigra;* and herbaceous species of *Galium verum, Solidago spp.,* and *Microstegium vimineum.* I identified twenty 5m x 5m plots, ten in each of the two early successional fields. These plots were chosen based upon varied abundances of buckthorn.

3.2.2 Question 1: Plant Species Composition

Plant species composition of each plot was determined using a point-frame method, with a grid over the entire plot and 0.5 m space between each grid line, for a total of 121 measurement points (Figure 1). At each vertex, a dowel was dropped and every plant touching it was recorded, and relative frequencies were calculated.

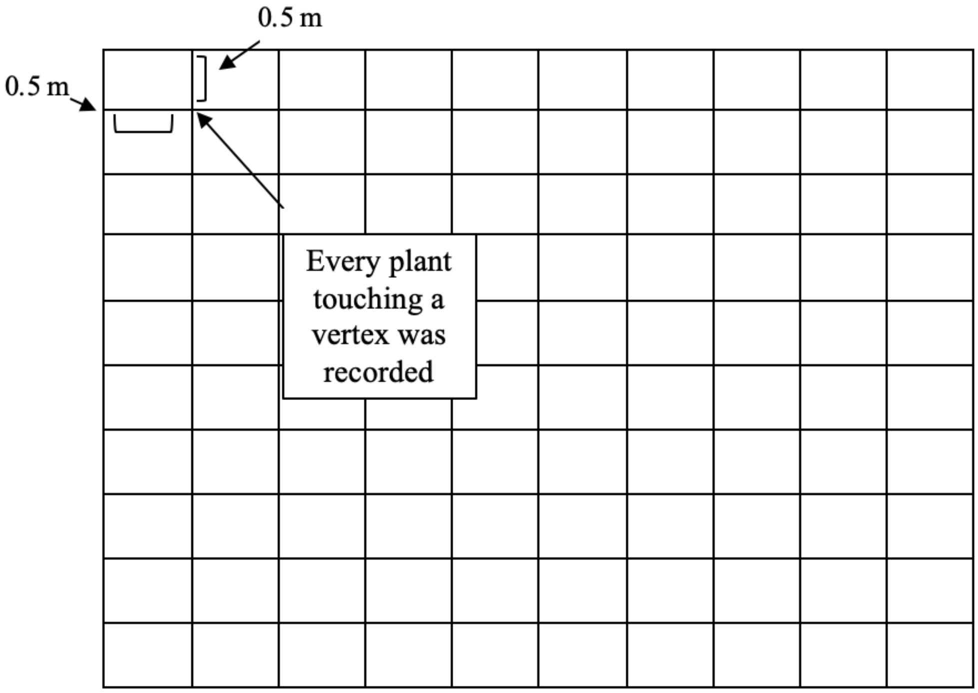


Figure 1.) An example of the point-frame, plant composition sampling method used in this study.

Following species composition sampling, a "buckthorn index" (none, low, medium, high) was developed based on the frequency distribution of *Rhamnus davurica* abundances; 5 plots contained no *Rhamnus davurica,* 5 plots contained 0.34%-4.68% (low), 4 plots contained 7.78%-19.1% (medium), and 6 contained 28.8%-78.3% (high) (Figure 2).

3.2.3 Question 1: Light Levels

Light levels below the canopy were recorded every three hours in 12 of the 20 plots, with three plots in each of the four buckthorn index categories, using a HOBO Pendant MX Temperature/Light Data Logger in June – August of 2019 and 2020. The sensors were connected to a plank of wood as a backing and PVC pipe to support the sensor facing upwards. The loggers were placed ~10 cm above the ground under a buckthorn thicket or among native species.

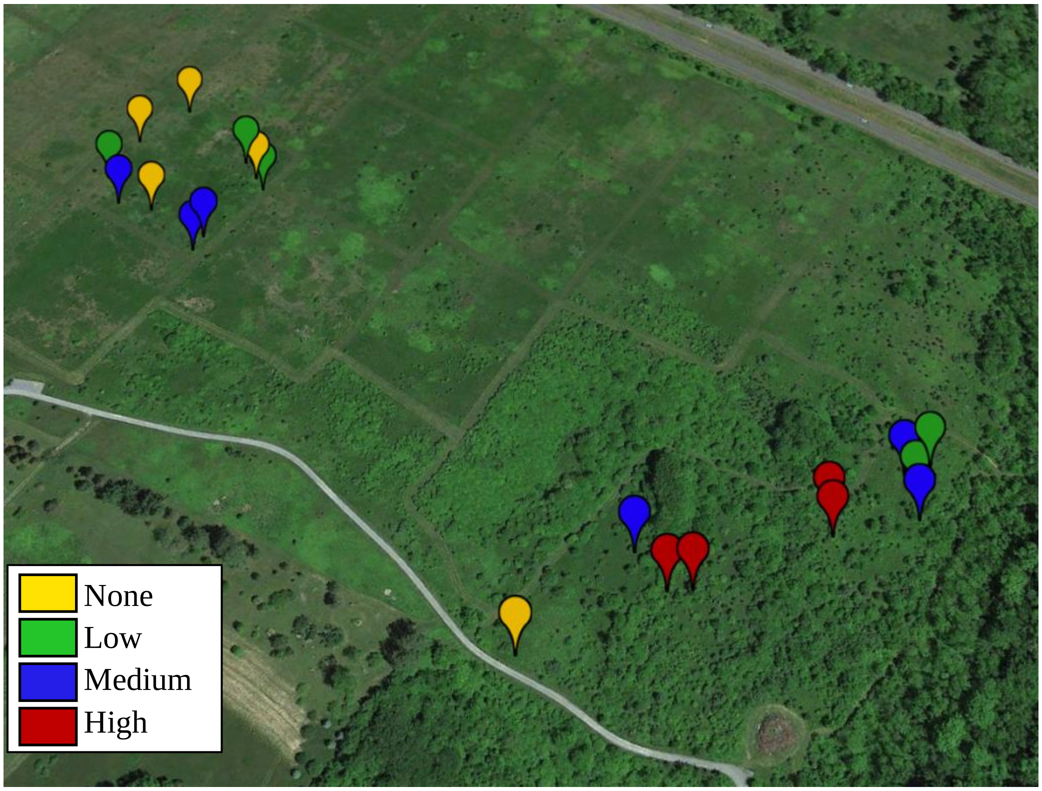


Figure 2.) Locations of 5m x 5m plots at Blandy Experimental Farm, the upper left 10 plots were abandoned for regeneration in 2003, the lower right 10 plots were abandoned in 1997. Within the plots there are varying levels of *R. davurica* presence including: none (0%), low (0.34% - 4.68%), medium (7.78% - 19.1%), and high (28.8% - 78.3%).

3.2.4 Question 2: Soil Properties

During the peak growing season (May – August) of 2019 and 2020, soil temperature, moisture, and soil respiration were measured on a weekly basis at three randomly selected locations within each plot. The three random locations in each plot were flagged, and soil properties were measured at flagged points for the remainder of the growing season. Soil temperature was measured using a digital 10 cm soil probe. Volumetric soil moisture was measured to 12 cm depth using a hand held Hydrosense time domain reflectometer (Campbell Scientific Inc., Logan, UT). Soil CO_2 efflux, as a proxy for soil respiration, was measured using a PP Systems EGM-5 Portable CO_2 Gas Analyzer (PP Systems, Amesbury, MA).

A soil core of 10 cm depth was taken in each plot; the soil was dried and then sieved with a 2 mm sieve to separate soil matter from non-soil matter. The non-soil matter was separated further into mineral (gravel) and organic matter (litter). The soil volume was calculated using the theoretical densities of organic matter (0.2 g/cm^3) and mineral matter (2.65 g/cm^3). Last, the <2 mm soil fraction was weighed, and bulk density was calculated using Equation 1:

$$\text{Equation 1: Bulk density} = \frac{\text{Dry weight of } <2 \text{ mm fraction}}{\text{Total volume of soil core} - \text{calculated volume of } >2 \text{ mm fraction}}$$

Small aluminum packets were filled with 20 – 30 mg of soil, and soil C and N content were analyzed using a 1112 Series Flash EA Carbon and Nitrogen Analyzer (Thermo Fisher Scientific, Waltham, MA).

3.2.5 Question 2: Nitrogen Cycling

Net nitrogen mineralization and net nitrification were estimated using the paired soil core and resin bag method twice during the growing season of 2019 (Strader et al, 1989). Each soil incubation took place over one month. First, a 3 cm diameter soil sample (“initial”) was taken to a 10 cm depth; this sample was immediately bagged, frozen, and returned to the lab. There were three “initial” soil samples taken from each plot at the same randomly selected locations where soil properties were measured. Another 3 cm diameter, 10 cm depth soil sample was taken at an adjacent location (within a few cm of the “initial” sample); this sample was contained in a clear plastic core with a cation-anion exchange resin bag bound to the bottom. This second or “final” core and resin bag was inserted back into the ground, from where it was initially taken. The anion-cation exchange resin bag adsorbs ammonium and nitrate leached through the soil core, as it “incubates” for 30 days. After 30 days the “final” cores and resin bags were removed, and frozen in the lab, until further processing. One incubation was started in mid-June and ended in mid-July, the second incubation started in mid-July and ended in mid-August (240 total soil samples – 20 plots x 3 replicates x 2 time periods x 2 [initial-final] cores).

In the lab, the soil samples were thawed and pushed through a 2 mm sieve to remove non-soil matter (gravel and litter). To determine gravimetric soil water content, 10 grams of the sieved soil was set aside to be dried and re-weighed. Another 10 g of sieved soil was mixed with 40 mL of 2.0 M potassium chloride (KCl) for ammonium and nitrate extraction into solution. Once the KCL was added to the soil, it was shaken for one hour and then set to rest for one hour. Resin bags were treated similarly to the soil samples. The supernatant of the samples was then filtered and diluted to ensure the dissolved compounds were in a measurable range, and then run through a Lachat flow-injection analyzer (Hach Company, Loveland, CO) to determine NH_4^+ and NO_3^- levels.

Net N mineralization was calculated as the change in NH_4^+ and NO_3^- from the initial to final core plus the resin bag (Equation 2). Net nitrification was calculated as the change in NO_3^- from the initial to final core plus the resin bag (Equation 3):

Equation 2:

$$\frac{(NH_4^+{}_{\text{final core}} + NH_4^+{}_{\text{resin bag}} + NO_3^-{}_{\text{final core}} + NO_3^-{}_{\text{resin bag}}) - (NH_4^+{}_{\text{initial core}} + NO_3^-{}_{\text{intial core}})}{1\ month}$$

Equation 3:

$$\frac{(NO_3^-{}_{\text{final core}} + NO_3^-{}_{\text{resin bag}}) - (NO_3^-{}_{\text{initial core}})}{1\ month}$$

3.2.6 Question 2: Decomposition Rate

Quercus alba is present at BEF along with *R. davurica*, and was used as a comparison for native species litter decomposition, and mixed bags of litter were used to elucidate the effect of litter interactions on decomposition rate. To determine rates of litter decomposition, sets of 2 mm mesh litterbags with 2.5 g of litter were placed on the ground surface in each plot. *R. davurica* and *Quercus alba* were used for the litter decomposition analysis, and each bag contained litter from one of the two species, or in the case of a mixed bag, 1.25 g of each species litter. The bags were heat-sealed shut and placed randomly with a grid pattern within each plot. The litterbags were anchored to the soil in order to ensure contact with the soil surface. All of the leaf litter was collected in October of 2020, and all litterbags were put out on November 5th; each plot had at least 16 litterbags (8 of each species), with a subset of plots (high and no buckthorn) receiving 8 additional bags of mixed litter, for a total of 368 litter bags. The 2 mm mesh litterbags allowed micro- and mesofauna to interact with the litter. Once all litter bags were put out on November 5th, two bags of each litter type in each plot were collected on the fifth of every proceeding month for four months; the last set of litterbags was collected on March 5th, 2021. After collection, the litter was dried at 50°C for 24 hours. The fraction of litter mass loss was calculated using Equation 4.

Equation 4:

$$1 - (\mathrm{Mass}_{\mathrm{final}}/\mathrm{Mass}_{\mathrm{initial}})$$

Last, a portion of the dried initial and all retrieved litter was ground using a ball mill (Cianflone Scientific LLC, Pittsburgh, PA, USA) and combustion analyzed using a Fisher Scientific Flash 2000 Elemental Analyzer (Fisher Scientific™, Hampton NH, USA) to determine N content.

3.2.7 Statistical Analyses

For this study, I used a categorical variable (*R. davurica* index) in analyses of the dependent variables, with mixed model ANOVAs and repeated measures ANOVAs where applicable, using the statistical program R Version 1.4.1103 (R Core Team, 2020).

The soil temperature, moisture, and respiration were analyzed using a repeated measures ANOVA with buckthorn index as the independent variable and soil temperature, moisture or respiration as the dependent variable. The repeated measure used was the specific site in the plot that was measured each week; the timing of data recorded was included in the model as a fixed factor.

