Biotic Interaction of Invasive, Early-Succession Trees and Their Effects on Community Diversity: a Multi-Scale Study Using the Exotic Invasive Ailanthus altissima and the Native Robinia pseudoacacia in the Mid-Appalachian Forest of Eastern United States

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Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY IN BIOLOGICAL SCIENCES

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JANUARY 30, 2015
BLACKSBURG, VIRGINIA

KEYWORDS: COMPETITION, FACILITATION, INVASIVE SPECIES, INTERSPECIFIC COMPETITION, INTRASPECIFIC COMPETITION, BELOWGROUND, COMPETITION FOR NUTRIENTS, DIMENSION ANALYSIS, ADDITIVE-REPLACEMENT SERIES, REPLACEMENT SERIES, NODULATION, ALLELOPATHY, RAPID EVOLUTION, COMMUNITY STRUCTURE, DIVERSITY, COMMUNITY EVENNESS, VEGETATION SURVEY, CANOPY TREE, REGENERATION, COMMUNITY ORDINATION, NONMETRIC MULTIDIMENSIONAL SCALING, GEOGRAPHIC SCALE, SOIL NITROGEN, SPECIES DISTRIBUTION, FOREST INVENTORY AND ANALYSIS PROGRAM, SPATIAL ASSOCIATION, BASAL AREA
Biotic Interaction of Invasive, Early-Succession Trees and Their Effects on Community Diversity: a Multi-Scale Study Using the Exotic Invasive *Ailanthus altissima* and the Native *Robinia pseudoacacia* in the Mid-Appalachian Forest of Eastern United States

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ABSTRACT

Invasive plants can displace native species, deteriorate native forest, and change plant communities and ecosystem functions. Native plant populations are fundamentally impacted by invasive species because of the interactions between invasive species and native plants. This study focuses on understanding the extent, mechanisms and consequences of interaction between a non-indigenous invader *Ailanthus altissima* and its functionally similar native species *Robinia pseudoacacia* in the Mid-Appalachian region, from an individual scale to a regional scale. These two subject species are common and coexist in early-successional eastern deciduous forest. The interactions between these two common species are important to community structure and canopy tree regeneration.

To address the type and extent of interactions of these two species, a greenhouse experiment utilizing various species proportions, nutrient levels and seed sources was performed. In addition, a common-garden experiment with various species densities and proportions over three consecutive growing seasons was performed in a more natural condition than that of the greenhouse experiment. We found at the seedling stage, the dominant interaction was competition, and *R. pseudoacacia* was the winner both above- and belowground. The
allelopathic compounds of *A. altissima* may have inhibited nodulation of *R. pseudoacacia*. *Ailanthus altissima* seedlings from its native region had slightly stronger competitive abilities compared with the seedlings from its invaded range. In the common garden experiment, *R. pseudoacacia* plants grew quicker than *A. altissima*, but *A. altissima* inhibited the growth of *R. pseudoacacia* by interspecific competition. The negative impact of *A. altissima* on *R. pseudoacacia* became larger as time progressed.

To assess the community-level consequences of the two species, we conducted a forest mapping and a complete target-tree-based forest survey, and analyzed regional-scale data from the Forest Inventory Analysis Data Base. The two target species were significantly associated with themselves and with each other. Community species composition and diversity were significantly different across sites. A negative impact of both species on the understory community diversity and tree regeneration at the neighborhood scale was detected; while at a regional level, tree diversity in the FIA plots with either *A. altissima* or *R. pseudoacacia* was higher than the reference plots.
ACKNOWLEDGEMENTS

First, I want to thank Dr. Erik Nilsen, my major advisor, for his tremendous help during the four and half years of my graduate studies. His wisdom, patience, kindness, and encouragement have been the most important support for me in graduate school, and inspired me in many ways. I appreciate not only the freedom he has given to me and made me to be an independent researcher, but also the timely, detailed advice and help he has given when I needed them. He evolved in the design and conduct of experiments, and the writing for each chapter. His support was extremely important during the last year of my graduate school when my life went through tremendous changes. Besides academic advice, I benefit tremendously from his noble character, and will cherish that in my future journey.

I thank my committee: Drs. Robert Jones, Lisa Belden, and Jacob Barney. Their critical questioning, generous suggestions and support, were very important for my research. I am thankful for their time and patience attending committee meetings, as well as reading and commenting on this dissertation. A special thanks to Dr. Robert Jones who had to travel back for these events and always gave me warm encouragement, thoughtful support, and insightful suggestions. Also, I thank Drs. Belden and Barney for your unique contributions to my intellectual development in the classroom.

I thank Drs. David E. Carr, T’ai Roulston, Kyle Haynes and the kind staff at Blandy Experimental Farm for sponsoring part of my research, providing field sites and resources and generous assistance. David Carr served as an adviser while I was at my field sites in the summer of 2011-2013. His suggestions and connections, and his knowledge about the farm, the region, plants and nature in general, were critical for the accomplishment of my field-work. Ground workers, staff, researchers and students I encountered at Blandy were all truly amazing people,
who made a fun and productive field season memory.

I thank my collaborator Dr. Cynthia D. Huebner in USDA forest service, northern research station, for sponsoring a large amount of my work. Cynthia conducted the target-tree-based field survey at two WV sites in summer 2013, and helped with the design and data analysis of this part of study. My gratitude also goes to Dr. William McShea at Smithsonian Conservation Biology Center and staff there to give access and help me locate field site there. Special thanks to the generous lady Alison Teetor at Clark County, who let me use her private property for the forest mapping study.

I want to give my sincere thanks to field assistants that helped with experiment plot set up, maintenance, and field survey in northern Virginia, and the greenhouse and lab assistants at Virginia Tech: Adam Ovens, Jackson Mitchell, Chris Brassell, Carl Wepking, Nigel Temple, Tere Williams, Nicholas Smith and Shengchen Su. Thanks to Austin Jackson for continuously dedicating his time for the long hours of hard lab and field work. My research would not be accomplished on time without the hard work of all of you.

Thank Tom Wieboldt from department of biology at VT for his tremendous help with plant identification. Thanks to Kai Zhu from Duke University for providing FIA data. Thanks to my biology and statistics colleagues at VT, Tatpong Tulyananda, Jingjing Liu, Matt Dittler, Angang Zhang for their friendship, and inspiring discussions and debates. Finally, I would like to thank my family for unconditional love, support, and encouragement throughout my graduate school. I am especially thankful for Rui Sun, who I met at Virginia Tech, and became my husband. He was the best unpaid field/lab assistant, data typist, and scientific topic debater I have ever met in graduate school. Thank you for being an amazing company, and lightening my life.
ATTRIBUTIONS

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Biological Sciences at Virginia Tech. Dr. Nilsen assisted with the experimental design and handling samples, will be a co-author on this manuscript when it is submitted.

Chapter 5- Distribution patterns of invasive species *Ailanthus altissima* and native *Robinia pseudoacacia* in Appalachian Mountain forests using Forest Inventory and Analysis plots: 1) Angang Zhang, is currently a PhD student in Department of Statistics, who helped with statistical analysis and will be a co-author on this manuscript when it is submitted. 2) Erik T. Nilsen, PhD, is currently a Professor of Plant Ecology in the Department of Biological Sciences at Virginia Tech. Dr. Nilsen assisted with the data analysis, will be a co-author on this manuscript when it is submitted.
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CHAPTER 1

Literature Review and Introduction

1 Plant competition and coexistence

Competition is a mutually harmful interaction between two or more organisms when there is at least one shared limiting resource (Begon et al. 2006). Plants compete with each other for resources such as light, water, nutrients, and physical space. Plant competition can happen both aboveground and belowground, and for more than one resource at the same time (Booth et al. 2011).

1.1 Direct and indirect interference

Competition is traditionally divided into two broad categories by mechanism: direct interference and indirect exploitation of shared resources. Direct interference includes a species harming its neighbor in various ways such as toxic substances (Muller et al. 1968), direct overgrowth (Connell 1990) and mechanical abrasion (Rebertus 1988). Indirect interaction occurs via a shared resource as an intermediate, or occurs between two prey having a same predator (Holt 1977, Menge 1995). Indirect is usually tested by removal experiments in the field (Tilman 1984), but this kind of experiment may also have impacts on other interactions (i.e. with microorganisms and herbivores).

Connell (1990) included a category called apparent competition, including indirect interaction via a shared enemy, and via other species on the same trophic level (Connell 1990). Since few studies of competition have demonstrated the mechanisms underlying the interaction, it is quite possible that some cases of competition may be apparent, not real competition (Tilman 1987).
1.2 Interspecific competition and intraspecific competition

Intraspecific competition occurs between individuals of the same species when they vie for the same resources. Individuals of the same plant species, which grow close enough, will compete with each other for light, water, nutrients, space and pollinators. Individual plants growing by themselves usually perform better than when grown in a mixture. Plants may respond to intraspecific competition by growing taller with deeper root systems (Casper and Jackson 1997, Stoll and Prati 2001).

Interspecific competition occurs between individuals of two different species in the same area. If the amount of available resource cannot support populations of both species, then competition may result in mutually negative effects on both species. Interspecific competition has the potential to alter populations, communities and the evolution of the interacting species (Goldberg and Barton 1992, Aerts 1999). Competition has been observed between individuals, populations and species. Interspecific competition has been viewed as a powerful evolutionary force and species sharing ‘too similar’ resource requirements cannot coexist over long periods of time (Schoener 1982).

1.3 Impact of mycorrhizae, nitrogen-fixing bacteria, parasites and herbivores on plant competition

Communities and ecosystems consist of species at different trophic levels that interact through competition, predation, pathogenicity, and mutualism. Interactions of plants and plant communities may be changed by the actions of herbivores (Bentley and Whittaker 1979, Bardgett et al. 1998, Carson and Root 2000) and microorganisms (Fitter 1977, Burdon et al. 1984, Packer and Clay 2000).

1.3.1 Mycorrhizae and nitrogen-fixing bacteria
Mycorrhizal fungi have numerous physiological effects on individual plants, which are important to community processes such as competition and ecosystem processes (Harley and Smith 1983, Bever et al. 2010, Philippot et al. 2013). There are three major groups of mycorrhizae discussed here: arbuscular mycorrhizae, ectomycorrhizae, and ericoid mycorrhizae. Though some of the mechanisms differ among the three groups, all of them could increase water and nutrient uptake, increase drought stress tolerance, and increase the rate of growth and total annual production (Chapin III 1980, Koide 1991, Schützendübel and Polle 2002). In order to change the competitive balance between neighboring plants, mycorrhizae must have different physiological effects on the different plants. Mycorrhizal-mediated competition may be more important in some biomes than others, and the degree of mycotrophy of plants in each biome changes with successional stages and environmental conditions (Allen and Allen 1989). For instance, endomycorrhizal colonization of Ailanthus altissima seedlings from a non-forested site (65.2%) was significantly greater than that of the seedlings from the forested site (37.9%) (Huebner et al. 2007). Plants are less dependent on mycorrhizal fungi during early succession even though myorrhization is higher. Nutrient sharing has been demonstrated for plants of the same and different species when they are connected by mycorrhizal hyphae and hyphal nutrient transport may be important when there is a significant nutrient gradient between those plants (Allen and Allen 1989).

In a nutrient-limited ecosystem, resource availability could be a primary factor determining competition and invasion success. Nitrogen fixing plant species, primarily legumes, could influence local nutrient pools, benefitting native species diversity, but also invasion by other exotic species. Higher establishment and growth rates, in areas with higher nutrition, may give the non-native a greater advantage in competition (Goergen 2009). Inversely, competition...
may influence nodule numbers, size and acetylene reduction activity per nodule weight
(Kitamura et al. 1981).

1.3.2 Parasitism

Plants infected by parasitic fungi or other pathogens are weakened compared with uninfected plants, and have reduced growth and reduced competitive ability (Clay 1990, Carson and Root 2000). The effect of parasites could change competitive interactions. For instance, in mixed plantation of barley and wheat, barley had a competitive advantage and tended to exclude wheat, but if powdery mildew was present, the two crops tended to coexist (Burdon and Chilvers 1977). A shared parasite can cause “apparent competition” between hosts even though no direct competition occurs. In a plant community, the infected plant species might be gradually replaced by uninfected species with similar niche over time (Clay 1990).

1.3.3 Herbivory

Herbivores can affect plant abundance and distribution by consuming plant living parts selectively. Herbivory could mediate the results of plant competitive interaction: 1) by changing a plant’s relative ability to acquire limited resources, or 2) by decreasing growth and fecundity, even eliminating one of the plant competitors (Louda et al. 1990). Experimental studies show that, in general, herbivore consumption is variable in both space and time, and among species (Crawley 1983); herbivory could change plant abundance or modify distribution of some species (Parker and Root 1981), thereby changing resources for competitors. However, very few studies has evaluated explicitly the effects of herbivory and competition separately. The impact of herbivory depends on the density of insects, response and compensation of plants and populations, and resource availability in the site (Louda et al. 1990).

2 Invasive ecology theories
2.1 Definition of invasive species and how introduced species become invasive

The invasion of exotic plant and animal species into regions that were previously separated by biogeographic barriers is one key problem of land use and cover change (D’Antonio and Vitousek 1992). Frequently, plant species are introduced into new areas because of their economic value as crop species, timber trees, forage plants, or for ornamental use and environmental protection (Heywood 1989). One commonly used definition of an invader is a species that has colonized, successfully persisted, and spread into ecosystems in which it did not exist before (Mooney and Drake 1989, Reichard and Hamilton 1997). The federal definition of invasive species is an exotic species that has successfully naturalized into introduced areas and causes harm to the environment, the economy or human health (Federal Executive Order 13112).

Only a small fraction of alien species have become invasive. The process of invasion appears to occur in stages. Some researchers have characterized four stages of invasion: arrival, escape and establishment, naturalization and spread, and achieving pest or weed status (Williamson and Fitter 1996); some have defined the stages as: arrival, establishment, spread and adjustment (Reise et al. 2006). Plant invasion in particular, is suggested to be a three step process:

1) Introduction; as a result of dispersal, propagules arrive at a site beyond their previous geographical range and establish populations of adult plants; 2) Colonization; the plants in the founding population reproduce and increase in number to form a colony that is self-perpetuating; and 3) Naturalization; the species establishes new self-perpetuating populations, that undergo widespread dispersal and become incorporated within the resident flora (Richardson et al. 2000).

The success of invasive species depends on the traits of the introduced plants, the nature of the habitat at the time of invasion, and the relationships with other species after the first establishment. If a species has been observed to invade some regions in the world, there is a high
probability that it will be an invasive plant somewhere as well (Reichard & Hamilton 1997).

According to a comparative plant study, invasive plants exhibit characteristics of R-selected plants with short juvenile periods, short intervals between large seed crops, and small seed masses. These qualities allow an invasive plant to reproduce quickly and consistently (Rejmanek and Richardson 1996). Invasive plants have also been suggested to have “general purpose genotypes”, which is one genotype exhibits an ability to produce different phenotypes in order to adapt many different conditions. The large phenotypic plasticity is thought to be necessary for successful establishment of invasive species in a variety of environmental condition (Baker 1965). One study found that of a list of invasive and noninvasive species, 86% of the invasive species could be identified from these traits (i.e., R-selected and phenotype plasticity) alone (Kolar and Lodge 2001). However, another study found that invasive species tended only to have a small subset of the suggested invasive traits, and that many of these invasive traits were found in noninvasive species as well, indicating that invasiveness involves complex interactions not easily categorized (Thébaud et al. 1996, Reichard and Hamilton 1997)

Some argued that environmental conditions of the habitat in which certain alien species arrive also determine the results of whether the introduced species becomes an invader or not. Charles S. Elton argued ecosystems with higher species diversity were less subject to invasive species because of fewer available niches (Elton 1958). Since then, other ecologists have argued ecosystems with high species diversity seem to be heavily invaded and more susceptible to invasion (Stohlgren et al. 1999). This debate seems largely dependent on the spatial scale at which invasion studies are performed. Small-scale studies tend to show a negative relationship between native diversity and invasion success, while large-scale studies tend to show a positive relationship (Byers and Noonburg 2003). The latter result may be an artifact of non-native
species capitalizing on increased resource availability and weaker overall species interactions
when larger samples are considered (Levine 2000). Also, some researchers tried to adapt species-
area relationships in island biogeography theory into invasive ecology. New colonists should
decline as the number of species already in the patch increase; and exotic species should increase
with area. But previous evidence indicts that the fraction of exotic species actually declines with
area (Lonsdale 1999).

2.2 Hypotheses to explain invasive success

Despite substantial research, little is known about why some species can dominate new
habitats and displace native plants. Here I include several theories about how introduced alien
species become invasive or fail to become invasive that are related to my research.

The Biotic Resistance Hypothesis (BRH) states that species-rich communities are more
resistant to invasion because available resources in these types of communities are less than in
low-richness communities (Levine et al. 2004). But biotic resistance has been found to be scale-
dependent, with the native and exotic species diversity often negatively correlated at small-scales
but positively related at large-scales (Byers and Noonburg 2003).

The Fluctuating Resource hypothesis (FRH) purports that resource fluctuations promote
invasions by creating resource openings for new species, or reduces competition from residents
at certain times. Land disturbances (typically generated by humans) cause the environment to
become more susceptible to invasion due to the immediate fluctuation of resources following a
disturbance. The sharp change in available resources such as water, light, and available space can
open an “opportunity window” for non-native species and facilitate an exotic plant invasion
(Davis et al. 2000). Other resource increase such as nitrogen addition also have been shown to
increase the success of invaders in California prairie (Maron and Connors 1996).
The Superior Competitor Hypothesis (SCH) explains invasive success based on the resource capture ability of individual species in competition with other species. Tilman (Tilman 1982) suggested that an invader could establish if it was more efficient on obtaining resources \((R^*)\) than native species and thereby result in competitive exclusion of less-fit residents that have higher \(R^*\). Shea and Chesson re-discussed Tilman’s \(R^*\) theory and proposed that invasion will be favored if a resident species’ \(R^*\) is greater than an invader’s (Shea and Chesson 2002). SCH may still need to combine with other theories to explain the mechanism by which an exotic species becomes a superior competitor.

The natural enemies of invaders (i.e. parasite, pathogens, herbivores or predators), may be reduced in the introduced region, giving the non-indigenous species an advantage compared to its native range. Kean and Crawley suggested that Enemy Release Hypothesis (ERH) could account for the success of an invader if specialist enemies are absent in the introduced range and if generalist enemies present in that range have a greater impact on native hosts (Keane and Crawley 2002). However, observations of the level of attack from herbivores on introduced plants were found to be equal to or significantly higher than attack on native residents (Agrawal and Kotanen 2003). Biogeographical studies comparing enemies in native and introduced populations of the same species usually supported ERH, but results from community studies comparing the levels of attack between introduced and native species were controversial (Colautti et al. 2004). There are several alternate explanations of the relationship between invaders and pathogens or herbivores: 1) Enemy of my Enemy Hypothesis (EEH), when a co-introduced enemy does not damage native plant species as much as the invaders; 2) Enemy Inversion Hypothesis (EIH), describes a situation when an introduced enemy of an invasive increases the dispersal of native species (Pearson et al. 2000); 3) Increased susceptibility
hypothesis (ISH), which predicts the genetic bottlenecks of introduced plants may make them more susceptible to pathogens in the new habitat.

A modification of the BRH and ERH, Invasional Facilitation Hypothesis (IFH), predicts that invasion rates increase through time. The term ‘invasional meltdown’ is used to describe the situation in which the invasion of one non-indigenous species facilitates additional invasion by other non-indigenous species (Simberloff and Von Holle 1999). In addition to individual examples like zebra mussel in the Laurentian Great Lakes (Ricciardi 2001), accelerating rates of invasion have been noticed in some of the best-studied ecosystems (Cohen and Carlton 1998).

The Weapons of Mass Destruction hypothesis (WMD) suggests that some introduced plant species possess ‘novel weapons’ such as allelopathic chemicals (Callaway and Ridenour 2004). Root exudates of a non-indigenous plant may have little negative effects on competitors in its native range, but can have big effects on native species in the invaded community (He et al. 2009).

2.3 Impact of invasive plants on forest structure and dynamics

The introduction of an non-indigenous species is often responsible for an increase in predation and competition, habitat reduction, a variety of diseases, extinction of native plants or animals and genetic change in populations (Rejmánek 2000). Introduced non-indigenous species have been documented to displace native plant species, deteriorate the quality of native forests, threaten the integrity of native forest ecosystems (Kohli et al. 2006) as well as change soil chemical properties (Gray 2008).

One study suggests that invasive non-indigenous species are able to successfully compete with native species because natural competitors of the invasive species are not present, which enables the invader to maximize its competitive potential (Callaway and Aschehoug 2000).
Interspecific competition between exotic and native species can be a driving force in the shifts of plant community during succession. An experimental approach to test the competitive abilities of invasive species is to examine the response of native species to the removal of the invasive species (Brooks 2000).

Plant invaders can alter basic ecosystem processes such as hydrology, nutrient cycling, soil erosion rates (Vitousek and Walker 1989), and fire frequency and intensity (D'Antonio and Vitousek 1992). Changes made in key ecosystem processes can have a feedback effect on global changes such as climate and atmospheric composition (D'Antonio and Vitousek 1992). Invasive species can also prevent recruitment of native species (Mooney and Drake 1989) by negatively affecting the fecundity, survival, and fitness of native plants (Gould and Gorchov 2000). These alterations of the ecosystem can cause the invasive species to become dominant over native species in the ecosystem (Vitousek 1990). For example, through the process of actively fixing nitrogen, *Myrica faya* has been a successful invader and its dominance in the ecosystem has surpassed that of the native *Myrica polymorpha* (Walker and Vitousek 1991).

3 Robinia pseudoacacia

3.1 Species Description

*Robinia pseudoacacia* L. (Black locust) is a fast-growing deciduous tree in the pea family: Fabaceae. This tree is capable of reaching 30m in height, but is more often found to be between 12 to 25m tall with a canopy spread of 8 to 14m (Gilman and Watson 1993). The trunk can be up to 0.8m in diameter, covered with thick, deeply furrowed black bark. The leaves of *R. pseudoacacia* are 10-25cm long alternate odd-pinnately compound, with oval leaflets. Leaves barely fade to a yellow/green color before abscising early in the autumn (Gilman and Watson 1993). The white flowers are intensely fragrant and borne in pendulous racemes, which bloom
between May and June. Black locust has a typical legume pod fruit, 5-10 cm long, dry and brown, with four to ten small black seeds, finally splitting into two wind-carried valves with seeds attached (Brown and Brown 1972) (Figure 1). The heavy and hard wood of black locust has been successfully used as fence posts, boat frames and firewood. Most *R. pseudoacacia* will have a decreased growth rate after 10-20 years and have a high mortality, mainly due to stem damage by the locust borer (*Megacyllene robiniae*) (Boring and Swank 1984b).

3.2 Invasion History:

*Robinia pseudoacacia* is native to the Appalachian region of Southeastern North America, but now is considered invasive in the United States because it has recently increased its range outside of its long-term traditional range in the Appalachian mountains (Boring 1984a). Black locust has been widely planted throughout the world for its valued resistant wood, distinctive flowers, nitrogen-fixing root nodules, and rapid growth (Young and Young 1992). *Robinia pseudoacacia* was brought to Europe in the 17th century and has spread throughout Central Europe (Holzener 1982) and is considered a noxious invasive tree in Europe and China (Castro-Diez et al. 2009). Currently, *R. pseudoacacia* is naturalized in Europe, the Middle East, Australia, most Asian countries, New Zealand and all but one of the lower contiguous 48 United States (Cronk et al. 2001, Mehrhoff et al. 2003).

3.3 Habitat preferences

*Robinia pseudoacacia* is a pioneer species, which usually aggressively enters into disturbed areas such as clear-cut areas, old pastures, disturbed road-sides and post-fire locations (Chapman 1935, Boring and Swank 1984b, Elliott et al. 1997). Habitats in which *R. pseudoacacia* thrives include moist through wet cool-temperate and dry through moist subtropical forest life zones. Mean annual temperature of 7.6 to 20.3 °C, soil pH of 6.0 to 7.0 are
tolerated by *R. pseudoacacia*. Moreover, *R. pseudoacacia* is able to tolerate drought, aerosol salt and poor soils except soils that are permanently wet (Duke 1979). *R. pseudoacacia* will grow better in full sun and is considered intolerant of shade (Ford et al. 2006). It is highly tolerant of pollution. These trees are not for general use in urban forest due to the stem-borer problem and damage by a leaf miner. The leaf miner is a universal problem for *R. pseudoacacia*, causing trees along the highways in the south to be riddled with damage from this pest during the summer (Boring and Swank 1984b).

3.4 Reproduction and invasiveness

Black locust has both sexual and asexual reproduction. Therefore, this species reproduces and spreads by both seeds and root sprouts. The seeds are widely dispersed by birds and other wildlife. Black locust is a fast-growing tree with an optimal annual average of 1.22 meter of growth on productive sites (USDA 1965), but the growth rate will gradually decline over time because of pest and disease damage (Chittenden 1904, USDA 1999), or inhibition by shade tolerant plant species later in succession (Boring and Swank 1984b, Elliott et al. 1997).

Although *R. pseudoacacia* allelopathy is not as famous as the allelopathic effect of *A. altissima*, the inner bark, root sprouts, and wilted leaves of *R. pseudoacacia* have been found to contain a phytotoxin called “robinetin” and also myricetin and quercetin (Nasir et al. 2005). Waks (1936) reported that park stands of *R. pseudoacacia* are nearly void of all other vegetation, and the bark and wood contain toxins that inhibit the growth of barley (*Hordeum*). His results suggested that the failure of herbs to grow under *R. pseudoacacia* trees might be allelopathy (Rice 1984). *R. pseudoacacia* commonly inhabits early successional sites, has fast growth rate and high reproductive potential, plus allelopathic potential, therefore, in U.S.A., it has been considered invasive in Massachusetts (Weatherbee et al. 1998), Michigan (Voss 1985), and in
some habitats such as meadows and riparian habitats (Dudley 1998). It is one of top 100 woody plant invaders worldwide (Cronk et al. 2001).

3.5 Importance for forest succession - Nitrogen-fixation

*Rhinia pseudoacacia* is a woody legume species that forms a symbiosis with a bacteria—rhizobium—within root nodules that fix nitrogen (Chapman 1935, Bormann et al. 1993, Von Holle et al. 2006). After site disturbance, *R. pseudoacacia* can have a significant increasing effect on soil nitrogen availability and organic matter accumulation, because of its nitrogen fixation capabilities (Boring et al. 1981). In addition, symbiotic N fixation increases the concentration of NO$_3$ in the soil, which may due to increased fluxes of N from litter mineralization and nitrification (Boring and Swank 1984b). In temperate soil, particularly sandy soil, nitrogen is a limiting nutrient (Dzwonko and Loster 1997).

The soil enrichment properties of this species are thought to facilitate invasion of other nonnative species into these stands (Mehrhoff et al. 2003). Researchers in uplands of Cape Cod, MA (Von Holle et al. 2006) found greater richness and abundance of understory nonnative species in *R. pseudoacacia* stands than other dominant tree stands.

*Rhinia pseudoacacia* dominates secondary successional habitats in temperate southern Appalachian deciduous forests and controls forest regeneration (Boring et al. 1981). Boring and Swank proposed that the high mortality of *R. pseudoacacia* is an early successional mechanism that releases co-dominant species such as *Liriodendron tulipifera*, and creates canopy gaps favorable for growth of longer-lived individuals (Boring and Swank 1984b). They also found that nitrogen fixation contributed to biomass accumulation in 4, 17 and 38-year-old *R. pseudoacacia* stands, but total soil N addition by *R. pseudoacacia* was only apparent in the 38-year-old stand. However, average annual total stand N increase rate and nodule biomass reached
a peak in 17-year-old stands and declined with later successional development. This accretion of nutrients may be a mechanism to minimize nutrient leaching from soil following disturbance in southern Appalachian forest (Johnson and Swank 1973). The leaves produced by black locust have a low decomposition rate. White and Hanies (1988) found after 863 days $R.\ pseudoacacia$ leaflets still contain 81% of original nitrogen. The retention of nitrogen and great potential to form recalcitrant material during decomposition of black locust indicate it can have a long-term effect on N storage in forest floor and soil.

**4 Ailanthus altissima**

4.1 Species description

*Ailanthus altissima* (Mill.) Swingle (stinking ash, Tree-of-Heaven, Chinese sumac) is a deciduous tree in the Simaroubaceae family (Quassi family). It can grow up to 17-27 meters tall (Miller 1990), with a truck diameter up to 1m; it has smooth gray bark and alternately-arranged, compound, odd-pinnate leaves, and heart-shaped leaf scars. Flowers occur in panicles at the ends of braches in late spring to middle summer, and they are dioecious (male and female plants).

Male flowers and crushed leaves can produce a distinctive disagreeable odor. Seeds are centered in a papery samara, and dispersed mainly by wind (Hu 1979), but also by water and vehicle transportation (Figure 2). Unlike the other 14 members of the genus *Ailanthus*, *A. altissima* is found in temperate rather than tropical climates (Brizicky 1962).

4.2 Invasion History

Native to central China, *A. altissima* has been considered to be an invasive species worldwide. The species was apparently introduced into North America by two different routes. The first route began with Pierre d'Incarville who mistook it for a lacquer tree in China and sent seeds to England around 1751 (Feret 1974, Hu 1979). It was then introduced into North America
as an ornamental by William Hamilton, a Philadelphia gardener in 1784 (Hu 1979). There was a second introduction made by W. Prince, a Long Island horticulturalist, in response to the demand of small, pollution tolerant trees for New York City in the 1820’s (Davies 1942). Because of the rapid growth and ability to grow in unfavorable conditions, it became a common stock plant in eastern nurseries by 1840 (Feret et al. 1974, Hu 1979). The third route was through Chinese miners who used the plant for its medicinal properties in the 1800s (Feret 1985). Escaping from cultivation and quickly becoming established on both coasts, this species has expanded its range to most states in the USA since its initial introductions. Through two hundred years of invasion, American *A. altissima* has gone through more genetic drift than its Chinese counterpart, which perhaps was accentuated by man during selection for ornamental or medicinal purposes (Feret et al. 1974).