Light curves were also analyzed using a repeated measures ANOVA. The net nitrification and mineralization data were analyzed with a standard one factor ANOVA with buckthorn index as the independent variable and nitrification or mineralization as the dependent variable.

Last, the litter bag data were analyzed using a standard one factor ANOVA.

3.3.0 Results

3.3.1 Question 1: Plant Species Composition

For each of the 20 plots, the relative frequencies of all species were calculated using data from the grid point-intercept method. The six most common invasive species found were *Rhamnus davurica* (Dahurian buckthorn) with an average of ~16% relative frequency per plot, *Galium verum* (yellow bedstraw) with an average of ~15% relative frequency per plot, *Elaeagnus umbellata* (autumn olive) with an average of ~7%, *Microstegium vimineum* (stiltgrass), *Celastrus orbiculatus* (Oriental bittersweet), and *Ligustrum vulgare* (wild privet) with averages of ~1%. As *R. davurica* increased in frequency, *E. umbellata* also increased, while the other four common invasive species exhibited a decline in frequency.

The five most common native species were *Rubus* spp. (blackberry or raspberry) with an average of ~18% relative frequency per plot, *Symphoricarpos orbiculatus* (coralberry) with an average of ~9%, *Solidago* spp. (goldenrod) with an average of ~3%, *Maclura pomifera* (Osage orange) at 2.5%, and *Cirsium vulgare* (spear thistle) at 0.9%. The native species all decreased in frequency as *R. davurica* frequency increased (Table 1; Figure 3).

Native species made up higher proportions of the overall composition in no buckthorn plots, but at high levels of buckthorn, the invasive species were more abundant than the native species (Figure 3).

Table 1.) Comprehensive list of plant species found in the 20 plots at BEF used in this study.

Plant Species	**Dahurian Buckthon Index**			
	None	**Low**	**Medium**	**High**
Achillea millefolium	X	X		X
Allium vineale	X			
Ambrosia spp.				X
Ampelopsis glandulosa			X	
Asclepias syriaca	X	X	X	X
Celastrus orbiculatus		X	X	X
Cirsium canadensis	X	X	X	X
Cirsium vulgare		X		
Clinopodium vulgare				X
Desmodium canadense		X		
Elaeagnus umbellata		X	X	X
Erigeron annuus	X	X		X

Fraxinus pennsylvanica				
Galium verum	X	X	X	X
Gleditsia triacanthos	X			
Grasses (other)	X	X	X	X
Juniperus virginiana		X	X	X
Leucanthemum vulgare		X	X	X
Ligustrum vulgare	X	X		
Linaria vulgaris		X	X	
Lonicera japonica	X	X	X	X
Lonicera mackii		X	X	X
Maclura pomifera		X	X	X
Microstegium vimineum	X	X	X	X
Nepeta cataria	X	X	X	X
Phytolacca americana	X			
Plantago lanceolata	X	X		X
Polygenum convolvulus		X		
Rhamnus davurica		X	X	X
Rosa multiflora			X	X
Rubus spp.	X	X	X	X
Ruellia				X
Solanum carolinense	X	X	X	
Solidago spp.	X	X		X
Symphoricarpos orbiculatus	X	X	X	X

Toxicodendron radicans	X		X
Trifolium campestre	X		X
Verbascum	X	X	X
Vitis vinifera	X		

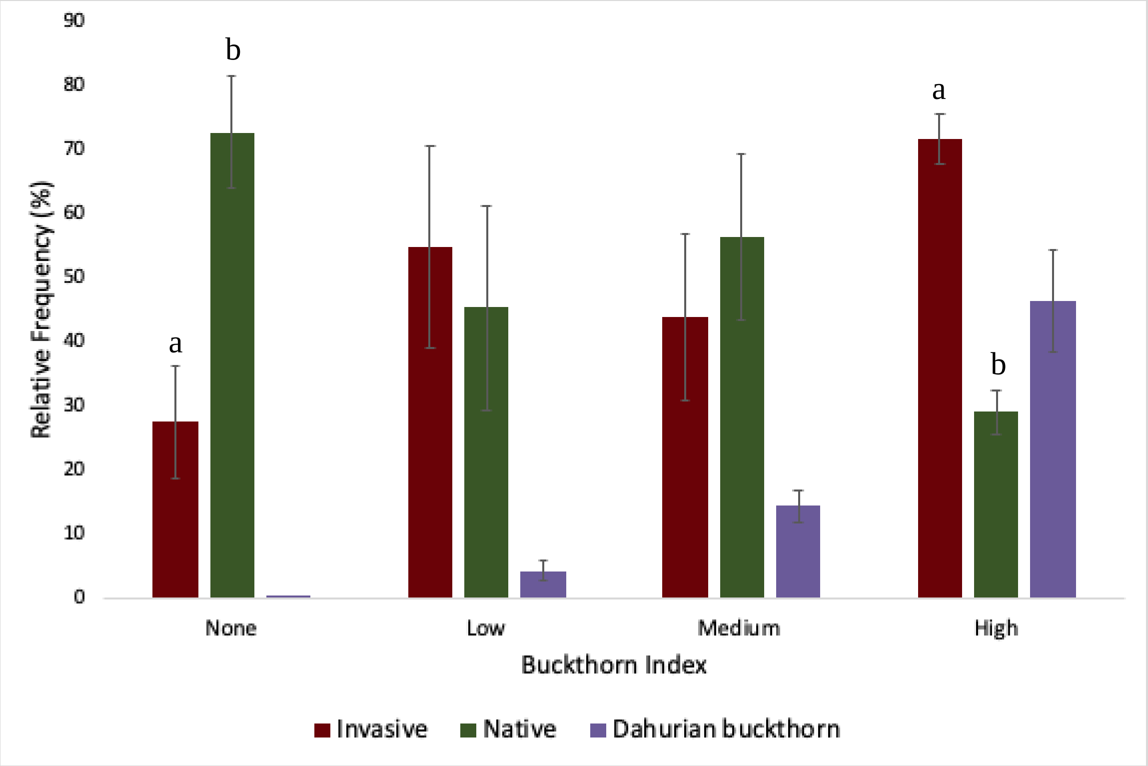


Figure 3.) Relative frequency (%) of invasive (red) versus native (green) plant species in 20 plots (5m x 5m) at Blandy Experimental Farm during the summer of 2020. The purple bars represent the amount of Dahurian buckthorn in each plot. Error bars represent standard error. The lower case letters represent significant differences between invasive versus native species in each index found using a t-test (None p = 0.007, Low p = 0.684, Medium p = 0.523, High p = 1.025 x 10^{-5}).

3.3.2 Question 1: Light Levels

In plots with high levels of *R. davurica*, PPFD (Photosynthetic Photon Flux Density) at ~15 cm above the ground was lower in May, June, July, August, and September of 2020 than in plots with no buckthorn, low, or medium levels of buckthorn (Figure 4). Although there were monthly variances among plots, the trends follow the pattern expected with a gradient moving from high light levels in no buckthorn plots to extremely low light levels in high buckthorn plots. The average daily light level across all months was 171.7±16.1μmol $m^{-2}s^{-1}$ in no buckthorn, 132±13.9μmol $m^{-2}s^{-1}$ in low levels, 80.2±8.5μmol $m^{-2}s^{-1}$ in medium levels, and 14.4±1.6μmol $m^{-2}s^{-1}$ in high levels. A RMANOVA was run on all 5 months for the average noon light. In July, low buckthorn indices had significantly higher light levels than medium and high buckthorn indices ($p < 0.05$, $F_{[3, 7]} = 5.76$) (Figure 4). In May, no buckthorn plots has significantly higher light levels than medium and high buckthorn indices ($p < 0.02$, $F_{[3, 7]} = 8.20$) (Figure 4).

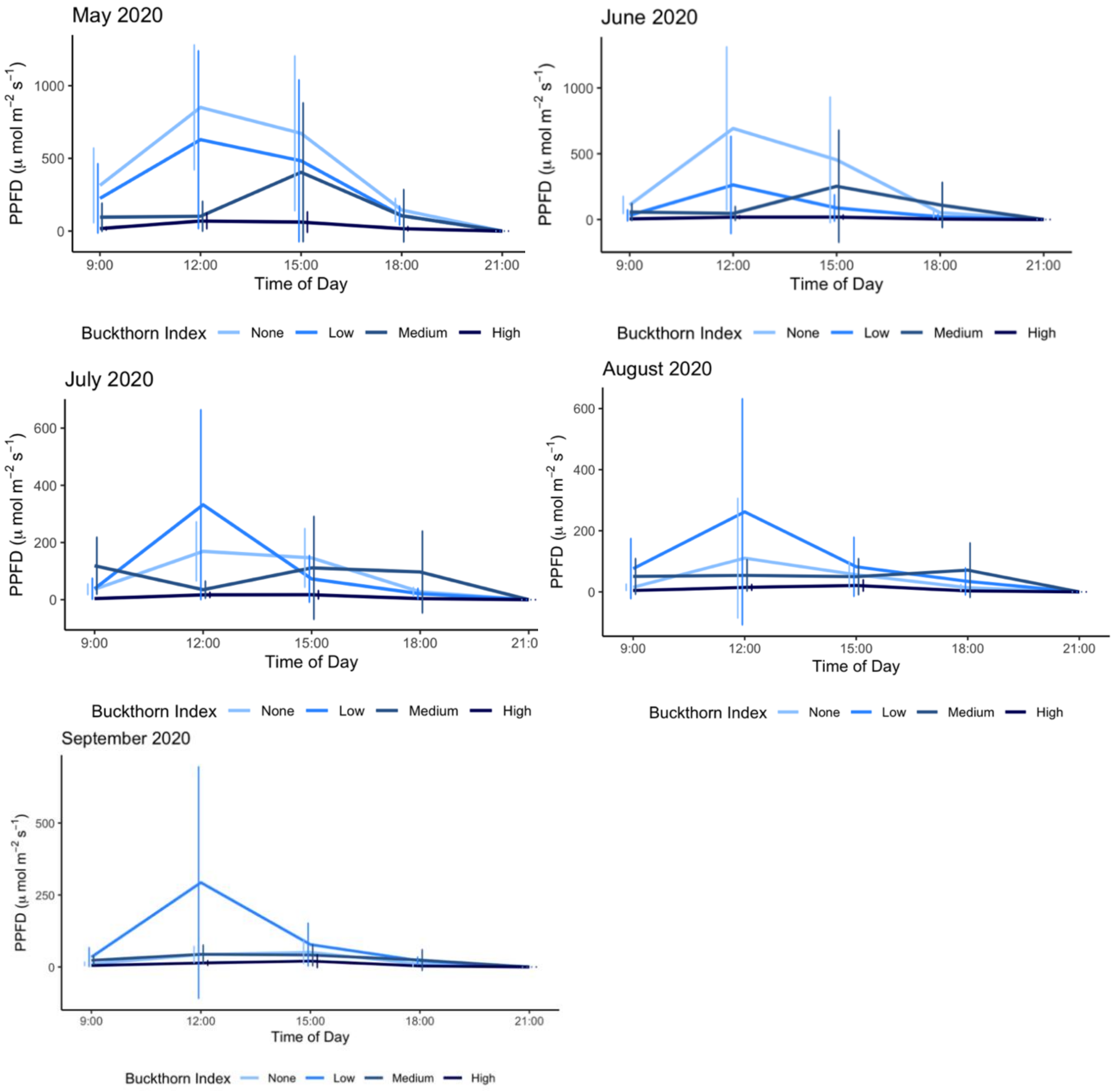


Figure 4.) Average light levels in Photosynthetic Photon Flux Density (PPFD; µmol photons m^{-2} s^{-1}) at 3 hour time increments for each month of May-September at Blandy Experimental Farm. HOBO Pendant MX Temperature/Light Data Loggers were used to measure the light levels.

3.3.3 Question 2: Soil Properties

Average soil temperatures in low level buckthorn (20.5°C) plots were significantly greater than medium (19.9°C) and high (19.6°C) buckthorn plots during the growing season of 2019 ($p < 0.03$, $F_{[3, 56]} = 7.31$) (Figure 5). In the growing season of 2020, plots with no buckthorn (20.3°C) and low levels (20.5°C) of buckthorn had significantly greater soil temperatures than high buckthorn (19.4°C) plots ($p < 0.02$, $F_{[3, 56]} = 5.75$) (Figure 5).