4.3 Habitat preferences

In the eastern United States, the frequency of *A. altissima* increases as one nears the cities. In neglected urban areas, *A. altissima* grows "as trees close to buildings, as hedges, or as bushy aggregates along railroad tracks, highway embankments, walls at the ends of bridges and overpasses, or in cracks of sidewalks and along fences" (Hu 1979). For example, in southwestern Virginia, 30% of the mileage along the interstate highways has been colonized by *A. altissima* (Burch and Zedaker 2003). In a roadside survey of Virginia, mean density of tree-of-heaven throughout the roads surveyed was 39 km$^{-1}$ (McAvoy et al. 2012). Kowarik viewed human settlements as centers of its distribution and roads as migration routes (Kowarik 1983). Although *A. altissima* is usually found in disturbed areas, it occasionally spreads to undisturbed areas. In North America, *A. altissima* has invaded into hemlock, oak-hickory and maple-birch forests, mostly as a result of disturbance (Miller 1990, Huebner 2003). Also, *A. altissima* has invaded
river banks and stream habitats (Kowarik 1983, Gutte et al. 1987). In wastelands and North American hardwood forest, *A. altissima* was found associated with *R. pseudoacacia or Acer* species (Gutte et al. 1987, Call and Nilsen 2005). Although *A. altissima* is primarily an urban weed, it has become a problem in forested areas as well (Kowarik and Säumel 2007).

4.4 Response to abiotic factors

Although *A. altissima* may suffer from root competition by other trees already established in an area, it usually competes successfully with other plants and is considered a weed in forests (Hu 1979, Hoshovsky 1988). It is an aggressive pioneer, even able to grow in the cracks in concrete. *Ailanthus altissima* is an opportunistic species, growing quickly in full sun in disturbed areas or open gaps in forest (Kowarik 1995, Knapp and Canham 2000). Grime listed it as shade intolerant, but it does exhibit some degree of shade tolerance, giving its competitive edge over other plant species (Grime 1965). *Ailanthus altissima* is considered among the most tolerant trees to pollution, including sulfur dioxide (Liu and Ding 2008). *Ailanthus altissima* does well on very poor soils, and dry soils are probably more suitable for its growth than wet soils (Hoshovsky 1988). The tree has been used in re-vegetating acid mine spoils, tolerating a pH of less than 4.1, soluble salt concentrations up to 0.25 mmhos/cm and phosphorus levels as low as 1.8 ppm (Plass 1975). *Ailanthus altissima* has been planted widely in urban areas because of its ability to tolerate atmospheric pollution. Since the late 1900s, people have noted its ability to adapt to "the dirt and smoke, the dust and drought of cities" (Sargent 1888). Leaves of *A. altissima* can absorb significant amounts of sulfur in areas of high traffic flow (Hoshovsky 1988); it can accumulate high levels of mercury in its tissues (Smith 1972); and it is somewhat resistant to ozone exposure (Davis et al. 1978).

4.5 Reproduction and invasiveness
Ailanthus altissima reproduces both sexually and asexually. Asexual reproduction of A. altissima is mainly by vegetative sprouting from stumps or roots, and repeated cuttings will promote vegetative growth (Hu 1979). Ailanthus altissima has the longest winter dormancy of all the trees in its native Chinese habitat, flowering in late spring to middle summer (Hu 1979). Precocious flowering is not a rare occurrence in this species and has been observed in seedlings only 6 weeks after germination (Feret 1973). Seeds ripen in large crowded clusters from September to October of the same year and may persist on the tree through the following winter (Little 1974, Hu 1979). Ailanthus altissima is a prolific seed producer. An individual tree can produce 325,000 seeds per year which are easily wind-dispersed, but seed germination rates may vary widely among populations (Bory and Clair-Maczulajtys 1980).

4.6 Importance for forest succession – Allelopathy

Ailanthus altissima is a prolific seed producer, grows rapidly and can successfully compete with the native vegetation. The root system of A. altissima is also aggressive enough to cause damage to sewers and foundations (Hu 1979). In addition to these invasive traits, A. altissima can produce toxins which prevent the establishment of other plant species (Mergen 1959).

The term allelopathy was defined as biochemical interactions between plants (Molisch 1937). An important point to note is that allelopathy effects depend on a chemical compound being added to the environment, thereby separating it from competition for resources (Rice 1984). Mergen (1959) reported that succession appeared to be considerably slow in the A. altissima stands, with virtually pure stands remaining a monoculture for a long period. Also, he found alcohol extracts of the rachis, leaflets, and stem of the tree-of-heaven caused rapid wilting of other plants when applied to the cut surface of the stems of the test species (Mergen 1959).
The test species included 35 species of gymnosperms and 11 species of angiosperms, which makes the toxicity of wide significance. The toxicity levels of *A. altissima* are highest in the leaves during the early part of the growing season and are maintained at high levels at least until October (Voight and Mergen 1962). Heisey (1990) found that inhibition by *A. altissima* is highest in bark, roots, leaflets, and the inhibitors can transfer from bark to the new leaves. The allelochemicals can inhibit seed germination and seedling growth, and can work as herbicides in the field (Heisey 1990, Heisey and Kish Heisey 2003). Heisey further extracted and identified the structure of allelochemical in *A. altissima*, ailanthone, a quassinoid compound, which could stay in soil for 3 days (Heisey 1996).

Allelopathy may have important influences on plant community patterning, which refers to the spatial arrangements of organisms within a community. Most ecologists have attempted to explain the patterning of vegetation and the general distribution of plants largely on the basis of competition. However, there is growing evidence that allelopathy could play a role in plant-plant competition and influence spatial distribution of plants (Muller 1966, Wilson and Rice 1968, Rasmussen and Rice 1971, Stowe 1979, Schenk et al. 1999, Qasem and Foy 2001).

Much evidence exists indicating that allelochemicals strongly inhibit nitrification in soils of many ecosystems (Rice 1984, Paavolainen et al. 1998, Singh et al. 2006, Castaldi et al. 2009). In addition, a marked allelopathic effect on plant roots is the inhibition of infection of root hairs by nitrogen fixation bacteria, thus decreasing nodulation and nitrogen fixation (Jobidon and Thibault 1981, Jobidon and Thibault 1982, Mallik and Tesfai 1988). Much research has been reported on allelopathic effects against *Rhizobium* (Rice 1972, Murthy and Nagodra 1977, Alsaadawi et al. 1983, TzuPATHI et al. 2000, Weston and Mathesius 2013), less on effects against the N$_2$-fixing blue-green algae (Graneli et al. 2008), and nodulation and symbiotic N$_2$
fixation by non-legumes (Rice 1972, Murthy and Nagodra 1977). It is doubtful that allelopathy has any appreciable effect on aminization and ammonification, because these processes are carried out by a very heterogeneous group of soil organisms (Brady 1974, Rice 1992).

5 Introduction

Invasive alien species have become one of the five main reasons for loss of biodiversity worldwide (IUCN definition, also see (Pyšek and Richardson 2010). Some invasive plants can alter indigenous plant populations (Zavaleta et al. 2001), community structure (Cushman and Gaffney 2010), food webs (Aravind et al. 2010), and ecosystem processes (Sharma and Raghubanshi 2009). Specific interactions between indigenous plants and non-indigenous invaders are the fundamental processes leading to impacts of the non-indigenous invaders on indigenous populations, communities and ecosystems. Therefore, it is critical to understand the mechanisms of those interactions to enable control of current non-indigenous plant invasions and prevent future invasions.

Plants of similar functional type are most likely to interact because they reside in the same types of habitats and utilize the same resources in similar ways (Hooper and Dukes 2010). Ailanthus altissima is an invasive tree native to China that has naturalized in a majority of North America (Ding et al. 2006) and Europe (Sheppard et al. 2006). It is characteristically found in high-light, disturbed habitats and has putative allelopathic potential (Gomez-Aparicio and Canham 2008), which may reduce native species regeneration (Albright et al. 2010), alter soil chemistry and change ecosystem nutrient dynamics. Robinia pseudoacacia is co-located with A. altissima in many early succession sites in the Appalachian region (Call and Nilsen 2003). It is also an early-successional species with some degree of invasive traits and nitrogen fixation.

We proposed that A. altissima and R. pseudoacacia will interact with each other because
they are the same functional types and are co-located in early successional sites. The result of the interaction between these two trees is likely to have important consequences to community structure and ecosystem function. Both species have global significance because they are both invasive in many parts of the world. In fact, *R. pseudoacacia* is considered the most abundant tree in the global biosphere (Boring and Swank 1984b) and *A. altissima* is not far behind. The allelopathic effect of *A. altissima* has potential to inhibit the growth of, and competition between, these two species. Cao et al. (2009) used extracts from *A. altissima* root to treat *R. pseudoacacia* seedlings, and found that height growth of *R. pseudoacacia* seedlings was inhibited compared to controlled group; leaf chlorophyll content and whole individual biomass of the treated group of *R. pseudoacacia* seedlings were also reduced (Cao B. 2009).

I focused my research on these two species because of their global significance, their varying effects on community and ecosystem processes, and their likelihood for interaction in the Appalachian region of the United States. The consequence of the interference between these two species will not only help us understand the mechanisms underlying the competition or facilitation of invasive and native species, but also help to predict forest succession in the eastern region of the US.

To address this question, we designed both experimental and observational studies to understand the result, mechanism and impact of the interaction between *A. altissima* and *R. pseudoacacia* at the individual scale (Chapter 2), local scale (Chapter 3), community and ecosystem scale (Chapter 4) and the regional scale (Chapter 5). To understand how they interact and by which mechanisms they interact, we need data from experiments in which both species coexist at local and individual scale. To understand how the two species and their interactions influence forest structure and succession, we need information from community scale field
vegetation surveys in their natural habitat and regional scale database analysis.

The overall purpose of this study is to determine if: 1) The distribution of *Ailanthus altissima* and *Robinia pseudoacacia* are highly associated in Appalachian Mountains region (Chapter 4 and 5); 2) Interspecific and intraspecific competition occurs between and within these two species (Chapter 2 and 3); 3) The aboveground and belowground growth and nodule production of *Robinia pseudoacacia* are inhibited by *Ailanthus altissima* in comparison to monoculture stands (Chapter 2); 4) The impact of both species on community structure, regeneration and ecosystem process will be changed by their interaction (Chapter 4 and 5).

Overall, we are interested in how their interaction results and impacts change across various scales. “Conceptually there is no single natural scale at which ecological patterns should be studied. Rather, the appropriate scale is dictated by the study goals, the system, and available data.” (Elith and Leathwick 2009)
Literature cited:


Brooks, M. L. (2000). "Competition between alien annual grasses and native annual plants in the


24:185.


perspective." Biological Invasions 8(7): 1439-1450.


American naturalist: 771-801.


Biological Invasions: a Global Perspective. J. A. D. e. al., John Wiley & Sons Ltd: 491-505.


Figure 1. Illustration of *R. pseudoacacia*. A) front view of flower, B) side view of flower, C) longitudinal section of flower showing diadelphous pistils, D) legumes, E) seed, F) leaflet, and G) stipules. (Adapted from Call 2002)
Figure 2. Illustration of *A. altissima*. A) pistillate flower, B) staminate flower, C) unopened flower bud, D) samaras, E) individual seed, F) leaflet, and G) gland underneath leaflet. (Adapted from Call 2002)
Figure 3. Range of *Ailanthus altissima*, with a differentiation of the native Chinese range (hatched), and the secondary world-wide distribution (black) resulting from the introduction since 1740s. (Mapped by E. J. Jäger & E. Welk, AG Chorology, Institute for Biology Halle/Saale, adapted from Kowarik and Säumel 2007)
CHAPTER 2

Aboveground and belowground interactions between seedlings of the exotic invasive

*Ailanthus altissima* and the native *Robinia pseudoacacia*

Abstract:

The interactions between exotic invasive species and native species can determine whether the invasive species can become established, impact community structure, and alter ecosystem processes. In the U.S., the invasive tree *Ailanthus altissima* and the native tree *Robinia pseudoacacia* are often associated in early-successional forests, and have similar growth and reproduction characteristics. The allelopathic effect of *A. altissima* and the nitrogen fixing ability of *R. pseudoacacia* make the interaction between these two species important to community dynamics. A replacement series greenhouse experiment was used to investigate the type of interaction that occurs between seedlings of *A. altissima* and *R. pseudoacacia* at high and low soil nutrition states, and to assess the underlying mechanisms of that interaction. Also, seeds of *A. altissima* from its native (China) and invasive ranges (U.S.) were used to compare the effect of the different seed sources on the interaction with *R. pseudoacacia*. We found *R. pseudoacacia* was better in performance both above- and below-ground, especially in the lower soil nutrient state. The presence of *A. altissima* significantly inhibited nodulation of *R. pseudoacacia* roots, suggesting that an allelopathic effect of *A. altissima* existed. *Ailanthus altissima* from the U.S. seed lot had less biomass increment, bigger root/shoot ratio, lower soil nutrient
needs and slightly stronger allelopathic effect on *R. pseudoacacia* nodulation compared
with *A. altissima* from the Chinese seed lot. In summary, the exotic invasive tree *A.
altissima* did not out compete the native invasive tree *R. pseudoacacia*, especially under
the low nutrient treatments.

**Keywords:** allelopathy, belowground competition, competition for nutrients, invasive
species, nodulation, rapid evolution, replacement series.

**Introduction:**

Many mechanisms can contribute to the impact of invasive species on native flora,
but competition is often central (Wyckoff and Webb 1996, Gentle and Duggin 1997,
2013). Moreover, invasive and native species that have similar habitat preferences are
most likely to interact and compete because of their similar resource requirements.
Invasive species are a focus of research because they may have negative impacts on
ecosystem processes compared with native species (Barney et al. 2013). Therefore,
understanding the competitive interaction between invasive and native species that have
similar functional traits, yet influence the ecosystem differently, is an important aspect of
invasive ecology research.

Our research program concerns the mechanisms of interaction between one important
invasive species *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae) and a similar
species, *Robinia pseudoacacia* L. (Fabaceae), native to the mid-latitude Appalachian
mountains. These two species were selected because they have comparable ecological
traits. Both species are abundant in early-successional temperate forests, and both can
survive and thrive in relatively nutrient-poor soils (Hu 1979, Hoshovsky 1988, Vlachodimos et al. 2013). Both species grow rapidly and reproduce by vegetative cloning
and prolific seed production. However, the two species have an important difference that
causes divergent impacts on forest succession and ecosystem processes. Ailanthus
altissima is recognized as an invasive species with a novel weapon (allelochemicals)
because of its negative effect on native species germination, which can reduce plant
diversity and forest succession after disturbance (Mergen 1959, Heisey 1990, Heisey
pseudoacacia is the most important nitrogen-fixing tree in the eastern forests of the
United States, which facilitates forest succession. Robinia pseudoacacia is used for
restoring vegetation on post-disturbance sites (Boring and Swank 1984b, Von Holle et al.
2006, Vlachodimos et al. 2013). Thus, the result of competition between these two
species may have a significant effect on native community diversity and succession. One
possibility is that allelochemicals produced by A. altissima may reduce the ability of root
formation and nodulation of R. pseudoacacia (Halsall et al. 1995), hence changing
nitrogen cycling, succession and community structure in eastern forests of the U.S.
Therefore, understanding the result of competition between these two species is important
for understanding the relationship between invasive-native species interactions and
community dynamics.

There have been many theories proposed about why an invasive species is more
successful in its invaded range than its native range, such as being a superior competitor (Tilman 1982, Shea and Chesson 2002), escaping from natural enemies (Keane and Crawley 2002), having novel weapons (Callaway and Ridenour 2004) and inducing invasive meltdown (Simberloff and Von Holle 1999). One aspect of that theoretical discussion is the effect of lower genetic diversity for the invasive species in the invaded habitat compared with its native habitat. This can result from establishment of only a small number of genotypes introduced in the new habitat that initiated the invasion (Lee 2000, Tsutsui et al. 2000). Particularly vigorous genotypes are usually selected for use in horticultural introductions. Thus, genotypes with high fitness dominate the introduced population more so than the native populations. Therefore, it is logical to posit that the invasive species population in the invaded ecosystem may be selected to be a more competitive group than the populations of invasive species in their native ecosystem. In this study, the competitive ability of seedlings derived from a seed lot of *A. altissima* from its native range (eastern China) and a seed lot from the invasive range (eastern U.S.) were evaluated.

The overall goal of this study is to understand the interaction between *R. pseudoacacia* and *A. altissima* seedlings in relatively low nutrient environments, and to assess if there is a difference between the competitive ability of *A. altissima* genotypes growing in the U.S. vs. those from its native China. We focused on both belowground processes and aboveground competition in a greenhouse setting, which facilitated harvesting the entire root system, counting and collecting nodules from *R. pseudoacacia* seedlings, and manipulating nutrient conditions.
We used a full-factorial additive design greenhouse experiment to address the following questions: 1) Which species gains the most belowground and aboveground biomass at the seedling stage over the defined growth period? 2) Does whole plant biomass and allocation to roots and shoots for both species increase from low to high nutrient levels used in this study? 3) Does the presence of the other species change the whole plant biomass and allocation patterns? 4) Does the presence of *A. altissima* inhibit nodule number per root mass of *R. pseudoacacia*? 5) Is the competitive ability of *A. altissima* seedlings from the invaded range (U.S.) greater than that of *A. altissima* seedlings from its native region (China)?

**Materials and Methods:**

*Seed germination*

The *A. altissima* seeds from the invaded ecosystems were collected from Mineral, Hampshire, Hardy, and Grant Counties of West Virginia, U.S.A from multiple trees along highways in 2009 and 2010. The *A. altissima* seeds from its native range were bought from Shuang Ye Seeds Wholesale Co., Ltd. in 2011, and were collected in 2009 from multiple trees in JiangSu province, China. Both seed lots were stored at 4°C until being used in January 2013. The samaras were cut off, and about 500 seeds of *A. altissima* from each seed lot were soaked in 100 mL 500 ppm Gibberellic Acid (GA) solution in a dark incubator at 40 °C for 24h to break dormancy. We used the GA method to break dormancy instead of the traditional warm water incubation because we found very low germination rate in preliminary attempts with the traditional technique. *Robinia*
pseudoacacia seeds purchased from Sheffield's Seed Co. Inc. (Locke, New York, U.S.A.) were collected from multiple trees in Kentucky, U.S.A. The R. pseudoacacia seeds were stratified at 4 °C for 60 days, soaked in water and put in a dark incubator at 40 °C for 12h. Following incubations, seeds of both species were sown in germination flats with Metro Mix® in the BIOL/VBI plant growth service center at Virginia Tech. Air temperature in the BIOL/VBI plant growth bay used for this study ranged from 22 – 28 °C during the experiment, light intensity (photosynthetic photon flux density) peaked at 1600 – 1800 μmol m⁻² s⁻¹ and relative humidity ranged from 65-80% on a daily basis.

Experimental design

After one month in flats in the greenhouse, seedlings were transplanted into 8 L pots (approximately 25 cm in diameter, 35 cm in height), which contained washed masonry sand mixed with 10g of soil collected from under planted R. pseudoacacia stands at Blandy Experimental Farm, Boyce, Virginia, U.S. to stimulate nodulation. There were 5 levels of species combinations (defined as “proportion” treatments hereafter), 2 levels of nutrient (defined as “nutrient” treatments hereafter), and 2 sources of A. altissima seed (defined as “seed source” treatments hereafter), with 8 replicates per treatment, for a total of 160 pots in the experiment. The planting density was held constant at 6 plants per pot. Mixtures consisted of the following species proportions: 1) monoculture of A. altissima (6A), 2) monoculture of R. pseudoacacia (6R), 3) 2 A. altissima and 4 R. pseudoacacia (2A4R), 4) 3 A. altissima and 3 R. pseudoacacia (3A3R), and 5) 4 A. altissima and 2 R. pseudoacacia (4A2R). Randomly selected similar sized seedlings from each species were planted into pots based on our experimental design.
After transplantation, nutrient treatments were randomly assigned to each pot. Either 0.936 or 3.744 grams (i.e. 39.5 and 158kg N ha\(^{-1}\) yr\(^{-1}\)) of Osmocote® 14-14-14 slow-release fertilizer was added to each pot to create the low and high nutrient treatments respectively. These two nutrient treatments were selected to represent low soil nutrient conditions in a natural temperate forest (Finzi et al. 1998) and the upper limit of fertilizer added to agriculture land (158kg N ha\(^{-1}\) yr\(^{-1}\)). Fertilizer was reapplied at the same rates after 5 months because the slow release fertilizer has a predicted lifespan of 5-6 months at 21 ºC. After all treatments were established, pots were completely randomized on two greenhouse benches and were rotated along the benches every month to minimize the effect of spatial variation in greenhouse microclimate.

Due to the difference in germination and growth rate, the replicates using Chinese A. altissima seedlings were transplanted about 40 days earlier than the replicates using U.S. A. altissima seedlings. Hence, pots with the two different seed sources were harvested at different times, to keep the same number of growing days. The plants were harvested after about 6 months, when the seedlings reached the maximum capacity of the pots without significant root binding. The aboveground parts were harvested first on the same day. Height and basal diameter of each plant were measured. Stems and leaves were separated and oven-dried at 65 ºC for approximately 4 days to insure constant dry weight. Roots were harvested during a 30-day period after shoot collection, and the pots with roots waiting to be harvested were kept unwatered in the greenhouse to make sure no additional growth occurred. Roots from each of the six plants in one pot were separated carefully, and washed out from sand. The extra roots, which were broken during the
separation process and left in sand were either sieved or washed out carefully for each pot. The number of nodules on *R. pseudoacacia* root systems was determined. All nodules greater than 1 mm in size were picked off and dried to constant weight. All other root tissues were also oven-dried at 60 °C for 7 days to constant weight. We used the number of nodules (number of infection sites) as a proxy for the amount of nitrogen fixed by the plant based on a positive correlation between nodule number and the isotopic signature of nitrogen ($\Delta^{15}$N) for *R. pseudoacacia* growing in sand fertilized with Hoaglands solution – N (Supplementary figure 1).

**Data analysis**

During the harvest, it was unavoidable to have broken fine roots left in each pot. In this experiment, the total amount of extra roots left in the sand after separating the root systems of the six plants averaged 28% of the total root biomass in each pot. In a previous study, we found it was impossible to distinguish these two species’ fine roots based on their morphological characteristics (unpublished data). Since these extra roots could not be separated between the 6 original plants, we assigned a proportion of the extra root biomass to each of the 6 plants based on the relative harvested root biomass of each plant in the pot. For example, if a plant had 33% of the total extracted root mass in a pot, then 33% of the extra roots sieved and washed from the sand was added to that plants’ root mass.

Aboveground biomass (leaf plus shoot), leaf biomass, belowground biomass, total biomass and root/shoot (i.e., belowground biomass/aboveground biomass) ratio were calculated, and the correlations among aboveground, belowground, and total biomass
were evaluated. Aboveground biomass and below ground biomass were found to be autocorrelated \((r = 0.76)\), and they were both highly correlated with total biomass \((r=0.95\) and 0.92). Therefore, three-way ANOVA was only presented for total biomass, which reflects plant growth, leaf biomass, which reflects investment into carbon gain and photosynthesis, and root/shoot ratio, which reflects the relative investment in nutrient accumulation. Three-way ANOVAs for each species were performed separately, to evaluate the main effects of seed source, nutrient level, species proportion, the interactions between each pair of these three factors and the three-way interaction.

Subsample error was considered into this ANOVA, because there was more than one plant (subsample) in each pot (replicate). All the factors were treated as fixed effects. If a significant ANOVA result was identified, multiple-comparisons between all possible pairs of treatments in the particular effect group were evaluated using Tukey’s HSD.

To compare our results with a prior study (Call and Nilsen 2005), the Relative Yield (RY) was calculated to determine if the species were sharing resources or competing with each other for resources (Fowler 1982, Williams and McCarthy 2001). These indices were calculated separately based on total biomass of each species. The equations of calculating RY for the two species are:

\[
RY_A = \frac{Y_{AR}}{p_A Y_A}
\]

\[
RY_R = \frac{Y_{RA}}{p_R Y_R}
\]

Where:

\(Y_A\) = yield (dry biomass) of \(A. \text{altissima}\) in monoculture,
\[ Y_R = \text{yield of } R. \text{pseudoacacia in monoculture}, \]
\[ Y_{AR} = \text{yield of } A. \text{altissima in the presence of } R. \text{pseudoacacia}, \]
\[ Y_{RA} = \text{yield of } R. \text{pseudoacacia in the presence of } A. \text{altissima}, \]
\[ p_A = \text{proportion of } A. \text{altissima sown in one pot}, \]
\[ p_R = \text{proportion of } R. \text{pseudoacacia sown in same pot}, \text{ and } p_A + p_R = 1. \]

A RY less than 1 indicates that the species is experiencing competition from the other species. A RY more than 1 indicates that the species is experiencing more competition from itself than from the other species.

A three-way ANOVA was applied to the calculated RY\_A and RY\_R using total biomass to compare the difference of competition intensity under different treatment combinations. There were only 3 levels in the proportion treatment for RY\_A and RY\_R, because both indices could only be calculated for mixtures. There was no subsample error in the RY\_A and RY\_R ANOVA, because both indices can only be calculated once for each pot.

To determine whether A. altissima affected nodulation of R. pseudoacacia, the relationship between nodule number and nodule weight under different nutrient levels was assessed by general linear regression. Poisson regressions of the numbers of nodules per root mass were fitted for R. pseudoacacia plants under high and low nutrient levels. Also, the average nodule number per R. pseudoacacia root mass was used as the response variable, and the relationships with number of neighboring A. altissima and A. altissima seed source were evaluated to explore the possibility of a negative effect of A. altissima roots on R. pseudoacacia nodulation. A three-way ANOVA was used to detect the effect
of seed source, nutrient level and species proportion on nodule number per root weight. Also, a three-way ANOVA was used for nodule weight, but we suggest that nodule number (number of infection points) is a better representation of the amount of nitrogen fixation by *R. pseudoacacia*, because a previous study showed nodule nitrogen fixing activity is the highest for mid-size nodules (1-2mm), which are the most frequent size of nodules. Moreover, the nitrogen-fixation activity doesn’t increase as size of the nodules increases for nodules that are larger than the mid-size category (Tajima et al. 2007). Thus, increased size of the nodule does not represent effectively the amount of nitrogen fixed by the nodule.

**Results:**

In general, *R. pseudoacacia* seedlings became significantly larger (p<0.0001) than *A. altissima* seedlings. The average total dry biomass per plant for *R. pseudoacacia* in all the treatments combined was almost 7 times the total dry biomass per plant of *A. altissima* (Table 1).

**Results for Ailanthus altissima biomass and allocation**

All the main effects of the three factors (seed source, nutrient, proportion) on the average total biomass of *A. altissima* were significant at the α=0.05 level (Table 2). The average total biomass of Chinese *A. altissima* plants was significantly greater than that of U.S. *A. altissima* (Table 1, 2). Also, in the germination stage, the Chinese seed lot of *A. altissima* had a much faster germination and higher germination rate than those from U.S. seed lot, indicating a faster growth rate of Chinese *A. altissima* (personal observation).
The effect of nutrient level on average total biomass per plant at high nutrient level was significantly greater than that at low nutrient level (Table 1, 2). *Ailanthus altissima* seedlings in monoculture had a significantly greater total biomass than those grown in mixed pots with *R. pseudoacacia* (Table 1). In general, the bigger the proportion of *R. pseudoacacia* seedlings in a pot was, the lower the average total biomass of *A. altissima* seedlings was in the pot; but there was no significant difference among the different proportions in mixtures (Figure 1).

All the three factors (seed source, nutrient, proportion) significantly affected the average leaf biomass of *A. altissima* at the α=0.05 level (Table 2). The average leaf biomass of Chinese *A. altissima* plants was significantly greater than that of U.S. *A. altissima* (Table 1, 2). *Ailanthus altissima* seedlings grown at the high nutrient level had a significantly greater leaf biomass than that of *A. altissima* grown at low nutrient level (Table 1, 2). Although the proportion was a significant effect (Table 2), only the *A. altissima* seedlings in monoculture had a significantly greater leaf biomass than those grown in 3A3R treatment (Table 1). In general, the bigger the proportion of *R. pseudoacacia* seedlings in a pot was, the lower the average leaf biomass of *A. altissima* seedlings was in the pot (Table 1). The interaction between seed source and nutrient was barely significant (Table 2), because Chinese *A. altissima* had a bigger difference of leaf biomass between the high and low nutrient levels than that for U.S. *A. altissima*.

In general, *A. altissima* seedlings produced more root biomass compared with shoot biomass because, in most treatment groups, root/shoot ratio >1 (Table 1, Figure 1). The root/shoot ratio of *A. altissima* was significantly altered by seed source and proportion,
and there was also a significant interaction between these two factors (Table 2).

Root/shoot ratio was significantly greater for *A. altissima* seedlings from the U.S. seed lot compared with those from the Chinese seed lot (Table 1, 2). The monoculture group had significantly higher root/shoot ratio than the 4A2R and 3A3R groups (Table 1). As the number of *R. pseudoacacia* increased, root/shoot ratio of Chinese *A. altissima* seedlings increased slowly. However, U.S. *A. altissima* seedlings had the highest root/shoot ratio in monoculture compared with those grown in mixtures with *R. pseudoacacia* (Figure 1).

Results for *Robinia pseudoacacia* biomass and allocation

Nutrient and proportion significantly affected the average total biomass of *R. pseudoacacia* and there was a significant two-way interaction between them (Table 3). There was no significant difference in total biomass of *R. pseudoacacia* seedlings when grown with either of the two seed sources of *A. altissima* (Table 1, 3). Average total biomass of *R. pseudoacacia* seedlings was significantly greater when grown in the high nutrient treatment than when grown in the low nutrient treatment (Table 3). Average total biomass of *R. pseudoacacia* seedlings increased significantly as the number of *A. altissima* seedlings increased in the pot, and there were significant differences between each pair of the proportion treatments (Table 1, 3). In the high nutrient treatment, average biomass decreased more rapidly as the proportion of *A. altissima* decreased. Also, the group with the fewest *R. pseudoacacia* seedlings in the pot (4A2R), at the low nutrient level, had more total biomass (8.71±1.22g) than the monoculture group (6R) at the high nutrient level (7.23±0.69g) (Figure 2).

The main effects of all three factors in this experiment were highly significant for the
average leaf biomass of *R. pseudoacacia* (Table 3). There were significant two-way
interactions between seed source and nutrient, seed source and proportion, and nutrient
and proportion (Table 3). When grown with *A. altissima* from the Chinese seed lot,
*Robinia pseudoacacia* had a significantly higher leaf biomass than when grown with *A.
altissima* from the U.S. seed lot (Table 1, 3). Average leaf biomass of *R. pseudoacacia*
seedlings was significantly greater when grown in the high nutrient treatment than when
grown in the low nutrient treatment (Table 1, 3). Average leaf biomass of *R. pseudoacacia*
seedlings increased significantly as the number of *A. altissima* seedlings increased in the
pot, resulting in a significant species proportion effect (Table 1, 3). The 2R4A group had
significantly more leaf biomass than the other groups, and the 3R3A group had
significantly more leaf biomass than the 4R2A and 6R groups (Table 1). The significant
interaction between seed source and nutrient was likely due to *R. pseudoacacia* producing
greater leaf biomass in the high nutrient level than in the low nutrient level when grown
with Chinese *A. altissima*, but less leaf biomass in the high nutrient level than in the low
nutrient level when grown with U.S. *A. altissima*. The significant interaction between
seed source and species proportion was because *R. pseudoacacia* leaf biomass increased
more rapidly as the number of *A. altissima* increased when grown with Chinese *A.
altissima* than when grown with U.S. *A. altissima*. Also, the increase in leaf biomass of *R.
pseudoacacia* as *A. altissima* number increased in the pot was slightly faster in the high
nutrient level than in the low nutrient level.

In contrast to *A. altissima*, *R. pseudoacacia* seedlings had similar production of
aboveground and belowground biomass (Figure 2). The three-way ANOVA results for
root/shoot ratio of *R. pseudoacacia* showed a highly significant main effect of seed source, and a significant interaction between seed source and nutrient (Table 3). The root/shoot ratio of *R. pseudoacacia* seedlings was significantly greater when *R. pseudoacacia* seedlings were grown with *A. altissima* seedlings from the U.S. seed lot than when grown with *A. altissima* seedlings from the Chinese seed lot (Table 1, 3). When grown with Chinese *A. altissima* seedlings, *R. pseudoacacia* produced more root biomass in the high nutrient soil than in the low nutrient treatment; but this pattern was reversed for *R. pseudoacacia* grown with *A. altissima* seedlings from the U.S. seed lot (Figure 2, Table 3).

Intraspecific and interspecific competition

Relative yield results for *A. altissima* (RYA) were mostly less than 1 (Table 4), indicating interspecific competitive inferences from neighboring *R. pseudoacacia* seedlings was stronger than the intraspecific competition. Three-way ANOVA showed that RYA was significantly different between the two seed sources (Table 5), and neighboring *R. pseudoacacia* seedlings had stronger negative impact on *A. altissima* seedlings from the U.S. seed lot than those from the Chinese seed lot (Table 4, 5). RYA was also significantly different between nutrient levels (Table 5), and *A. altissima* seedlings in low nutrient group had lower RYA, indicating relatively stronger competition from *R. pseudoacacia* than from itself (Table 4). There were no significant differences among the three proportion treatments of mixture (Table 5). There was a significant two-way interaction between seed source and nutrient (Table 5), because the increase in RYA from the low nutrient level to the high nutrient level for Chinese *A. altissima* was bigger than that of U.S. *A. altissima*.
Relative yield results for *R. pseudoacacia* seedlings (RY<sub>R</sub>) were mostly larger than 1, indicating more intraspecific competition than the negative effect from neighboring *A. altissima* seedlings (Table 4). The three-way ANOVA result showed both nutrient and species proportion treatments had a strongly significant effect, but not the seed source (Table 5). The greater the number of *A. altissima* seedlings in the pot, the larger was the RY<sub>R</sub> and the stronger was intraspecific competition (Table 4). The high nutrient level group had a larger RY<sub>R</sub> than the low nutrient level (Table 4).

*Nodulation of Robinia pseudoacacia*

There was a positive linear relationship between nodule number and nodule weight, and this relationship was not statistically different for the two nutrient levels (p=0.124). Therefore, in the following analysis of nodulation among different treatments, only the results using nodule number will be shown.

We found that nodule number was strongly positively related to root biomass ($R^2=0.42$, p<0.0001, Figure 3). There was no significant difference for this regression relationship between the two nutrient treatments (p=0.19).

In both nutrient levels of our study, *R. pseudoacacia* formed a fair amount of nodules, with an average number of 50.6±3.6 and 81.6±3.6 nodules per plant in the low and high nutrient level respectively. The response variable nodule number per unit root weight (g⁻¹) was chosen to determine the relative nodule number.

The number of nodules per root weight was significantly different between nutrient groups (p=0.03). *Robinia pseudoacacia* produced more nodules per unit root mass at the low nutrient level (34.36±2.01 g⁻¹) than that at the high nutrient level (26.35±2.04 g⁻¹).
When grown with *A. altissima*, the number of nodules per root mass of *R. pseudoacacia* seedling was lower (27.12±1.80 g⁻¹) than that of *R. pseudoacacia* seedlings in monoculture (35.31±2.36 g⁻¹, p=0.005). *Robinia pseudoacacia* seedlings grown with *A. altissima* seedlings from the U.S. seed lot had fewer nodules per root weight (18.38±0.87 g⁻¹), compared with *R. pseudoacacia* seedlings grown with *A. altissima* seedlings from the Chinese seed lot (42.51±2.53 g⁻¹, p<0.0001). When total nodule number was evaluated instead of nodule number per unit root mass, the effect of *A. altissima* seed lot became insignificant, because *R. pseudoacacia* seedlings grown with *A. altissima* seedlings from the U.S. seed lot had larger root systems.

**Discussion:**

Understanding the nature of competitive interactions among plants is essential to understanding changes in plant community structure and dynamics in response to invasion. In response to our first question of which species gains the most belowground and aboveground biomass, we found that *R. pseudoacacia* gained the most total, aboveground, and belowground biomass in competition with *A. altissima* under the two nutrient levels of this study. This result contradicts previous research (Call and Nilsen 2005), in which the invasive species *A. altissima* was the winner in competition with *R. pseudoacacia* when planted in a higher nutrient level than is typically found under natural conditions. In both nutrient levels of our study, *R. pseudoacacia* formed nodules, which was greater in the higher nutrient treatment, which seems to disagree with the hypothesis that *R. pseudoacacia* forms more nodules when soil nutrients are scarce. However, nodule
number per root weight was higher in the low nutrient level treatment, suggesting the low
average number of nodules was due to low root biomass production in the low nutrient
treatment. In contrast, *R. pseudoacacia* seedlings in the experiment of Call and Nilsen
(2005) didn’t form nodules because nutrients were so high. Therefore, their conclusion
about the advantage of invasive *A. altissima* in competition with *R. pseudoacacia* is
restricted to the very high nutrient level conditions used in their study. In natural
ecosystems, which our present study tried to simulate, we found that the nitrogen fixing
native plant, *R. pseudoacacia*, had the advantage in the interaction with *A. altissima*.
Therefore, at the seedling stage, the exotic species doesn’t necessarily have a competitive
advantage over the native species in this greenhouse study with natural early succession
site conditions.

Also, the extremely small size of *A. altissima* seedlings suggested the low nutrient
level we used was too low for *A. altissima* to thrive in a sandy environment. For both
species, the two nutrient levels we used in this experiment were designed to be close to a
natural system, but not high enough to let the plants achieve optimal growth at the
seedling stage, which was supported by the significant increases in leaf biomass and total
biomass of both species from low nutrient level to high nutrient level (Table 1, 2, 3).
Therefore, our conclusion that *R. pseudoacacia* has a competitive advantage over *A.
altissima* should be limited to relatively low soil nutrient conditions.

Our second question addressed whether *R. pseudoacacia* would be more
competitive when soil nutrients are limiting due to its ability to fix nitrogen, while *A.
altissima* would have a competitive edge when the nutrient levels are relatively high, as in
Call and Nilsen (2005). We found that in lower nutrient conditions, *R. pseudoacacia* was the stronger competitor (smaller $R_Y$ in low nutrient treatment). In fact, the ability to tolerate a low nitrogen environment is essential to the strong competitive ability of *R. pseudoacacia* (Boring 1984a, Gioria and Osborne 2014), and is a main reason why *R. pseudoacacia* is considered to be a good invasive species itself outside of its native range in the eastern U.S. (Castro-Díez et al. 2008).

In response to question three of how intraspecific and interspecific interaction impact biomass production, we identified both competition and facilitation between these species. However, we think that the “facilitation” observed in this experiment from *A. altissima* on *R. pseudoacacia* ($R_Y > 1$) was mainly due to the small size of *A. altissima* under these experimental conditions, which minimized the competitive effect of *A. altissima* on *R. pseudoacacia*. The latter had more resources available for growth when there were more *A. altissima* in the pot. The main effect that influenced *R. pseudoacacia* biomass traits in this study was intraspecific competition. We found $R_Y$ was smaller under the lowest nutrient condition, which is also due to weaker intraspecific competition under the low nutrient level.

Also, in response to interaction between question two and three, we found a significant interaction between species proportion and nutrient for the belowground biomass of *A. altissima*; as the number of *R. pseudoacacia* increased in the pot, the difference between high and low nutrient treatments became smaller. This might suggest that, in low nutrient conditions, greater *R. pseudoacacia* density in the pot led to more of a facilitation effect on *A. altissima* through nitrogen fixation. However, as $R_Y$ did not
increase as the number of *R. pseudoacacia* increased, this explanation seems unlikely. So this result may simply relate to the dominance of *R. pseudoacacia* in the belowground biomass under higher nutrient conditions, which led to stronger competition with *A. altissima* as the number of *R. pseudoacacia* increased.

Previous research found *A. altissima* (seedlings from the U.S. seed lot) had the competitive advantage primarily because of a dominant root biomass (Call and Nilsen 2005). The results from our study support this finding. Although *A. altissima* was not the winner in competition, it did produce more roots when competing with *R. pseudoacacia*. Significantly higher root/shoot ratio was found in the *A. altissima* seedlings from the U.S. seed lot as compared to those from the Chinese seed lot, suggesting high root/shoot ratio might be a mechanism of successful invasion for *A. altissima*. Some suggest that the superior competitive ability of invasive species over native species is closely related to more efficient resource acquisition or resource conservation (Tecco et al. 2010, Gioria and Osborne 2014). A variety of invasive species have higher resource-use efficiency than native species, especially in low-resource environments (Funk and Vitousek 2007). *Ailanthus altissima* has high physiological plasticity (Mou et al. 2013), which is an important physiological trait for this exotic invasive species to adapt to changing environments and be an “opportunist” when resource supply is variable. However, when grown in stable nutrient supply environments, similar to the experimental design used in our study, *A. altissima* didn’t show a strong plasticity to make it more competitive (Mou et al. 2013).

*Ailanthus altissima* has an allelopathic effect on many species (Mergen 1959), and
this could be one mechanism that determines the successful invasion of *A. altissima* (novel weapons; Rice 1984, Callaway and Ridenour 2004). Allelochemicals from some allelopathic invasive species have been found to have negative impacts on microbial mutualists of native species and nodulation of nitrogen fixing plants (Batish et al. 2007, Callaway et al. 2008, Wurst and Beersum 2009), including *A. altissima* (Lincoln 2012). In another bioassay study (Binninger and Bao, unpublished result), we found the water extraction of *A. altissima* leaves significantly decreased both the germination rate and radicle growth of *R. pseudoacacia* seeds, suggesting that the allelochemicals of *A. altissima* could negatively influence *R. pseudoacacia* growth.

In response to question four, we found that the presence of *A. altissima* reduced the number of nodules per root mass for *R. pseudoacacia*. In addition, our study found *R. pseudoacacia* seedlings grown with *A. altissima* seedlings from the U.S. seed lot had significantly fewer nodules per root weight compared with *R. pseudoacacia* seedlings grown with *A. altissima* seedlings from the Chinese seed lot. *Ailanthus altissima* from the U.S. seed lot seemed to have a stronger negative (perhaps allopathic) effect on *R. pseudoacacia* nodulation compared with that of the Chinese seed lot, which also answered question five.

The two seed sources of *A. altissima* had different growth rates, different responses to nutrient level and different competitive ability in the interaction with *R. pseudoacacia*. In response to question five of whether the Chinese *A. altissima* seed lot has a stronger competitive ability, we only found that the positive effect of nutrient increase on biomass production was more obvious for *A. altissima* seedlings from the
Chinese seed lot than those from the U.S. seed lot. This suggests the *A. altissima* genotypes in the U.S. may not need as high of a nutrient level to thrive as would the Chinese *A. altissima* genotypes. This could be related to rapid evolution of *A. altissima* in the U.S. populations. Also, it is possible that the introduced population of *A. altissima* 300 years ago was better-adapted to poor nutrient conditions than the population in China. We didn’t find that U.S. *A. altissima* had a higher competitive ability than the Chinese *A. altissima* when challenged with *R. pseudoacacia*. This is because there was a lower biomass and a lower RY_A for U.S. *A. altissima*, and no difference in RY_R, based on total biomass production, between the two seed sources. But the resource allocation (root/shoot ratio and leaf biomass production) of *R. pseudoacacia* was changed significantly when grown with different seed sources of *A. altissima*. *Ailanthus altissima* from its invasive range seemed to stimulate more root production of *R. pseudoacacia*. However, the seeds we used in this experiment were collected from a relatively small area in the U.S. and China, and therefore our conclusions only represent the differences between these two specific seed lots. There have been no studies so far on genetic differences between Chinese *A. altissima* populations and those in the U.S.A.

Contradicted to the hypothesis of *A. altissima* from the invasive region has stronger competitive ability, we found that the biomass production of *A. altissima* from the U.S. seed lot was in general smaller than the seedlings from the Chinese seed lot. We did notice that *A. altissima* seedlings from the U.S. seed lot produced more root than shoot biomass, whereas *A. altissima* seedlings from the Chinese seed lot produced equal amount or less root biomass than shoot biomass, especially in monoculture. This result suggests
that there is a different resource allocation strategy between *A. altissima* from its native and invasive range. However, U.S. *A. altissima* experienced more intense competition from *R. pseudoacacia* and the higher root/shoot ratio didn’t result in a better growth for U.S. *A. altissima*.

In summary, this study revealed that the exotic invasive species was not always a winner at the seedling stage, especially when competing with a nitrogen-fixing, fast-growing native plant in low nutrient environments. Our findings support the idea that *A. altissima* roots can inhibit nodulation and growth of nitrogen-fixing species, perhaps by allelochemical means. Also there was evidence to support the theory that invasive species have high nutrient acquisition ability. We found some evidence for different phenotypic response patterns between the invasive species from their invaded ecosystem compared with the invasive species from their native ecosystem. However, further analysis would be required to support the suggestion that there are genetically determined differences in response patterns.


Table 1. Mean ± 1 standard error of total biomass, leaf biomass, and root/shoot ratio per plant of *Ailanthus altissima* and *Robinia pseudoacacia* seedlings with different seed source, nutrient and proportion treatments. Seed sources are either a Chinese or a U.S. *Ailanthus altissima* seed lot. Nutrients are either high (3.744 g Osmocote® 14-14-14 slower-released fertilizer) or low (0.936 g). Proportion refers to the composition of species in each pot (For example, 6A = 6 *A. altissima* seedlings and 4A2R = 4 *A. altissima* and 2 *R. pseudoacacia* seedlings in the pot). Different letters following the means and standard errors indicate significant difference at the α = 0.05 level.

<table>
<thead>
<tr>
<th>Seed Source</th>
<th>Chinese <em>A. altissima</em></th>
<th>U.S. <em>A. altissima</em></th>
<th>Chinese <em>A. altissima</em></th>
<th>U.S. <em>A. altissima</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Biomass (g)</td>
<td>1.429±0.166 a</td>
<td>0.862±0.057 b</td>
<td>7.816±0.513 a</td>
<td>8.116±0.504 a</td>
</tr>
<tr>
<td>Leaf biomass (g)</td>
<td>0.503±0.066 a</td>
<td>0.151±0.008 b</td>
<td>3.343±0.191 a</td>
<td>1.413±0.084 b</td>
</tr>
<tr>
<td>Root/Shoot</td>
<td>1.167±0.050 a</td>
<td>2.238±0.091 b</td>
<td>0.477±0.021 a</td>
<td>1.564±0.033 b</td>
</tr>
</tbody>
</table>

| A. altissima | R. pseudoacacia |
|--------------|----------------|----------------|----------------|----------------|
| **Total Biomass (g)** | 1.15±0.09g a | 7.97±0.36g b |
| **Nutrient level** | **High nutrient** | **Low nutrient** | **High nutrient** | **Low nutrient** |
| **Total Biomass (g)** | 1.588±0.170 a | 0.711±0.036 b | 10.902±0.622 a | 5.091±0.260 b |
| **Leaf biomass (g)** | 0.464±0.067 a | 0.195±0.014 b | 3.140±0.198 a | 1.638±0.091 b |
| **Root/Shoot** | 1.718±0.081 a | 1.677±0.080 a | 1.037±0.042 a | 1.003±0.047 a |
| **Proportion** | **6A** | **4A2R** | **3A3R** | **2A4R** | **6R** | **4R2A** | **3R3A** | **2R4A** |
| **Total Biomass (g)** | 1.635±0.120 a | 1.027±0.255 b | 0.653±0.087 b | 0.679±0.119 b | 5.572±0.423 a | 7.623±0.631 b | 9.635±0.823 c | 13.416±1.349 d |
| **Leaf biomass (g)** | 0.444±0.044 a | 0.333±0.099 ab | 0.153±0.021 b | 0.242±0.086 ab | 1.761±0.140 a | 2.273±0.204 ab | 2.738±0.258 b | 3.921±0.424 c |
| **Root/Shoot** | 2.047±0.112 a | 1.390±0.066 b | 1.417±0.072 b | 1.696±0.182 ab | 0.952±0.044 a | 1.063±0.065 a | 1.064±0.081 a | 1.069±0.087 a |
Table 2. Results from three-way ANOVAs of seedling growth metrics for *Ailanthus altissima* growing in a full factorial additive design competition experiment with *Robinia pseudoacacia* in a greenhouse environment. Effects are *A. altissima* seed source (Chinese and U.S.), nutrient level (high and low) and proportion. Proportion refers to the composition of species in each pot. Four proportions of species were used (6A, 4A2R, 3A3R, 4A2R). n=8 replicates for each composition/nutrient/seed source, which totals 160 pots in the experiment. Significant effects are shown in bold.

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>Type III Sums of squares</th>
<th>F ratio</th>
<th>P value</th>
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<td></td>
</tr>
<tr>
<td>Seed source</td>
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<td>9.75</td>
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<tr>
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<td>1</td>
<td>3.208</td>
<td>5.89</td>
<td><strong>0.0157</strong></td>
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<tr>
<td>Proportion</td>
<td>3</td>
<td>5.615</td>
<td>3.44</td>
<td><strong>0.0170</strong></td>
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<tr>
<td>Source * nutrient</td>
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<td>2.124</td>
<td>3.90</td>
<td><strong>0.0490</strong></td>
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<tr>
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<td>2.05</td>
<td>0.1065</td>
</tr>
<tr>
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<td>0.857</td>
<td>0.52</td>
<td>0.6657</td>
</tr>
<tr>
<td>Source<em>nutrient</em>proportion</td>
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<td>1.084</td>
<td>0.66</td>
<td>0.5751</td>
</tr>
<tr>
<td><strong>Total Biomass (g)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>16.446</td>
<td>4.47</td>
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<tr>
<td>Nutrient</td>
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<td>34.412</td>
<td>9.35</td>
<td><strong>0.0024</strong></td>
</tr>
<tr>
<td>Proportion</td>
<td>3</td>
<td>80.541</td>
<td>7.30</td>
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</tr>
<tr>
<td>Source * nutrient</td>
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<td>12.339</td>
<td>3.35</td>
<td>0.0678</td>
</tr>
<tr>
<td>Source * proportion</td>
<td>3</td>
<td>6.782</td>
<td>0.61</td>
<td>0.6060</td>
</tr>
<tr>
<td>---------------------</td>
<td>----</td>
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<td>------</td>
<td>--------</td>
</tr>
<tr>
<td>Nutrient * proportion</td>
<td>3</td>
<td>15.092</td>
<td>1.37</td>
<td>0.2523</td>
</tr>
<tr>
<td>Source<em>nutrient</em>proportion</td>
<td>3</td>
<td>2.721</td>
<td>0.25</td>
<td>0.8638</td>
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**Root/Shoot (g)**

<table>
<thead>
<tr>
<th>Seed source</th>
<th>1</th>
<th>36.649</th>
<th>38.54</th>
<th>&lt;0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient</td>
<td>1</td>
<td>0.070</td>
<td>0.07</td>
<td>0.7857</td>
</tr>
<tr>
<td>Proportion</td>
<td>3</td>
<td>35.333</td>
<td>12.39</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Source * nutrient</td>
<td>1</td>
<td>0.394</td>
<td>0.41</td>
<td>0.5201</td>
</tr>
<tr>
<td>Source * proportion</td>
<td>3</td>
<td>107.718</td>
<td>37.76</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Nutrient * proportion</td>
<td>3</td>
<td>3.504</td>
<td>1.23</td>
<td>0.2991</td>
</tr>
<tr>
<td>Source<em>nutrient</em>proportion</td>
<td>3</td>
<td>2.307</td>
<td>0.81</td>
<td>0.4895</td>
</tr>
</tbody>
</table>
Table 3. Results from three-way ANOVAs of seedling growth metrics for *Robinia pseudoacacia* growing in a full factorial additive design competition experiment with *Ailanthus altissima* in a greenhouse environment. Effects are *A. altissima* seed source (Chinese and U.S.), nutrient level (high and low) and proportion. The proportion effect refers to the proportion of species in each pot. Four proportions were used (6A, 4A2R, 3A3R, 4A2R). n=8 replicates for each proportion/nutrient/seed source, which totals 160 pots in the experiment. Significant effects are shown in bold.

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>Sums of squares</th>
<th>F ratio</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leaf Biomass (g)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed source</td>
<td>1</td>
<td>407.519</td>
<td>104.92</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Nutrient</td>
<td>1</td>
<td>261.346</td>
<td>67.29</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Proportion</td>
<td>3</td>
<td>190.456</td>
<td>16.35</td>
<td>&lt;0.0001</td>
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<tr>
<td>Source * nutrient</td>
<td>1</td>
<td>66.565</td>
<td>17.14</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Source * proportion</td>
<td>3</td>
<td>45.431</td>
<td>3.90</td>
<td>0.0091</td>
</tr>
<tr>
<td>Nutrient * proportion</td>
<td>3</td>
<td>37.011</td>
<td>3.18</td>
<td>0.0241</td>
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<tr>
<td>Source<em>nutrient</em>proportion</td>
<td>3</td>
<td>6.801</td>
<td>0.58</td>
<td>0.6260</td>
</tr>
<tr>
<td><em>Total Biomass (g)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed source</td>
<td>1</td>
<td>2.434</td>
<td>0.06</td>
<td>0.8110</td>
</tr>
<tr>
<td>Nutrient</td>
<td>1</td>
<td>3869.860</td>
<td>91.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proportion</td>
<td>3</td>
<td>2495.848</td>
<td>19.58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Source * nutrient</td>
<td>1</td>
<td>26.178</td>
<td>0.62</td>
<td>0.4330</td>
</tr>
<tr>
<td>Source * proportion</td>
<td>3</td>
<td>50.526</td>
<td>0.40</td>
<td>0.7557</td>
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<td>---------------------</td>
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</tr>
<tr>
<td>Nutrient * proportion</td>
<td>3</td>
<td>679.104</td>
<td>5.33</td>
<td><strong>0.0013</strong></td>
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<tr>
<td>Source<em>nutrient</em>proportion</td>
<td>3</td>
<td>23.932</td>
<td>0.19</td>
<td>0.9048</td>
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**Root/Shoot (g)**

<table>
<thead>
<tr>
<th>Seed source</th>
<th>1</th>
<th>83.072</th>
<th>481.72</th>
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<tr>
<td>Nutrient</td>
<td>1</td>
<td>0.060</td>
<td>0.35</td>
<td>0.5568</td>
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<tr>
<td>Proportion</td>
<td>3</td>
<td>1.138</td>
<td>2.20</td>
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<tr>
<td>Source * nutrient</td>
<td>1</td>
<td>1.265</td>
<td>7.34</td>
<td><strong>0.0071</strong></td>
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<tr>
<td>Source * proportion</td>
<td>3</td>
<td>0.411</td>
<td>0.79</td>
<td>0.4980</td>
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<td>Nutrient * proportion</td>
<td>3</td>
<td>0.167</td>
<td>0.32</td>
<td>0.8095</td>
</tr>
<tr>
<td>Source<em>nutrient</em>proportion</td>
<td>3</td>
<td>0.784</td>
<td>1.52</td>
<td>0.2100</td>
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</table>
Table 4. Relative Yield for *Ailanthus altissima* (RY<sub>A</sub>) and *Robinia pseudoacacia* (RY<sub>R</sub>) in a replacement series experiment at a fixed density of 6 seedlings per 8L pot. L and H represented low and high nutrient levels; CH-AA and US-AA indicates *A. altissima* seedlings germinated from a Chinese seed lot or a U.S. seed lot; Treatment refers to the ratio of the number of seedlings of each species in the pot (e.g. 4A2R = 4 *A. altissima* and 2 *R. pseudoacacia* seedlings in the pot).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>L</th>
<th>H</th>
<th>L</th>
<th>H</th>
<th>L</th>
<th>H</th>
<th>L</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>4A2R</td>
<td>0.425</td>
<td>1.470</td>
<td>0.254</td>
<td>0.346</td>
<td>1.366</td>
<td>3.251</td>
<td>1.799</td>
<td>3.120</td>
</tr>
<tr>
<td>3A3R</td>
<td>0.252</td>
<td>0.734</td>
<td>0.295</td>
<td>0.377</td>
<td>1.047</td>
<td>2.370</td>
<td>0.980</td>
<td>2.685</td>
</tr>
<tr>
<td>2A4R</td>
<td>0.308</td>
<td>0.727</td>
<td>0.306</td>
<td>0.320</td>
<td>0.776</td>
<td>2.018</td>
<td>0.895</td>
<td>1.782</td>
</tr>
</tbody>
</table>
Table 5. Results of a three-way ANOVAs on Relative Yield for *Ailanthus altissima* (RY\textsubscript{A}) and *Robinia pseudoacacia* (RY\textsubscript{R}) based on the final, total plant dry weight in a replacement series experiment at a fixed density of 6 seedlings per 8L pot. Effects are *A. altissima* seed source (Chinese and U.S.), nutrient level (high and low) and proportion. The proportion effect refers to the proportion of species in each pot. Three proportions were involved in the RY calculation for each species (4A2R, 3A3R, 4A2R). n=8 replicates for each proportion/nutrient/seed source, which totals 160 pots in the experiment. Significant effects are shown in bold.

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>Sums of squares</th>
<th>F ratio</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Relative Yield Ailanthus (RY\textsubscript{A})</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed source</td>
<td>1</td>
<td>2.633</td>
<td>5.68</td>
<td>0.0195</td>
</tr>
<tr>
<td>Nutrient</td>
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<td>2.947</td>
<td>6.35</td>
<td>0.0137</td>
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<tr>
<td>Proportion</td>
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<td>0.882</td>
<td>0.95</td>
<td>0.3908</td>
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<tr>
<td>Source * nutrient</td>
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<td>2.000</td>
<td>4.31</td>
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<td>1.106</td>
<td>1.19</td>
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<td>Nutrient * proportion</td>
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<td>0.531</td>
<td>0.57</td>
<td>0.5665</td>
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<tr>
<td>Source<em>nutrient</em>proportion</td>
<td>2</td>
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<td>0.6642</td>
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<tr>
<td><strong>Relative Yield Robinia (RY\textsubscript{R})</strong></td>
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<tr>
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<tr>
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<tr>
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<tr>
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<td>0.853</td>
<td>1.45</td>
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Figure 1. Aboveground, belowground and total biomass of *Ailanthus altissima* in a replacement series experiment with *Robinia pseudoacacia* at constant density (6 plants per 8 L pot). The solid bars and error bars represent the average biomass per *A. altissima* plant and the standard error within each treatment group. The upper graph represents the results from an experiment using *A. altissima* seedlings from a Chinese seed lot, and the lower graph represents *A. altissima* seedlings grown from a West Virginia, U.S. seed lot. Two nutrient levels (low and high) were used for the experiment, along with the 4 different proportions of *A. altissima* and *R. pseudoacacia*. (e.g. 2A4R = 2 *A. altissima* and 4 *R. pseudoacacia* seedlings; 3A3R = 3 *A. altissima* and 3 *R. pseudoacacia* seedlings).
Figure 2. Aboveground, belowground and total biomass of *Robinia pseudoacacia* seedlings in a replacement series experiment with *Ailanthus altissima* at a constant density (6 plants per 8 L pot). The solid bars and error bars represent the average biomass per *R. pseudoacacia* plant and the standard error within each treatment group. The upper graph represents the results from an experiment using *A. altissima* seedlings from a Chinese seed lot, and the lower graph represents using *A. altissima* seedlings grown from a West Virginia, U.S. seed lot. Two nutrient levels were used for the experiment, along with 4 different proportions of *R. pseudoacacia* and *A. altissima* seedlings. (e.g. 2R4A = 2 *R. pseudoacacia* and 4 *A. altissima* seedlings; 3R3A = 3 *R. pseudoacacia* and 3 *A. altissima* seedlings).
Figure 3. The linear relationship between nodule number on *R. pseudoacacia* seedling root systems and total root system mass under two nutrient levels in a replacement series experiment with *Ailanthus altissima* seedlings. The red crosses are *R. pseudoacacia* plants in the high nutrient treatment, and the dashed line shows the regression fitting. The blue circles are *R. pseudoacacia* plants in the low nutrient treatment, and the solid line shows the regression fitting for this group. The grey area is the 95% confidence interval of the regression line.
Appendix:

Supplementary Figure 1. The $\Delta^{15}N_{\%}$ in leaf tissue and number of nodules on 30 *R. pseudoacacia* seedling root systems grown in monoculture in Virginia Tech greenhouse, 2012. The only source of N in *R. pseudoacacia* tissues came from nitrogen fixation by nodules.
CHAPTER 3

Interaction between saplings of exotic invasive Ailanthus altissima and native Robinia pseudoacacia in a 3-year common garden experiment

Abstract:

Interactions between exotic invasive plants and native plants can influence native plant population dynamics, communities and ecosystems. This study focuses on understanding the extent of interaction between a non-indigenous invader Ailanthus altissima and its coexisting native Robinia pseudoacacia in the Mid-Appalachian region. An additive-replacement series common garden experiment was established at the Blandy Experimental Farm, VA to identify the type and extent of interactions between A. altissima and R. pseudoacacia over a three-year period. Height and basal diameter of all plants were measured at the beginning and end of each field season. Extra plants of both species growing at the same site, but not in the experiment, were harvested for dimension analysis to estimate biomass. Competition indices were calculated to indicate the type and intensity of interaction. Both A. altissima and R. pseudoacacia growth in monocultures were inhibited by intraspecific competition. When the two species were grown together, competition rather than facilitation or no interaction were demonstrated. In the first year, A. altissima grown with R. pseudoacacia tended to be larger than A. altissima in monoculture, suggesting that R. pseudoacacia facilitated the growth of A. altissima. At the same time A. altissima had no negative influence on R. pseudoacacia. However, after the second year, R. pseudoacacia growth decreased as the proportion of coexisting A. altissima increased, suggesting an inhibition on R. pseudoacacia by A. altissima. Concurrently, there was no longer
any sign of *R. pseudoacacia* facilitating *A. altissima* growth. When there were more *A. altissima* than *R. pseudoacacia* in the plot, interspecific competition caused a reduction of biomass increment of *R. pseudoacacia* more so than *A. altissima*; in all other combinations, intraspecific competition affected biomass increment of *R. pseudoacacia* more than interspecific competition. Soil pH, the concentration of major elements, and the concentration of NH$_4^+$-N and NO$_3^-$-N were significantly changed during the experiment period, however, no significant differences were found among treatments. In conclusion, intraspecific competition occurred for both species at the sapling stage; *R. pseudoacacia* was influenced more from interspecific competition than *A. altissima*, and the intensity of competition changed across time.