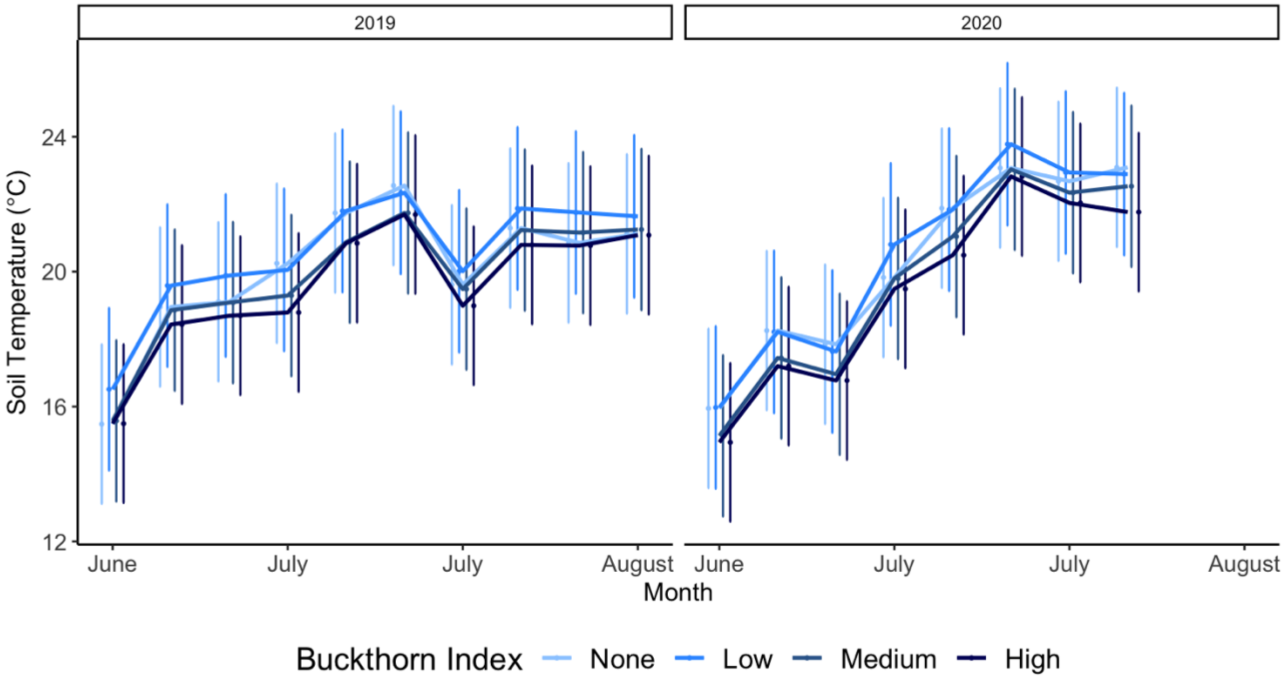


Figure 5.) Average soil temperature (°C) at 10 cm depth in 20 plots at Blandy Experimental Farm. The plots range from no Dahurian buckthorn presence (lightest blue) to high Dahurian buckthorn presence (darkest blue). These data were measured for both the summer of 2019 (left) and the summer of 2020 (right); in 2019 low buckthorn index plots had significantly higher soil temperatures than medium and high index, in 2020 no buckthorn and low buckthorn had significantly higher soil temperatures than high buckthorn index ($p < 0.03$). Error bars represent standard error.

During the 2019 growing season the average soil moisture was significantly lower in the medium level buckthorn (10.4%) plots than all three other plot types (~13%) ($p < 0.05$, $F_{[3, 56]} =$

4.41) (Figure 6). While in 2020 medium level (13.7%) had significantly lower soil moisture than high levels plots (16.7%) ($p < 0.02$, $F_{[3, 56]} = 3.44$)(Figure 6).

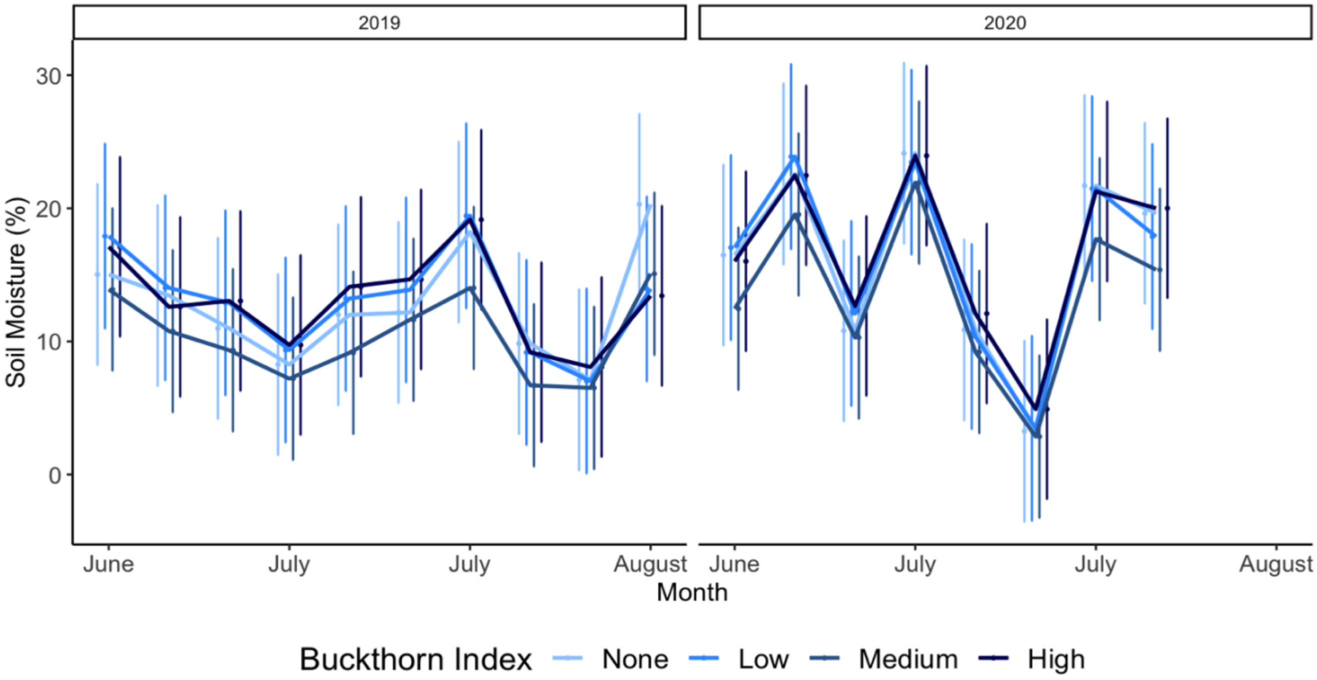


Figure 6.) Average soil moisture (%) at 12cm in 20 plots at Blandy Experimental Farm. The plots range from no Dahurian buckthorn presence (lightest blue) to high Dahurian buckthorn presence (darkest blue). These data were measured for both the summer of 2019 (left) and the summer of 2020 (right); medium buckthorn had significantly lower soil moisture than all other plot types in 2019 and significantly lower soil moisture than high buckthorn in 2020 ($p < 0.05$). Error bars represent standard error.

Soil respiration was greatest in high buckthorn plots (average in 2019 = 1.05 µmol CO_2 $m^{-2}s^{-1}$, average in 2020 = 1.18µmol CO_2 $m^{-2}s^{-1}$) and lowest in the medium level plots (average in 2019 = 0.96µmol CO_2 $m^{-2}s^{-1}$, average in 2020 = 1.08µmol CO_2 $m^{-2}s^{-1}$) (Figure 7), although, there were no significant differences.

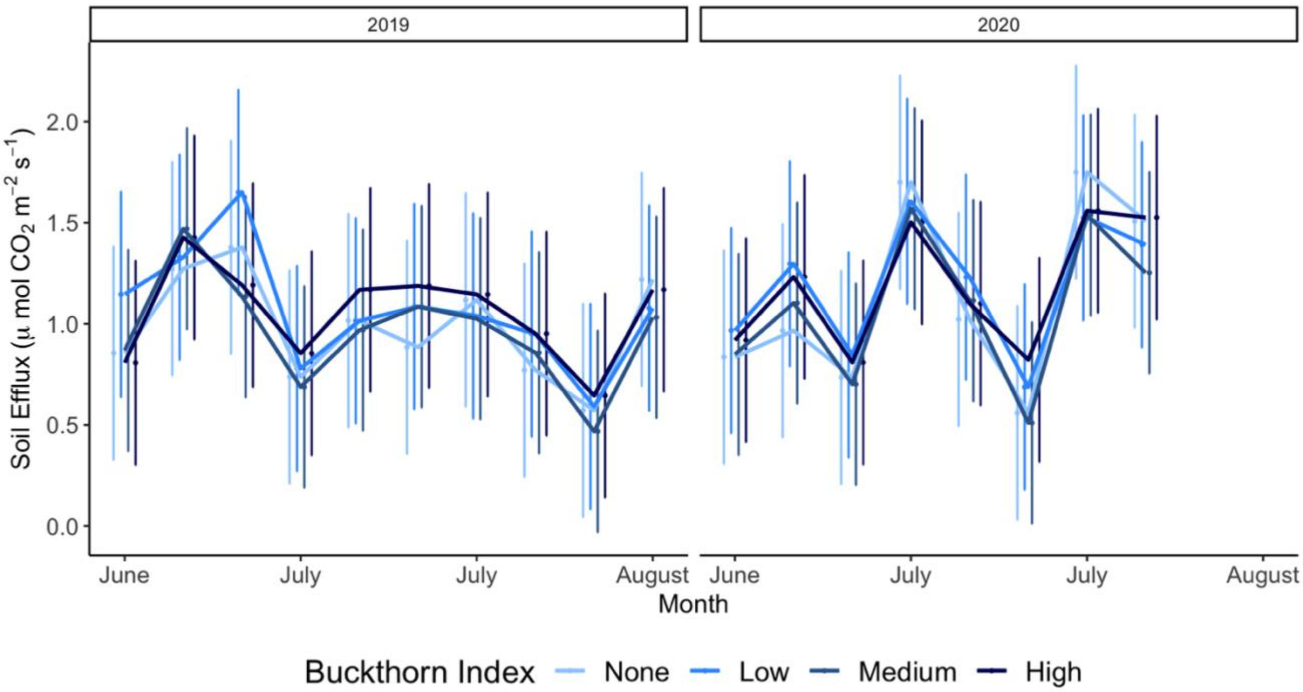
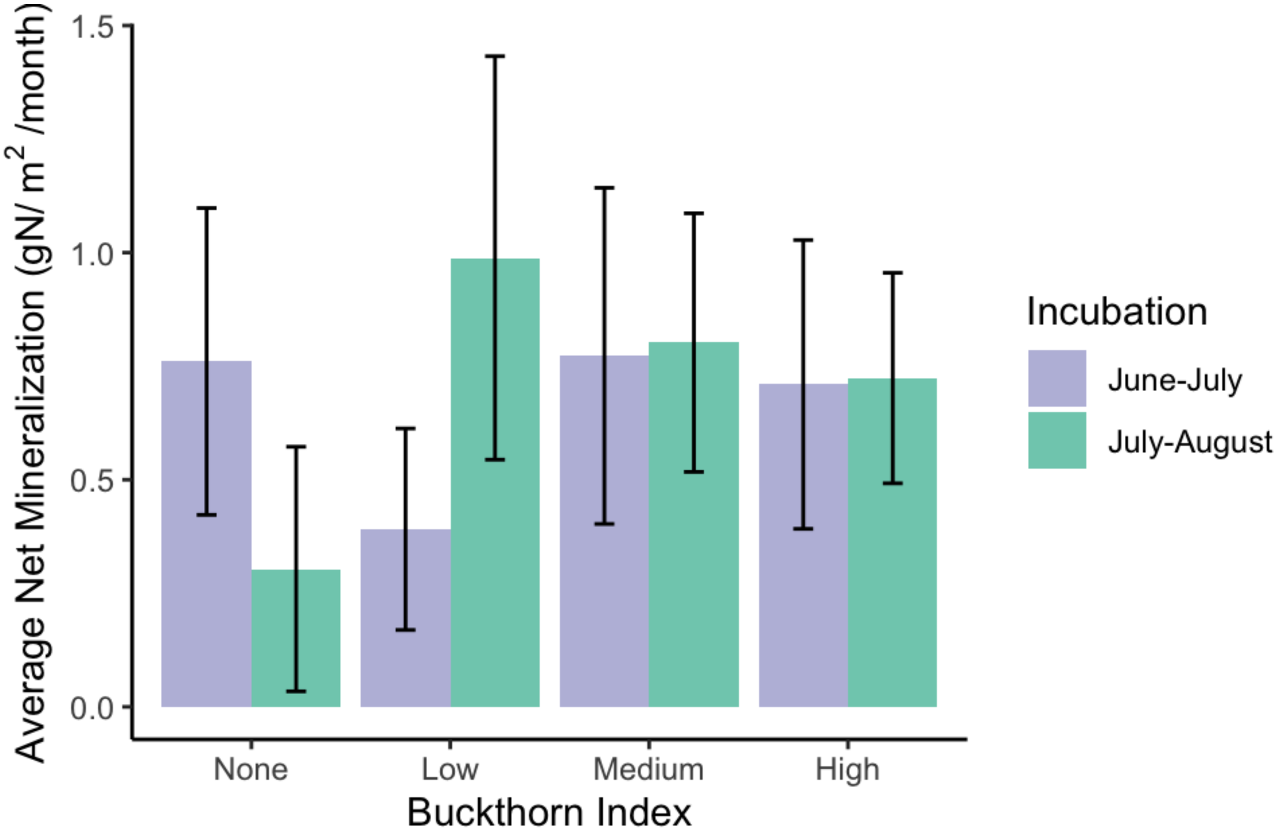


Figure 7.) Average soil respiration (µmol CO_2 m^{-2} s^{-1}) in 20 plots at Blandy Experimental Farm. The plots range from no Dahurian buckthorn presence (lightest blue) to high Dahurian buckthorn presence (darkest blue). This data was measured for both the summer of 2019 (left) and the summer of 2020 (right). Error bars represent standard error.

Net N mineralization was lowest in the June-July incubation within low buckthorn plots (0.391 gN/m^2), and the no buckthorn (0.303 gN/m^2) plots had the lowest mineralization in the July-August incubation (Figure 8). In the incubation for June-July of 2019, there was a significantly greater rate of nitrification in the no buckthorn plots (0.152 gN/m^2) than the high buckthorn plots (0.103 gN/m^2) ($p = 0.012$, $F_{[3, 44]} = 3.58$) (Figure 8). Ammonium concentrations were lower in the no buckthorn and low buckthorn plots than the medium and high buckthorn plots (Figure 9). Nitrate concentrations were significantly higher in the no buckthorn than the low and high buckthorn indices (Figure 9; $p = 0.004$, $F_{[3, 97]} = 4.73$).


Average Net Mineralization (gN/ m2 /month)
1.5
1.0
0.5
0.0
None
Low
Medium
High
Buckthorn Index
Incubation
June-July
July-August

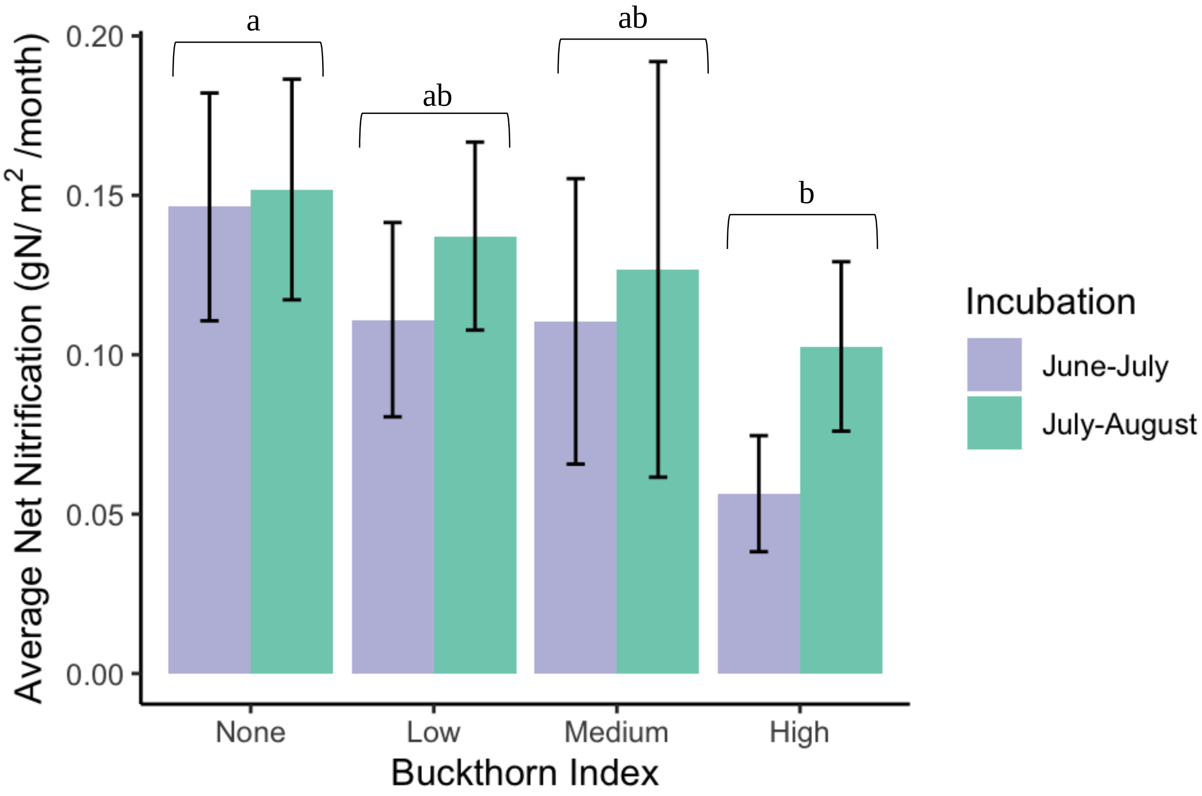
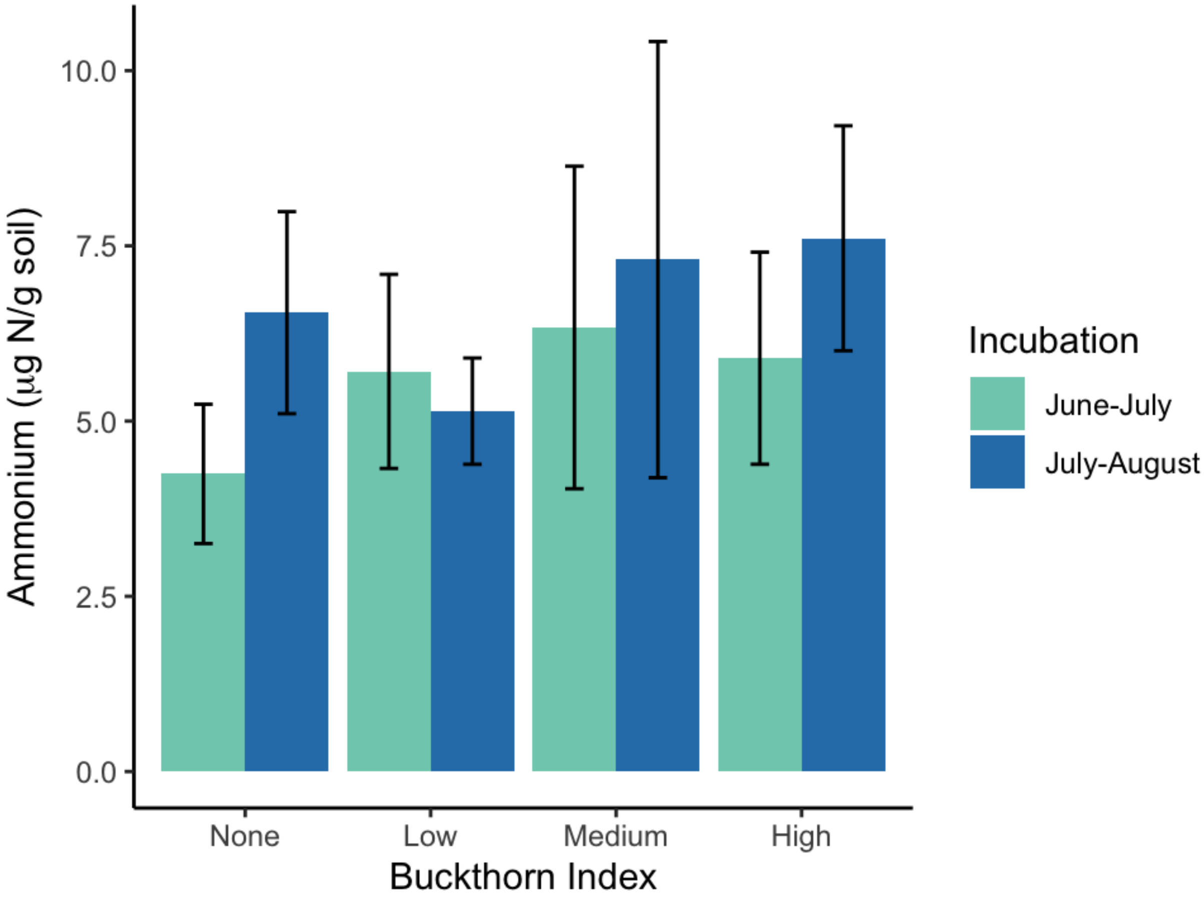


Figure 8.) Average net mineralization (top) and net nitrification (bottom) across 20 plots of varying buckthorn presence during the months of June-August (2 incubations total) of 2019 at Blandy Experimental Farm. Lower case letters indicate significant differences (p = 0.012). Error bars represent standard error.


Ammonium (µg N/g soil)
10.0
7.5
5.0
2.5
0.0
None
Low
Medium
High
Buckthorn Index
Incubation
June-July
July-August

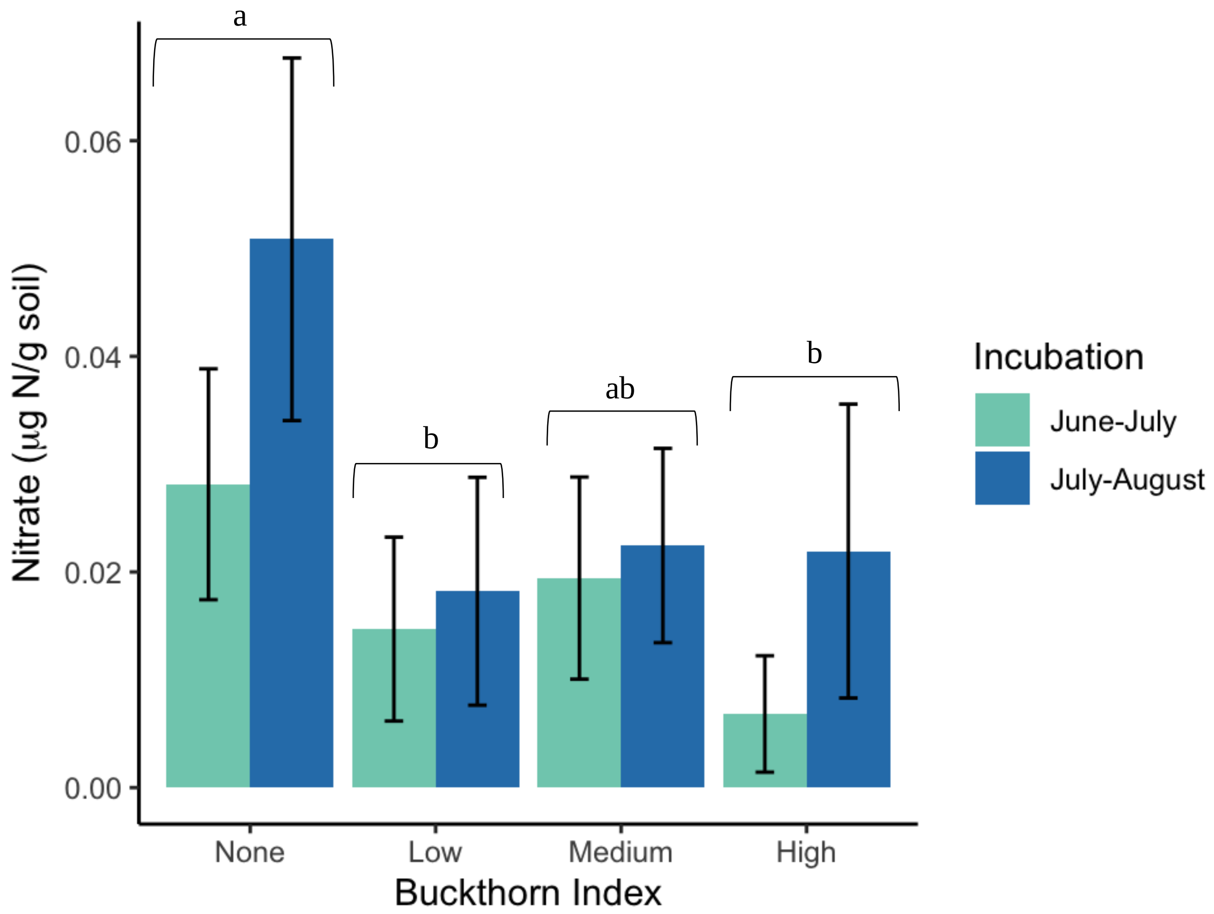


Figure 9.) The average concentration of ammonium (µg N/g soil; top) and nitrate (µg N/g soil; bottom) in the soil across 20 plots with varying levels of buckthorn presence. The light green bar denotes the collection period during June of 2019 and the darker blue bar denotes the collection period during July of 2019. Lowercase letters signify significant differences between buckthorn indices ($p = 0.004$). Error bars represent standard error.