**Keywords:** additive-replacement series, competition, dimension analysis, facilitation, interspecific competition, intraspecific competition, invasive species

**Introduction:**

Negative impacts of invasive plant species on native plant populations, plant community structure, and ecosystem nutrient recycling are well documented. Invasive plants have displaced native plant species (Stinson et al. 2006), deteriorated the quality of native forests (Alvarez and Cushman 2002, Kohli et al. 2006) and changed soil chemical properties (Gray 2008). The success of invasive species depends on the nature of the habitat at the time of invasion, the functional traits of introduced plants, and the interactions with other species after the first establishment of the invasive species. Some invasive plants become dominant in forest systems by outcompeting other species through interference competition, such as *Microstegium vimineum* and *Alliaria petiolata* (Belote and Weltzin 2006, Morrison et al. 2007). Other invasive plants are less-
dominant (distributed sparsely in the forest), but still have measurable effects on forest regeneration and understory diversity, such as *Lythrum salicaria* L. and *Ailanthus altissima* (Eckert et al. 1996, Gómez-Aparicio and Canham 2008a). In general, many introduced invasive plants will encounter and interact with native plants that have similar habitat requirements and similar functional traits. The success of each particular introduction will, in part, be dependent upon the ability of the invasive plant species to successfully compete with native plants for resources (Seabloom et al. 2003).

The interactions between introduced plants and native plants is an important filter to biological invasion. Invasive species are often thought to be better competitors compared with native species. Indirect competition can be defined as a negative (reduction in the performance, such as growth, reproduction) interaction between individuals caused by at least one common limited resource (Weigelt and Jolliffe 2003). Therefore, indirect competition is most likely to occur between closely related species sharing similar niche space (Harper et al. 1961). There are often members of the native species pool that have similar traits as the invading species. Consequently, it is most likely that invasive species will encounter and compete with a member of the native species pool that has invasive properties as well. However, it is also possible that the success of the exotic invasive species is due to facilitation by native species (Rodriguez 2006). The possibility of facilitation is more likely to occur when the invading species is at relatively low density. Furthermore, the interaction between particular invasive species and native species could switch between facilitation and competition depending on the environmental conditions, plant developmental stage, and the neighborhood of other species in the community (Stultz et al. 2007). We are interested in understanding how exotic invasive plants of low relative abundance interact (Competition, facilitation, or both) with native plants, especially
those native species that occupy a similar niche space as the invasive species.

We selected two tree species that are both fast-growing, early-successional species, which reproduce by both a large-amount of seeds and root sprouts: *Ailanthus altissima* (Mill.) Swingle (Tree of Heaven) and *Robinia pseudoacacia* L. (Black Locust). Both species prefer disturbed sites with high light intensity, and coexist in the eastern U.S. forest (Call and Nilsen 2003).

*Ailanthus altissima* has been an invasive tree in U.S. forest for more than 200 years, yet is found at relatively low density compared to some other invasive species (Feret 1973); *Robinia pseudoacacia* is native to the Mid-Appalachian mountain region, but also is viewed as an invasive species worldwide (Castro-Díez et al. 2008). Both species are among the most abundant trees on the planet because of their ability to grow fast, their high reproduction rate, their tolerance to extreme environment, and their use in horticulture, forestry, agriculture and medicine, by several cultures.

We propose that that these two species will encounter each other frequently in early successional stands of the Mid-Appalachian mountain region. In fact, a previous study has indicated that these species are contagious in selected post-logging sites (Call and Nilsen 2003). Their interaction could result in competition for early succession resources. However, *R. pseudoacacia*, a nitrogen fixing tree, increases the availability of nitrogen in post disturbance sites (Boring and Swank 1984) and increases non-native species richness and abundance (Von Holle et al. 2006). Thus, it is also possible that *R. pseudoacacia* will facilitate the growth and success of *A. altissima* by providing nitrogen to the higher nitrogen demanding *A. altissima*.

Based on our understanding of these two species, our overall goal was to address the following questions concerning the type, intensity and mechanisms of the interactions between these two target species: 1) What is the type of interaction between these two species,
competition, facilitation or neutral? 2) Does the intensity or type of interaction change during different stages from seedling to sapling? 3) Is aboveground leaf production (relevant to competition for light) different between the two target species and consistent with the competitive interaction between species? 4) Is the impact of the each species and their combination on native soil nutrients reflective of competition or facilitation? In this study, we evaluate the type of interaction between the two selected tree species at the level of seedling through sapling. Our approach was to establish a common garden study in which seedlings of both species were planted in an additive-replacement design and their growth patterns were followed for three years during which time the trees became saplings.

**Materials and methods:**

**Study species and site description**

The seeds of *A. altissima* were collected in early November, 2010 from existing trees in Blandy experimental farm (Boyce, VA, N 39° 3.82’ W 78° 3.87’), where this experiment was conducted. The seeds of *R. pseudoacacia* were collected in October, 2010 from roadside trees near Jefferson National Forest near Blacksburg, VA. Seeds of both species were germinated in a Virginia Tech greenhouse in April 2011 after stratification, and then transplanted to 25.4 cm deep tree seedling sleeves in the greenhouse. In June 2011, the seedlings were transplanted into the field site at the Blandy experimental farm.

This field site was originally a post agricultural field that had been fallow for at least 10 years. The particular area used for this study had been used previously to investigate the systematics and growth characteristics of morning glory. The site was sprayed with herbicide (Round-up) twice over a three-week period to control the previous plants before the site was
prepared for planting. The site was fenced during the three years of the experiment to reduce herbivory by deer and rabbits.

A weather station was installed in the experimental site for continuous monitoring of ground and leaf-height air temperature, and precipitation. The average growing season (May-October) monthly high/low temperature and total precipitation in the three years of the study were: 24.5°C / 12.9°C, 65.6cm in 2011; 25°C /11.9°C, 81.7cm in 2012; 24.4°C /11.4°C, 73.4cm in 2013.

Experimental design

The experimental design was an additive-replacement series (Figure 1A). Each experiment unit was a 2m*2m square plot, separated by at least 1m space on all sides of each plot. The plots were laid out into 6 rows with each row separated by 2m (Figure 1B). The experimental plot design was completely random design, resulting in 14 treatments that included four different tree densities (1-4 plants per plot), all possible species combinations under each density, and five replicates for each treatment. Within the plots that had more than one tree, a constant distance of 1m was kept between neighboring trees and the trees were arranged as far away from the border as possible. The spatial arrangement of neighboring plants in each plot was set up randomly to avoid the effect of different layouts. All the treatments and locations were randomly assigned to the 70 experimental plots. The seedlings of *A. altissima* and *R. pseudoacacia* (about 20-30cm in height) were transplanted and established successfully in June 2011.

In addition to the experimental plots, 12 extra plots were established for growing trees, destined to be harvested, to formulate a dimensional analysis after each growing season. We included these extra plots because the experimental trees could not be harvested for biomass
estimates during the three-year study. The extra plots were monocultures of 3 or 4 plants per plot resulting in 21 extra trees of each species. In the early fall of each year 7 plants of each species were randomly selected for harvest in order to formulate annual dimension analyses.

**Measurements**

The experiment ran from June 2011 to November 2013. No additional watering or nutrients were applied to the plots except some watering during the establishment period in the first growth year. Weeds were mowed around experimental plots and removed from within the plots on regular weekly basis during the growth season. After three seasons of growth the trees were large enough for the canopies of adjacent plots to touch. Therefore, the experiment was terminated after three years because plots would not be independent in future years.

At the beginning and end of each growing season, height and basal diameter were measured for every tree in all plots. Also, at the end of each growth season, before leaf fall, the seven randomly selected extra trees of each species were harvested at ground level, separated into leaf and stem, and put into a 60 °C oven until completely dry (typically 4-7 days for leaves, 15-35 days for stems) to get dry biomass measurements. Subsamples of leaves were taken from randomly selected plants for calculating specific leaf area during the first year harvest.

Four soil samples were taken from each experimental plot at the beginning and the end of the experimental period. For each experimental plot, we randomly selected four locations near the corners and collected a 10cm deep, 1cm diameter soil core at each location. Then big plant tissues in the soil core were picked out, and the four samples are completely mixed into one box, kept air-dry and sent to the Virginia Tech soil testing lab for general nutrients test (CEC, pH, BpH, P, K, Ca, Mg, Zn, Mn, Cu, Fe, B etc.). In addition, an anaerobic incubation with KCl extraction method (Maynard et al. 2008) was used to extract total soluble nitrogen. Nitrate and
ammonium ion concentrations in the filtered soil extractions were measured by the stream ecology lab at the Department of Biological Sciences, Virginia Tech (Lachat QuikChem 8500 Flow Injection Analyzer).

*Dimension Analysis, Competition Indices and Data Analysis*

Annual above-ground biomass increment, defined as end biomass minus beginning biomass in each growing season, was chosen as the measurement of plant fitness for the competition analysis. At the end of each growing season, a dimension analysis was performed for *A. altissima* and *R. pseudoacacia* respectively in order to establish the relationship between biomass, plant height and basal diameter. After obtaining the best regression equation for each species, biomass for each individual of *A. altissima* and *R. pseudoacacia* in the experimental plots was estimated based on its height and basal diameter measurements. The annual above-ground biomass increment for each tree was determined by subtracting the spring estimated aboveground biomass value from the fall estimated aboveground biomass value.

The aboveground biomass increment in the three growing seasons were used as response variable, and a repeated measurement ANOVA were used to detect the impacts of density, proportion of the other species and time. Students’ T-tests were applied to pairwise comparison among different density and proportion of other species treatments in each year. The final aboveground biomass after the three growing seasons was analyzed by two-way ANOVA with density and the proportion of the other species in the plot as two factors. Then Students’ T-tests were also applied to pairwise comparison.

The interpretation of the outcome of competition depends critically on how the competition was measured and which indices were used (Freckleton and Watkinson 1999). We evaluated two ways of quantifying the neighboring plant effect on target species biomass
increment. The first, and most common method used in the literature, was an evaluation of biomass increment in relation to the number of neighboring plants of the other species. The second way was an evaluation of target species biomass increment in relation to the total biomass of the neighboring species. The second method has been considered more appropriate when two species with large size difference are compared (Thomas and Weiner 1989, Ramseier and Weiner 2006). In our study, although both species started with similar size, \textit{R. pseudoacacia} was characteristically larger than \textit{A. altissima} later in the experimental period.

To evaluate the competition effect and intensity, we reviewed the existing indices in the literatures and chose two competition indices that were appropriate for our experimental design—1) substitution rate/competition coefficient (S), and 2) relative interaction intensity (RII) to evaluate the type and extent of interaction between these two species.

Substitution rate is computed based on multivariate regression coefficients to evaluate the relative intensity of intraspecific and interspecific competition, which are obtained by the following equations:

\begin{equation}
\text{Mean biomass increment per plot } A. \text{altissima} = B_0 + B_{aa} (D_{aa}) + B_{aarp} (D_{rp})
\end{equation}

\begin{equation}
\text{Mean biomass increment per plot } R. \text{pseudoacacia} = B_0 + B_{rp} (D_{rp}) + B_{rpaa} (D_{aa})
\end{equation}

Where

\begin{itemize}
\item $D_{aa}$: density of \textit{A. altissima} in the plot
\item $D_{rp}$: density of \textit{R. pseudoacacia} in the plot
\item $B_0$: estimated intercept in the model
\item $B_{aa}$: estimated coefficient for intraspecific competition of \textit{A. altissima}
\item $B_{rp}$: estimated coefficient for intraspecific competition of \textit{R. pseudoacacia}
\item $B_{aarp}$: estimated coefficient for interspecific competition of \textit{R. pseudoacacia} on \textit{A. altissima}
\end{itemize}
*altissima*

\[ B_{rpa} : \text{estimated coefficient for interspecific competition of } A. \text{altissima on } R. \]

*pseudoacacia*

Then, the Substitution Rate (also called the Competition Coefficient) for *A. altissima*: \( S_{aa} = \frac{|B_{arp}/B_{aa}|}{B_{rpa}/B_{rp}} \) was calculated (Spitters 1983, Firbank and Watkinson 1985).

To measure the interaction type and intensity in these two plants mixtures, Relative Interaction Intensity (RII) was calculated.

\[
RII = \frac{B_w - B_o}{B_w + B_o}
\]

Where

\( B_w : \text{Biomass observed for target plant when growing with other plants} \)

\( B_o : \text{Biomass of target plant growing in absence of inter/intraspecific interactions} \)

RII was chosen over other frequently used competition indices because it has robust mathematical and statistical properties, and clear interpretation. RII has a continuous range from -1 (competitive exclusion) to 1 (symbiosis), and a positive number indicates facilitation while a negative number suggests competition. RII offers the most consistent results among the other interaction indices that are commonly used (Armas et al. 2004).

Since there were only 5 replicates for the monoculture plots in our experimental design, the usual calculation of a mean \( B_o \) could result in a bias when calculating the RII for the other 45 plots. In order to reduce the possibility of this bias, we used a non-parametric bootstrap to make sure the \( B_o \) we used was randomly selected (Efron and Tibshirani 1993). For each mixture plot, we first computed the average of calculated \( B_w \), then computed RII 2000 times by bootstrapping the \( B_o \) value from the 5 different biomass increment values from the monoculture plots, and
obtained the average RII value for this plot. After computing RII for each mixed species plot, a
repeated measurement two-way ANOVA was applied to analyze the competition indices. Also,
we used a rank order technique to analyze the competitive effect of various treatments. For each
year, we ranked the treatments by RII values from lowest (more competition) to highest (possibly
facilitation) to perform a rank order analysis. The three rank numbers (from 2011, 2012 and 2013)
were added together for each treatment to get the overall rank, which was used to indicate
competition intensity.

Results:

At the end of the first growing season, plants were relatively small with an average (mean
\[ \pm \text{standard deviation} \] height of 1.24±0.71 and 0.52±0.43m for *R. pseudoacacia* and *A. altissima*
respectively. The plants had not formed multi-branched architecture at this stage. We harvested 6
individuals per species from the extra plots. Only 6 were harvested, instead of 7 as we planned,
in order to keep a larger number of extra plants available for harvesting in years two and three
when the plant sizes were going to have larger variance. Without subsampling, the resulting
sample size and the range of size were too small to effectively estimate the biomass of the other
100 plants in the experimental plots using the first year regression data alone. After the second
growing season the trees attained 3.85±1.49m and 1.48±1.63m height on average for *R.
pseudoacacia* and *A. altissima* respectively. The final average heights of trees after three years
of growth were 4.58±2.04m for *R. pseudoacacia* and 2.03±2.12m for *A. altissima*. Following a
late freeze in the spring of the 2nd year, *A. altissima* became heavily multi-branched. Therefore,
we measured the height and diameter for all branches of each plant to increase the accuracy of
our estimated biomass increment. Consequently, the height and diameter of *A. altissima* had
increased variance in later years. So starting in the 2nd year, we expanded the sample size for the
dimension analysis by harvesting 7 trees per species. Additionally, we included whole branch
subsamples as data points to develop a regression that had a large enough range in basal diameter
and height to include most of the trees in the experimental plots. The biomass of trees in the first
growing season was estimated using combined data from 2011 and 2012 to formulate our
regressions between biomass, height and diameter for both species (Table 1). The fitting power,
of the combined regressions, was improved compared with the regressions formed by using data
from each year alone. In order to fit the regression equations for biomass estimation, square root
transformation was needed for A. altissima in all three years, while log transformation was
needed for fitting linear regressions for R. pseudoacacia in the first two years to meet the
normality assumption (Table 1). Adjusted R^2 for these four equations are all ≥0.95, which
suggested that our estimate of biomass was robust. Basal diameter was more important in
predicting biomass than height, which is reasonable because of the wide-spread nature for the
canopies of both species.

There was a tremendous size difference in biomass increment between these two species
(p<0.0001) and across the three years (p<0.0001, Table 2). The biomass increment for R.
pseudoacacia in the first growing season was about double that of A. altissima, but both plants
were still small at the end of 2011 (Figure 2). In the second growth season, biomass increment
for both species significantly increased by 6-8 times that of the first growth season (p<0.0001).
This biomass increment during the third growing season was smaller than that of the second
growth season (p=0.0017).

Evidence for intraspecific competition was found for both species (Figure 2). Both
species had the largest individual biomass increment in the lowest density plots (density=1) in all
three years (Figure 2), but the effect of density was not significant at the $\alpha = 0.05$ level. Pairwise comparison showed for *A. altissima*, that the biomass increment was significantly larger in the lowest density monoculture than that of all other monoculture densities (density=2, 3, or 4 trees per plot) in year 1 (p=0.032, 0.047, and 0.037 respectively) and year 2 (p=0.030, 0.023, and 0.013 respectively). For year 3, the difference in biomass accumulation was not significant between any pair of monoculture density of *A. altissima* (Figure 2A). Surprisingly, in monoculture, *R. pseudoacacia* showed a trend of increasing biomass growth as density increased in year 1, but the trend was not significant. This trend was reversed in the next two growing seasons (Figure 2B). The difference in average biomass increment between monoculture densities was only significant between the highest density and lowest density group in year 2 (p=0.032). There was a significant or barely significant difference in biomass increment in pairwise comparison between the lowest density monoculture and other density monocultures for *R. pseudoacacia* in year 3 (p=0.026, 0.053, 0.035 respectively) (Figure 2B). In general, increasing density from 2 trees per plot to 3 and 4 trees per plot in monoculture did not affect biomass increment significantly in all three years for either species (Figure 2).

Aboveground individual biomass increment of *A. altissima* tended to be higher in the presence of *R. pseudoacacia* than in the presence of itself at density 2 in all three years, indicating facilitation of *A. altissima* by the presence of *R. pseudoacacia* (Figure 3A). However, this trend was not statistically significant (p=0.65, 0.42, 0.57 in year 1, 2 and 3). There was no significant difference in biomass increment of *A. altissima* among different proportions of *R. pseudoacacia* treatments at higher densities. In the year 2 and year 3, the differences among the groups with different neighboring *R. pseudoacacia* proportion became less and less identifiable (Figure 3A). Also, in year 1 and year 2, the trend of increasing *A. altissima* biomass increment by
neighboring *R. pseudoacacia* occurred when the proportion of the latter was no more than 50% in the plot, and it disappeared when *R. pseudoacacia* became dominant (more than 50% in number) in the plot (Figure 3A). This was not true for year 3, when there was a trend for increasing aboveground biomass increment for *A. altissima* at density 4 (four trees per plot) when *R. pseudoacacia* was dominant (Figure 3A). The effect of density and proportion of *R. pseudoacacia* on *A. altissima* were not significant at the α=0.05 level when all treatments and years were combined (Table 2).

Aboveground individual biomass increment of *R. pseudoacacia* slightly increased in the presence of *A. altissima* at density 2 in all three years (Figure 3), but that increase was not statistically significant (p=0.27, 0.31, 0.42, in three years respectively). However, aboveground biomass increment of *R. pseudoacacia* decreased slightly, as the proportion of *A. altissima* increased at density 3 (Figure 3B). The effect of *A. altissima* on *R. pseudoacacia* tended to change from a positive relationship to a negative relationship in year 1 and year 2 at density 4 (Figure 3B). There was one tree with negative biomass increment in both species at density 4 monoculture group, which may have caused the monoculture group to have a lower observed average value than actual. The effect of *A. altissima* on the aboveground biomass increment of *R. pseudoacacia* was not significant at the α=0.05 level when all treatments and years were combined (Table 2). It is important to note that the biomass increments in the mixture treatments were the result of combined effects of both intra- and inter-specific interactions.

The final aboveground biomass of both species after three years of the experiment (Figure 4) was analyzed to evaluate the accumulation of three years of plant interaction on the final fitness of each species. For *A. altissima*, the density and proportion of *R. pseudoacacia* had no significant effect for the final aboveground biomass (Table 3). But plants in density 1 had
significant biomasses compared with plants in density 2, 3 and 4 (p=0.043, 0.022 and 0.008 respectively). The final biomass of monoculture in density 1 was significantly greater than monocultures with the other three densities (p<0.0001), also there was no significant differences among the other three monocultures (Figure 4). For *R. pseudoacacia*, density was a significant factor, while the proportion of *A. altissima* was not significant for the final aboveground biomass (Table 3). Plants in density 1 had significantly greater biomasses when compared with plants in density 2, 3 and 4 (p=0.003, 0.018 and 0.001). *Robinia pseudoacacia* grown in the 50-50 mixture with *A. altissima* had significantly greater final biomass than those grown with 33% or 67% *A. altissima* (p=0.024, 0.035). In monocultures of *R. pseudoacacia*, there was no significant density effect; but *R. pseudoacacia* monocultures in density one had significantly higher biomass than those in density 2 and 4 (p=0.0098, 0.0099).

In order to separate the effects of intra- and inter-specific interaction, we calculated the competition coefficients using aboveground biomass increment in each year (Table 4). In all three years, all the coefficients of regression had a negative sign, indicating that competitive interactions occurred either between or within both species. Although intraspecific competition intensity of *A. altissima*, indicated by $B_{aa}$, were not significant at the $\alpha = 0.05$ level, the intraspecific competition intensity tended to increase from year 1 to year 2 and year 3 (Table 4). Intraspecific competition intensity for *R. pseudoacacia*, increased from years 1 to 2, but then decreased at year 3. Only *R. pseudoacacia* in year 2 had a significant $B_{rp}$ at the $\alpha = 0.05$ level (Table 4). The interspecific competitive effect of *R. pseudoacacia* on *A. altissima*, indicated by $B_{aarp}$, tended to increase from year 1 through year 3, although none of them were significant at the $\alpha = 0.05$ level (Table 4). The interspecific competitive effect of *A. altissima* on *R. pseudoacacia*, indicated by $B_{rpaa}$ also increased from year 1 through year 3. The $B_{rpaa}$ was
significant at the $\alpha = 0.1$ level in year 1, and at the $\alpha = 0.05$ level for year 2 and 3, suggesting a strong interspecific competition of A. altissima on R. pseudoacacia, and that competitive effect increased through time. Substitution rate (S) values suggested intraspecific competition was dominant in A. altissima, but interspecific competition became more important as time passed ($S_{aa}$ were smaller than 1 and increased in three years, Table 4). For R. pseudoacacia, interspecific competition was significant and the dominant factor in all three years, especially years 1 and 3 ($S_{np}$ was bigger than 1, Table 4). Robinia pseudoacacia experienced an equally significant and severer intraspecific competition and interspecific competition in year 2, and intraspecific competition was slightly more important (Table 4).

Despite the general high p-values, multiple comparisons of RII did reveal consistent patterns for both species across treatments (Table 5). Most RII values were negative for A. altissima, suggesting a competitive effect, either intraspecific or interspecific had occurred. Two-way ANOVA results suggested density had a significant effect on RII of A. altissima in year 1. Specifically, RII at density 2 was significantly lower than at density 3 ($p=0.0376$). On the other hand, the proportion of the neighboring R. pseudoacacia trees was not a significant factor in year 1 ($p=0.091$). Neither the effect of density nor proportion of the R. pseudoacacia was significant in year 2 and 3. Although, there was a trend that higher density groups had lower RII, suggesting more intense competition as tree density increased in both years. In all three years, the 50%-50% group had the biggest RII (close to 0). For year 1 and 2, there was a trend that if there were more R. pseudoacacia than A. altissima in the plots, RII was lower than other treatments at the same total density level for A. altissima. For the yearly change of interaction intensity, the order of average RII value of A. altissima across all treatments from lowest to highest was year 2 (average RII= -0.175), year 1 (RII= -0.13) and year 3 (RII= -0.061).
Values of RII for *R. pseudoacacia* were similar across the three density levels (Table 5). And the competitive effects were the strongest in 2nd year, and were slightly bigger in 1st year than 3rd year (Table 5). There were consistent patterns showing various competitive effects from *A. altissima* to *R. pseudoacacia* in three years: *R. pseudoacacia* grown with 66% and 75% of *A. altissima* had the largest competitive effect, followed by 0% plots (intraspecific competition only), and 50%, 33% and 25% (Table 5). This pattern indicated that *R. pseudoacacia* trees were inhibited by *A. altissima* if the latter was dominant in the neighborhood, otherwise, *R. pseudoacacia* trees suffered more from competition with themselves. The rank order analysis of competition intensity showed RII was the biggest in 2A1R treatment, followed by 2R and 3A1R treatments, then 2A2R and 3R. This pattern also suggested that *A. altissima* may have a bigger interspecific competition effect on *R. pseudoacacia*, than the intraspecific competition from *R. pseudoacacia* when there are at least equal number of both species.

The biomass of neighboring *R. pseudoacacia*, instead of the number of neighboring *R. pseudoacacia*, did not have a significant negative effect on *A. altissima* biomass increment in all years. The regression of target tree biomass increment vs. neighbor tree total biomass showed no relationship between these two variables (Figure 5). The biomass of neighboring *A. altissima* also did not have a significant negative effect on the biomass increment of *R. pseudoacacia*. But in year 1, as size of neighboring *A. altissima* increased, the biomass increment of *R. pseudoacacia* tended to decrease (Figure 5).

Since the architecture of *R. pseudoacacia* and *A. altissima* were quite different, and aboveground biomass difference might not give enough information on their competition ability, we further tested if there was any difference in the amount of leaf mass one branch can produce between these two species. We hypothesized that the leaf production per branch diameter could
explain why a certain species had an advantage in aboveground biomass increment. Using the
extra harvest trees in year 3, we made regressions of leaf biomass vs. branch basal diameter. A
square root transformation was made to response variable leaf biomass in order to satisfy the
linear regression assumptions. We found that as branch diameter increased, leaf biomass of *R.
pseudoacacia* increased faster than that of *A. altissima* (Figure 5). The two regression lines are
significantly different (p=0.028) based on ANCOVA test.

We further tested the difference in specific leaf area for both species from the data
collected in year 1. The average SLA for *A. altissima* was 146.03 ± 12.83 cm²/g, and for *R.
pseudoacacia* it was 185.27 ± 9.66 cm²/g. Combining this result with the previous relationship
between branch diameter and leaf biomass, we concluded that *R. pseudoacacia* produced more
leaf area than *A. altissima* at the same size of branch.

Soil pH, CEC and major soil nutrient elements were not statistically different among
treatment groups at the beginning of experiment. Among all tested ions, only potassium
concentration had a slightly significant difference among treatment groups at the beginning of
experiment (p=0.0455). This could be caused by the previous use of this same field for
experiment involving fertilization. At the end of the three-year growing period, soil pH increased
to 6.65 ± 0.29 from 5.57 ± 0.20 (p<0.0001). This increase in pH is consistent among all
treatment groups. Potassium concentration had significantly increased by the end of the three-
year experiment (p<0.0001). Although potassium concentration was still significantly different
among treatment groups at the end (p=0.0385), there was no difference in potassium
concentration increase during the three years among groups (p=0.1223). K, Ca, Mg, Zn, Mn and
B concentrations were all significantly increased (p<0.0001) after the three-year experiment,
while Cu and Fe concentrations were significantly decreased (p<0.0001). None of these changes
was significantly different among treatment groups. CEC significantly increased from 3.34 to 9.92 meq/100 (p<0.0001). \(NH_4^+\)-N concentration significantly increased from 6469.83 ug/L to 12800.1 ug/L (p<0.0001). Meanwhile, \(NO_3^-\)-N concentration significantly decreased from 290.17 ug/L to 193.73 ug/L (p<0.0001). None of these changes were significantly different among treatment groups. However, at density 1, 2 and 3, there was a pattern that that plots with more \(R.\) pseudoacacia trees had less of a decrease in \(NO_3^-\)-N and a larger increase in \(NH_4^+\)-N. At the plot level, we noticed that there was a much thicker leaf litter layer under pure \(R.\) pseudoacacia stands or in the plots dominated by \(R.\) pseudoacacia in year 2 and 3 than in other plots (Bao, personal observation).

**Discussion:**

The native \(R.\) pseudoacacia had more than twice the biomass increment compared with the invasive \(A.\) altissima in the first three years of establishment. Intraspecific competition occurred for both species because both species grew best in the one plant per plot condition compared with all the other monoculture densities. However, as the density increased above one tree per plot, intraspecific competition intensity remained the same. Also, intraspecific competition became stronger as the years passed, but was only significantly different among densities above one tree per plot in the second growing season for \(R.\) pseudoacacia. Along with the big jump in size for both species in the second growing season, the intraspecific competition coefficient also increased significantly from the 1st year to the 2nd year. The intensity of intraspecific competition was highly correlated with plant size, which suggests that indirect competition for the same resources such as light and nutrients was the cause of intraspecific competition.
In response to question one, the type of interaction between these two species was mainly competition rather than facilitation. The negative effect of competition on plant growth affected *R. pseudoacacia* more than it did *A. altissima*. In the three years, the influence of *R. pseudoacacia* on vegetative growth of *A. altissima* was either neutral or slightly facilitated, while in most treatments, vegetative growth of *R. pseudoacacia* trees was inhibited by neighboring *A. altissima*. For *R. pseudoacacia*, the competition impact from *A. altissima* was stronger than intraspecific competition only when *A. altissima* was dominant in number (Table 3.). For *A. altissima*, there was less intraspecific competition, which could explain why there are more pure stands found for this invasive tree in the forest compared with that of *R. pseudoacacia* (personal observation). The interspecific competition outcomes from this experiment were not as clear as intraspecific competition results in Figure 2, because of larger variation within each treatment group, and also because the outcomes reflected the combination effects of both intraspecific and interspecific competition. The competition coefficient result (Table 3) supported an interspecific competitive interaction between these two species. Overall, our results are consistent with previous theories of density-dependent regulation in plants. We believe that *R. pseudoacacia* in the eastern forest could be negatively impacted by the neighboring *A. altissima*, and this negative impact on *R. pseudoacacia* growth may make the native *R. pseudoacacia* more vulnerable to herbivory by leaf miners, which could further weaken the performance of *R. pseudoacacia* in the presence of *A. altissima* and other common invasive species in the Mid-Appalachian forest.