3.3.4 Question 2: Decomposition Rate

Decomposition rate was estimated with litter bags deployed in the field. Decomposition rates among the three different litter bag treatments were significantly different, although there was no plot type significance. Litter bags filled with buckthorn litter ($k = 0.0054$) decomposed significantly faster than the mixed litter ($k = 0.0031$), which decomposed significantly faster than the white oak litter ($k = 0.0019$) ($p < 0.005$, $F_{[2, 42]} = 89.11$) (Figure 10). After 120 days (or 4 months) Dahurian buckthorn litter lost ~37% of leaf mass, mixed litter lost ~23%, and white oak lost ~13% ($p < 0.001$, $F_{[2, 42]} = 105.7$) (Figure 11). Dahurian buckthorn litter started with the highest amount of nitrogen in the leaf litter at day 0 (0.038g) and ended with the lowest amount of nitrogen at day 120 (0.027g) (Figure 12).

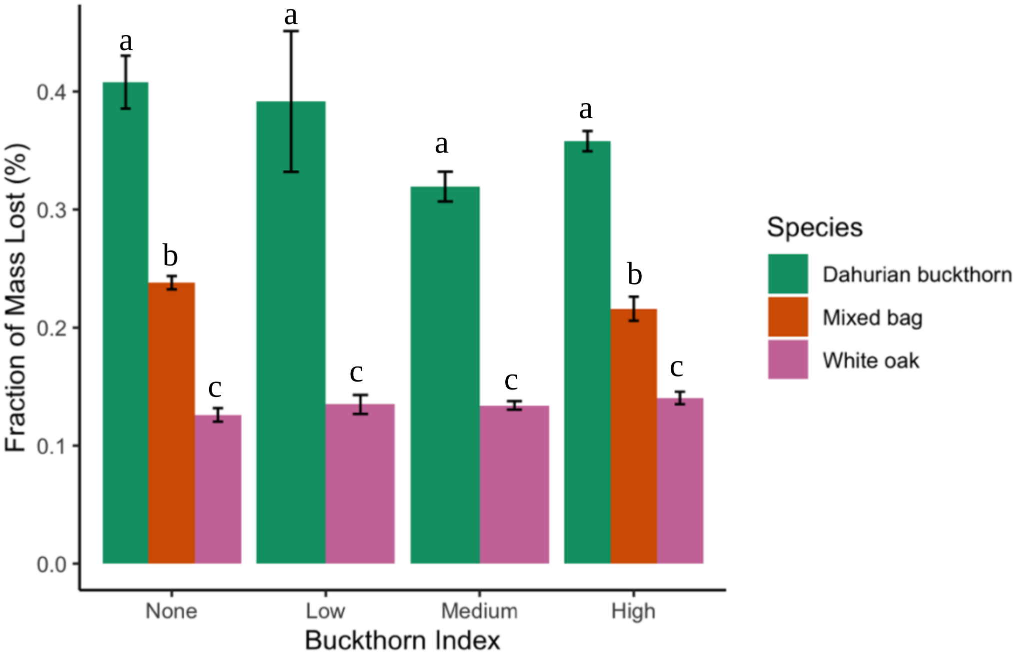


Figure 10.) Average total mass lost (%) during a litter decomposition experiment over 4 months (120 days) at Blandy Experimental Farm during the fall of 2020-2021 (November-March) using the litter from Dahurian buckthorn, white oak and mixed litter which included 50% of buckthorn and 50% of oak. Lower case letters represent significant differences ($p < 0.0009$) among species of litter; there were no significant differences among buckthorn indices. Error bars represent standard error.

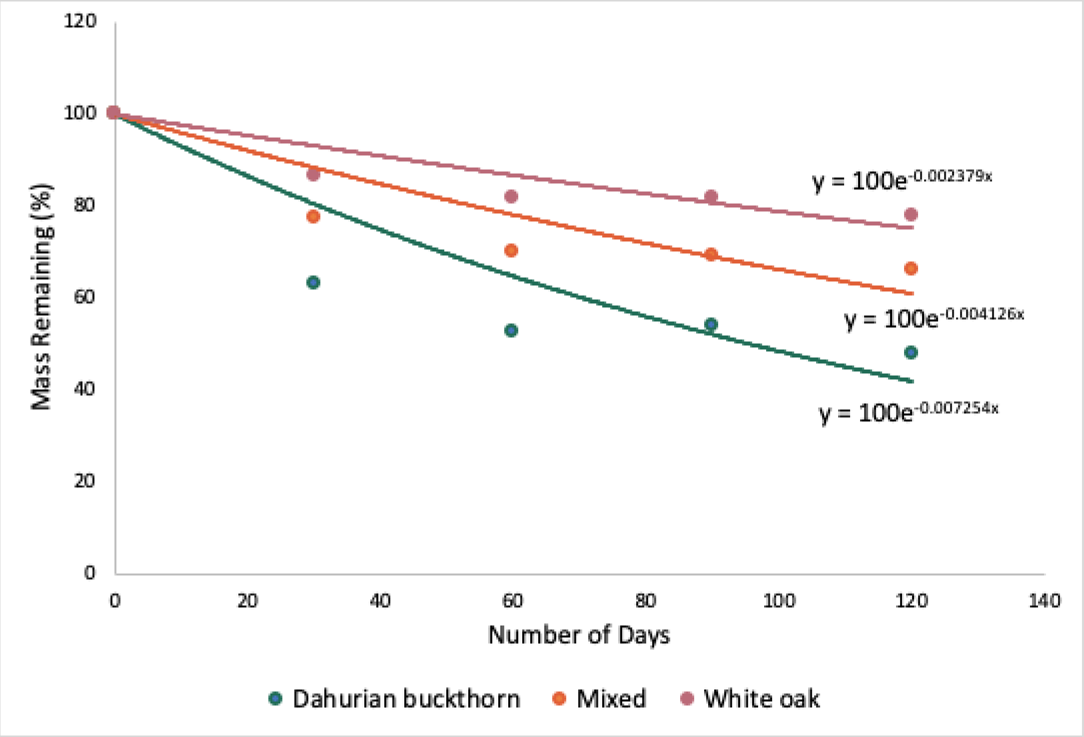


Figure 11.) The average decay rate for the 3 litter bag types (Dahurian buckthorn, white oak, and mixed litter (50% buckthorn and 50% oak) over 120 days. Litter bags were picked up from Blandy Experimental Farm, dried and weighed consecutively every 30 days. Lines are negative exponential fits.

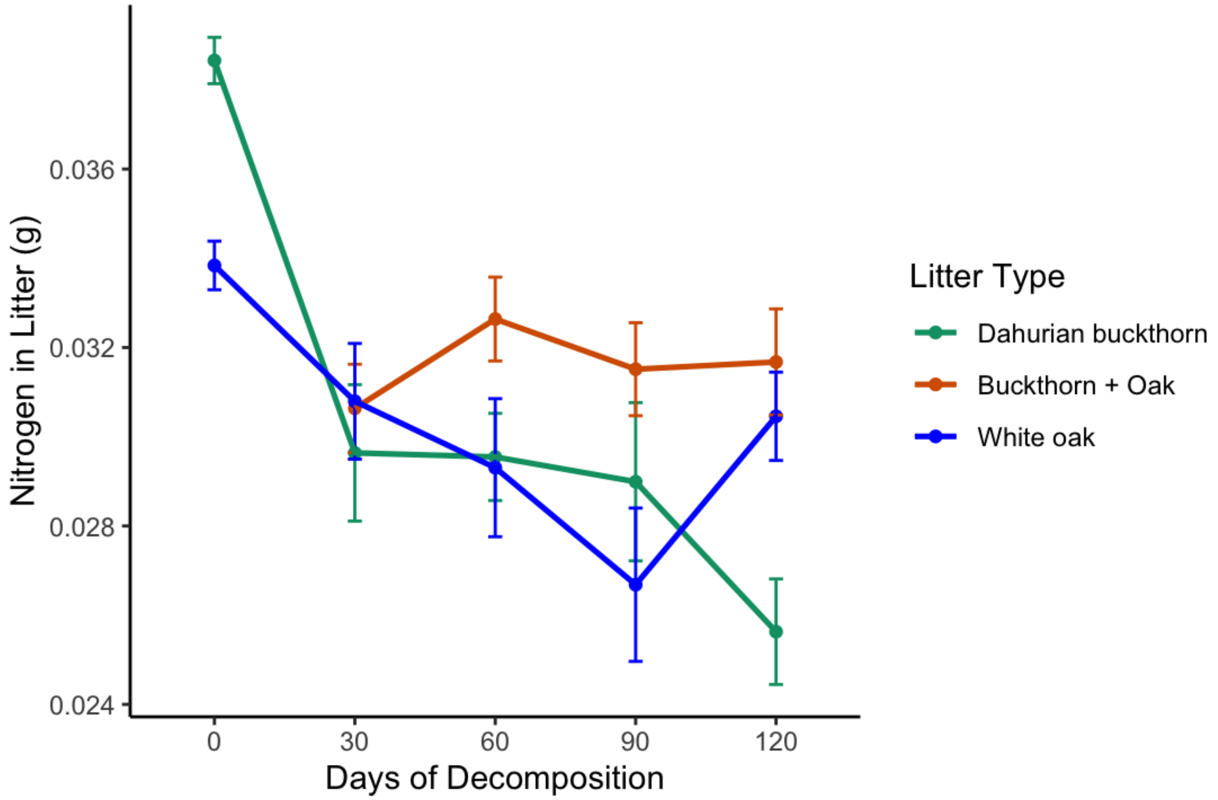


Figure 12.) The average amount of nitrogen (g) found in the litter of Dahurian buckthorn (green), mixed bag (orange), and white oak (blue) at 0, 30, 60, 90, and 120 days in the field. The error bars represent standard error.

3.4.0 Discussion

Invasive species are causing unprecedented changes in our ecosystems, especially as climate change shifts weather patterns and environmental conditions. In the past decade, we have learned that just one invasive species (such as chestnut blight, autumn olive, and buckthorn) can alter the species composition, nutrient cycling, and the overall functionality of an ecosystem (Vitousek et al., 1997; Gordon, 1998; Vila and Weiner, 2004; Peltzer et al., 2010). The early successional fields of Blandy Experimental Farm (BEF) offer some insight on the initial invasion pattern and eventual ecological consequences that result from plant invasions of abandoned agricultural fields. In this study at BEF, I observed the differences in early successional fields as Dahurian buckthorn abundance increased. I found evidence that buckthorn is affecting succession by changing soil properties (temperature and moisture), shifting nutrient cycling, and altering species composition.

3.4.1 Question 1: Plant species composition

In a regenerating forest, there is likely a relatively wide range of plant species present. As the landscape changes over time there are distinct stages in vegetation, i.e. herbaceous cover in the beginning followed by shrubs and young trees to an eventual climax community forest (Grime, 1979). However, when an invader such as Dahurian buckthorn comes in, it can put the system in arrested succession (Gill and Marks, 1991). A successful invader can dominate the landscape and decrease native species richness, with a positive feedback in which more invasive species can become established (Vila and Weiner, 2004; Kuebbing et al., 2015; Woodworth et al., 2020).

At BEF, the fields with high levels of buckthorn had a much larger number of invasive species than areas that had no buckthorn present (Figure 3). Invasive species can alter the microbial community, the arthropod community, and nutrients present in the soil, impacting the suitability of the soil environment for native species (Knight and Reich, 2005; Rodrigues et al., 2015). Across all four plot types (no buckthorn, low, medium, and high) the invasive species did not fully overtake the native species until high levels of buckthorn were reached, which may suggest an invasive species feedback on soil-plant interactions, although not definitively (Heneghan et al., 2006). Buckthorn can also have allelopathic effects which further alter the soil and deter establishment of other species (Klionsky et al., 2011; Warren et al., 2017; Pinzone et al., 2018; Bergman et al., 2021). Whereas most invasive species are linked to negative impacts on local species richness, they can potentially increase primary productivity in plant communities, although this is disputed (Peltzer et al., 2010; Vila et al., 2011)

3.4.2 Question 1: Light resources

In early stages of buckthorn invasions, there is often an abundance of light; buckthorn grows rapidly in high light environments such as forest edges or fields (Qaderi et al., 2009; Schulte et al., 2011). In a forest environment, buckthorn can grow structurally like a small tree with a high leaf area to production ratio, while in open fields the shrubs make monospecific thickets with dense canopy cover (Harringtion et al, 1989; Schulte et al., 2011; Charles – Dominique, 2012; Whitfeld et al., 2014; Kalkman et al., 2019). At BEF, the shrubs have grown into dense thickets in abandoned agricultural fields, and I found a direct negative relationship between light availability at the forest floor and an increase in buckthorn abundance.

In no buckthorn plots, the average daily light reached 171.7µmol/m^2/s and in high buckthorn plots, it was as low as 14.4µmol/m^2/s (Figure 4). Among other competitive traits, invasive species decrease light resources for seedlings and other sprouting vegetation through dense canopy cover (Gordon, 1998; Fagan and Peart, 2004; Whitfeld et al., 2014). In buckthorn thickets at BEF, there is little to no regeneration of native species and even low regeneration of buckthorn itself underneath the dense, older buckthorn canopies; most of the successful buckthorn seedlings are found on the thicket edges or in gaps. Another interesting observation at Blandy is the absence of buckthorn in mature forests; the shrub needs disturbed, high light environments to establish at a level of dominance (Charles-Dominique, 2012, Dey et al., 2019; Kalkman et al., 2019).

Native plants face competition from invasive species that have no natural enemies or pathogens and fast growth rates; by also reducing light resources the invaders are completely excluding native species in some areas (Fagan and Peart, 2004). These effects can cause economic harm, environmental consequences, and even contribute to climate change as invasives change the functionality and services provided by ecosystems (Vitousek et al., 1997).

3.4.3 Question 2: Soil Properties

My study looked at the soil temperature, moisture, and respiration over 18 total weeks during the growing seasons of 2019 and 2020. These soil properties and processes play important roles in the plant community through microclimate establishment and impact other processes such as ecosystem respiration (Breshears et al., 1998). In high buckthorn plots, I expected lower soil temperature and higher soil moisture due to intense shade from the dense canopies, which reduce evaporative radiation. I hypothesized that there would be greater soil respiration from the more mature shrubs than with less buckthorn (Pierson and Wight, 1991; Breshears et al., 1998), since in higher moisture environments, decomposition can occur more rapidly, especially when the high-quality buckthorn leaf litter is present (Heneghan et al., 2002; Heneghan et al., 2004; Heneghan et al., 2006; Metcalfe et al., 2011). While I did see increased moisture in the higher levels of buckthorn and warmer temperatures in the lower levels of buckthorn, respiration did not differ significantly across the buckthorn indices (Miller, 2010). This response of respiration could be due to the balance between increasing soil moisture but lower soil temperatures as buckthorn increases. In 2019 the medium index buckthorn plots had significantly lower soil moisture than all other indices, which was an unexpected result, this result could portray the balance between evaporative processes and moisture uptake by the rapidly growing shrubs; although this is speculation.

These data were collected in May-August of 2019 and 2020, results may have been different if data had been collected in the fall months. The summer respiration could be largely driven by temperature and moisture of the soil and less so by buckthorn litter; the fall decomposition of newly senesced leaves may be more affected by the high quality litter.

3.4.4 Question 2: Decomposition Rates

The Dahurian buckthorn leaf litter had a significantly faster decomposition rate (0.00725) than white oak (0.00238) and the mixed litter (0.00413). I expected this result due to buckthorn leaves possessing a higher nitrogen content (1.54%) and lower C:N ratio of 23.13, compared to 35.13 for white oak and 26.90 for mixed litter (Woodworth et al., 2020). Over 120 days, the buckthorn leaf litter lost an average of 37% of mass, followed by the mixed litter (23%), and the white oak litter (13%) (Figure 10). The mixed bag, with equal weights of buckthorn and white oak litter, had a decomposition rate of 0.00413 which was inbetween the buckthorn and white oak decomposition rate. I had expected buckthorn's high nitrogen litter to have an accelerated effect on the decomposition of the mixed bag but we I did not observe that. Woodworth et al. (2020) found that Dahurian buckthorn at BEF had a C:N ratio of 25.8 and a nitrogen content of 1.5%, which are very close to my findings. In comparison, common buckthorn (*Rhamnus cathartica*) has been studied more extensively than Dahurian buckthorn and has similar litter properties with fast decomposition rates (22% leaf litter mass lost at ~120 days) and a nitrogen content of 2.2% (Heneghan et al., 2002; Heneghan et al., 2004; Heneghan et al., 2006).

We know that in high buckthorn plots there is a shaded canopy with greater soil moisture which can increase decomposition rates, but I saw no significant relationships between the buckhorn index plot types and decomposition rates. In contrast to our study, invaded sites typically had higher decomposition rates than non-invaded sites regardless of litter type in two studies done with common buckthorn and other invasive species (Heneghan et al., 2002; Ashton et al., 2005). In previous studies, buckthorn species have been found to alter the microbial and arthropod communities, which can in turn alter decomposition rates and nutrient cycling; these can act as a legacy effect on the landscape (Ehrenfeld, 2003; Elgersma et al., 2011; Woodworth et al., 2020).

In comparison to the native hardwoods that may have grown in the place of buckthorn, the high nutrient litter leads to faster decomposition rates and higher respiration levels (Metcalfe et al., 2011). Higher nutrient leaf litter should increase nitrogen for neighboring plants, but buckthorn soils have actually been shown to lower levels of soil nitrogen by 11% due to their re-uptake abilities (Jo et al., 2015).

3.4.5 Question 2: Nitrogen Cycling

Exotic species often enter a novel ecosystem with different physiological traits than the native plants. Most invasive plants have higher nitrogen content and decompose faster than native species, which impacts nutrient cycling (Ehrenfeld, 2003; Vila et al., 2011)(Figure 12). During the growing season of 2019, two *in situ* nitrogen cycling incubations were performed during June-July and July-August. I found that in fields with high levels of buckthorn, net nitrification was significantly lower (by 32%) than the fields with no buckthorn (Figure 8), although this difference in nitrification rates only occurred in the months of June-July and was not present in July-August. Invasive species that keep ecosystems in a stable arrested vegetation state can cause lasting effects on the landscape even after they have been removed (Milchunas and Lauenroth, 1995; Corbin and D'Antonio, 2004). For example, ammonium and nitrate turnover at BEF was greater in soils associated with plant invasions in comparison with areas not invaded (Rodrigues et al., 2015). At BEF, Dahurian buckthorn has taken over large swathes of land experiencing secondary succession and has arrested this process, which can alter long-term N pools and the native species that access them. In areas with high levels of common buckthorn, soils have had lower ammonium concentrations than in areas with no buckthorn or "open" habitat (Heneghan et al., 2004); my study found significantly lower levels of nitrate in the high buckthorn plots than in areas with no buckthorn, but no difference in ammonium. Buckthorn could be rapidly taking up nitrate or buckthorn may prefer nitrate to ammonium which is reducing the amount of nitrate in areas with high levels of buckthorn (Figure 9). In Heneghan et al. (2002, 2004, 2006) the soil C:N ratio was significantly lower in sites with common buckthorn than open sites.

Most invasive species have more rapid nitrogen uptake than their native competitors (Mueller et al., 2017). Since nitrogen is one of the main limiting factors for primary productivity in mesic, temperate ecosystems, when invaders are better at taking up nitrogen than native species, it can cause large shifts in plant-available nitrogen and ecosystem nitrogen pools (LeBauer and Treseder, 2008).

4.0 Allelopathy in *Rhamnus davurica* (Dahurian buckthorn) and implications for secondary succession (Chapter 2)

4.1.0 Introduction

4.1.1 Background on Rhamnus davurica and allelopathy

Over the past 150 years, land-use in the northeastern United States has shifted from active agriculture to abandoned fields as large-scale farming moved to the midwestern U.S. (Compton and Boone, 200). The disturbance from which these fields are recovering provides optimal conditions for regrowth through low initial competition and plenty of sunlight; although, with the northeastern U.S. transitioning from agriculture to secondary successional ecosystems, invasive species represent a widespread threat to native species regeneration (Dey et al., 2019). Invasive plant species often share similar characteristics, such as high degrees of plasticity to variable environmental conditions, rapid growth rates, long growing seasons, widespread seed dispersal, prolific seed viability, and secondary compound production. These aggressive traits increase the competitive advantage of invasive species, and can decrease the productivity and regeneration of native species (Mascaro and Schnitzner, 2007; Fridley, 2012; Smith, 2013; Jo et al., 2015; Pinzone et al., 2018). High severity disturbances, such as agriculture (and subsequent abandonment) and clear-cutting, present these species with a “get rich quick” scheme, as the high light and low competition of a disturbed area allows invasive species to explode in growth (Charles-Dominique, 2012, Dey et al., 2019). Increases in invasive species over the past decade have led to reduced carbon sequestration due to the decreased biomass accumulation of invasive shrubs versus native hardwood trees, which can lead to long-term shifts in C and N pools, and changes in nutrient availability (Gordon, 1998; Mascaro and Schnitzner, 2011; Larkin et al., 2014). The proliferation of invasive species can also drive loss of biodiversity and the habitat heterogeneity on which many animal species rely (Whitfield et al., 2014; Schuh and Larsen, 2015; Bartowitz and Orock, 2016). When non-native, potential invasives are introduced to a novel ecosystem, they have escaped their native predators and co-evolved competitors, leaving them to grow, reproduce, and spread unchecked (Callaway and Aschehoug, 2000). These invasive species can then quickly alter aspects of the ecosystem to reduce regeneration of native competitors (Harrington et al., 1989).

When invasive species are established, we often observe monospecific stands rather than a more diverse community of pioneer species, as we would typically see with native regeneration (Kurylo et al., 2007). These monospecific thickets could be the result of various strategies, including high photosynthetic capacity and rapid growth rates, prolific seed dispersal and sustained viability, and/or production of secondary allelopathic compounds (Gill and Marks, 1991; Heneghan, 2006; Rodrigues et al., 2015; Jo et al., 2015; Bartowitz and Orrock, 2016; Guiden and Orrock, 2017; Woodworth et al., 2019). Within the invasive species literature, there are two main theories of successful invasions: the ‘enemy release’ hypothesis and the ‘novel weaponry’ hypothesis. The former involves the escape of non-native species from their native predators and competitors; the latter highlights the use of novel strategies to which native plants have never been exposed and are therefore unequipped to handle (Keane and Crawley, 2002; Callaway and Ridenour, 2004). Allelopathy is a common novel strategy in invasive species, as the native community has not coevolved with the secondary compound production of the invader (Pisula and Meiners, 2010).

Allelopathy describes the process by which plants produce certain secondary chemical compounds, which are not used for normal metabolic pathways or reproduction; they are however used to defend the plant and its surrounding area from predators and/or competitors, essentially creating a hostile or poisonous environment for individuals of other species (Hierro and Callaway, 2003). Using this method combined with other invasive species properties, such as dense canopies (reduction in light resource availability) and longer growing seasons, invaders can dominate entire landscapes (Dey et al., 2019).

Whereas *R. davurica* has not been studied with regard to allelopathic effects, its close invasive relative, *Rhamnus cathartica* (common buckthorn), is known to harbor emodin, which is a secondary compound that can deter the consumption of its berries and leaves by frugivores (e.g. birds), herbivores, and micro-organisms; undigested emodin within the fleshy fruit could also be inhibiting germination of its own seeds (Izhaki, 2002). Whether emodin is causing decreased growth or inhibition of the germination of other plant species is unknown.

Common buckthorn has also been previously studied specifically with regard to allelopathic outcomes (Seltzner and Eddy, 2003; Klionsky et al., 2011; Warren et al., 2017; Pinzone et al., 2018). In common buckthorn, the leaves and berries had their greatest allelopathic effects on meadow rue, Calico aster, large-leaved aster, and alfalfa (Klionosky et al., 2011; Seltzner and Eddy, 2003). Other studies have found common buckthorn to have no effect on the germination of other plant species (Archibold et al., 1997; Warren II et al., 2017). Secondary compounds can also alter microbial and arthropod communities, causing changes to nutrient cycling; by preventing the growth of other plant species, they can lead to lower diversity and leave the community more vulnerable to local extinctions and disruptions to the decomposition food web (Callaway and Aschehoug, 2000; Hierro and Callaway, 2002; Callaway and Ridenour, 2004; Grunzweig et al., 2015).

In 1939, both *Rhamnus cathartica* (common buckthorn), an invasive shrub/small tree from Europe, and *Rhamnus davurica* (Dahurian buckthorn), an invasive shrub/small tree originating from Asia, were introduced to the State Arboretum of Virginia at the Blandy Experimental Farm (BEF) in Boyce, north-central Virginia. Although both buckthorn species were intentionally planted around the same time, Dahurian buckthorn has become the top invader of the landscape.

A few previous buckthorn studies have been carried out at Blandy, and found that areas invaded by buckthorn had increased rates of nitrogen mineralization and nitrification as well as increased inputs from high quality leaf litter compared to areas without buckthorn (Rodrigues et al., 2015). Litter from invasive plant species (*R. davruica* and *L. mackii*) at Blandy had higher nitrogen concentrations than native species litter, which caused an increase in arthropod abundance but with the added effect of diminished litter resources by mid-summer (Woodoworth et al., 2020). In comparison to native species communities, areas with high levels of buckthorn have much lower carbon sequestration capacity, plant species diversity, and arthropod abundance (Heneghan et al., 2002; Mascaro and Schnitzner, 2011; Whitfeld et al., 2014; Larkin et al., 2014; Grunzweig et al., 2015; Schuh and Larsen, 2015). Many invasive species found at the Blandy Experimental Farm exhibit allelopathy, including tree of heaven, garlic mustard, oriental bittersweet, and stilt grass (Pisula and Meiners, 2010). However, it has yet to be determined if *Rhamnus davurica*, (Dahurian buckthorn) has this property.