We found that the growth of *R. pseudoacacia* was inhibited by *A. altissima* the most when the latter was dominant in the neighborhood. Otherwise, intraspecific competition might be more important than interspecific competition for *R. pseudoacacia*. This revealed the importance of early-stage removal for the invasive species *A. altissima* in eastern native forest, especially
around *R. pseudoacacia*, to eliminate the negative impact of this invasive species on the native nitrogen fixing *R. pseudoacacia* and its influence on ecosystem nitrogen cycling. This finding supports the “early detection, rapid response” approach for invasive species control (Westbrooks 2004).

Our results suggest that studying different developmental stages of interactions between species is important. Most previous studies of the competition between native and invasive plants have focused on grasses and forbs, because of their short life cycle and because they are much easier to manipulate compared with trees (Vila and Weiner 2004). However, interactions between invasive trees could be very different from the results of studies with invasive herbaceous species (Tecco et al. 2010, Goria and Osborne 2014), and shape the community structure and function and impact other species more significantly in the understory (Siemann and Rogers 2003, Kueffer et al. 2007, Hejda et al. 2009). The impact of invasive trees on native trees occurs for a long time period after disturbance (Strayer et al. 2006, Atwood et al. 2010, Vilà et al. 2011). Studies like ours should occur over an even longer time period to better understand the interactions between these invasive and native trees. Although we only conducted this experiment for three years, we have discovered a shift of interaction type and variation in competition intensity for trees from the seedling stage to the sapling stage. In response to our second posed question, we found that the negative effect of the invasive tree *A. altissima* on native *R. pseudoacacia* became stronger as plant developed from seedlings to saplings. One possible reason would be *A. altissima* grew bigger and as a result had a stronger interaction with neighbors in year 2 and year 3. Also, intraspecific competition became stronger for both species as time passed, especially during the second growing season when the growth was most rapid. For *A. altissima*, the competitive impact from *R. pseudoacacia* and itself were both the strongest
in second year. We propose that substantial negative impact of *A. altissima* on *R. pseudoacacia* was dependent upon the size of the invasive species becoming large enough to influence other neighboring saplings. These results support an early control strategy for the invasive *A. altissima*.

Previous studies have shown that the type of interaction between a pair of plant species can switch quickly corresponding to environment conditions, even on a daily basis (Tielbörger and Kadmon 2000). At any particular time the overall result of competition or facilitation is a cumulative effect. Therefore, it is possible that *R. pseudoacacia* has a facilitative effect (due to the effect of nitrogen fixation on soil nitrogen availability) on the growth of *A. altissima* when the soil nutrient level is low. Moreover, at times or places in which the population of locust leaf miners are low, the competitive effects from *A. altissima* may also be low (refer to Chapter 2). Although the intensity of competition or the possibility for facilitation may change within a season, our analyses were limited to the overall effect of a whole growing season. In our case, competition is a dominant aspect of the interaction between *R. pseudoacacia* and *A. altissima*.

One hypothesis posed in the literature about interspecific competition is that the negative effect of a neighbor increases as its size enlarges (Goldberg and Werner 1983). However, in contradiction to the results of the previous study (Ramseier and Weiner 2006), we found no strong negative relationship between competitive effect and the size (in terms of biomass) of its neighbor. Our result may be a consequence of the longer experimental period of our study compared with the one month experimental period used by Ramseier and Weiner. For example, we did find a trend for a size effect of *A. altissima* on *R. pseudoacacia* in the first year in our study when the plants were small, but when plants were bigger this trend became unclear.

In theory, tree biomass growth rate follows an exponential curve, and we think one possible reason for a slowing annual biomass increment in our study between year 2 and 3 could
be due to an increase of competition intensity among plants in the plots. We observed that the
individual trees of both species were too small to have a lot of interactions above and
belowground (competition for light) in the first growing season. However, tree biomass got much
larger at the end of the 2nd year, and the branches started to shade each other heavily within the
plots, suggesting that competition for light could be occurring commonly in all plots starting in
the 2nd year. Also, we determined that *R. pseudoacacia* produced a greater amount of leaf area
per branch diameter than *A. altissima*. Thus, the larger leaf area production and the more
branched canopy architecture of *R. pseudoacacia* was consistent with its increase in intraspecific
competition from seedling to sapling stage. However, in response to question 3, the higher leaf
area of *R. pseudoacacia* was not consistent with the increasing negative effect of *A. altissima* on
*R. pseudoacacia* measured in this study. Therefore, it is likely that a competitive advantage
below ground or a direct competition effect by allelopathy is inducing the increasing negative
influence of *A. altissima* on *R. pseudoacacia* with time.

We found that almost all soil characteristics we measured have been significantly
changed in the three-year period, which demonstrated the potential change to soil nutrients and
environment in the presence of these two species. The increase in soil pH and NH$_4^+$-N agreed
with the findings in a forest survey study where plots under *R. pseudoacacia* and *A. altissima* had
higher soil pH and NH$_4^+$-N compared with control plots (Bao and Nilsen, unpublished result). In
response to our fourth question, each species affected the soil nutrition, but there wasn’t any
difference in those effects among different treatments in our experimental settings. Our plot was
only 4 m$^2$ in area, so the change in density and species combinations we set up for different
treatments might be not big enough to demonstrate differences in the influences of each species
on soil nutrition. Also, it is possible that both species effect soil nutrition similarly, but for
different reasons. One time test at the end of the experiment might not provide enough
information to explain the mechanisms by which the soil conditions have been changed during
the three years of the study.

We knew that both species would grow vigorously and quickly during young ages,
because both are considered invasive in their non-native geographic regions. However, native
herbivores could influence the interaction between these two species. In native Appalachian
forest in VA, *R. pseudoacacia* seems to be less healthy than adjacent *A. altissima*, mainly due to
the effects of herbivory by locust leaf miner (Bao, personal observation). Our experimental
design did not include any treatment for herbivores. Leaf miners were found on *R. pseudoacacia*
saplings in this experiment and those leaf miners did induce defoliation for some experimental
trees. However, *R. pseudoacacia* still had a stronger overall performance that *A. altissima* based
on the larger biomass at the end of the experiment. Therefore, *R. pseudoacacia* appears to be a
more vigorously growing species than *A. altissima*, even with the negative effects of native
herbivores, in old agricultural fields in the Mid-Appalachian Mountains. Further studies on the
specific effects of herbivory on the interaction between these two species is ongoing.

In summary, small intraspecific competition, effective belowground competition, possible
allelopathic effects and the ability to form monoculture stands could all be mechanisms to
explain the invasability of *A. altissima*. The rapid growth, capacity to fix nitrogen, and large leaf
production explains the competitive ability of *R. pseudoacacia*, and why it is considered to be an
invasive tree worldwide. When the two species co-exist, our findings suggest that the growth of
invasive *A. altissima* would not be negatively affected by *R. pseudoacacia*; conversely, the
native *R. pseudoacacia* was suppressed by *A. altissima*, which could be a result of negative effect
of allelopathy on nodule formation and nitrogen fixation of *R. pseudoacacia* (Batish et al. 2007).
Understanding the potential implications of competition between these two species in the Mid-
Appalachian forest will require study over a longer time scale, and larger spatial scales such as local forest stands and the region level. These studies are currently underway, which will provide a broader prospective on the consequence of the interaction between these species on community structure.
Literature cited:


(Lythraceae)." **Evolution** *50*(4): 1512-1519.


Stultz, C. M., C. A. Gehring and T. G. Whitham (2007). "Shifts from competition to facilitation


Table 1. Dimension Analysis of total above ground dry weight (biomass) for *Ailanthus altissima* and *Robinia pseudoacacia* based on basal diameter and height for three growing seasons 2011-2013. All plants were grown in a common garden setting in Boyce, Virginia, USA. Regression equations and adjusted $R^2$ of those equations are presented for the combined data set (2011 and 2012) and the data set for 2013 alone.

<table>
<thead>
<tr>
<th>Biomass Estimation Equation and Adjusted $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Ailanthus altissima</strong></td>
</tr>
<tr>
<td><strong>2011</strong> Biomass (g) = ($-4.94247+1.974 \times \text{Height (m)}+7.266 \times \text{Diameter (cm)} +0.74 \times (\text{Height}−1.4963) \times (\text{Diameter}−2.5354))^2$</td>
</tr>
<tr>
<td>$R^2=0.951$</td>
</tr>
<tr>
<td><strong>2012</strong> Biomass (g) = (2.5812+1.6176 \times \text{Diameter (cm)}−0.1 \times (\text{Diameter (cm)}^2)</td>
</tr>
<tr>
<td>$R^2=0.947$</td>
</tr>
<tr>
<td><strong>2013</strong> Biomass (g) = ($-10.43578+1.952572 \times \text{Height (m)} +9.263966 \times \text{Diameter (cm)}+0.795541 \times (\text{Height}−2.37) \times (\text{Diameter}−3.7924)^2$</td>
</tr>
<tr>
<td>$R^2=0.976$</td>
</tr>
</tbody>
</table>

| **Robinia pseudoacacia**                     |
| **2013** Biomass (g) = $-5847.114−361.3571 \times \text{Height (m)}−2052.2315 \times \text{Diameter (cm)}+287.6451 \times (\text{Height}−3.358) \times (\text{Diameter}−5.871)$ |
| $R^2=0.966$                                   |
Table 2. Repeated measurement ANOVA result of total above ground dry biomass increment for *Ailanthus altissima* and *Robinia pseudoacacia* from a three-year common garden competition experiment, 2011-2013. All plants were grown in a common garden setting in Boyce, Virginia, USA. Only three density levels: 2, 3, 4 trees per plot were included in the analysis of intra- and interspecific interactions. Num DF = degrees of freedom of numerator; Den DF = degree of freedom of denominator; Density = effect of number of trees per plot on mean biomass increment; Year = effect of different years on mean biomass increment.

<table>
<thead>
<tr>
<th>Fix Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. altissima</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>2</td>
<td>85</td>
<td>0.43</td>
<td>0.6546</td>
</tr>
<tr>
<td>% of <em>R. pseudoacacia</em></td>
<td>5</td>
<td>85</td>
<td>0.28</td>
<td>0.9235</td>
</tr>
<tr>
<td>Density * % of <em>R. pseudoacacia</em></td>
<td>10</td>
<td>85</td>
<td>0.40</td>
<td>0.5300</td>
</tr>
<tr>
<td>Year</td>
<td>2</td>
<td>186</td>
<td>118.48</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>R. pseudoacacia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>2</td>
<td>86</td>
<td>0.94</td>
<td>0.3942</td>
</tr>
<tr>
<td>% of <em>A. altissima</em></td>
<td>5</td>
<td>86</td>
<td>0.93</td>
<td>0.4665</td>
</tr>
<tr>
<td>Density * % of <em>A. altissima</em></td>
<td>10</td>
<td>86</td>
<td>0.67</td>
<td>0.4170</td>
</tr>
<tr>
<td>Year</td>
<td>2</td>
<td>187</td>
<td>152.39</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 3. Two-way ANOVA result of final aboveground dry biomass for *Ailanthus altissima* and *Robinia pseudoacacia* from a three-year common garden competition experiment, 2011-2013.

All plants were grown in a common garden setting in Boyce, Virginia, USA. Four density levels: 1, 2, 3, 4 trees per plot (4m$^2$) were included in the analysis of intra- and inter-specific interactions. Interactions were not included.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. altissima</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>3</td>
<td>27277437</td>
<td>2.19</td>
<td>0.1033</td>
</tr>
<tr>
<td>% of <em>R. pseudoacacia</em></td>
<td>5</td>
<td>5662554</td>
<td>0.27</td>
<td>0.9251</td>
</tr>
<tr>
<td>Subsample</td>
<td>49</td>
<td>218657903</td>
<td>1.10</td>
<td>0.3802</td>
</tr>
<tr>
<td><em>R. pseudoacacia</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>3</td>
<td>132342523</td>
<td>3.68</td>
<td>0.0192</td>
</tr>
<tr>
<td>% of <em>A. altissima</em></td>
<td>5</td>
<td>76479701.8</td>
<td>1.28</td>
<td>0.2916</td>
</tr>
<tr>
<td>Subsample</td>
<td>48</td>
<td>1015353577</td>
<td>1.73</td>
<td>0.0357</td>
</tr>
</tbody>
</table>
Table 4. Coefficients of interspecific and intraspecific competition intensity between *Ailanthus altissima* and *Robinia pseudoacacia* growing in a common garden experiment in Boyce, VA, USA over a three year period (2011 – 2013). $B_{aa} = \text{coefficient of intraspecific competition for } A. altissima; B_{rp} = \text{coefficient of intraspecific competition for } R. pseudoacacia; B_{aarp} = \text{interspecific competitive effect of } A. altissima \text{ on } R. pseudoacacia; B_{rpaa} = \text{the interspecific competitive effect of } R. pseudoacacia \text{ on } A. altissima; S_{aa} = \text{substitution index for } A. altissima; S_{rp} = \text{Substitution index for } R. pseudoacacia. (* indicates } 0.05 < p < 0.1, ** \text{ indicates } p < 0.05)

<table>
<thead>
<tr>
<th>Year</th>
<th>$B_{aa}$</th>
<th>$B_{aarp}$</th>
<th>$S_{aa}$</th>
<th>$B_{rp}$</th>
<th>$B_{rpaa}$</th>
<th>$S_{rp}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-17.229</td>
<td>-3.536</td>
<td>0.205</td>
<td>-10.381</td>
<td>-63.985 *</td>
<td>6.614</td>
</tr>
<tr>
<td>2</td>
<td>-176.314</td>
<td>-70.239</td>
<td>0.398</td>
<td>-634.314 **</td>
<td>-582.3113 **</td>
<td>0.918</td>
</tr>
<tr>
<td>3</td>
<td>-193.282</td>
<td>-174.142</td>
<td>0.901</td>
<td>-256.952</td>
<td>-598.274**</td>
<td>2.328</td>
</tr>
</tbody>
</table>
Table 5. Mean Relative Interaction Intensity (RII) for the 12 mixture treatments (2A, 2R, 1A1R, 3A, 3R, 1A2R, 2A1R, 4A, 4R, 1A3R, 2A2R, 3A1R) of *Ailanthus altissima* (“A”) and *Robinia pseudoacacia* (“R”) growing in a common garden addition series experiment in Boyce, VA, USA over a three year period (2011 – 2013). Also, data are grouped by tree density (2, 3, or 4 m$^2$) and by the proportion of the other species in the plot. The mixture treatments with no shared letter are the ones that had significant difference in Tukey’s HSD test.

\[\text{A. altissima}\]

<table>
<thead>
<tr>
<th>Density (No. Tree per plot)</th>
<th>Proportion of <em>R. pseudoacacia</em> in plot</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Year1</td>
<td>-0.175</td>
</tr>
<tr>
<td>Year2</td>
<td>-0.096</td>
</tr>
<tr>
<td>Year3</td>
<td>0.013</td>
</tr>
<tr>
<td>2A</td>
<td>1A1R</td>
</tr>
<tr>
<td>C</td>
<td>AB</td>
</tr>
<tr>
<td>Year1</td>
<td>-0.296</td>
</tr>
<tr>
<td>Year2</td>
<td>-0.195</td>
</tr>
<tr>
<td>Year3</td>
<td>-0.047</td>
</tr>
<tr>
<td>AB</td>
<td>A</td>
</tr>
</tbody>
</table>

\[\text{R. pseudoacacia}\]

<table>
<thead>
<tr>
<th>Density (No. Tree per plot)</th>
<th>Proportion of <em>R. pseudoacacia</em> in plot</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Year1</td>
<td>-0.077</td>
</tr>
<tr>
<td>Year2</td>
<td>-0.149</td>
</tr>
<tr>
<td>Year3</td>
<td>-0.223</td>
</tr>
<tr>
<td>2R</td>
<td>1A1R</td>
</tr>
<tr>
<td>AB</td>
<td>A</td>
</tr>
<tr>
<td>Year1</td>
<td>-0.233</td>
</tr>
<tr>
<td>Year2</td>
<td>-0.233</td>
</tr>
<tr>
<td>Year3</td>
<td>-0.321</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
</tr>
</tbody>
</table>
Figure 1. Common garden competition experiment layout. Left panel: design of the additive-replacement treatments. The design consists of three replacement series (SW-NE diagonal directions in the left panel) at three densities 2, 3, 4 plants per plot. Each replacement series is flanked by monocultures for each species. Right Panel: the size and layout of the plots at the field site. Each of the 5 rows of experimental plots contains 14 evenly distributed plots. Treatments are randomly assigned to plots. The shaded row contains extra plots of plants destined for harvesting to determine the relationship between plant dimensions and plant dry weight.
Figure 2. Mean annual total above-ground dry-weight (biomass) increment per tree for monoculture plots (area = 4m$^2$) of *Ailanthus altissima* (A) and *Robinia pseudoacacia* (B) growing in a common garden in Boyce VA, USA during three consecutive growing seasons. Note the difference in the biomass increment scale between panel A and panel B. Density 1 = 0.25 tree m$^{-2}$; Density 2 = 0.5 tree m$^{-2}$; Density 3 = 0.75 tree m$^{-2}$; Density 4 = 1 tree m$^{-2}$. Error bars represent 1 standard error on each side of the mean. N = 5 plots per treatment.
Figure 3. Mean annual total above-ground dry-weight (biomass) increment per tree for *Ailanthus altissima* (A) and *Robinia pseudoacacia* (B) growing in substitutive combinations at three densities in a common garden in Boyce VA, USA during three consecutive growing seasons. Left side, from top to the bottom are total biomass increments of *A. altissima* in the presence of different proportions of *R. pseudoacacia* for years 1, 2 and 3, respectively. Experimental plots were 4 m$^2$ in area. The density of trees in the plot were 2 (0.5 trees m$^{-2}$), 3 (0.75 trees m$^{-2}$), and 4 (1 tree m$^{-2}$). 0 R.p. = a monoculture of *A. altissima*; 1 R.p. = 1 *R. pseudoacacia* plot$^{-1}$; 2 R.p. = 2 *R. pseudoacacia* plot$^{-1}$; 3 R.p. = 3 *R. pseudoacacia* plot$^{-1}$. Right side, from top to the bottom are biomass growth of *R. pseudoacacia* in year 1, 2 and 3, respectively. 0 A.a. = a monoculture of *R. pseudoacacia*; 1 A.a. = 1 *A. altissima* plot$^{-1}$; 2 A.a. = 2 *A. altissima* plot$^{-1}$; 3 A.a. = 3 *A. altissima* plot$^{-1}$. Error bars represent 1 standard error on each side of the mean. N = 5 plots per treatment.
Figure 4. The total aboveground dry biomass per tree for *Ailanthus altissima* (A) and *Robinia pseudoacacia* (B) growing in substitutive combinations at four densities in a common garden in Boyce VA, USA at the end of three consecutive growing seasons. Left panel is total biomass of *A. altissima* in the presence of different proportions of *R. pseudoacacia*, and right panel is total biomass of *R. pseudoacacia* in the presence of different proportions of *A. altissima*.

Experimental plots were 4 m$^2$ in area. The density of trees in the plot were 1 (0.25 trees m$^{-2}$), 2 (0.5 trees m$^{-2}$), 3 (0.75 trees m$^{-2}$), and 4 (1 tree m$^{-2}$). 0 R.p. = a monoculture of *A. altissima*; 1 R.p. = 1 *R. pseudoacacia* plot$^1$; 2 R.p. = 2 *R. pseudoacacia* plot$^1$; 3 R.p. = 3 *R. pseudoacacia* plot$^1$. 0 A.a. = a monoculture of *R. pseudoacacia*; 1 A.a. = 1 *A. altissima* plot$^1$; 2 A.a. = 2 *A. altissima* plot$^1$; 3 A.a. = 3 *A. altissima* plot$^1$. Error bars represent 1 standard error on each side of the mean. N = 5 plots per treatment.
Figure 5. Mean annual total above-ground dry biomass increment per tree for *Ailanthus altissima* and *Robinia pseudoacacia* as a function of total biomass of neighbor trees of other species per plot, growing in mixed species substitutive combinations, across three densities (density=2, 3, 4 tree/plot) in a common garden in Boyce VA, USA during three consecutive growing seasons. Left side, from top to the bottom are total biomass increments of *A. altissima* as target in the presence of different biomass of *R. pseudoacacia* for years 1, 2 and 3, respectively. Right side, from top to the bottom are total biomass increments of *R. pseudoacacia* as target in the presence of different biomass of *A. altissima* for years 1, 2 and 3, respectively.
Figure 6. The relationship between leaf dry biomass and the basal diameter of two species, in harvesting plots at the 3\textsuperscript{rd} growing season in a common garden competition experiment in Boyce VA, USA. Solid and open circles represent the actual branch diameter and square root of dry leaf biomass of that branch for \textit{A. altissima} and \textit{R. pseudoacacia} respectively. The solid line and dash line shows the estimated regression between square root of leaf biomass and basal diameter for the two species respectively.
Chapter 4

Influences of exotic invasive *Ailanthus altissima* and native *Robinia pseudoacacia* on understory diversity, tree seedling regeneration and community structure in Mid-Appalachian Mountain forests, USA

Abstract:

Invasive plants can displace native species, deteriorate native forest, and have long-term impacts on plant communities and ecosystems. To assess the community-level consequences of the invasion of *Ailanthus altissima*, and how the interaction between *A. altissima* and native *R. pseudoacacia* influences forest communities, we compared tree regeneration, understory composition, community structure, associated environmental conditions and the soil nutrients under *A. altissima* and *R. pseudoacacia* trees within hardwood forests. First, in the summers of 2011 and 2012, comprehensive forest mapping was done for trees, saplings and seedlings of woody species in 20m*50m* forest plots that contained *A. altissima* and/or *R. pseudoacacia* near Boyce, VA. In 2013, a complete forest survey including vegetation inventory and environmental factors around selected trees of these two species and reference trees was conducted in VA and WV. In the forest mapping study, the two target species tended to be associated with themselves mostly and with each other as well, and they both negatively influenced neighboring tree seedling regeneration. In the multiple-location vegetation survey study, a strong negative relationship between *A. altissima* and understory community richness, diversity, and evenness at the neighborhood scale was detected. *Robinia pseudoacacia* had a positive impact on soil N pool, but slightly negative impact on associated plant community richness, diversity, and evenness compared with the control sites. When the two species co-located, the impact on herbaceous
layer community diversity was the average effect of both species, but on tree regeneration the impact was dominated by the negative effect of *A. altissima*. Community coordination suggested the effect of these two species on plant community composition was less than the location effect. In conclusion, *Ailanthus altissima* trees impact associated plant community richness and diversity more negatively than *R. pseudoacacia*.

Key words: canopy tree, community evenness, community ordination, community structure, diversity, geographic scale, invasive species, nonmetric multidimensional scaling, regeneration, soil nitrogen, vegetation survey

Introduction:

Biological invasion can cause extinction of native species (Mooney and Cleland 2001, Gurevitch and Padilla 2004), genetic change in populations (Rejmánek 2000), decline of community diversity and evenness (Kohli et al. 2006) and change in ecosystem function (Hooper and Vitousek 1997, Zavaleta et al. 2001). Some invasive species have fundamental influences on the entire community, such as keystone predators and some dominant trees and shrubs (Simberloff 1990). Trees are usually considered to be foundation species of forest ecosystems (Ellison et al. 2005). Canopy trees provide physical and functional structure for understory plants in forest communities, determine the species composition of communities, and are essential to the stability and/or regeneration of communities. Loss or decreased abundance of native trees can dramatically change community structure and ecosystem processes (Ellison et al. 2005).
Examples include the degradation of *Tsuga canadensis* (eastern hemlock) due to the hemlock woolly adelgid (Jenkins et al. 1999, Orwig et al. 2002, Ellison et al. 2005) and the collapse of *Castanea dentata* (America chestnut) population due to chestnut blight (Smock and MacGregor 1988, Paillet 2002). Introduced invasive trees can create new habitats for the associated understory community (D'Antonio and Meyerson 2002, Hejda et al. 2009), change soil nutrients and the soil microbial community and thereby influence the structure and succession of forest communities (Vitousek 1990, Ehrenfeld and Scott 2001, Richardson and Rejmánek 2011).

Although some invasive plants are less-dominant (distributed sparsely in the forest), they can still have measurable effects on forest regeneration and understory diversity because of their allelopathic chemicals. Examples of the latter include the invasive species *Alliaria petiolate*, *Lythrum salicaria* and *Ailanthus altissima* (Eckert et al. 1996, Morrison et al. 2007, Gómez-Aparicio and Canham 2008a).

The relationship between invasive plants and invaded community diversity has been studied frequently over the past few decades with contradicting conclusions. At the large landscape-scale, a positive relationship between native diversity and invasibility contradicts observations at smaller-scale, where a negative relationship has been documented (Reed et al. 1993, Stohlgren et al. 1999, Davis 2003, Gilbert and Lechowicz 2005, Maskell et al. 2006). The large-scale positive relationship between invasive species and community diversity may be the result of a facilitation cascade by which ecosystem engineering species improve desirable habitat for native species (Altieri et al. 2010), or because favorable conditions and resource heterogeneity have a greater effect on diversity than competition at the large scale (Stohlgren et al. 2003, Davies et al. 2005). At smaller local-scales, where interactions between invasive species and native species are more likely to influence community structure, the invasive species can significantly decrease community diversity (Parker et al. 1999, Levine et al. 2003, Hejda et al.).
Also, a loss of community diversity due to one invader can enhance invasion by others (Levine 2000, Byers and Noonburg 2003).

Our study focused on one of the most common invasive trees *Ailanthus altissima* (Mill.) Swingle (Tree of Heaven) and the most important nitrogen fixing tree *Robinia pseudoacacia* L. (Black Locust) in the Mid-Appalachian region of eastern North America. Both species are fast-growing, early-successional trees, which reproduce by both a large-amount of seeds and root sprouts. Both species prefer disturbed sites with high light intensity, and coexist in the eastern U.S. forest (Call and Nilsen 2003). *Ailanthus altissima*, from China, has been an invasive tree in U.S. forest for more than 200 years (Feret 1973). *Robinia pseudoacacia* is native to the Mid-Appalachian mountain region, but also is viewed as an invasive species worldwide (Castro-Díez et al. 2008). Both species are among the most abundant trees on the planet because of their ability to grow fast, their high reproduction rate, their tolerance of extreme environments, and their widespread use in horticulture, forestry, agriculture and medicine. Although both species are considered invasive, the consequences of their invasion to the overall plant community may differ because the allelopathic effect of *A. altissima* is predicted to have a negative effect on diversity while the nitrogen fixing ability of *R. pseudoacacia* ought to increase diversity.

In previous research, we found that *A. altissima* and *R. pseudoacacia* are spatially associated with each other in the Mid-Appalachian mountains on a regional scale (Chapter 5) and at the local scale (Call and Nilsen 2003). Both species can thrive in early-successional forest after disturbance, and are likely to encounter each other. Also, we found evidence for both interspecific competition and intraspecific competition for these two species when grown in a common garden experiment (Chapter 3). However, the potential effect of each species, as individuals and when growing together on community composition is unknown.

We propose that these two species will encounter each other in early successional forest
of the Mid-Appalachian mountain region, and consequently be spatially associated in mid-age forest. Also, we propose that there are fewer tree species seedlings around *A. altissima* trees and more seedlings around *R. pseudoacacia* in the forest understory compared with other native dominant tree species. A forest mapping approach was used to evaluate these two propositions. Although forest mapping over a large area (1000 m$^2$) provided exact spatial locations of trees and seedlings, it was time-consuming, which limits the sample size for statistical comparison. Therefore, to further study the influence of *A. altissima* and *R. pseudoacacia* trees on forest regeneration and understory community structure, we utilized a target species approach at 4 sites, with 4 types of plots within each site. Our overall questions were: 1) Is there a difference in community composition, species richness and community diversity between plots dominated by *A. altissima*, *R. pseudoacacia*, and other reference trees? 2) Can the coexistence of *A. altissima* and *R. pseudoacacia* change their individual influence on forest regeneration and understory diversity? and 3) What is the relationship between environmental variables, such as soil characteristics in forests with *A. altissima*, *R. pseudoacacia*, *A. altissima* and *R. pseudoacacia* or neither species?

**Material and Methods:**

**Part I Forest mapping**

**Study Area and Experimental design**

In order to understand the spatial distribution of *A. altissima* and *R. pseudoacacia* trees at the community-level, and study the associated trees and understory woody seedlings, we conducted vegetation surveys at the Blandy experimental farm (BL), Boyce, VA and a nearby private forest (AL), in Berryville, VA. Blandy experimental farm, a 283 hectare property donated to the University of Virginia in the 1920s, includes a 69 hectare area of the Virginia State
Arboretum, and a broad range of habitats: woodlots, pastures, successional fields and small ponds. There were two main forest woodlands utilized at the Blandy experimental farm for this study—the southwest woodlot and northeast woodlot—which were plantations about 100 years ago, and were kept as unmanaged research forest without any large disturbances (logging, fire etc.) since 1937. The location of the forest mapping study was in the southwest woodland (39°3’41”N, 78°4’29”W), which contained early, middle, and late successional oak-hickory forest, that included typical species of the Mid-Appalachian mountain region. The soil types within our sampling area in the southwest woodland are Nicholson-Duffield silt loam and Poplimento silt loam. The elevation averages 176m for both woodlands, and the average annual precipitation averages 100cm (Data from 1984 to 2014). Monthly high and low temperature, cumulative precipitation and snowfall were collected by an on-site weather station. The private property (39°7’39”N, 78°5’41”W) at Berryville, VA was only 16 km away from the Blandy experimental farm. It contained two forested lands, which went through natural old-field succession without management for about 100 years except for the construction of several 1.5-2m wide trails. There is a nearby stream 100m distant from the forest in Berryville. Canopy diversity is greater at AL than BL, which is related to greater human activities at BL mid-successional forest.

All plots were located in early to middle successional forest sites. Stands (around 0.4 hectare) that were mostly dominated by either *A. altissima* or *R. pseudoacacia* or both species were chosen after surveying the entire forested site. At each site, three 20m × 50m rectangular plots were established. One plot in the stand with *A. altissima* but no *R. pseudoacacia* (AA), the second plot in the stand with *R. pseudoacacia* but no *A. altissima* (RP), and the third plot in the stand with both *A. altissima* and *R. pseudoacacia* (BOTH). A point of origin was selected by random number in each chosen stand to be the corner of the 20m × 50m rectangle plot. A
compass direction was randomly selected to establish the first 50m length. After all four edges were established, the sample plot area was divided into 25, 2m wide and 20m long rectangles (parallel to the short side of the sample plot). The entire plot was surveyed starting from the 0-2m rectangle to the 48-50m rectangle.

Measurements and data analysis in sample plots used for mapping vegetation

Every live tree, sapling, and seedling of all woody species was recorded. Trees were defined as those with a diameter at breast height (DBH, at the height of 1.3m above ground) greater than 10cm, and saplings were defined as small trees with a height more than 1m but a DBH smaller than 10cm. Seedlings had a height less than 1m. We considered the whole quadrat as an X-Y axis system. The start point was set as (0, 0), and the range of X and Y axis was 50m and 20m respectively. The locations of each tree, sapling, and seedlings in the X-Y coordinate system were determined. Also, the DBH of trees, height and DBH of saplings, and height and basal diameter of seedlings were recorded. All 3 plots at BL and 2 plots at AL were surveyed in July to August of 2011, and 1 plot at AL (plot type AA) was surveyed in July 2012. The AA plot at AL was only surveyed to 42m in length because no more A. altissima trees were found beyond that point, and the natural forest structure in the un-surveyed area was disturbed by the presence of discarded rusting machinery.

We mapped the spatial location of each tree, sapling and seedling in two-dimensional 20×50 (or 20×42) system in Matlab (8.1.0.604, Copyright 1984-2013, The MathWorks, Inc., Natick, MA). Nearest neighbor analyses were conducted in R (version 0.98.1091, Boston MA, RStudio, Inc) to determine the most associated tree species with A. altissima, R. pseudoacacia and other common tree species. In order to further detect the neighborhood influence of each tree species, we drew virtual circles around each mature tree or sapling, and calculated how many seedlings fell into these circles. We proposed that the impact of each mature tree/sapling should
be positively related to its size, which is evaluated by DBH in our study. We tried three different relationships to decide the radius of the virtual circle (influence distance): 1) 10×DBH of the center tree (in meters), 2) 20×DBH, and 3) 50×DBH. The number of seedlings that were located within these three influence distances was summarized for every tree and sapling individually. However, neighborhood seedling number would be an underestimate for the trees on the edge of each 20×50m plot, because we only surveyed the seedlings within the 20×50m plot. To minimize this edge bias, an edge correction was performed for every tree. The proportion of the area around the tree that was within the 20×50m area was approximated by generating 10,000 random points within the circular area around each tree and counting the proportion of the points that fall within the plot area vs. outside the edge of the plot. The original neighborhood seedling number was divided by the proportion of points in the sample plot to attain the edge corrected number of seedlings for each tree and each circle size.

Tree species richness and community similarity among all possible pairs of sampled plots was determined using Sorensen’s index:

\[ SCs = \frac{2C}{(A+B)} \]

where C is the number of common species in the two stands,

A is the total number of species in stand A,

B is the total number of species in stand B.

The Sorensen’s indexes for trees, saplings, and seedlings were calculated separately. No statistical tests were conducted because no meaningful conclusion could be generated from the small sample size (n=2).

**Part II Vegetation and environment survey around target trees**

**Study Area**

We conducted a vegetation and associated environment survey across different areas of
the Mid-Appalachian region in order to include different habitats and improve our hypothesis testing. Two locations in Virginia and two locations in West Virginia were selected and surveyed in July to early September of 2013. One location in Virginia was the Blandy experimental farm, Boyce, VA (VBLD) at the southwest woodland where we conducted the forest mapping plus one control site at the northeast woodland. The environmental conditions of the northeast woodland are similar to those at the southwest woodland, except the area of the northeast woodland is smaller, and the soil type was Poplimento silt loam and Timberville silt loam. We didn’t find a control site in the southwest woodland because the only forest area without *A. altissima* or *R. pseudoacacia* was obviously significantly older than the early to middle successional forest where our other sites were located. The other location in Virginia was within the Smithsonian Conservation Biology Institute (SCBI) property at Front Royal, VA. The SCBI location (VSCB) was secondary forest, last disturbed by logging 120 years ago, and located within the Oak-Chestnut/Appalachian Oak region at the intersection of the Blue Ridge, Ridge and Valley, and Piedmont physiographic provinces (38° 53’ 36.6” N, 78° 8’ 43.4” W) (Bourg et al. 2013). The entire area, where all of our sample plots were located, was fenced by SCBI in 1975-1980 to exclude deer. Primary soil types are Myersville and Montalto series silty loam soils, which are stony, well-drained and steep. The mean elevation is 302m. Dominant canopy trees include yellow poplar (*Liriodendron tulipifera*), hickories (*Carya* spp.), oaks (*Quercus* spp.), white ash (*Fraxinus americana*), and black gum (*Nyssa sylvatica*) (Bourg et al. 2013).

West Virginia sites (WV) were selected and surveyed by our collaborator Cynthia D. Huebner and her crew in USDA-FS. Both WV sample site locations were on private property in Hampshire County within the Ridge and Valley Province. The JDS L and LLC property (WJDS) is owned by Mr. Jeff Orndorff and is located near Pine Mountain, west of the town of Bloomer and the Cacapon River (39° 22’ 20” N 78° 26’ 00” W). It is 535 acres in size and the elevation
ranges from 305 to 536 m. Vegetation varies from *Ailanthus altissima*, *Robinia pseudoacacia*, and *Rubus phoenicolasius* dominated sections to adjacent, relatively mature stands of oak, mixed hardwoods, and mixed pines and hardwoods. The second property (WDH) is located off of North River Mountain near Moores Run and Warden Lake, west of the town of Yellow Spring (39° 07’ 50” N 78° 35’ 00” W). It is 15 acres in size and its elevation ranges from 396 to 596 m. Vegetation at this second property is similar to the first with dominant stands of *Ailanthus altissima* and *Robinia pseudoacacia* and with adjacent sections of mature mixed hardwoods; pines are less frequent than in WJDS.

**Sampling procedures and measurements**

Within each of the four locations stated above, areas with *A. altissima* and/or *R. pseudoacacia* were identified by traversing the forests by car and on foot. Four types of sites were chosen in each location: a) with *A. altissima*, no *R. pseudoacacia* (AA); b) with *R. pseudoacacia*, no *A. altissima* (RP); c) with both species (BOTH); d) without either of the two species (C, control). If there was more than one site available for AA, RP and BOTH in the general location, only one site for each type was selected by random number. The reference site was selected to be as close as possible to the three other site types based on geographic location and physical environment. The most common native species in the control site was selected as the reference species. Therefore, at different locations the reference tree species may be different.

Within each site, 10 target trees (*A. altissima* for AA site, *R. pseudoacacia* for RP site, both *A. altissima* and *R. pseudoacacia* for BOTH site) were chosen (if available) to be the center of a sampling plot. The following selection criteria were used when selecting sample plots: 1) the plot should be in lowland/broad valley early-successional forest, not on ridge tops; 2) the slope should be between 0-10 degrees; 3) the age of the plot should be approximately between 10-40 years, judging by the DBH of the largest target tree within the plot; 4) the plots should be at least
20 meters from trails or forest edges. Within each site, a target tree estimated to be central in the site area was selected as the target tree of the first sample plot. Additional trees were selected for plot centers by walking in random bearings from the first plot until the full set of trees were located. During the selection of sampling trees, we made sure that no two plots were within 10m of each other. For sites with both species present, target *A. altissima* and *R. pseudoacacia* trees in sample plots were no more than 5 meters from each other, and were sampled in a pairwise manner. An effort was made to keep paired plots at least 10m apart. However, 10 target tree plots per site type could not be universally achieved using this sampling criteria. There were 96 and 67 plots in total at the end of survey for WV and VA respectively.

A 5-meter diameter circular plot was laid out around each selected target tree, with target tree as plot center (Figure 1). The GPS point for each target tree as well as basic information such as slope and aspect of each plot were recorded. The DBH and distance from plot center of all live trees, including the target tree were measured within the 78.5 m² plot. The number of saplings was counted for each species within a 3 m circular plot (28.3 m² in area) with the target tree at plot center. A sapling was defined as height ≥1m and DBH <10cm. At the inside edge of the 3 m circular plot, a 1m² subplot was established at each cardinal direction. Percentage cover and number of tree seedlings, as well as percentage cover of herbaceous species, shrubs, and vines were measured within each subplot. Subplot surface percentage cover by moss/lichens, rock, live and dead woods, and litter were measured. Soil samples were collected just outside the 3m edge of each subplot at 10cm depth. The four soil cores were bulked and mixed for each target tree plot, dried naturally and sieved, resulting in one soil sample per sampled plot. A bank of elements, pH, and cation exchange capacity were measured on each soil sample (Virginia Tech Soil Testing lab). In addition, an anaerobic incubation followed by a KCl extraction and liquid chromatography was performed to determine the availability of nitrate and ammonium ions in
the soil solution (Maynard et al. 2008). For each site type and location (16 sites in total), the age of the site was estimated by tree core samples. The two largest target trees or reference species were selected for each site, and a 16 inch long, 4.3mm thin core was extracted by increment borer from the selected trees at breast height. Tree core samples were labeled, sealed in straws and sent to USDA-FS Northern Research Station lab for analysis.

**Data Analysis for target tree plot survey.**

Importance values were calculated for associated trees, saplings, seedlings, and understory herb/shrub/vine species in each site type and location combination. Due to our sampling procedure, relative frequency was not included in the importance value calculation. For trees and tree seedlings, importance value = relative density + relative dominance (DBH or cover). For saplings, importance value = relative density; for understory herb/shrub/vine species, importance value = relative dominance (cover).

Community ordinations using nonmetric multidimensional scaling (NMS) (McCune et al. 2002) were done for tree seedlings and understory herb/shrub/vine species by PC-ORD (Version 5.10, MjM Software Design, Glendened Beach, Oregon, USA). The ordinations were done based on herb/shrub/vine layer and tree seedlings separately, using both the combined data set across all sites, and for each site type/location combination separately. To detect the species-specific influence within the BOTH sites the data for plots with *A. altissima* target trees (BAA) were treated separately than data for plots with *R. pseudoacacia* target trees (BRP). Therefore, there were a total of 20 different data sets used for each ordination, which resulted in 20 points on the ordination. Sorensen’s distance measure was used in all community ordinations. Two-dimensional graphs were drawn using NMS axis to indicate community separation.

Community richness (S), evenness (E), Simpson’s diversity index (D) and Shannon’s diversity index (H) were also calculated for the combined data set across all sites, and for each
site type/location combination separately. Diversity and evenness were compared across the five
site types (AA, RP, AA in BOTH, RP in BOTH, C) to test the difference in plots around target
trees, by building a mixed effect model in JMP (Pro 11, SAS Institute Inc., Cary, NC, USA).
Each target tree was considered a sampling unit, and the subplots were subsamples. Site type was
treated as a fixed effect, and site location was the random effect in the model. Environmental
factors, such as soil PH and soil nutrient content were compared between site types and locations
using a similar approach.

Results:
Part I Forest mapping
From the maps of mature trees, saplings, and tree seedlings, it was clear that the three
plots at BL had both fewer species and fewer trees, saplings and seedlings than the three AL plots
(Figure 2). The total number of trees, saplings and seedlings were calculated (Table 1). Chi-
square test showed no significant difference in total tree number between the two sites (p=0.628),
but total sapling and seedling numbers were significantly greater in AL site than BL site
(p<0.0001). The total plot species richness was also significantly greater in AL plots than in BL
plots (p=0.0007). RP plots had a slightly greater total number of trees than BOTH and AA plots
(p=0.052), and a significantly greater total number of saplings than AA and BOTH type
(p=0.026). AA plots had significantly greater numbers of seedlings than RP and BOTH plots
(p<0.0001). However, species richness was not significantly different among the three site types
(p=0.952).

Although the canopy of plot BL-RP was dominated by *R. pseudoacacia* and *Juglans nigra*, there was only one *R. pseudoacacia* sapling, and 18 *R. pseudoacacia* seedlings in the 0.1
hectare area. The number of other tree species seedlings in BL-RP was also the least among the
six surveyed plots. The canopy of plot BL-BOTH was dominated by *R. pseudoacacia*, *A. altissima* and *J. nigra*. Although the dominance of *A. altissima* and *R. pseudoacacia* was similar in the canopy, there were many more *A. altissima* saplings/seedlings (26/196) than *R. pseudoacacia* saplings/seedlings (2/2) in the understory. The canopy of plot BL-AA was dominated by *A. altissima* and *J. nigra*. Interestingly, there were no *J. nigra* seedlings in this plot, while there were several *J. nigra* seedlings in the other two BL plots even though the number of *J. nigra* trees was similar among the three plots. About 90% of the seedlings at the BL site were *A. altissima*, and the other seedlings were mostly *Celtis occidentalis*. Species richness within each of the three layers was slightly higher in BL-BOTH and BL-AA plot than in the BL-RP plot.

The canopy of plot AL-RP was dominated by *R. pseudoacacia* and *J. nigra*, but there was also 7 other species, including *Juniperus virginiana* and *Gleditsia triacanthos*. *Celtis occidentalis* and *Amelanchier* spp. were common in the sapling layer in addition to *R. pseudoacacia* and *J. nigra*. Although the number of *R. pseudoacacia* and *J. nigra* trees and saplings were both the most abundant in the tree and sapling layer, there were very few *R. pseudoacacia* (4) and *J. nigra* (0) seedlings. In contrast, although *Prunus serotina* trees and saplings were not common, there were many *P. serotina* seedlings in the understory. There were very few mature trees in the AL-RP plot, but many saplings and seedlings of *C. occidentalis*, and seedlings of *Fraxinus Americana*. The canopy of AL-BOTH was dominated by *A. altissima*, *G. triacanthos*, *Maclura pomifera*, *J. nigra* and *R. pseudoacacia*. Seedlings were dominated by *A. altissima*, *F. Americana*, *C. occidentalis*, *G. triacanthos*, *P. serotina*, and *A. negundo*, with a few *R. pseudoacacia* (5) and no *J. nigra*. The canopy of AL-AA was dominated by *J. nigra*, *A. altissima*, *C. occidentalis*, *Elaeagnus angustifolia* and *Elaeagnus umbellata*. This plot had the greatest number of seedlings, but they were mostly dominated by *A. altissima*, *C. occidentalis* and *F. Americana*, with a moderate number of *Ulmus pumila*, *Elaeagnus angustifolia* and *Elaeagnus umbellate* seedlings.
Species richness of the three layers was slightly higher in AL-RP plot than in the other plots at the AL site.

The maps indicated that both *A. altissima* and *R. pseudoacacia* trees tended to be close to themselves, and in BOTH plots, trees of these two species were also found close to each other. A nearest neighbor analysis for a combined data set of all plots indicated that for trees and saplings of *R. pseudoacacia* (79 in total), the nearest species was itself (59.5%), and the average distance between neighbors was 1.35±0.13m. The other nearest associated species to *R. pseudoacacia* were *J. nigra* (27.8%), *A. altissima* (3.8%), *C. occidentalis* (2.5%), *G. triacanthos* (2.5%), *Amelanchier* spp. (1.3%), *P. serotina* (1.3%) and *Quercus rubra* (1.3%). For trees and saplings of *A. altissima* (119 in total), the nearest species was itself (79.8%), and the average distance was 1.26±0.09m. The other nearest associated species were *M. pomifera* (10.1%), *J. nigra* (3.4%), *R. pseudoacacia* (1.7%), *Morus rubra* (0.8%), *P. serotina* (0.8%), *Platanus occidentalis* (0.8%), *F. Americana* (0.8%), *G. triacanthos* (0.8%) and *C. occidentalis* (0.8%). Also, *C. occidentalis*, *J. nigra*, *M. pomifera* and *Asimina triloba* had the tendency to be mostly self-associated.

Although no statistical analysis could be done to compare seedling regeneration among the three types of plots, a comparison of neighborhood seedling numbers for each mature tree and sapling was performed. Within the 10×DBH (in meter) radius circle, there were significantly more seedlings around *M. rubra* (p<0.0001) than the mean for all other tree species; and there were significantly fewer seedlings around *J. nigra* (p<0.0001), *R. pseudoacacia* (p=0.0009), *C. occidentalis* (p=0.0008), *M. pomifera* (p=0.0019), *G triacanthos* (p=0.0111) and *J. virginiana* (p=0.0253) trees. For trees with the closest tree being *A. altissima*, there were significantly fewer seedlings in the circle (p=0.0112); when the nearby closet tree was *A. triloba* (p=0.0072) and *J. virginiana* (p=0.0101), there were more seedlings. Within the 20×DBH (in meter) radius circle, the number of seedlings was significantly greater around *M. rubra* (p<0.0001) than the mean for
all other species, and fewer seedlings were found around *J. nigra* (*p*=0.0018) and *R. pseudoacacia* (*p*=0.0488). When the nearby tree was *A. altissima*, there were fewer seedlings in the circle (*p*=0.017), and when the nearby tree was *A. triloba* (*p*=0.0049) and *J. virginiana* (*p*=0.0143), there were more seedlings. Within the 50×DBH (in meter) radius circle, the number of seedlings was greater around *M. rubra* (*p*=0.0014) and *A. altissima* (*p*=0.0206) than all the other species. When the nearby tree was *J. nigra* (*p*=0.0256) and *C. occidentalis* (*p*=0.0256), there were fewer seedlings; when the nearby tree was *A. triloba* (*p*<0.0001), there were more seedlings.

Community similarity was greatest for AL-RP and AL-BOTH, and least between BL-BOTH and AL-AA (Table 2). Within BL site, BL-BOTH and BL-AA were more similar than the other two pairs. Within the AL site, AL-BOTH and AL-RP were more similar than the other two pairs. The similarity was greater within sites than between sites for any plot type. When comparing 2 plots in different sites, the similarity was more likely to be higher for plots with the same plot type than plots with different types.

**Part II Vegetation and environment survey around target trees**

*Microstegium vimineum* appeared in all 16 sites, and in most sites it was one of the top 3 most abundant species in the herb/shrub/vine layer except for the VBLD-C, VSCB-RP and WJDS-C (Table 3). Other common species across the 16 sites were *Alliaria petiolata*, *Toxicodendron radicans*, *Rubus phoenicosilasius*, *Parthenocissus quinquefolia* and *Carex* spp. In the two Virginia locations, *Celastrus orbiculatus*, *Geum canadense*, *Lonicera maackii*, *Lonicera japonica*, *Parthenocissus quinquefolia*, *Persicaria virginiana*, *Pilea pumila* and *Lindera benzoin* were common besides the above listed species (Table 3). In the two West Virginia locations, there were fewer species on average, and the most common species for the 8 sites were *Ageratina altissima var. altissima*, *Berberis thunbergii*, *Vitis* spp. and *Persicaria posumbu*. The
control site type was less likely to be dominated by a single species (except in the WJDS site) (Table 3). Herb/Shrub/Vine species richness was not significantly different between both the location (p=0.1567) and the type of site (p=0.6670). The only significantly difference in species richness was between VSCB and WJDS locations (p=0.0190). The VSCB location had greatest species richness in the control site. In both the VSCB and WDH locations, the A. altissima site (AA) had the lowest understory herb/shrub/vine species number. In the WJDS sites, the number of species was generally low, except for the WJDS-RP site (Table 3). In the VBLD sites, the VBLD-RP site had the lowest number of species, which was consistent with the findings of the forest mapping study conducted in this area (Table 1, Figure 2a). In both the VBLD and the WDH locations, the number of species was greatest in the BOTH site type. Note that the results from the BOTH sites are the average of the data from the sample plots around both the trees of the paired A. altissima and R. pseudoacacia. (Table 3).

Tree seedling richness was significantly different between both the location (p=0.0167) and the type of site (p=0.0173). Tree seedling richness was the greatest in the control sites (except WJDS location in which WJDS-RP was greater than WJDS-C), and was significantly greater than AA (p=0.0036) and BOTH sites (p=0.0044). RP sites also had significantly greater tree seedling richness than the AA (p=0.0214) and BOTH (p=0.0269) sites (Table 4). VBLD-RP was different, because there were fewer plots surveyed, fewer overall tree species and no seedling regeneration. Ailanthus altissima was the most common species in the seedling regeneration across sites (12 out of 15 sites); seedlings of Fraxinus americana and Sassafras albidum were also common across sites. Sites at WDH and VSCB locations had significantly more tree seedling regeneration than VBLD (p=0.0015 and 0.0086). Seedlings of Celtis occidentalis and Prunus serotina were common in VA sites, but not in WV sites; seedlings of Fraxinus pennsylvanica, Acer saccharum, Nyssa sylvatica and Ulmus rubra were common in
WV sites, but not in VA sites.

Community ordinations based on herb/shrub/vine layer and tree seedlings were performed first using all 20 data sets combined (Figure 3). The number of runs was 50, the number of iterations was 250, and a maximum of six dimensions were used. The best solution was 2-dimensions for the herb/shrub/vine ordination, with the final stress at 11.73 and instability at 0.033. The best solution for the tree seedling ordination was 2-dimensions, with the final stress 13.59, and final instability 0.00821. In general, stress values 10 or lower are usually considered relatively good ordination results, though values between 10 and 20 are not uncommon in ecological data. Numbers greater than 10 may be unreliable because the observed pattern might be just by chance. Runs are considered stable if the instability value is < 0.0001.

The location of the data sets in the ordination is an indication of how similar the data sets are and that similarity can fall along multiple dimensions. Our ordination based on the herb/shrub/vine data separated the 20 data sets slightly better than the ordination using tree seedlings data, but the latter was more stable. Site location (2 in WV and 2 in VA) seemed to be a more important determinate for site separation than data set type (AA, RP, BAA, BRP or C). AA and BAA data sets had the greatest tendency for clustering, while RP and C data sets were distributed widely over the axis space (Figure 3), but this pattern was disrupted by the location effect. Monte Carlo tests showed both ordinations were better than random (p=0.008 for herb/shrub/vine data, and p=0.004 for tree seedling data).

Ordinations using the separate site location data were done to further detect site type differences within each location (Figure 4 – Figure 7). The VA-BLD location didn’t have any tree seedlings so the ordination was only done using herb/shrub/vine data (Figure 4). The best solution was 3-dimensional, with 124 iterations, the final stress was 9.16, and the final instability was 0.00001. The 2-dimensional graph is shown using 1st and 3rd axis because it had the best
separation on 2-dimensional graph. This ordination result was better than all locations together, it suggested that control plots were clustered, and BAA was closer to BRP than to AA (Figure 4).

The 1<sup>st</sup> and 3<sup>rd</sup> axis 2-dimensional graphs were selected for VA-SCB location ordinations (Figure 5). The best solution for herb/shrub/vine in the VA-SCB location was 3-dimensional, with 104 iterations, the final stress was 11.47, and the final instability was 0.00001. In the graph, AA and C plots seemed to cluster, while BAA and BRP were another cluster; RP plots were closer to the BAA and BRP clusters (Figure 5a). The best solution for tree seedlings in the VA-SCB location was also 3-dimensional, with 250 iterations, the final stress was 14.2, and final instability was 0.0025. The ordination result had a clear cluster of the C plots, and the RP, BRP plots were closer to control plots based on tree seedling regeneration (Figure 5b).

The best solution for herb/shrub/vine in WV-DH location was 2-dimensional, with 250 iterations, the final stress was 17.79, and final instability was 0.0069, which was not a strong ordination. There were 4 clusters in the graph; none appeared to be related to difference in data set type. BAA and BRP were closer to each other, and they were closer to RP data sets than to AA data sets (Figure 6a). The best solution for tree seedlings in WV-DH location was 3-dimensional, with 250 iterations, the final stress was 12.81, and final instability was 0.0005. The Axis 1 to Axis 2 dimensional graph is shown for WV-DH location tree seedling ordinations because data sets were better separated than Axis 1 to Axis 3, or Axis 2 to Axis 3 graphs (Figure 6b). The ordination result showed two clusters: one was between the C plots and most of the RP plots, and the other was most of the BAA plots with the BRP plots. AA plots were closer to BAA and BRP plots than control plots. (Figure 6b).

The best solution for herb/shrub/vine data in the WV-JDS location was 2-dimensional, with 250 iterations, the final stress was 15.74, and final instability was 0.00016. There was one big cluster in the graph, with most the RP plots, and all the AA, BAA, BRP plots close together.
Control plots were separate, with three controls and three of the RP plots close to each other (Figure 7a). The best solution for tree seedlings in the WV-JDS location was 2-dimensional, with 48 iterations, the final stress was 13.3, and final instability was less than 0.00001. The ordination result suggested that control plots were a different cluster than the others. The RP, BRP and BAA plots were closer to each other than the C plots (Figure 7b).

There were 172 species in the herb/shrub/vine layer among all sites. Herb/shrub/vine richness (S) was significantly different among the site types (p=0.0397). The average richness was 9.13±2.97 for AA, 11.16±2.99 for BAA, 10.61±2.98 for BRP, 11.57±2.97 for C, and 12.31±2.98 for RP (Figure 8). But the difference in richness between site types was only significant for AA and RP (p=0.0066). Shannon’s diversity (H) for the herb/shrub/vine data differed significantly among the 5 site types (p<0.0001). Specifically, no significant difference was found between BAA, BRP and RP; diversity in BAA and BRP was very similar and both slightly lower than RP site. There was significantly greater Shannon’s diversity in the C site (p=0.025), and significantly lower Shannon’s diversity in the AA site (p<0.0001). Simpson’s diversity (D) was significantly different among site types (p<0.0001), but this difference was mainly due to the lower diversity in AA plots (p<0.0001). Also, community evenness (E) was significantly different among site types (p<0.0001). Specifically, no significant difference in evenness was found between BAA, BRP and RP, with RP having slightly greater evenness. Community evenness was the greatest in the C site (p<0.0001), and the lowest in the AA site (p<0.0001). The conclusions from the two diversity indices and evenness index were consistent.

There were 47 species in the tree seedling layer among all sites. Tree seedling richness (S) was significantly different among the site types (p<0.0001). The average richness was 1.48±0.30 for AA, 1.43±0.30 for BAA, 1.53±0.32 for BRP, 4.10±0.27 for C, and 3.32±0.31 for RP (Figure 8). The difference in richness between AA and C, AA and RP, BAA and C, BAA and RP, BRP
and C, BRP and RP, C and RP were all significant (p<0.0001). Shannon’s diversity (H) was significantly different for tree seedlings among the 5 site types (p<0.0001). The order of Shannon’s diversity in these 5 site types was C > RP > BRP > AA > BAA. There was a significant difference between C and RP (p<0.0001), BRP and RP (p=0.0003), AA and RP (p<0.0001), BAA and RP (p<0.0001), AA and C (p<0.0001), BAA and C (p<0.0001), BRP and C (p<0.0001). The same results were found for Simpson’s diversity. Also, community evenness (E) had very similar results, with a slightly different order: C > RP > AA > BRP > BAA. Therefore, the control site had highest diversity and evenness based on either tree seedlings, or herb/shrub/vine. *R. pseudoacacia* understory had higher diversity and evenness than *A. altissima* and the two trees together.

Control sites (71 years on average) were older than the AA, RP and BOTH sites (22, 46, and 32 years on average), which is expected because *A. altissima* and *R. pseudoacacia* are commonly found in early to middle successional forest. The sites at DH location were older than the sites at JDS location in WV. Soil pH was significantly different among the 5 site types (p<0.0001). Soil pH was significantly lower in Control sites (5.06) than the RP, BRP, BAA and AA sites (5.67, 6.01, 6.09, and 6.14). Soil NH$_4^+$ concentration was significantly different among the 5 site types (p<0.0001). Soil samples from the BAA, BRP sites had the highest NH$_4^+$ concentration (p<0.001), followed by the RP and AA sites. The control sites had the lowest available NH$_4^+$. Soil NO$_3^-$ concentration was not significantly different among the five site types (p=0.2313).

**Discussion:**

**Mapping**

The mapping study was focused on tree association and the impacts of specific trees on
seedling abundance in a local region. Fewer seedlings were regenerating under *R. pseudoacacia* than other dominant tree species in the three Blandy plots (Figure 2, a-c) which was opposite to our hypothesis that more seedlings would be found around *R. pseudoacacia* due to its N-fixation ability. Fewer seedlings were regenerating under *J. nigra* as well. There were several possible reasons for this anomaly. Both these species are pioneer species growing in early-successional sites, but their seedlings are not shade-tolerant. Also, *R. pseudoacacia* seeds we collected in the Blandy experimental farm woodlots had a very low germination rate (Bao and Nilsen, unpublished result). Moreover, *J. nigra* is known to have allelopathic potential and negatively influence woody and herbaceous species regeneration (Rietveld 1983). *Robinia pseudoacacia* has been found to have allelopathic potentials on herbaceous species as well when evaluated in lab experiments (Nasir et al. 2005) although the allelopathic effect of *R. pseudoacacia* has not been documented in field studies. In addition, these two species were found to be significantly associated with each other, which means that their effects on understory seedling abundance would overlap. Also, the neighborhood-level analysis of BL plots found both species inhibited seedling regeneration, and the distance range of this negative effect was longer for *J. nigra* than *R. pseudoacacia*. Conversely, *Fraxinus Americana* had many seedlings but seldom became a canopy tree in our mapped area. This agrees with the general conclusion that *F. americana* is very common in the sapling layer but seldom becomes a dominant tree in these forests (USDA-FS, [http://www.na.fs.fed.us/pubs/silvics_manual/volume_2/fraxinus/americana.htm](http://www.na.fs.fed.us/pubs/silvics_manual/volume_2/fraxinus/americana.htm)). The pattern of fewer seedlings regenerating around *J. nigra* than *R. pseudoacacia* did not occur in AL plots (Figure 2, d-f).

AL plots had a more even, and diverse community than BL plots in the forest mapping study. However, in both sites, at the 1000m² plot scale, the number of species was similar between plots dominated by *A. altissima, R. pseudoacacia* or both species within each site. The
number of seedlings was significantly greater in the two AA plots compared with other plot types within the same site, but this difference was mainly caused by more *A. altissima* seedlings. At the tree neighborhood scale, there was evidence to support the supposition that *A. altissima* inhibited seedling regeneration within certain distance (the circle radius 10×DBH or 20×DBH). But this negative effect became positive when the distance increased to 50×DBH. This change in results across scales suggested that size of plot is important when evaluating the impact of certain tree species on their neighborhoods. In the target tree based vegetation survey, we found less tree seedling regeneration in the AA sites, in which the size of plot for seedling survey was closer to the scale of 10×DBH and 20×DBH. We noticed that AL-AA plot type had more invasive woody species (*Ulmus pumila*, *Elaeagnus angustifolia*, *Elaeagnus umbellata*), which agreed with invasive melt-down theory (Simberloff and Von Holle 1999). But this pattern was not obvious for BL site which had a limited number of woody species overall. Unexpectedly, we found fewer tree seedlings around *R. pseudoacacia* at the tree neighborhood scale (circle radius 10×DBH or 20×DBH). This finding contradicts the belief that nitrogen fixing tree *R. pseudoacacia* could increase soil nutrient level and facilitate the development of other species (Boring and Swank 1984b, Von Holle et al. 2006, Castro-Díez et al. 2008). But this result is limited to the two locations (BL, AL) in one region of Virginia. Furthermore, other neighborhood tree species would also influence seedling regeneration near *R. pseudoacacia* thereby diluting the effect of *R. pseudoacacia*. Thus, the reliability of this result needs further experiments, which could exclude the impact of other neighboring tree species on the effect of *R. pseudoacacia* on seedling regeneration.