In this study, I used a greenhouse experiment to observe the germination and growth success of three plant species commonly found at BEF alongside Dahurian buckthorn. I planted the seeds of *Quercus alba* (white oak), *Gleditsia triacanthos* (honey locust), *Fraxinus americana* (white ash), and *Rhamnus davurica* in individual pots with treatments of 1) buckthorn berries and leaves, 2) *Q. alba* leaves, and 3) a control with no leaves or berries. The goal of this study was to determine if Dahurian buckthorn had allelopathic qualities comparable to common buckthorn. These qualities could be contributing to the successful invasion of Dahurian buckthorn in secondary successional fields.

4.2.0 Methods

4.2.1 Question 3: Allelopathy in Dahurian buckthorn

I explored possible allelopathic properties of Dahurian buckthorn using a greenhouse study. I tested allelopathic effects on the germination and growth of four different woody species including: *Quercus alba, Gleditisia triacanthos, Fraxinus americana, and Rhamnus davurica*. The native tree species, *Q. alba, G. triachanthos,* and *F. americana* are present at Blandy Experimental Farm with *Rhamnus davurica*. In the fall of 2020, I ordered the acorns, pods, and seeds of the three native species from Sheffield's Seeds (Locke, NY). I collected *R. davurica* berries in October of 2020, as these are not sold online. Each seed was stratified using their known stratification time and temperature (Table 1), except for *G. triacanthos* which was scarified with concentrated sulfuric acid for two hours prior to planting.

After stratification/scarification, I planted the seeds in a 50/50 ratio of potting soil and high clay soil and maintained field capacity soil moisture conditions, and used average greenhouse light conditions. Clay was used in the soil to replicate the clay soils at Blandy. There were three treatment types: 1) soils mixed with *R. davurica* leaves and berries, 2) soils mixed with *Q. Alba* litter, and 3) control soils. For the *R. davurica* treated soils, I used allotted weights of macerated leaves and berries (0.24 g of crushed leaves per 1 g of soil, and 1 g of macerated berries per 1 g of soil) mixed in with sterile greenhouse soil. For the *Q. alba* treated soil, I used the same leaf to soil ratio as the buckthorn treatment. For each of the four target tree/shrub species, I had eight replicates for each treatment type for a total of 96 pots.

After 12 weeks, the seeds were checked for germination, and seedlings were measured for height, dried, and then weighed for dry biomass.

Table 1. Woody species seed stratification time and temperature

	Rhamnus davurica	*Fraxinus americana*	*Quercus alba*
Stratification Time	45 days	90 days	Not needed
Temperature	2-3°C	~23°C for 30 days and 2-3°C for 60 days	Not needed

4.2.2 Statistical Analyses

For this study, I used a mixed model ANOVA with independent variables of soil treatment and species type with the dependent variables of height of seedling and dry weight of seedling. All statistics were done in the statistical program R (R Core Team (2021).

4.3.0 Question 3: Results

In the greenhouse, the stratified or scarified seeds were left to germinate and grow for 12 weeks. At the end of the 12 weeks, no white ash seeds in the buckthorn treated soils germinated, 37.5% of the white oak seeds germinated, 62.5% of the Dahurian buckthorn seeds germinated, and 100% of honey locust seeds germinated (Table 2). Honey locust dry biomass was significantly different among the three treatments ($p < 0.001$, $F_{[2, 21]} = 35.48$) (Figure 1a). The mean biomass of honey locust was greatest in the control group (1.724 g ±0.148 g) followed by the white oak treatment (1.140 g ±0.175 g) and the buckthorn treatment (0.157 g ±0.023 g). Buckthorn itself had significantly lower biomass in the buckthorn treatment (0.00875 g ±0.005

g, p = 0.001) than both white oak and control, which were not significantly different from one another (0.663 g ±0.112 g and 0.609 g ±0.138, respectively) (Figure 1b). White oak seedlings had significantly lower biomass in the buckthorn treatment (0.884 g ±0.432 g, $p < 0.001$) and the greatest biomass in the control treatment (2.975 g ±0.164 g), although there were no significant differences between the white oak treatment and the control treatment (Figure 1c). White ash had no germination in the treatment with buckthorn leaves and berries but had greater biomass in the control than the white oak treatment, although these were not significantly different (1.014 g ±0.157 g and 0.554 g ±0.102 g) (Figure 1d).

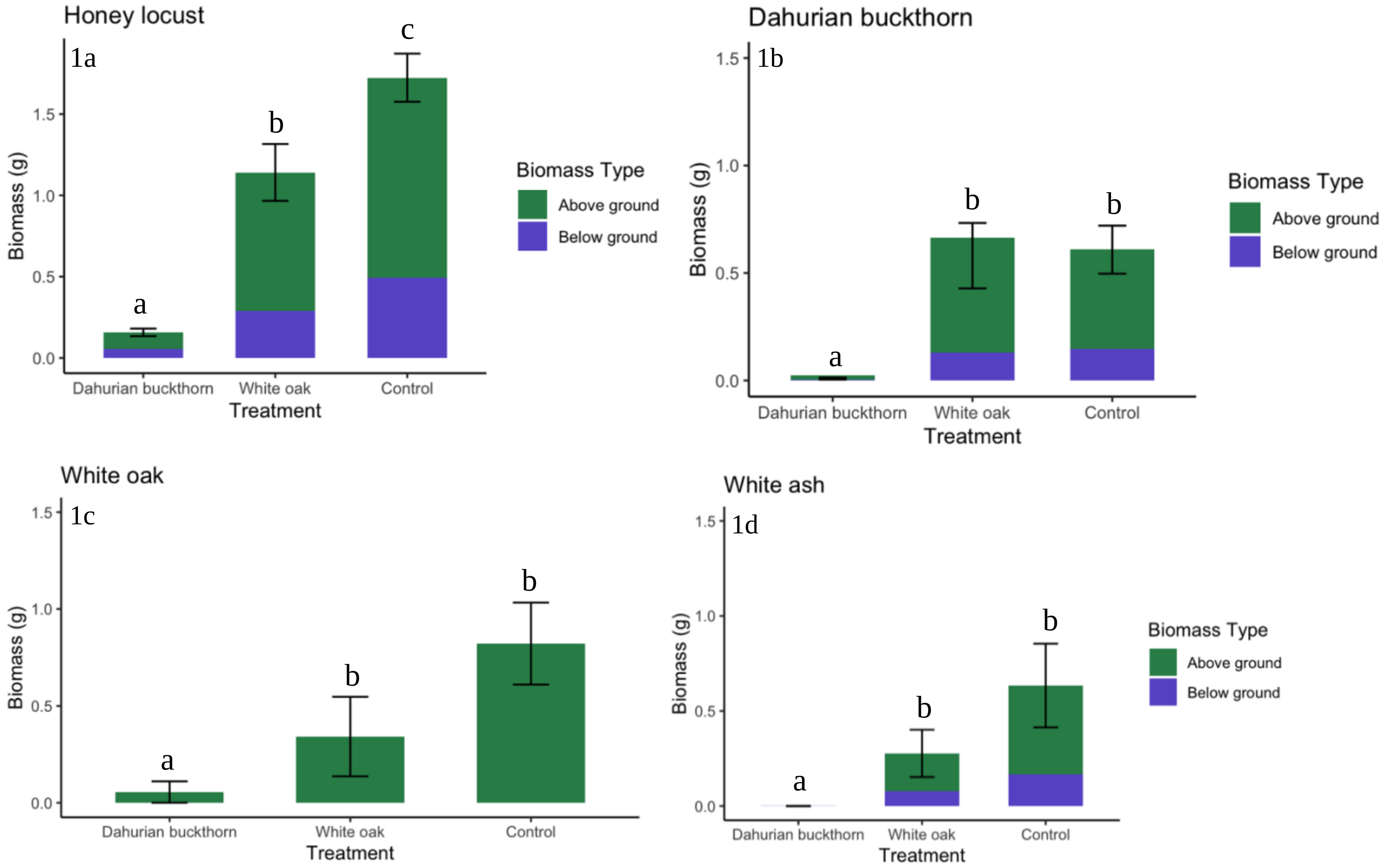


Figure 1) The mean biomass (g) of four different seedlings (honey locust, Dahurian buckthorn, white oak, and white ash, panels a-d respectively) after 12 weeks of growing in the greenhouse. Eight of each species were planted in three different treatments, including Dahurian buckthorn treated soils (0.24 g of crushed leaves per 1 g of soil, and 1 g of macerated berries per 1 g of soil), white oak treated soil (0.24 g of crushed leaves per 1 g of soil), and a control with no

additives. The green portion of the bar shows the aboveground biomass and the blue color shows the belowground biomass. For the white oak, only the aboveground biomass is shown due to the relatively large belowground mass of the acorn. Lower case letters represent significant differences ($\alpha = 0.05$). Error bars represent ± one standard error.

Table 2. Number of seeds germinated after 12 weeks and number of seedlings alive after 12 weeks (each species started out with eight plants in each treatment type with a total of 24 of each species)

	Honey locust	Dahurian buckthorn	White oak	White ash
# germinated in buckthorn	8	5	3	0
# germinated in white oak	8	8	8	4
# germinated in control	8	8	7	4
# alive in buckthorn	6	2	3	0
# alive in white oak	8	8	8	4
# alive in control	8	8	7	4

Honey locust had significantly different heights across all three treatments; mean honey locust height in the buckthorn treatment (3.275 cm ±1.10 cm) was significantly lower than both the white oak treatment (8.900 cm ±1.190) and the control (11.375 cm ±1.373 cm) ($p < 0.001$, $F_{[2, 21]} = 74.09$) (Figure 2). Dahurian buckthorn seedlings were significantly shorter in the buckthorn treatment (0.733 cm ±0.342 cm) than both the white oak (6.029 cm ±2.171 cm) and control (5.313 cm ±1.729 cm) treatments ($p = 0.005$, $F_{[2, 15]} = 7.654$) (Figure 2). In white ash and white oak, the buckthorn treated seedlings had significantly lower heights than the control treatment ($p = 0.0234$, $F_{[2, 21]} = 4.563$, $p = 0.011$, $F_{[2, 21]} = 5.391$, Figure 2).

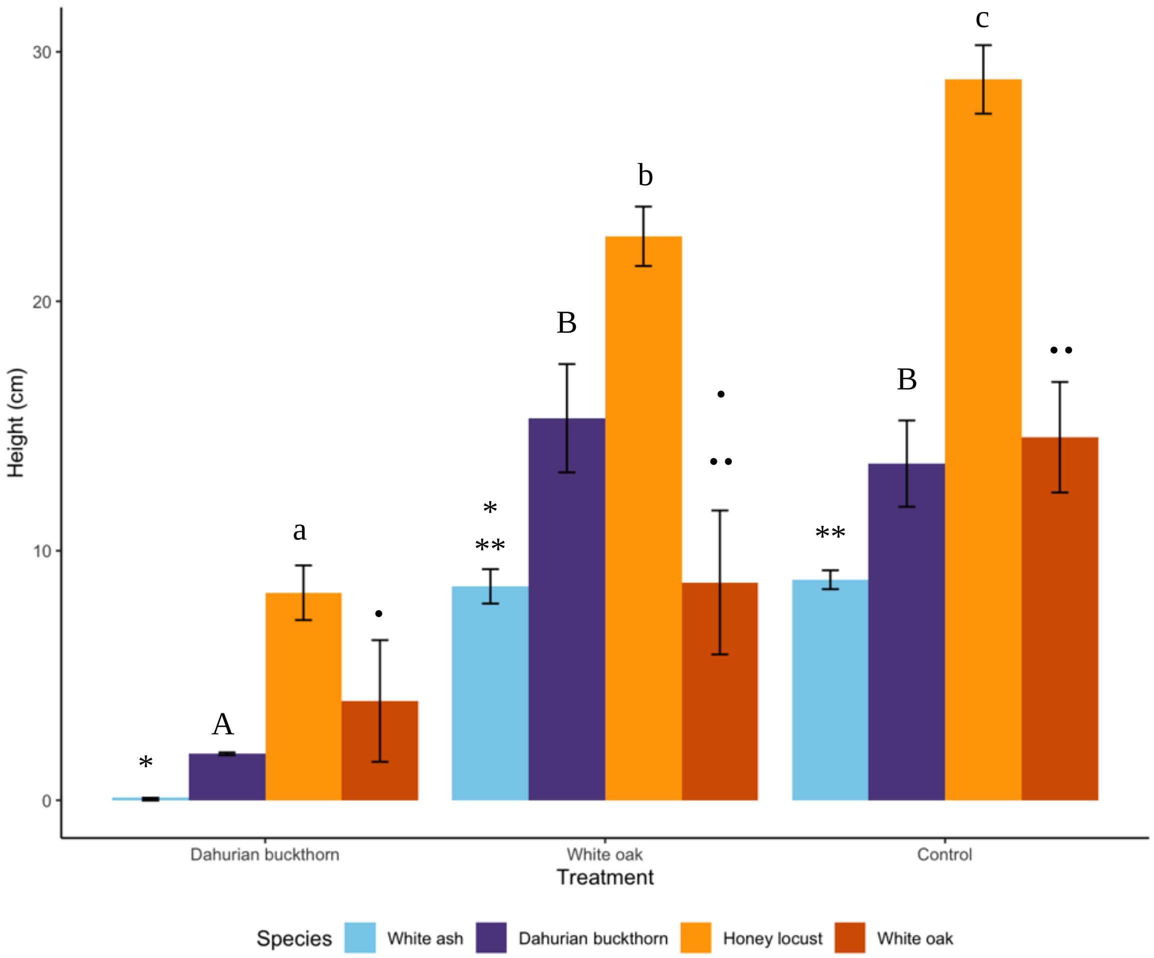


Figure 2) The average height (cm) of all four species (white ash, Dahurian buckthorn, honey locust, and white oak) after 12 weeks of growth in the greenhouse within three different treatments; Dahurian buckthorn treated soils (0.24 g of crushed leaves per 1 g of soil, and 1 g of macerated berries per 1 g of soil), white oak treated soil (0.24 g of crushed leaves per 1 g of soil), and a control with no additives. The uppercase letters signify significance among buckthorn seedlings in the three different treatments, the lower case letters indicate significance among honey locust seedlings in all three treatments, asterisks indicate significance among the white ash seedlings, and the dots signify significance of white oak between all 3 treatments. Error bars represent standard error.

4.4.0 Question 3: Discussion

In the greenhouse experiment, I saw strong evidence of allelopathy in Dahurian buckthorn. This is the first study to test the allelopathic effects of Dahurian buckthorn, rather than *Rhamnus cathartica* (common buckthorn) (Seltzner and Eddy, 2003; Klionsky et al., 2011; Warren et al., 2017). All four species (honey locust, white oak, white ash, and Dahurian buckthorn) had much lower success in the soils with high amounts of buckthorn litter (Figures 1,2). White oak and white ash had 37.5% and 0%, respectively, successful germinations in buckthorn treated soils; buckthorn fared better with 62.5% germinations in buckthorn soil, and honey locust had 100% seed germination in the buckthorn soil. In the control, 100% of the honey locust and buckthorn germinated, 87.5% of the white oak germinated, and 50% of the white ash germinated (Table 2). At the end of the 12-week study, 75% honey locust seedlings, 25% buckthorn seedlings, and 37.5% white oak seedlings were alive of the initial seeded sample; however, all were stunted in growth compared to the control treatments (Table 2, Figures 1,2).

Common buckthorn litter can inhibit germination in some species and hinder growth and survival post-germination in other species (Seltzner and Eddy, 2003; Klionsky et al., 2011; Pinzone et al., 2918). In other *Rhamnus* spp., emodin has been studied as the main secondary metabolite found in their tissues (Izhaki, 2002). Emodin has been noted to elicit numerous effects, such as acting as an herbivore deterrent (insect, mammal, bird), disrupting the microbial community, creating toxic soil conditions for other species, and inhibiting germination and growth of plant species (Izhaki, 2002).

The severity of common buckthorn leaf and berry allelopathy increased as the dosage was increased (Seltzner and Eddy, 2003). For this study I used realistically high doses of buckthorn leaves and berries to test for allelopathy. Other studies have found that common buckthorn had the highest allelopathic effect in their leaves and drupes, which were the only plant tissues used in my study (Seltzner and Eddy, 2003; Klionsky et al., 2011; Warren et al., 2017). The root tissue of common buckthorn species has been observed to have possible allelopathic effects as well, but this is debated in the literature (Warren et al., 2017). After 12 weeks of growth, even the seedlings that survived the buckthorn treatment were stunted and yellow with significant effects on honey locust and buckthorn itself (Figure 3).


1


2


3


4

Figure 3.) Seedlings after 12 weeks of growth: 1. Honey locust, 2. White oak, 3. Dahurian buckthorn, 4. White ash. The red circle outlines buckthorn treatment pots, the blue circle outlines white oak treated pots, and the yellow circle outlines the control pots.

White oak litter in the soil had a slight negative effect on biomass in honey locust, white oak, and white ash. This effect could have been due to crowding or lesser known effects of white oak litter (Klionsky et al., 2011). Dahurian buckthorn even had negative impacts on its own germination and growth, which is consistent with common buckthorn (Izhaki, 2002; Seltzner and Eddy, 2003; Klionsky et al., 2011; Warren et al., 2017). Seeds often need to be freed from the flesh of their berry through frugivore digestion or berry decomposition in order to germinate; when the soil has high quantities of buckthorn berries, it may be causing a germination inhibition effect on itself (Knight et al., 2007; Gourley, 1985; Archibold et al., 1997). At BEF, directly under large canopies of parent trees, there are high quantities of buckthorn berries dropped, however not many reach the seedling state. This inhibition could be adaptive to avoid competition with parent trees and other seedlings (Cipollini and Levey, 1997a). This habit further contributes to the spreading behavior of the shrub on thicket edges (Qaderi et al., 2009; Schulte et al., 2011; Kalkman et al., 2019).

In-situ studies have found similar allelopathic effects of common buckthorn; high levels of buckthorn decreased survival, flowering, and growth of four native forbs (Klionsky et al., 2011). Warren et al. (2017) tested the effect of low light conditions under buckthorn canopies to determine if that was contributing to seedling mortality; they found that light level alone was not depressing regeneration and concluded that allelopathy was likely a contributing factor (Warren et al., 2017). Buckthorn could also be disturbing the soil by altering the symbiont microbial community on which the native species rely (Rodrigues et al., 2015; Kuebbing et al., 2015); buckthorn can change the microclimate, and alter nutrient cycling regimes upon which arthropods and microbes depend (see Section 3). While I did not study changes in the microbial community, other studies have found differences between invaded and uninvaded sites (Callaway and Ridenour, 2004; Rodrigues et al., 2015; Pinzone et al., 2018).

Allelopathy is not an uncommon tool used by invasive species to outcompete native species. In their native ranges, potential invaders have evolved alongside other species so their secondary compounds are usually less potent and better adapted to by competitors and predators; however. when introduced to novel systems, these compounds can have severe consequences (Callaway and Ridenour, 2004). Both the enemy release hypothesis (Keane and Crawley, 2002) and novel weaponry hypothesis (Callaway and Ridenour, 2004) play a big role in buckthorn invasibility (Klionsky et al., 2011). Some traits that facilitate the success of Dahurian and common buckthorn as invaders are: not being a preferred browse for most herbivores; not having any known pests or strong competitors outside of their native ranges; having extended growing seasons; and wielding allelopathy as an advantage to develop into monocultured thickets (Klionsky et al., 2011; Fridley, 2012). *Rhamnus* spp. are not the only species at BEF that utilize secondary compounds; tree of heaven (*Ailanthus altissima*) has known allelopathy, as does oriental bittersweet (*Celastrus orbiculatus*) and even Japanese stilt grass (*Microstegium vimineum*) (Pisula and Meiners, 2010). Allelopathic qualities are just one of the many traits propelling invasive species forwards; it is a difficult trait to disentangle without fully analyzing the compounds utilized and carrying out various field and lab experiments. I aimed to establish a foundation for the presence of allelopathic properties in Dahurian buckthorn; thus, I did not apply different dosage amounts or conduct any field experiments. From my results, it can be strongly suggested that Dahurian buckthorn does inhibit germination and growth in some native tree species.

Future studies that would further the research on allelopathy in buckthorn could look at secondary compounds found in soils directly under fruiting buckthorn, conduct a full analysis of the buckthorn metabolites, and determine if there are any seasonal variations in allelopathy. It would also be helpful to determine if other invasive species are as affected by buckthorn tissues as the native plants; at BEF, buckthorn is often found growing with autumn olive, bush honeysuckle, and oriental bittersweet. Other speculative questions are: What is the energy/productivity trade-off of constructing these secondary compounds? Are the allelopathic effects prominent before a monospecific thicket of buckthorn is present, or do they only become important once the species is abundant and is dropping large amounts of leaves and berries?

Rhamnus spp., among other plant species, exhibit allelopathic qualities that hinder germination or growth and survival of competitor species. Novel allelopathy, without parallel adaptation, can change the composition of a landscape and in turn its functionality and services provided.

5.0.0 Conclusions

Dahurian buckthorn is rapidly changing the process of secondary succession at Blandy Experimental Farm. It has already altered soil temperature and soil moisture significantly, impacting microclimate and seedling establishment. The low light levels at the ground continue to hinder the regeneration of any plants underneath the dense buckthorn-dominant canopies. These canopies have high quality leaf litter that rapidly decomposes causing a boom-bust effect in the arthropod and microbial communities, while decreasing soil nitrogen available to other plants (Corbin and D’Antonio, 2004; Schuh and Larsen, 2015; Kuebbing et al., 2015; Woodworth et al., 2020). Both common buckthorn and Dahurian buckthorn were introduced to

Blandy Experimental Farm around the same time in the mid to late 1930s, but Dahurian buckthorn has spread and dominated the landscape.

Dahurian buckthorn is an excellent example of both the enemy release hypothesis and the novel weaponry hypothesis (Keane and Crawley, 2002; Callaway and Ridenour, 2004). Dahurian buckthorn is not a preferred browse for herbivores (Kurylo et al., 2007), has no known pests in the U.S., and possesses the novel weaponry (allelopathy) observed in the greenhouse portion of my study; the shrub is an important and formidable invasive species. We have observed shifts in nitrogen cycling, changes in soil properties, and reductions in light resource levels; buckthorn is able to dominate early regenerating fields, and so far succession has been arrested in a state of monoculture buckthorn thickets. This shrub is just one of the many invaders that have appeared, as international transportation has become affordable and easy. Invasive species are having large effects on whole ecosystems; they are an ever-present threat that has increased in prevalence over the last 50 years. Invasive species have the second largest impact on biodiversity loss across ecosystems, second only to habitat loss and fragmentation (Vitousek et al., 1996). When healthy ecosystems are degraded and fragmented, there are more opportunities for exotic species to establish and then outcompete native species, changing the trajectory of a landscape. Studying the impacts of invasive species and the effectiveness of mitigation efforts on ecosystems and their services offer support for the prevention of degradation and restoration of these affected areas.

Landscapes taken over by common buckthorn have a noticeable drop in carbon sequestration, adding another layer to how invaders contribute to climate change (Mascaro and Schnitzer, 2011); however, this is a disputed finding, as some studies have seen an increase in primary productivity with invasive species (Ehrenfeld, 2003). These mixed findings offer another reason that invasive species and their effects on climate change need to be explored further. Invasive species can have easily-observed, shorter-term, direct impacts on ecosystems. In the long-term, invaders could be altering carbon sequestration and nutrient pools, resulting in large impacts on primary production and ecosystem carbon storage (Peltzer et al., 2010).

5.1.0 Possible management of the invasive shrub

Some studies have found negative legacy effects with invasive species even post removal, but there have also been studies that show restoring a once-invaded buckthorn area can lead to high biodiversity and carbon storage (Larkin et al., 2014). For buckthorn specifically, there could be value in cutting and treating stumps or treating flowering branches with herbicide (Pijut and Juzwik, 1992; Delanoy and Archibold, 2007), preventing buckthorn invasion by reducing optimal growing conditions (McCay and McCay, 2008; Wragg et al., 2021), and utilizing tilling or frequent disturbances can decrease buckthorn seedling populations (Iannone et al., 2013). One interesting finding has been the reproductive ratio of Dahurian buckthorn; buckthorn populations in northeastern China were female biased, and fruit set was significantly impacted by the distance to male trees and pollen availability. By only treating the fewer male trees with herbicide, it could decrease the costs and increase the efficiency of buckthorn control in already invaded landscapes (Knight et al., 2007; Wang et al., 2013). On a global scale the management and restoration of biodiverse native forests and landscapes can actually keep out a lot of invaders simply by being resistant and/or resilient (Kennedy et al., 2002; McCay and McCay 2008; Whitfield et al. 2014). The real issues lie in the fragmented landscapes that are easily overtaken by invaders; if we can efficiently manage these systems for natural regeneration and restoration, we can start on a path to mitigate invasive species driven changes (Vitousek et al., 2008).

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