The invasive *A. altissima* had a strong capability to regenerate itself and dominated the woody species regeneration (called advance regeneration). However, we observed that *A. altissima* didn’t dominate the canopy, which might be because the selected sites were at an early
stage of *A. altissima* invasion into these communities, or that most *A. altissima* seedlings die
before becoming saplings. Assuming survival and development of *A. altissima* seedlings through
saplings into adults, there will be a higher abundance of *A. altissima* in the future canopies of
these woodlands. We did find a stand with almost pure *A. altissima* trees at another location
(SCBI-AA site in 2013 survey). Therefore, under certain conditions, *A. altissima* can become a
canopy dominant species. We don’t have historical data in the AL site to show if the *A. altissima*
invasion is at an early stage. *Robinia pseudoacacia* had many fewer seedlings/saplings of itself
in the mapped forest than did *A. altissima*. Therefore, in the sites with both species present, *A. al-
tissima* is likely to become more dominant than *R. pseudoacacia* in the future without further
disturbance of these woodlands.

**Tree-based Vegetation and environment survey**

This part of the study was based on multiple locations, across larger regions and with
more replicate plots than the forest mapping study. Therefore, the conclusions from the tree-
based vegetation survey were based on a larger scale and should be more effective at addressing
our three main questions. Question one was supported because we found significant effects of the
different target species on community composition, diversity and richness. *Ailanthus altissima*
had a significant negative impact on understory herb/shrub/vine richness, diversity and evenness.
Although the herb/shrub/vine community associated with *R. pseudoacacia* tree had the greatest
richness, diversity and evenness was significantly lower than the control sites. Herb/shrub/vine
community associated with BAA had significantly higher richness, diversity and evenness than
AA, which indicated a positive impact of *R. pseudoacacia* on communities that also had *A.
altissima* trees. On the other hand, diversity of herb/shrub/vine community associated with BRP
was slightly lower than RP, and very similar to BAA, indicating a slightly negative impact of *A.
altissima* on community metrics in forest that also had *R. pseudoacacia*. Thus, in response to
question two we found that co-existence of the two target species changed the individual effects of either species on understory herb/shrub/vine communities. We think that the impact on the herb/shrub/vine community where both target trees were present, was a result of the weighted average negative impacts of A. altissima and positive impacts of R. pseudoacacia, with higher weight on the effect of R. pseudoacacia.

The presence of A. altissima and R. pseudoacacia had negative impacts on tree seedling regeneration compared with the control sites again supporting our first question. Community richness, diversity and evenness of tree seedlings under R. pseudoacacia canopy trees were greater than that under R. pseudoacacia trees when a paired A. altissima canopy tree was nearby indicating a negative impact of A. altissima that potentially counteracted the positive effects of R. pseudoacacia. The BRP, BAA site types had similar, or even stronger negative impacts as that of AA site type on the tree seedling regeneration. This indicates that when the two species are co-located in the forest, the impacts on tree seedling regeneration was dominated by the impact of A. altissima, and the combined impacts could be worse than when they were alone. Thus, our second question was better supported by the seedling regeneration data than the herb/shrub/vine result. The effects of A. altissima, R. pseudoacacia and their coexistence were more significant to tree seedling regeneration than their effects on herbaceous diversity. Thus, the presence of A. altissima and/or R. pseudoacacia could influence community structure and tree diversity differently through succession. Further, the understory vegetation would be further influenced by changes in overstory composition, because of the associated change in light, water, soil nutrients, allelochemicals and microbial community (Barbier et al. 2008).

We found the influence of site locations on the understory community was greater than the influences of A. altissima or R. pseudoacacia, i.e., site type. For example, the herb/shrub/vine layers of WV sites were dominated by one or two species, and the dominant species was
Microstegium vimineum (Table 3), while *M. vimineum* was also abundant but hardly had more than 25% cover of the herb/shrub/vine layer in VA sites. Also, tree seedling regeneration in VA sites where *A. altissima* trees were present was dominated more by *A. altissima* seedlings than that of WV sites (Table 4). In the ordinations of both sites, control sites were separated from other sites more in the tree seedling ordination than herb/shrub/vine ordination. Therefore, the effects of region (larger scale) were more important than the effects of the neighborhood (smaller scale) on community traits. This finding agreed with previous findings that the impact of invasive species is site dependent (Gómez-Aparicio and Canham 2008b).

Exotic invasive species are known to have significant effects on native species diversity at local scales in many ecosystems (Wyckoff and Webb 1996, Alvarez and Cushman 2002, Hejda et al. 2009, Vilà et al. 2011). We found that species richness, diversity and evenness were reduced in invaded plots by *A. altissima*, which was consistent with other studies of community-level impact of invasive plants (Wyckoff and Webb 1996, Hejda et al. 2009). However, the negative relationship we observed between the invasive *A. altissima* and the associated community diversity could have been due to either: 1) the impact of *A. altissima* on the understory community, or 2) the environmental reasons why the invasive species was present in these communities (MacDougall and Turkington 2005, Huebner et al. 2009).

Control sites had the lowest NH$_4^+$ concentration, and sites with both target species present had the highest NH$_4^+$ concentration, which suggested that soil-N pool increased in the presence of *R. pseudoacacia* and *A. altissima* litter, or these two species are more likely to colonize high soil nitrogen habitats. The higher ammonium concentration in the soil solution was most likely due to both nitrogen fixation by *R. pseudoacacia* and rapid leaf decomposition of *A. altissima* leaves. Moreover, *R. pseudoacacia* sites had higher NH$_4^+$ concentration than *A. altissima* sites, which suggests that nitrogen fixation by *R. pseudoacacia* has a greater effect on
soil ammonium concentration than the effect of fast-decomposed *A. altissima* leaves. This finding is contradicted by a previous study conducted at a site in NW Spain invaded by *R. pseudoacacia* (Castro-Díez et al. 2008). The contradiction between studies was possibly due to a higher microbial activity in the native vs. exotic range of *R. pseudoacacia*. The increase of N in the soil pool was not associated with an increase in understory species diversity in our study. We suggest that allelopathic effects of the invasive species on soil and microbial processes could be one reason why the understory community and tree seeding regeneration was less diverse around *A. altissima* trees even though the soil N was higher. Sites richer in native or non-invasive exotic plants and with more alkaline soils were more likely to be invaded (Huebner et al. 2009). Therefore, addressing our third question, our data suggest that both target species were able to change the soil nutrition (particularly NH$_4^+$), but those changes were not associated with changes in understory community composition.

Previous studies have shown that native species diversity affects the establishment of invasive species. Higher diversity tends to make communities more resistant to invasive species at the neighborhood scale; but other factors such as propagule pressure may be more important on larger scales (Levine 2000). In our mapping study, the AL forest site had a greater diversity of native species than the BL forest site. However, the effects of *A. altissima* and *R. pseudoacacia* were similar among sites. Thus, in our study an increase in native species diversity was not associated with a decrease in the effects of invasive species on forest regeneration.

Our results from the tree-centered plot vegetation analysis showed that at a neighborhood scale (within site location) invasive species reduced native tree regeneration, but at a larger scale (cross location comparisons) the negative effect of invasive species on native tree regeneration was weak. The tree seedling abundance in different neighborhood size in the forest mapping study also supported this change of relationship between invasive species and diversity as scale...
changes. Previous studies support this result. For example, in British forests, the composition and ecological traits of communities which contained non-natives were very different from those that did not contain non-natives (Maskell et al. 2006). In fact, Maskell et al. suggested that the effects of invasive species on natives are limited to local scales and invasive species are not a big threat to biodiversity at large scales. The abundance of non-native species at the landscape level should be considered a result of disturbance and land-use change in Great Britain (Maskell et al. 2006).

A separate analysis of FIA data-base (Chapter 5) supported the concept that at a regional scale, the negative impact of A. altissima tended to be less significant than at a neighborhood scale.

Our study was an observational study, which reflects the current status of community associated with A. altissima and R. pseudoacacia. Without manipulative experiments, such as the removal of invasive species, or a long-term monitoring study, it would be hard to determine if the observed correlations between invasive species and native community diversity was a consequence of the invasive species or a reason for the presence of invasive species (Zavaleta et al. 2001).

In conclusion, we found a strong negative relationship between A. altissima and understory community richness, diversity, and evenness at several site locations at the neighborhood scale. The location of sites was a more important factor to determine site separation than the type of sites based on the community ordination results. Robinia pseudoacacia had a positive impact on soil N pool, but slightly negative impact on associated community richness, diversity, and evenness compared with the control sites. When the two species were together, the impact on herbaceous layer community diversity was the average effect of both species, but on tree regeneration the impact was dominated by the negative effect of A. altissima. The existence of both species is expected to change community diversity, structure and succession in Mid-Appalachian forest. The scale addressed in a vegetation survey
is important and could lead to different conclusions about the relationships between invasive species and native species diversity.
Literature cited:


Table 1. The total number of trees, saplings, seedlings, and species in the six 20m×50m plots surveyed in a forest mapping study conducted in the summer of 2011 and 2012. BL represents a forested site at the Blandy experimental farm, and AL represents a forested site on private property nearby the Blandy experimental farm. RP = plot with only *R. pseudoacacia*, no *A. altissima*; AA = plot with only *A. altissima*, no *R. pseudoacacia*; BOTH = plot with both species.

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<th>Plot type</th>
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<th># of Saplings</th>
<th># of Seedlings</th>
<th># of Species</th>
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Table 2. The community similarity (SCs) between each pair of the 6 plots surveyed in a forest mapping study conducted in the summer of 2011 and 2012. BL represents a forested site at the Blandy experimental farm, and AL represents a forested site on private property nearby the Blandy experimental farm. RP = plot with only *R. pseudoacacia*, no *A. altissima*; AA = plot with only *A. altissima*, no *R. pseudoacacia*; BOTH = plot with both species.

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<th>AL-RP</th>
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Table 3. Top 10 herb/shrub/vine layer species in the 16 sites surveyed in Virginia and West Virginia, ordered by importance value from a vegetation survey study conducted in summer 2013. The total number of species for each site is shown in brackets after the site name. VBLD: Virginia, Blandy experimental farm sites; VSCB: Virginia, Smithsonian Conservation Biology Institute sites; WDH: West Virginia, Dutch Hollow Hunt Club sites; WJDS: West Virginia, JDS Land LLC property sites. RP = site with only R. pseudoacacia, no A. altissima; AA = site with only A. altissima, no R. pseudoacacia; BOTH = site with both species; C = site with no R. pseudoacacia and no A. altissima.

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<th>VBLD_C (35)</th>
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<td>Microstegium vimineum 29.06</td>
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Table 4. The top 5 tree seedling dominants in the 16 sites surveyed at Virginia and West Virginia, ordered by importance value from a vegetation survey study conducted in summer 2013. The total species number of tree seedlings in each site is shown in brackets after the site name. VBLD: Virginia, Blandy experimental farm sites; VSCB: Virginia, Smithsonian Conservation Biology Institute sites; WDH: West Virginia, Dutch Hollow Hunt Club sites; WJDS: West Virginia, JDS Land LLC property sites. RP = site with only *R. pseudoacacia*, no *A. altissima*; AA = site with only *A. altissima*, no *R. pseudoacacia*; BOTH = site with both species; C = site with no *R. pseudoacacia* and no *A. altissima*. VBLD_RP site doesn’t have any seedlings and was not listed in the table.

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Figure 1. Diagram of the sampling plot design around each target tree (center black circle) in a forest vegetation survey study conducted in the summer of 2013 at 16 sites in the mid-Appalachian region of Virginia and West Virginia USA.

- 5 m plot for trees
- 3m plot for saplings
- 1m$^2$ plot for tree seedlings and herbaceous species /shrubs/vines
- Soil sample locations
Figure 2. Maps for locations of all trees, saplings, and seedlings in 20m×50m forested plots that contain *Ailanthus altissima*, *Robinia pseudoacacia*, or both species. The plots were either in the southwest woodlot of the Blandy Experimental farm (39°3’41”N, 78°4’29”W), Boyce, Virginia, USA (BL), or in a private property (39°7’39”N, 78°5’41”W) at Berryville, Virginia, USA (AL). From top to bottom are: (a) *R. pseudoacacia* only plot at BL; (b) both species plot at BL; (c) *A. altissima* only plot at BL; (d) *R. pseudoacacia* only plot at AL; (e) both species plot at AL; (f) *A. altissima* only plot at AL (this plot only surveyed from 0-42m). Data were from a forest woody species mapping survey conducted in summer 2011 and 2012 at the two locations. The symbols of the 32 woody species are shown in the legend; the red color symbols represent trees, the blue color symbols represent saplings, and the black color symbols represent seedlings.
Figure 3. Community ordinations using nonmetric multidimensional scaling (NMS) for 20 different WV and VA sites in a forest vegetation survey study conducted in summer 2013. Above
(a): ordination using herb/shrub/vine data; below (b): ordination using tree seedling data. VBLD: Virginia, Blandy experimental farm sites; VSCB: Virginia, Smithsonian Conservation Biology Institute sites; WDH: West Virginia, Dutch Hollow Hunt Club sites; WJDS: West Virginia, JDS Land LLC property sites. RP = data sets for sites with only *R. pseudoacacia*, no *A. altissima*; AA = data sets for sites with only *A. altissima*, no *R. pseudoacacia*; BRP = data sets for sites with both species, but the survey was done around *R. pseudoacacia* trees; BAA = data sets for sites with both species, but the survey was done around *A. altissima* trees; C = data sets for plots with no *R. pseudoacacia* and no *A. altissima*. 
Figure 4. Community ordinations by NMS using herb/shrub/vine data for VA-BLD sites in a forest vegetation survey study conducted in summer 2013 at the Blandy Experimental Farm, Boyce, VA. RP = data sets for sites with only *R. pseudoacacia*, no *A. altissima*; AA = data sets for sites with only *A. altissima*, no *R. pseudoacacia*; BRP = data sets for sites with both species, but the survey was done around *R. pseudoacacia* trees; BAA = data sets for sites with both species, but the survey was done around *A. altissima* trees; C = data sets for plots with no *R. pseudoacacia* and no *A. altissima*. 
Figure 5. Community ordinations by NMS for the VA-SCB sites in a forest vegetation survey study conducted in summer 2013 at the Smithsonian Conservation Biology Institute, Front Royal, VA. Above (a): ordination using herb/shrub/vine data; below (b): ordination using tree seedling data. RP = data sets for sites with only *R. pseudoacacia*, no *A. altissima*; AA = data sets for sites with only *A. altissima*, no *R. pseudoacacia*; BRP = data sets for sites with both species, but the survey was done around *R. pseudoacacia* trees; BAA = data sets for sites with both species, but the survey was done around *A. altissima* trees; C = data sets for plots with no *R. pseudoacacia* and no *A. altissima*. 
Figure 6. Community ordinations by NMS for the WV-DH location in a forest vegetation survey study conducted in summer 2013 at Dutch Hollow Hunt Club, West Virginia. Above (a): ordination using herb/shrub/vine data; below (b): ordination using tree seedling data. RP = data sets for sites with only *R. pseudoacacia*, no *A. altissima*; AA = data sets for sites with only *A. altissima*, no *R. pseudoacacia*; BRP = data sets for sites with both species, but the survey was done around *R. pseudoacacia* trees; BAA = data sets for sites with both species, but the survey was done around *A. altissima* trees; C = data sets for plots with no *R. pseudoacacia* and no *A. altissima*. 
Figure 7. Community ordinations by NMS for the WV-JDS location in a forest vegetation survey study conducted in summer 2013 at the JDS Land LLC property, West Virginia. Above (a): ordination using herb/shrub/vine data; below (b): ordination using tree seedling data. RP = data sets for sites with only *R. pseudoacacia*, no *A. altissima*; AA = data sets for sites with only *A. altissima*, no *R. pseudoacacia*; BRP = data sets for sites with both species, but the survey was done around *R. pseudoacacia* trees; BAA = data sets for sites with both species, but the survey was done around *A. altissima* trees; C = data sets for plots with no *R. pseudoacacia* and no *A. altissima*. 
Figure 8. Herb/shrub/vine species richness, evenness, Shannon’s and Simpson’s diversity for 20 WV and VA data sets in a forest vegetation survey study conducted in summer 2013. VBLD: Virginia, Blandy experimental farm sites; VSCB: Virginia, Smithsonian Conservation Biology Institute sites; WDH: West Virginia, Dutch Hollow Hunt Club sites; WJDS: West Virginia, JDS Land LLC property sites. RP = data sets for sites with only *R. pseudoacacia*, no *A. altissima*; AA = data sets for sites with only *A. altissima*, no *R. pseudoacacia*; BRP = data sets for sites with both species, but the survey was done around *R. pseudoacacia* trees; BAA = data sets for sites with both species, but the survey was done around *A. altissima* trees; C = data sets for plots with no *R. pseudoacacia* and no *A. altissima*. 
Figure 9. Tree seedling richness, evenness, Shannon’s and Simpson’s diversity for 20 WV and VA data sets in a forest vegetation survey study conducted in summer 2013. VBLD: Virginia, Blandy experimental farm sites; VSCB: Virginia, Smithsonian Conservation Biology Institute sites; WDH: West Virginia, Dutch Hollow Hunt Club sites; WJDS: West Virginia, JDS Land LLC property sites. RP = data sets for sites with only *R. pseudoacacia*, no *A. altissima*; AA = data sets for sites with only *A. altissima*, no *R. pseudoacacia*; BRP = data sets for sites with both species, but the survey was done around *R. pseudoacacia* trees; BAA = data sets for sites with both species, but the survey was done around *A. altissima* trees; C = data sets for plots with no *R. pseudoacacia* and no *A. altissima*. 
Chapter 5

Distribution patterns of exotic invasive *Ailanthus altissima* and native *Robinia pseudoacacia* in Mid-Appalachian Mountain forests using Forest Inventory and Analysis plots

Abstract:

We used permanent-plot data from the USDA Forest Service’s Forest Inventory and Analysis (FIA) program to analyze the spatial distribution pattern of the exotic invasive tree *Ailanthus altissima* and the native nitrogen-fixing tree *Robinia pseudoacacia* in forested land in the Mid-Appalachian mountain region, USA. The focus of this study was to assess mature trees and major environmental factors in FIA Phase 2 plots from 2010. A total of 4958 plots within the native range of *R. pseudoacacia* in the Appalachian Mountain region, covering 10 states were included in the analysis. By rank-based test and Chi-square test, we found a positive association between the spatial distributions of these two species. We found *R. pseudoacacia* to be more common in this region than the exotic invasive species. *Ailanthus altissima* is more likely to form dense stands, and to reduce neighborhood diversity than *R. pseudoacacia*. The effect of both species on neighborhood diversity is significant until the dominance of each species reaches beyond 40% of the whole plot. Site diversity, evaluated by Simpson’s index, showed that plots with *R. pseudoacacia* had higher diversity than reference plots in this region. At the regional scale of this study, the presence of *A. altissima* doesn’t negate the facilitative effect of *R. pseudoacacia* on community basal area and diversity.
Keywords: Basal area, diversity, forest inventory and analysis program (FIA), invasive species, spatial association, species distribution

Introduction:

Invasive species are exotic species that successfully naturalize in introduced areas and cause harm to the environment, the economy or human health (Federal Executive Order 13112). Previous research has shown that invasive plants can displace native plant species (D’Antonio and Vitousek 1992, Stinson KA 2006), deteriorate the quality of native forest (Alvarez and Cushman 2002), threaten the integrity of native forest ecosystem (Vitousek 1990) and change soil chemical properties (Musil 1993, Parker et al. 1999). Moreover, invasive plants can have substantial long-term impacts on plant communities and ecosystems globally because they can decrease suitable habitat for native species, alter soil microbial community and nutrient cycling differently than native species, and alter the established plant-plant, plant-insect or plant-microbes interactions (Mack et al. 2000, Simberloff 2006, Weidenhamer and Callaway 2010, Richardson and Rejmánek 2011). Invasive species are arguably considered to be one of the major causes for the decline and loss of long-term resident species in invaded ecosystems (Fritts and Rodda 1998, Gurevitch and Padilla 2004).

The success of invasive species depends on the nature of the habitat at the time of invasion, the functional traits of introduced plants, and the interactions with other species after the first establishment of the invasive species (Mack et al. 2000, Richardson et al. 2000, Pyšek et al. 2009). Native plants in the invaded community, which have similar growth form and share similar resource with the invasive plant, will interact the most with the invader during succession. Many invasive species colonize early succession sites in which they interact with early successional native species. The outcome of the competition between invasive species and early
successional native species may have a lasting effect on community composition and succession.

Our study focused on the most common invasive tree species *Ailanthus altissima* (Mill.) Swingle (Tree of Heaven) and the most important nitrogen fixing tree in the Appalachian region of the U.S., *Robinia pseudoacacia* L. (Black Locust). These two species are both fast-growing, early-successional species, which reproduce by an abundance of seeds and root sprouts. Both species prefer disturbed sites with high light intensity, and coexist in the eastern U.S. forest (Call and Nilsen 2003). *Ailanthus altissima*, from China, has been an invasive tree in U.S. forests for more than 200 years (Feret 1973). *Robinia pseudoacacia* is native to the Mid-Appalachian mountain region, but also is viewed as an invasive species worldwide (Castro-Díez et al. 2008). Both species are the most abundant trees on the planet because of their ability to grow fast, their high reproduction rate, their tolerance to extreme environment, and their use in horticulture, forestry, agriculture and medicine, by several cultures.

We propose that that these two species will encounter each other frequently in early successional forest of the Mid-Appalachian mountain region. In fact, a previous study has indicated that these species are contagious in selected post-logging sites (Call and Nilsen 2003). This previous study was done by surveying 10 plots in a silvicultural experiment at Blacksburg, VA and did not represent the association of these two species in other regions over a larger geographic scale. A regional approach is required to address the long-term relationships between these species and the rest of the community. Because *R. pseudoacacia* is a nitrogen fixing tree (Boring and Swank 1984) whereas *A. altissima* has an allelopathic effect on other forest species (Gómez-Aparicio and Canham 2008a), we propose that forest communities dominated by these two species will have a different diversity than other communities in the same region of similar age that do not have either species.
The overall goal of this study is to understand the result of the interaction between these two species on community composition over a large geographic scale in the Mid-Appalachian mountain region within the native range of *R. pseudoacacia*. The aims of this study are: 1) To test the association between *R. pseudoacacia* and *A. altissima* trees over a regional scale. We hypothesize that these two species would be positively associated rather than randomly distributed. 2) To determine the long-term impact of these two species on forest community composition. We hypothesize that plots with *A. altissima* trees will have lower diversity and basal area of other species compared with the reference plots, but plots with *R. pseudoacacia* trees will have higher diversity and basal area of other species compared with the reference plots. Also, we hypothesize that when *R. pseudoacacia* and *A. altissima* are both in the community, the facilitation role of *R. pseudoacacia* on other forest diversity and basal area will be absent. 3) To determine what environmental traits are associated with the abundances of each species. We hypothesize that *A. altissima* will be more associated with lower elevation, lower slope plots with wetter habitats and *R. pseudoacacia* will be best associated with higher elevation, steeper plots with drier habitats.

Our approach was to use species abundance data (trees) from the Forest Inventory and Analysis database (FIADB) compiled by the USDA Forest Service. USDA started the FIA program in the 1920s to conduct periodic forest inventories on a state-by-state basis, and annual inventories were started in 1999 (Woudenberg et al. 2010). The forest surveys today are comprehensive, and utilize a random, systematic sampling grid, which permits calculation of statistical confidence intervals for area estimates (O’Brien et al. 2003). The FIA program allows internal users and researchers to evaluate and monitor forest composition, total forest volume, forest health and change in forest structure across the United States.
Material and Methods:

Data source: Forest Inventory and Analysis Database (FIADB)

The USDA FIA program is the primary source for the status of historical and current forest resources in the United States (Smith et al. 2009). FIA uses a consistent quasi-systematic design protocol across all the states of U.S.A. and uses classified satellite images to stratify the sampling design (Bechtold and Patterson 2005). The Phase 2 data collection used the basic plot condition and tree measurements taken on the standard FIA base grid, which is roughly 1 sample location per 6,000 acres (Woudenberg et al. 2010). Forested land is defined as areas with more than 10% cover by tree species canopies, at least 0.4 ha in size and at least 36.6 m wide (Zhu et al. 2014). FIA plots consist of four fixed-radius subplots of 7.2 m in diameter, spaced 36.6 m apart in a triangular arrangement (Supplementary Figure 1) with one subplot in the center (Bechtold & Patterson, 2005). All trees (live and dead) with a diameter at breast height (DBH) of at least 12.7 cm are inventoried on all forested subplots. Within each subplot, a 2.07 m radius microplot 3.66 m at an azimuth of 90 degrees from the subplot center is established. Within each microplot, live tree saplings with a DBH between 2.5 and 12.7 cm and all live tree seedlings are inventoried (Woudenberg et al. 2010).

In this analysis, we used FIA data from recent annual inventories (1999–2008) in 31 eastern states extracted by Kai Zhu (Duke university, personal communication), from FIADB version 4.0 published on 16 March 2010 (http://fia.fs.fed.us/), which resulted in a total of 43396 inventory plots (Zhu et al. 2012, Zhu et al. 2014). To compare species abundance in different life stages, Zhu et al. followed the FIA sampling design and divided the data into two size classes: (i) seedling (DBH < 2.54 cm) and (ii) tree (DBH ≥ 2.54 cm). There is also a data set containing
environmental information, such as average annual temperature, precipitation, elevation, aspect and slope of each plot. Because we were interested in how these two tree species associated within the Mid-Appalachian mountain region within the native range of *R. pseudoacacia*, we selected the FIA plots within the native range of *R. pseudoacacia* in the Mid-Appalachian mountain region based on the distribution map of *R. pseudoacacia* (Flora of North America Editorial Committee, 1993+) in Arc GIS (ArcMAP 10.2.2). We focused on the count and basal diameter data of trees on the level of subplots and plots. Plots of 0.6 ha in area are commonly used for studying forest structure and diversity; however, because the subplot level in the FIADB correspond to local competition between these two trees, the subplot level in the FIADB may be more appropriate for our questions than the aggregated information at plot level.

**Statistical analysis methods**

All the analyses were done in R (version 0.98.1091, Boston MA, RStudio, Inc.). To test the association between *A. altissima* and *R. pseudoacacia*, we created a rank based p-value using the quantiles of the number of subplots in which the two subject species co-exist. That is, we first calculated the total numbers of subplots that *A. altissima* coexists with any other species in the data set and ranked the other species based on the number of subplots in which that species co-exists with *A. altissima*. Subsequently, we determined the quantile (percentage) of the number of subplots in which *A. altissima* co-exists with *R. pseudoacacia*. Similarly, the rank based p-value was calculated for *A. altissima* coexisting with *R. pseudoacacia*.

In order to compute the relationships among species on a larger scale (plot level), the above statistical analysis method was applied on the plot level as well by treating each subplot within site as an individual measurement unit. When performing analysis on each plot, we simply added up the number of each species in the four subplots together. We list all the frequent
species that coexisted with either *R. pseudoacacia* or *A. altissima*.

In addition, a two by two contingency table was constructed with one variable being the presence of *A. altissima* in each subplot and the other being whether *R. pseudoacacia* was present in each subplot. The chi-square test was then applied on the contingency table to determine the dependency of the presence of the two species. Another measure of association, phi-coefficient (Cramér 1999) was also calculated. The Phi coefficient is a method for evaluating the indicator value (presence/absence) of a species with respect to a one-way grouping of sample units. The Phi coefficient test results on plot level were examined as well as that for the subplot level.

In order to evaluate the effect of each species and their combination on community metrics we divided the subplots and plots into four types: 1) *A. altissima* is present but *R. pseudoacacia* is not present (“Aa”); 2) *R. pseudoacacia* is present but *A. altissima* is not present (“Rp”); 3) *A. altissima* and *R. pseudoacacia* are both present (“AaRp”); 4) Neither *A. altissima* nor *R. pseudoacacia* are present (“Ref”). The total number and total basal area of the other species besides the two target species were then totaled for each subplot or plot. A Student’s T-test was used to compare if there were differences in the total number of tree stems or total basal area of other trees between the Rp and Aa plots. The dominance of *A. altissima* or *R. pseudoacacia* in the plot was evaluated by relative basal area of both species. In order to test how the abundance and total basal area of other species in the plot varied as the dominance of the two target species varied, the plots were stratified based on *A. altissima* or *R. pseudoacacia*. Stratifications in 5% intervals from 5% basal area to a maximum of 50% basal area were used. The abundance and total basal area of other species was calculated for each of the strata levels. Significant differences in the mean response variable between the two target species were
evaluated for each strata by Student’s T-test. In addition, regressions were formed with the total basal area of other species in the plot as response variable, and the percentage of basal area of *A. altissima* or *R. pseudoacacia* in the plot as the independent variable. Then we tested if this relationship was different between *A. altissima* and *R. pseudoacacia* by ANCOVA.

Simpson’s index of diversity (D) was used to describe plot diversity because there are a lot of rare species (appear less than 10 plots) in our data set (Pielou 1969, Hill 1973). We used the equation below:

$$D = 1 - \sum_{i=1}^{k} \left( \frac{n_i}{N} \right)^2$$

where $n_i$ is the number of the ith species in the plot,

$N$ is the number all the species in the plot,

$k$ is the total species number in the plot.

The value of Simpson’s index of diversity ranges from 0 to 1, and a larger value for D corresponds to a higher diversity. A one-way ANOVA was used to compare the difference in diversity among the four types of plots.

To investigate the relationship between species distribution and environmental conditions provided by the FIA, we used a logistic regression model between the presence/absence of *A. altissima* or *R. pseudoacacia* and all the environmental variables available in the data set: latitude, longitude, elevation, aspect, slope, average annual temperature, average annual precipitation and physiographic class code. The categorical analysis and regression tree (CART) method was also adopted to predict the presence of both species by the environmental independent variables.

**Results:**
The association between *A. altissima* and *R. pseudoacacia*

Plots with either *A. altissima* or *R. pseudoacacia* or both were visualized on a states map in Arc GIS (Figure 1). Since each of the four subplots had the same location information in the data set, the spatial figure shows the distribution of *A. altissima* and *R. pseudoacacia* at the plot level. In the region we considered, the locations that *R. pseudoacacia* occurred alone tended to be denser on the bottom and southeast side of the semi-major axis of the ellipse to the southeast (Figure 1). In contrast, the places that *A. altissima* occurred alone or both *A. altissima* and *R. pseudoacacia* coexisted were uniformly distributed over the observed region. *Ailanthus altissima* also appeared frequently along the Appalachian Trail in Virginia.

Overall, there were many more plots that had *R. pseudoacacia* than *A. altissima*. In total, there were 4958 plots selected within the native range of *R. pseudoacacia*. Most of the plots did not contain *A. altissima* or *R. pseudoacacia*. *Robinia pseudoacacia* was a more common species compared with *A. altissima* over the sampled range. In this region, in terms of the frequency of appearance in the plots, *R. pseudoacacia* ranked 21st of all the tree species. The most common species in this region are *Quercus prinus*, *Acer rubrum* and *Liriodendron tulipifera*. More than 5% of the plots sampled in this region had *R. pseudoacacia*, while less than 1% of the plots sampled contained *A. altissima* (Table 1). The plots in which *A. altissima* and *R. pseudoacacia* coexisted were less frequent than plots with either species present alone. There were 50 plots that contained both species, while there were only 32 subplots that contain both species when subplots are not combined. This result is expected because if any one of the 4 subplots had *A. altissima* and *R. pseudoacacia* then this plot was counted as containing both. Although *A. altissima* appeared in less than 1% of the total plots, 5.2% of the subplots had a pure stand of *A. altissima*, and in 23% of the subplots *A. altissima* accounted for more than 50% of total basal
Robinia pseudoacacia had a similar percentage of pure stand subplots (5.5%), but only 15.1% of the subplots were dominated by *R. pseudoacacia*.

Both the chi-square independence test and the Phi coefficient test were significant (Table 2). This indicated that the presence of *A. altissima* and *R. pseudoacacia* were not independent throughout the native range of *R. pseudoacacia*.

There was significant evidence that the two species were more likely to be associated with each other than with other species based on rank-based p-value. The plant species that had stronger association with *A. altissima* than *R. pseudoacacia* and vice versa for *R. pseudoacacia* are presented (Table 3). In the case of *A. altissima*, *R. pseudoacacia* was the top 2.06% species on the subplot level and was the top 1.65% species on the plot level. In the case of *R. pseudoacacia*, *Ailanthus altissima* was the top 10.29% on the subplot level and 12.76% on plot level among all species that co-occurred (Table 3). More species are strongly correlated with *R. pseudoacacia* compared with *A. altissima* because there are many more subplots or plots that contain *R. pseudoacacia* than *A. altissima*.

The **Influences of *A. altissima* and *R. pseudoacacia* on diversity**

The average, median and max number of *A. altissima* trees in each subplot where it was present were 2.2, 1 and 15 respectively. The average, median and max number of *R. pseudoacacia* individuals in each subplot where it was present were 1.7, 1 and 12 respectively. Plots with *A. altissima* tended to have more stems of itself than that of plots with *R. pseudoacacia*. But, the density of other tree species coexisting with *R. pseudoacacia* (2.948 ± 1.605) was higher than other trees coexisting than *A. altissima* (2.376 ± 1.439).

The number of stems of other species and the total basal area of other trees decreased as the dominance of either *A. altissima* or *R. pseudoacacia* increased among stratified samples.
(Figure 2). The total basal area of other species in the plot was significant until the target species had more than 45% dominance (Table 4). Plots with R. pseudoacacia had a higher species diversity than plots with A. altissima at lower strata but, the total number of other species in the plot was not significant when the target species had more than 25% dominance (Table 4).

Similarly, regression analysis indicated that the total basal area of other species besides these two target species in a plot decreased as the dominance of A. altissima or R. pseudoacacia increased (Figure 3). The other species basal area dropped rapidly when the percentage of basal area of either A. altissima or R. pseudoacacia was less than 20%. But when either target species became dominant in the plot, the abundance of other species decreased slowly. Both the intercept and slope of this regression relationship for A. altissima and R. pseudoacacia were significantly different (p<0.0001), and the abundance of the other species decreased more as the dominance of A. altissima increased (steeper regression slope) than that for R. pseudoacacia.

The average ± standard deviation of Simpson’s diversity index for the four types of plot were 0.7869 ± 0.0806 for Aa plots, 0.7955 ± 0.0925 for Rp plots, 0.7995 ± 0.0894 for AaRp plots, and 0.7647 ± 0.1280 for Ref plots. The difference in Simpson’s diversity index was significantly different among these plot types (p=5.86e-12). However, this difference was strongly influenced by the difference between Ref and Rp plots (p=6.4e-10). There was no significant difference at the α = 0.05 level between all the other pairs of plot types, especially among Aa, Rp and AaRp plots.

The presence/absence of A. altissima was highly related to annual temperature (p<0.0001), longitude and latitude (p<0.0001), slope (p=0.0035), physiographic class code (p=0.0131) and elevation (p=0.0671). Ailanthus altissima was more likely to be found in plots with higher temperature, lower slope, lower elevation and moister soil. Based on physiographic
classes, *A. altissima* preferred mesic sites, especially flatwoods and narrow flood plains/bottomlands. The CART method selected latitude, temperature, longitude, elevation, aspect and precipitation as important variables (Figure 4a). The results of CART were more complicated and slightly different than the logistic regression results. Based on CART, as long as the latitude was more than 35.8169° and average annual temperature was beyond the lowest boundary (10.05 °C), plots with higher longitude, higher precipitation, and higher elevation were more likely to have *A. altissima* present.

Logistic regression result showed that *R. pseudoacacia* was more likely to be found in areas with cooler temperature (p<0.0001), lower elevation (p=0.0021), and steeper slope (p<0.0001) within its native range. Among physiographic classes, *R. pseudoacacia* preferred small drains (p=0.0032). The general pattern was that *R. pseudoacacia* preferred plots with steeper slope and moister habitats than *A. altissima*. The decision tree CART built for *R. pseudoacacia* was not very informative because there was only one node (Figure 4b). The lack of more nodes in the decision tree for *R. pseudoacacia* was because the deviance didn’t improve much as the tree divided further (Ritschard 2006).

**Discussion:**

Within the native range of *R. pseudoacacia* in the Appalachian mountain region, *A. altissima* is not a very common species. *R. pseudoacacia* is one of the top 2% of species most associated with *A. altissima*. Our hypothesis that these two species would be positively associated was supported because the association between *A. altissima* and *R. pseudoacacia* was significantly stronger than random. About 20% of all the plots with *A. altissima* also contained *R. pseudoacacia*. Thus, we are confident that these two species will encounter each other in early-
successional forest and interact through mid-age forest sites such as those in the FIADB.

Evidence for the likelihood of intraspecific competition for these two species varies among studies. In this study, at a regional scale, *A. altissima* formed higher density stands than *R. pseudoacacia*, which suggests that there is a greater potential for intraspecific competition in *A. altissima* than in *R. pseudoacacia*. However, in a complementary mapping study (Chapter 4) both species were found to have clumped distributions in 20 x 50m plots suggesting equal opportunity to intraspecific competition. In contrast, a previous study (Call and Nilsen 2003), *A. altissima* saplings were found distributed randomly whereas *R. pseudoacacia* had a clumped distributed in 50m×50m quadrats suggesting that *R. pseudoacacia* was more likely to experience intraspecific competition. The data set used in this study doesn’t have spatial information within subplots for either species. Thus, the data set used in this study, at a regional scale, cannot be used to evaluate spatial distributions at the neighborhood scale because our findings are limited to comparisons of density and basal area among *A. altissima*, *R. pseudoacacia*, and other species at the sub-plot and plot-level. However, based on the significant likelihood of these species being found together there is ample opportunity for interspecific competition or facilitation to occur.

Our hypothesis that *A. altissima* would decrease forest diversity compared with reference species was not supported by our data. In contrast to our hypothesis, the plots that had *A. altissima* present had slightly higher diversity compared with other reference plots. One possible mechanism for this effect is because *A. altissima* litter decomposes fast and can increase soil available nutrients, such as N and Ca in its neighborhood (Castro-Díez et al. 2008). The impact of *A. altissima* on other species is a result of the balance of positive facilitation (higher nutrient flux) and negative allelopathic effect, and can be highly species-specific (Gómez-Aparicio and Canham 2008b) rather than at the community diversity level. Also, a previous study found that
young *A. altissima* saplings produce more inhibitory compounds than mature trees (Lawrence et al. 1991), so for our plots where mature *A. altissima* occurs, the allelopathic effect should be less important. This result supports the importance of enhanced nutrient flux as the most likely mechanism by which *A. altissima* increased community diversity. However, we found that plots with *A. altissima* were less diverse than plots with *R. pseudoacacia*, based on either abundance or basal area of other species. This difference disappeared when the target species became dominant in the plot (relative basal area beyond 40%). Thus, when comparing the impact of these two species to forest community diversity and dominance, it was better to avoid plots that were highly dominated by the target species because this biased the diversity and dominance of other species simply by space occupied by the target species. Moreover, to prevent bias in the conclusion, plots where both species were rare (less than 2%) should be avoided as well.

Our findings rejected the hypothesis that invasive *A. altissima* reduces local diversity, yet it has been suggested that invaders (such as *A. altissima*) will reduce global diversity in the short term, but increase local diversity (Davis 2003). Actually, most invaded ecosystems are associated with higher disturbance rates and habitat loss, and there was evidence that invasive species were rather a passenger than the driver for community and ecosystem changes (MacDougall and Turkington 2005). Also, the impact of invasive species on plant community diversity depends on the characteristics and role of invasive species, as well as the environment context (Davis 2003, Mason and French 2008, Richardson et al. 2012). Our finding that plots with invasive *A. altissima* were associated with slightly higher diversity than other reference plots at a regional scale supports the theory of positive relationship between alien species diversity and native species diversity on a larger scale (Stohlgren et al. 1999, Maskell et al. 2006). But this positive association could be either a result of invasion or a reason these areas were invaded. A study
using FIA Phase 3 sites about invasive species distribution patterns (Huebner et al. 2009) found that younger forests, forests with non-forest patches present (disturbance), and forests with high native species richness were more likely to be occupied by exotic plants. Our findings are limited because our data only contains trees and sapling information, and other herbaceous invasive species in the plot should be considered to better test the relationship between invasive tree species and native/invasive species diversity.

In support of our hypothesis, plots with *R. pseudoacacia* had higher diversity than reference plots. Although *R. pseudoacacia* is not the most common and dominant tree in this region, plots having *R. pseudoacacia* had significantly higher diversity than plots dominated by other common species such as maple, yellow-poplar, hickory and oak. This evidence supports the theory that the nitrogen fixing tree *R. pseudoacacia* can facilitate forest succession after disturbance by increasing soil available nitrogen (Boring 1984a, Boring and Swank 1984b). In contrast to the expected result, plots with both target species present had slightly higher diversity than plots that had only *R. pseudoacacia* present. Thus, coexistence with *A. altissima* didn’t reverse this pattern of higher diversity in plots with *R. pseudoacacia*.

Abiotic conditions, together with species interactions and dispersal abilities determine the distribution of species (Soberon and Peterson 2005). In previous research on the distribution of common invasive species in southern U.S.A. using FIA data, minimum temperature was the most useful of all the dependent variables, followed by rainfall in the wettest month, elevation and temperature in the driest quarter (Lemke et al. 2012). Also, among the 22 invasive species studied in the southern Appalachian region, change in pine was an important predictor only for *A. altissima* (Lemke et al. 2012). The distribution model of *A. altissima* created from our data set generally agreed with Lemke et al. (2012). However, we don’t have climate change or
disturbance information in our data set to evaluate species changes as in Lemke’s model.

In conclusion, we found a positive association between *A. altissima* and *R. pseudoacacia* in the mid-Appalachian mountain region within the native range of *R. pseudoacacia* using FIA plots. Thus, it is likely these two species interact at local scales. Environmental factors, such as temperature, latitude and longitude, elevation, slope and habitat influence the presence of both species in different way. We also found that mature *A. altissima* has higher tendency to form pure stands than *R. pseudoacacia* suggesting that intraspecific competition is more likely to occur for the former species. Yet this conclusion was not supported by other research on the same species. The impact of *R. pseudoacacia* on other tree species was positive and forest plots with *R. pseudoacacia* had higher species richness than plots with *A. altissima* or plots dominated by other reference species. At the regional scale of this study, the presence of *A. altissima* doesn’t negate the facilitative effect of *R. pseudoacacia* on community basal area and diversity.
Literature Cited:


Lemke, D., J. W. Coulston, P. Hulme, C. Paterson and J. A. Brown (2012). *Invasive potential of invasive plants in the forest of the southern region, United States*. Moving from Status to
Trends: Forest Inventory and Analysis Symposium 2012.


Table 1: Two by two contingency tables of number of subplots (a) and plots (subplots combined) (b) containing *A. altissima* or *R. pseudoacacia* in FIA plots within the native range of *R. pseudoacacia*.

(a)

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td><em>A. altissima</em></td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>R. pseudoacacia</em></td>
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<td>133</td>
</tr>
<tr>
<td>&gt;0</td>
<td>1031</td>
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(b)

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<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>R. pseudoacacia</em></td>
<td>4007</td>
<td>94</td>
</tr>
<tr>
<td>&gt;0</td>
<td>807</td>
<td>50</td>
</tr>
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</table>
Table 2: Chi-square test ($\chi^2$) and Phi coefficient results of the association between *A. altissima* and *R. pseudoacacia* based on contingency tables of number of plots with either or both species present in the FIADB within the native range of *R. pseudoacacia*.

<table>
<thead>
<tr>
<th></th>
<th>Pearson $\chi^2$</th>
<th>$\chi^2$ p-value</th>
<th>Phi coefficient</th>
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</thead>
<tbody>
<tr>
<td>Subplots not combined</td>
<td>64.6</td>
<td>&lt;0.0001</td>
<td>0.06</td>
</tr>
<tr>
<td>Subplots combined</td>
<td>31.54</td>
<td>&lt;0.0001</td>
<td>0.08</td>
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</table>
Table 3: Plant species that have strong association with *A. altissima* or *R. pseudoacacia* in the FIADB within the native range of *R. pseudoacacia*. Species names were consistent with FIA inventory data standard, and the full scientific names and common names can be found in supplementary table 1. The number below each species name is how many subplots or plots had both the stated species and the target species present.

**Strong Association with target species *A. altissima***

<table>
<thead>
<tr>
<th>Subplots</th>
<th>ROPS ACSA3 LITU AIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>32  33  41  165</td>
</tr>
<tr>
<td>Plots</td>
<td>ROPS QURU FRAM2 ACSA3 ACRU LITU AIAL</td>
</tr>
<tr>
<td></td>
<td>50  54  57  68  83  86  144</td>
</tr>
</tbody>
</table>

**Strong Association with target species *R. pseudoacacia***

<table>
<thead>
<tr>
<th>Subplots</th>
<th>AIAL JUVI MAAC TSCA CACO15 TIAM PIST CAOV2 JUNI QUCO2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>32  32  33  35  37  37  43  52  59  59</td>
</tr>
<tr>
<td></td>
<td>NYSY CAAL27 SAAL5 OXAR ULAM QUVE QUAL ULRU CAGL8</td>
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<td>64  67  70  74  74  75  81  82  101</td>
</tr>
<tr>
<td></td>
<td>BELE FRAM2 QURU QUPR2 PRSE2 ACSA3 LITU ACRU ROPS</td>
</tr>
<tr>
<td></td>
<td>118 131 155 160 207 212 278 356 1063</td>
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<tr>
<td>Plots</td>
<td>AIAL AMELA CEOC JUVI MAAC PIVI2 CACO15 TIAM TSCA FAGR</td>
</tr>
<tr>
<td></td>
<td>50  54  54  59  93  95  97  107 112 120</td>
</tr>
<tr>
<td></td>
<td>CAOV2 JUNI ULAM ULRU PIST SAAL5 CAAL27 NYSY OXAR BELE</td>
</tr>
<tr>
<td></td>
<td>123 128 129 129 133 143 178 200 215 231</td>
</tr>
<tr>
<td></td>
<td>QUCO2 QUVE FRAM2 CAGL8 PRSE2 QUAL ACSA3 QURU QUPR2</td>
</tr>
<tr>
<td></td>
<td>232 243 259 262 301 306 375 376 407</td>
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<tr>
<td></td>
<td>LITU ACRU ROPS</td>
</tr>
<tr>
<td></td>
<td>459 590 857</td>
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Table 4: The Welch Two sample T-test results of total number and basal area of other species in the plots stratified based on 5% increments in total basal area of *A. altissima* (Aa plot) or *R. pseudoacacia* (Rp plot) using FIA DB plots within the native range of *R. pseudoacacia*. Significant p-values are shown in bold.

<table>
<thead>
<tr>
<th>Minimum relative basal area of <em>A. altissima</em> or <em>R. pseudoacacia</em> in the plot</th>
<th>p-value for t-test of total basal area of other species</th>
<th>p-value for t-test of total number of other species</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td><strong>1.096e-10</strong></td>
<td><strong>2.41e-7</strong></td>
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<tr>
<td>10%</td>
<td><strong>8.146e-9</strong></td>
<td><strong>3.044e-4</strong></td>
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<tr>
<td>15%</td>
<td><strong>9.143e-8</strong></td>
<td><strong>4.794e-3</strong></td>
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<tr>
<td>20%</td>
<td><strong>3.244e-5</strong></td>
<td><strong>6.707e-3</strong></td>
</tr>
<tr>
<td>25%</td>
<td><strong>2.722e-4</strong></td>
<td><strong>0.032</strong></td>
</tr>
<tr>
<td>30%</td>
<td><strong>4.55e-3</strong></td>
<td><strong>0.222</strong></td>
</tr>
<tr>
<td>35%</td>
<td><strong>5.135e-3</strong></td>
<td><strong>0.358</strong></td>
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<td>40%</td>
<td>0.011</td>
<td>0.596</td>
</tr>
<tr>
<td>45%</td>
<td>0.172</td>
<td>0.746</td>
</tr>
<tr>
<td>50%</td>
<td>0.788</td>
<td>0.397</td>
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</table>
Figure 1: Spatial distribution of *A. altissima* and *R. pseudoacacia* on a states map based on the FIA DB plots within the native range of *R. pseudoacacia*. Red circles are the plots where both species were present, blue circles are the plots where *A. altissima* was present but there were no *R. pseudoacacia*, and grey circles are the plots where *R. pseudoacacia* was present but no *A. altissima* was present.
Figure 2. The average total basal area (a) and number (b) of other species (besides *A. altissima* and *R. pseudoacacia*) in the plots within various strata defined by basal area percentage of *A. altissima* and *R. pseudoacacia*. The solid line indicates this relationship for *A. altissima*, the dotted line indicates this relationship for *R. pseudoacacia*. The bars are 95% confidence interval for the mean.
Figure 3. Regressions between total basal area of other species (besides *A. altissima* and *R. pseudoacacia*) and the basal area percentage of *A. altissima* and *R. pseudoacacia* in FIA plots from the native range of *R. pseudoacacia* in the Mid-Appalachian region of the United States. The solid line and black circles indicate the regression (Adjusted $R^2=0.125$) and data points for *A. altissima*; the dotted line and open circles indicate the regression (Adjusted $R^2=0.171$) and data points for *R. pseudoacacia*. 
Figure 4. The decision tree results by CART for (a) the distribution of *A. altissima* in relationship with environmental variables (above) and (b) the distribution of *R. pseudoacacia* in relationship with environmental variables (below) in FIA plots within the native range of *R. pseudoacacia*. 

lat.1 = latitude; tmp = average annual temperature; lon.1 = longitude; ppt = average annual precipitation; elev = elevation.
Appendices:

Supplementary Table 1. Species names referred to in Table 3.

<table>
<thead>
<tr>
<th>Species Symbol</th>
<th>Common Name</th>
<th>Scientific Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACRU</td>
<td>red maple</td>
<td>Acer rubrum</td>
</tr>
<tr>
<td>ACSA3</td>
<td>sugar maple</td>
<td>Acer saccharum</td>
</tr>
<tr>
<td>AIAL</td>
<td>tree of heaven</td>
<td>Ailanthus altissima</td>
</tr>
<tr>
<td>AMELA</td>
<td>serviceberry</td>
<td>Amelanchier spp.</td>
</tr>
<tr>
<td>BELE</td>
<td>sweet birch</td>
<td>Betula lenta</td>
</tr>
<tr>
<td>CAAL27</td>
<td>mockernut hickory</td>
<td>Carya alba</td>
</tr>
<tr>
<td>CACO15</td>
<td>bitternut hickory</td>
<td>Carya cordiformis</td>
</tr>
<tr>
<td>CAGL8</td>
<td>pignut hickory</td>
<td>Carya glabra</td>
</tr>
<tr>
<td>CAOV2</td>
<td>shagbark hickory</td>
<td>Carya ovata</td>
</tr>
<tr>
<td>CEOC</td>
<td>hackberry</td>
<td>Celtis occidentalis</td>
</tr>
<tr>
<td>FRAM2</td>
<td>white ash</td>
<td>Fraxinus americana</td>
</tr>
<tr>
<td>FAGR</td>
<td>American beech</td>
<td>Fagus grandifolia</td>
</tr>
<tr>
<td>JUNI</td>
<td>black walnut</td>
<td>Juglans nigra</td>
</tr>
<tr>
<td>JUVI</td>
<td>eastern redcedar</td>
<td>Juniperus virginiana</td>
</tr>
<tr>
<td>LITU</td>
<td>yellow-poplar</td>
<td>Liriodendron tulipifera</td>
</tr>
<tr>
<td>MAAC</td>
<td>Cucumber tree</td>
<td>Magnolia acuminata</td>
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<tr>
<td>NYSY</td>
<td>blackgum</td>
<td>Nyssa sylvatica</td>
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<tr>
<td>OXAR</td>
<td>sourwood</td>
<td>Oxydendrum arboreum</td>
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<tr>
<td>PIST</td>
<td>eastern white pine</td>
<td>Pinus strobus</td>
</tr>
<tr>
<td>PIVI2</td>
<td>Virginia pine</td>
<td>Pinus virginiana</td>
</tr>
<tr>
<td>PRSE2</td>
<td>black cherry</td>
<td>Prunus serotina</td>
</tr>
<tr>
<td>Code</td>
<td>Common Name</td>
<td>Scientific Name</td>
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<td>-------</td>
<td>-----------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>QUAL</td>
<td>white oak</td>
<td><em>Quercus alba</em></td>
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<tr>
<td>QUCO2</td>
<td>scarlet oak</td>
<td><em>Quercus coccinea</em></td>
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<td>QUPR2</td>
<td>chestnut oak</td>
<td><em>Quercus prinus</em></td>
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<tr>
<td>QURU</td>
<td>northern red oak</td>
<td><em>Quercus rubra</em></td>
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<tr>
<td>QUVE</td>
<td>black oak</td>
<td><em>Quercus velutina</em></td>
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<tr>
<td>ROPS</td>
<td>black locust</td>
<td><em>Robinia pseudoacacia</em></td>
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<tr>
<td>SAAL5</td>
<td>sassafras</td>
<td><em>Sassafras albidum</em></td>
</tr>
<tr>
<td>TIAM</td>
<td>American basswood</td>
<td><em>Tilia americana</em></td>
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<tr>
<td>TSCA</td>
<td>eastern hemlock</td>
<td><em>Tsuga canadensis</em></td>
</tr>
<tr>
<td>ULAM</td>
<td>American elm</td>
<td><em>Ulmus americana</em></td>
</tr>
<tr>
<td>ULRU</td>
<td>slippery elm</td>
<td><em>Ulmus rubra</em></td>
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</table>
Supplement Figure 1. FIA Plot Design. Adopted from (Bechtold and Patterson 2005)
CHAPTER 6

Conclusions

Summary:

A series of manipulative experiments and observational studies was used to answer three main questions: (1) Which type of interaction occurs between the co-located invasive *Ailanthus altissima* and native *Robinia pseudoacacia* during seedling and sapling stages and what are the results of that interaction? (2) Do other factors, such as density, species composition, soil nutrients, and seed source change the results of the interaction between the two subject species? (3) What are the consequences of the presence of *A. altissima* and/or *R. pseudoacacia* and their interaction on community structure, diversity, and regeneration on a large geographic scale?

To address the type and extent of local scale interactions of these two species (Question 1 and 2), a greenhouse experiment utilizing various species compositions, nutrient levels and seed sources at seedling stage was performed (Chapter 2). In addition, a common-garden experiment with various species density and species composition over three consecutive growing seasons (Chapter 3) was performed to evaluate inter and intraspecific interaction at the sapling level.

Both experiments were manipulative, but each had a different focus: the greenhouse experiment focused on belowground competition and the mechanisms underlying the interaction between these two species, while the common-garden experiment focused on being closer to the natural conditions for the growth of these two species (local weather, natural soil, microbes and herbivories) and following changes of interactions over a three year period after establishment.

Both experiments were full-factorial designs, with at least 5 replications.
At the seedling stage under relatively low nutrient conditions, the dominant interaction was competition, and *R. pseudoacacia* was the winner both above- and belowground. *Ailanthus altissima* did not benefit significantly from the nitrogen fixed by neighboring *R. pseudoacacia* seedlings. *Ailanthus altissima* inhibited nodulation of *R. pseudoacacia* roots perhaps by allelopathic means. *Ailanthus altissima* seedlings germinated from Chinese seed source (the native genotype) had weaker competitive abilities (slower growth, higher demand for nutrients, less inhibition effect) compared with the ones gminated from U.S. seed source. *Robinia pseudoacacia* could adapt to a nutrient-poor environment better than *A. altissima*. However, at the sapling stage under a more natural condition, *R. pseudoacacia* plants grew quicker and bigger than *A. altissima*, but the invasive *A. altissima* inhibited the growth of *R. pseudoacacia* significantly by interspecific competition. There was also less intraspecific competition in *A. altissima* monocultures than *R. pseudoacacia* monocultures under the same density, suggesting that *A. altissima* could form pure stands more easily than *R. pseudoacacia* and thereby excluding other species. The negative impact of *A. altissima* on *R. pseudoacacia* became larger as time passed, while the trend for a slight facilitation of *A. altissima* by *R. pseudoacacia* disappeared as time progressed.

To address how *A. altissima*, *R. pseudoacacia* and their interaction affect community structure, diversity, and regeneration (Question 3), two different types of forest vegetation survey studies (mapping and target tree-based) at the community scale were performed (Chapter 4). In addition, the association of these two species at the regional spatial scale was evaluated by probing the FIA data base (Chapter 5). Both the vegetation survey plots and the FIA plots were selected to be within the natural habitats and range of *R. pseudoacacia* in the Mid-Appalachian mountain region. Four types of plots were selected in both studies: 1) with *A. altissima*, no *R.*
with R. pseudoacacia, no A. altissima; 3) with both species; 4) without either of the two species. The community-level vegetation survey studies focused on the impact of both species on the associated understory community structure, tree species seedling regeneration, and ecosystem nutrient cycling. The FIA plot analysis focused on the spatial distribution of these two species, the relationship of their presence and diversity of other trees, and how their spatial distribution was associated with environmental conditions at the regional-level.

At both the community and the regional scale, we found A. altissima and R. pseudoacacia were closely associated with each other, and with themselves, indicating both interspecific competition and intraspecific competition could happen frequently without competitive exclusion between these two species. The impact of A. altissima on the understory community diversity and tree seedling regeneration was strongly negative compared with the control species, while the impact of R. pseudoacacia was neutral or slightly negative. Both species had positive impacts on the soil N pool and increased soil pH significantly. When the two species were co-located, the impact to understory community diversity and tree seedling regeneration was more similar to the impact of A. altissima alone, suggesting a negative influence of A. altissima on the effects of R. pseudoacacia on community structure, particularly seedling regeneration. The regional-scale analysis results agreed that plots dominated by R. pseudoacacia had higher richness and abundance of other tree species than the plots dominated by A. altissima. However, the regional study also suggested that the presence of both A. altissima and R. pseudoacacia was positively related to the diversity of other trees in the plots compared with randomly selected reference plots. The regional-scale data base analysis also found that A. altissima was not a common species in the Mid-Appalachian mountain region, however, this species, if present, was more likely to dominate the plots than was R. pseudoacacia.
Conclusions:

Ecosystem functions depend on the composition of communities, biodiversity of functional species, and the temporal and spatial distribution of organisms. Exotic invasive species which arrive and establish in a community could change the performance of local native individuals, and their interactions would have fundamental impacts on community structure, succession, ecosystem functioning, and hence impact human well-being (Parker et al. 1999, Levine et al. 2003, Hejda et al. 2009, Vilà et al. 2011). The two subject species of this multiple-scale study, *A. altissima* and *R. pseudoacacia*, constitute a good example of how two similar early-successional pioneer species, which have different functional characteristics, interact, and influence plant community, succession, and ecosystem process. Experimental, observational, and analytic approaches were undertaken to expand our understandings of these two species and their interaction from individual scale to regional scale, and from early seedling to mature tree.

The negative impacts of the exotic invasive species *A. altissima* on native coexisting tree *R. pseudoacacia*, the neighboring plant community, and forest regeneration were confirmed. However, in contradiction to the negative opinion in the literature of this invader, *A. altissima* was not always harmful, and not always the winner in competition with native species. First, seedlings of *A. altissima* did not thrive in low nutrient environments, meanwhile, native nitrogen fixing *R. pseudoacacia* seedlings grew well under the same conditions. Second, until the second year after germination, *A. altissima* had interspecific competition with *R. pseudoacacia*; and the intensity of this interspecific competition did not increase significantly as the number of *A. altissima* saplings increased. Although an allelopathic effect of *A. altissima* may have inhibited nodulation of *R. pseudoacacia* in the seedling experiment, *A. altissima* grew much more slowly
than *R. pseudoacacia* through the sapling stage in the field. Third, at the regional level, when the plots were not only limited to certain early-successional woodlots, there was no significant negative relationship between the presence of *A. altissima* and the diversity of other trees. The native nitrogen-fixing *R. pseudoacacia* could have negative impacts on understory community diversity when compared with other dominant native species. As an invasive species, the negative influences of *A. altissima* were not universal, and highly depend on the choice of study site, study scale and other confounding conditions.

Our study supported the new weapon hypothesis, biotic resistance theory and rapid evolution theory, which explain the success of invasive species. *Ailanthus altissima* is not a new invasive species to U.S. ecosystems, and it has brought changes to the invaded forest. At the community level, we did observe the negative impact of *A. altissima* on tree regeneration and diversity, and its negative effect often overwhelmed the positive influence of *R. pseudoacacia* when they co-existed. These findings suggest that an early management of the invasive species *A. altissima* could most effectively reduce its negative impact on native species performance and community diversity. From the results of our studies, we believe that *A. altissima* could weaken or eliminate the positive influences *R. pseudoacacia* has on succession in the early-successional forests of Mid-Appalachian Mountains.
Literature cited:


