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RESEARCH ARTICLE

Interactions between seedlings of the invasive tree *Ailanthus altissima* and the native tree *Robinia pseudoacacia* under low nutrient conditions

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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 between seedlings of *A. altissima* and *R. pseudoacacia* at high and low soil nutrition states. Also, seeds of *A. altissima* from its native (China) and invasive ranges (USA) were used to compare the effect of the different seed sources on the interaction with *R. pseudoacacia*. *Robinia pseudoacacia* was the better competitor. The presence of *A. altissima* significantly inhibited nodulation of *R. pseudoacacia* roots. In summary, in low nutrient early succession sites, seedlings of *R. pseudoacacia* and *A. altissima* will coexist and *R. pseudoacacia* will be the better competitor. However, *A. altissima* can increase its competitive ability by inhibiting nitrogen fixation by *R. pseudoacacia*. Differences between the competitive ability of the Chinese and US seed lots of *A. altissima* did not support the rapid evolution theory for invasive species success.

Keywords: Belowground competition; competition for nutrients; facilitation; invasive species; nodulation; replacement series

1. Introduction

Many mechanisms can contribute to the impact of invasive species on native flora, but competition is often central (Wyckoff & Webb 1996; Gentle & Duggin 1997; Levine et al. 2003; Besaw et al. 2011; Vilà et al. 2011; Dostál et al. 2013; Ricciardi et al. 2013). Moreover, invasive and native species that have similar habitat preferences are most likely to interact during early succession because of their similar likelihood to germinate early following disturbance. Early-successional, native species may compete with the invader for the same available resources, facilitate the invader by creating a favorable environment, both compete and facilitate an invader under different environmental conditions, or neither compete with or facilitate an invader. Invasive species are a focus of research because they may have negative impacts on ecosystem processes compared with native species (Barney et al. 2013). The interaction between exotic invasive species and early succession fast-growing native species may regulate the success of the invader and such interactions first occur at the seedling level. Therefore, understanding the competitive interaction between exotic invasive and native species seedlings that have similar functional traits, yet influence communities and ecosystems differently, is an important aspect of invasive ecology research.

The overall research program reported here concerns the mechanisms of interaction between one important exotic invasive species *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae) and a functionally 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 and growth, which can reduce plant diversity and forest succession after disturbance (Mergen 1959; Heisey 1990; Heisey 1996; Castro-Díez et al. 2008; Gómez-Aparicio & Canham 2008). *Robinia pseudoacacia* is one of the most important nitrogen-fixing trees in the eastern forests of the USA, which facilitates forest succession (Boring & Swank 1984b). Also, *R. pseudoacacia* is used for restoring vegetation on post-disturbance sites due to its ability to increase soil nitrogen content (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 USA. Another possibility is that *R. pseudoacacia* facilitates the growth

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of *A. altissima* by increasing soil nitrogen levels around the *A. altissima* roots through leakage from nearby nitrogen fixation. Therefore, understanding the result of competition or facilitation between these two species is important for understanding the relationship between invasive–native species interactions and its influence on 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 & Chesson 2002), escaping from natural enemies (Keane & Crawley 2002), having novel weapons (Callaway & Ridenour 2004) and inducing invasive meltdown (Simberloff & 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. Also, rapid evolution of highly adaptive traits could have occurred early in the introduction of *A. altissima* to the USA (Prentis et al. 2008). Therefore, it is logical to posit that the invasive species populations in the invaded ecosystem may be selected to be a more competitive group than the populations of invasive species in their native ecosystem.

The overall goal of this study was to investigate competition and facilitation between *R. pseudoacacia* and *A. altissima* seedlings in relatively low nutrient environments, and to assess if a difference between the competitive ability of *A. altissima* genotypes growing in the USA vs. those from its native China could be determined. We focused on both belowground and aboveground processes in a greenhouse setting, which facilitated harvesting the entire root system, counting and collecting nodules from *R. pseudoacacia* seedlings, and manipulating nutrient conditions.

A full-factorial substitutive design greenhouse experiment was used to address the following questions: (1) Does *A. altissima* produce the most belowground and aboveground biomass at the seedling stage? (2) Does *A. altissima* whole plant biomass and allocation to shoots increase significantly more from low to high nutrient levels than that for *R. pseudoacacia* seedlings? (3) Does the presence of *A. altissima* change the whole plant biomass and allocation patterns of *R. pseudoacacia*? (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 (USA) greater than that of *A. altissima* seedlings from its native region (China)? Answers to these questions will provide substantial insight to the possible mechanisms of interactions between these species in the early-successional forest.

2. Materials and methods

2.1. *Seed germination*

The *A. altissima* seeds from the invaded ecosystems were collected from Mineral, Hampshire, Hardy, and Grant Counties of West Virginia, USA 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 24 h 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, USA) were collected from multiple trees in Kentucky, USA. 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 12 h. 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 starting room used for establishing seedlings ranged from 22°C to 24°C during the experiment, light intensity (photosynthetic photon flux density) peaked at 1200–1500 μmol m^{-2} s^{-1} and relative humidity ranged from 75% to 80% on a daily basis.

2.2. *Experimental design*

After 3 weeks growing in flats in the greenhouse, six seedlings were transplanted into each 8 L pot (approximately 25 cm in diameter, 35 cm in height), which contained washed masonry sand mixed with 10 g of soil collected from under planted *R. pseudoacacia* stands at Blandy Experimental Farm, Boyce, Virginia, USA to stimulate nodulation. There were 2 levels of nutrient (defined as 'nutrient' treatments hereafter), 5 different species combinations (defined as 'proportion' 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 six plants per pot. Mixtures consisted of the following species proportions: (1) monoculture of *A. altissima* (6A), (2) monoculture of *R. pseudoacacia* (6R), (3) two *A. altissima* and four *R. pseudoacacia* (2A4R), (4) three *A. altissima* and three *R. pseudoacacia* (3A3R) and (5) four *A. altissima* and two *R. pseudoacacia* (4A2R). Randomly selected similar sized seedlings from each species were planted into pots based on our experimental design. Air temperature in the BIOL/VBI plant growth bay used for this study ranged from 22°C to 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% to 80% on a daily basis.

After transplantation, nutrient treatments were randomly assigned to each pot. Either 0.936 or 3.744 g (i.e. 39.5 and 158 kg 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 (158 kg N ha^{-1} yr^{-1}). Fertilizer was reapplied at the same rates after 4 months because the slow-release fertilizer has a predicted lifespan of 5–6 months at 21°C. 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 US *A. altissima* seedlings. Hence, pots with the two different seed sources were harvested at different times, to keep the same number of growing days. Day length varied between 13 and 14.74 h during the entire length of both experimental growth periods. The plants were harvested after about 5 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, from ground level to top of the main axis, and basal diameter of each plant were measured. Little to no branching occurred during the growth period. Stems and leaves were separated and oven-dried at 60°C 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 all sand was washed off the roots. The extra roots, which were broken during the separation process and left in sand were extracted and the sand was carefully washed off the root biomass 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 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).

2.3. *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 six original plants, we assigned a proportion of the extra root biomass to each of the six 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 belowground biomass were found to be autocorrelated ($r = .76$), and they were both highly correlated with total biomass ($r = .95$ and .92). Therefore, three-way analysis of variance (ANOVA) was only presented for total biomass, which reflected plant growth over the experimental period, 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 nutrient level, species proportion, seed source, 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 honest significant difference.

Regressions were used to test the relationships between height or basal diameter and aboveground or total biomass at the end of the experimental growth period. Differences in the regression of total biomass vs. height for *R. pseudoacacia* competing with *A. altissima* seedlings from the two seed sources was evaluated with analysis of covariance.

To compare our results with a prior study (Call & 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 & McCarthy 2001). These indices were calculated separately based on total biomass of each species. The equations of calculating RY for the two species are

$$\mathrm{RY_A} = \frac{Y_{\mathrm{AR}}}{p_{\mathrm{A}}\ Y_{\mathrm{A}}},$$

$$\mathrm{RY_R} = \frac{Y_{\mathrm{RA}}}{p_{\mathrm{R}}\ Y_{\mathrm{R}}},$$

where Y_A = yield (dry biomass) of *A. altissima* in monoculture, Y_R = yield of *R. pseudoacacia* in monoculture,

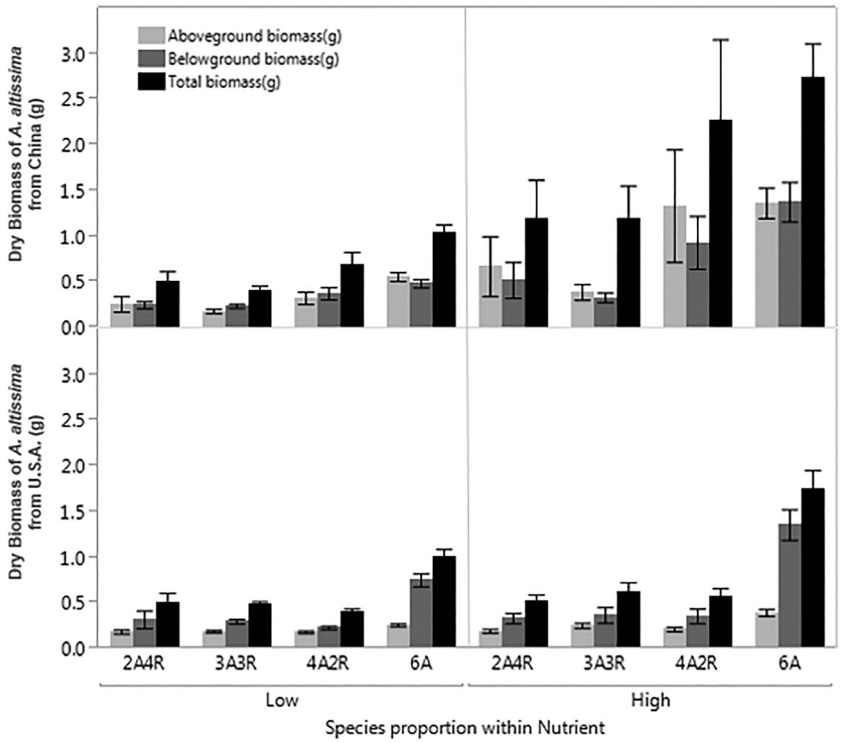

Figure 1. Aboveground, belowground and total biomass of *Ailanthus altissima* in a full-factorial substitutive design experiment with *Robinia pseudoacacia* at constant density (six plants per 8 L pot). The solid bars and error bars represent the average biomass per *A. altissima* plant and the standard 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, US seed lot. Two nutrient levels (low and high) were used for the experiment, along with the four different proportions of *A. altissima* and *R. pseudoacacia.* (e.g. 2A4R = two *A. altissima* and four *R. pseudoacacia* seedlings; 3A3R = three *A. altissima* and three *R. pseudoacacia* seedlings).

Y_{AR} = yield of *A. altissima* in the presence of *R. pseudoacacia*, Y_{RA} = yield of *R. pseudoacacia* in the presence of *A. altissima*, p_A = proportion of *A. altissima* sown in one pot, p_R = proportion of *R. pseudoacacia* sown in same pot, 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 three 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 (1–2 mm), which are the most frequent size of nodules. Moreover, the nitrogen fixation activity does not 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.

3. Results

In general, *R. pseudoacacia* seedlings had a significantly higher total biomass ($p < .0001$) than *A. altissima* seedlings. The average total dry biomass per plant for *R. pseudoacacia* in all the treatments combined was almost seven times the total dry biomass per plant of *A. altissima* (Table 1). *Ailanthus altissima* seedlings produced more root biomass compared with shoot biomass because, in most treatment groups, root/shoot ratio >1 (Table 1 and Figure 1). In contrast, seedlings of *R. pseudoacacia* produced similar amounts of

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.

	A. altissima				*R. pseudoacacia*			
Total biomass (g)	1.15 ± 0.09 g a				7.97 ± 0.36 g 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
Seed source	Chinese *A. altissima*		US *A. altissima*		Chinese *A. altissima*		US *A. altissima*	
Total biomass (g)	1.429 ± 0.166 a		0.862 ± 0.057 b		7.816 ± 0.513 a		8.116 ± 0.504 a	
Leaf biomass (g)	0.503 ± 0.066 a		0.151 ± 0.008b		3.343 ± 0.191 a		1.413 ± 0.084 b	
Root/shoot	1.167 ± 0.050 a		2.238 ± 0.091 b		0.477 ± 0.021 a		1.564 ± 0.033 b	

Notes: Seed sources are either a Chinese or a US *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 = six *A. altissima* seedlings and 4A2R = four *A. altissima* and two *R. pseudoacacia* seedlings in the pot). Different letters following the means and standard errors indicate significant difference at the α = .05 level.

aboveground and belowground biomass because their root/shoot ratios were approximately equal to 1.

The regressions of basal area with biomass (dependent variable) had a very low R^2 and are not presented for either species because they were insignificant. When grown with *A. altissima* seedlings from the Chinese seed lot the regression of height with aboveground ($R^2 = .37$) and total ($R^2 = .35$) for *R. pseudoacacia* were both significant at the $p < .0001$ level. Similarly, when grown with *A. altissima* seedlings from the US seed lot the regression of height with aboveground ($R^2 = .77$) and total ($R^2 = .76$) for *R. pseudoacacia* were both significant at the $p < .0001$ level. Although the slopes were steeper for the regressions calculated for *R. pseudoacacia* when growing with *A. altissima* seedlings from the Chinese seed source, those differences were not significant.

3.1. *Effect of nutrient level*

The effect of nutrient level on average total biomass per plant at high nutrient level was significantly greater than that at low nutrient level for both species (Tables 1–3). Total biomass of *A. altissima* increased by 1.23 times from low to high nutrients while that of *R. pseudoacacia* increased by 1.141 times. Leaf biomass increased significantly with increase of nutrient level for both species (Tables 1–3) and *A. altissima* leaf biomass increased by 1.38 times while that of *R. pseudoacacia* only increased by 0.92 times. Root/shoot ratio did not decrease significantly for either species from low to high nutrients (Tables 1–3). The only significant interaction for *A. altissima* between the nutrient effect and other effects was the interaction of nutrient with source for leaf biomass (Table 2). This interaction means that the effect of nutrient on leaf biomass was different for seedlings grown from the two seed sources. There was a significant interaction between the nutrient and proportion effects on total biomass and leaf biomass for *R. pseudoacacia* (Table 3). This interaction indicates that the increase in total biomass and leaf biomass induced by high nutrition varied among mixtures with *A. altissima*. Also, there was a significant interaction between nutrient level and *A. altissima* seed source on leaf biomass (Table 3). This interaction means that the increase in leaf biomass in high nutrient conditions is different when different sources of *A. altissima* seedlings are in the experiment. Although there was no significant effect of nutrients on root/shoot ratio, there was a significant interaction between nutrient and *A. altissima* seed source for root/shoot ratio (Section 3.3).

3.2. *Effect of proportional mixtures*

Ailanthus altissima seedlings in monoculture had a significantly greater total biomass than those grown in mixed pots (Table 1). However, the *R. pseudoacacia* seedlings in monoculture had a significantly lower average individual biomass than those in mixed culture. The effect of proportion was significant for both species (Tables 2 and 3). 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. Although, the effect of proportion was significant (Table 2), only the *A. altissima* seedlings in monoculture had a significantly greater leaf biomass than those grown in 3A3R treatment (Table 1). In contrast, the total average biomass per plant of *R. pseudoacacia* increased significantly as the proportion of *A. altissima* increased in the pot (Table 1 and Figure 1). In the high nutrient treatment, average biomass of *R. pseudoacacia* seedlings decreased more rapidly as the proportion of *A. altissima* decreased, which resulted in a significant interaction between nutrient and proportion (Table 3). The results for leaf biomass were similar among proportions to the results for total biomass for both species (Table 1). The monoculture group of *A. altissima* had significantly higher root/shoot ratio than the 4A2R and 3A3R groups (Table 1 and Figure 1), which resulted in a significant effect of proportion (Table 2). There was no significant effect of proportion on *R. pseudoacacia* root/shoot ratio (Tables 1 and 3).

3.3. *Effect of A. altissima seed source*

The average total biomass and leaf biomass of Chinese *A. altissima* seedlings was significantly greater than that of seedlings from the US *A. altissima* seed lot (Tables 1 and 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 US seed lot, indicating a faster growth rate of Chinese *A. altissima* (personal observation). There was no significant difference in total biomass of *R. pseudoacacia* seedlings when grown with either of the two seed sources of *A. altissima* (Tables 1 and 3). However, *R. pseudoacacia* seedlings produced significantly less leaf biomass when mixed with the *A. altissima* seedlings from the US seed lot (Table 1), which resulted in a significant interaction between source and proportion (Table 3). The significant interaction between seed source and nutrient on leaf biomass 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 US *A. altissima*.

The root/shoot ratio of *A. altissima* seedlings from the US seed lot was significantly higher than that of the seedlings from the Chinese seed lot (Tables 1 and 2). Moreover, there was a significant interaction with the proportion of *R. pseudoacacia* in the mixture (Tables 1 and 2). The interaction was significant because seedlings of *A. altissima* from the US seed lot had the highest root/shoot ratio in monoculture compared with those grown in mixtures with *R. pseudoacacia* (Figure 1). The root/shoot ratio of *R. pseudoacacia* seedlings was significantly greater when *R. pseudoacacia* seedlings were grown with *A. altissima* seedlings from the US seed lot than when grown with *A. altissima* seedlings from the

Table 2. Results from three-way ANOVAs of seedling growth metrics for *Ailanthus altissima* growing in a full-factorial substitutive design competition experiment with *Robinia pseudoacacia* in a greenhouse environment.

Effect	DF	Type III sums of squares	*F* ratio	*p*-Value
Total biomass (g)				
Nutrient	1	34.412	9.35	**.0024**
Proportion	3	80.541	7.30	**<.0001**
Source	1	16.446	4.47	**.0351**
Nutrient * proportion	3	15.092	1.37	.2523
Nutrient * source	1	12.339	3.35	.0678
Proportion * source	3	6.782	0.61	.6060
Nutrient * proportion * source	3	2.721	0.25	.8638
Leaf biomass (g)				
Nutrient	1	3.208	5.89	**.0157**
Proportion	3	5.615	3.44	**.0170**
Source	1	5.310	9.75	**.0019**
Nutrient * proportion	3	0.857	0.52	.6657
Nutrient * source	1	2.124	3.90	**.0490**
Proportion * source	3	3.348	2.05	.1065
Nutrient * proportion * source	3	1.084	0.66	.5751
Root/shoot (g)				
Nutrient	1	0.070	0.07	.7857
Proportion	3	35.333	12.39	**<.0001**
Source	1	36.649	38.54	**<.0001**
Nutrient * proportion	3	3.504	1.23	.2991
Nutrient * source	1	0.394	0.41	.5201
Proportion * source	3	107.718	37.76	**<.0001**
Nutrient * proportion * source	3	2.307	0.81	.4895

Notes: Effects are *A. altissima* seed source (Chinese and USA), 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 3. Results from three-way ANOVAs of seedling growth metrics for *Robinia pseudoacacia* growing in a full-factorial substitutive design competition experiment with *Ailanthus altissima* in a greenhouse environment.

Effect	DF	Sums of squares	*F* ratio	*p*-Value
Total biomass (g)				
Nutrient	1	3869.860	91.06	**<.0001**
Proportion	3	2495.848	19.58	**<.0001**
Source	1	2.434	0.06	.8110
Nutrient * proportion	3	679.104	5.33	**.0013**
Nutrient * source	1	26.178	0.62	.4330
Source * proportion	3	50.526	0.40	.7557
Nutrient * proportion * source	3	23.932	0.19	.9048
Leaf biomass (g)				
Nutrient	1	261.346	67.29	**<.0001**
Proportion	3	190.456	16.35	**<.0001**
Source	1	407.519	104.92	**<.0001**
Nutrient * proportion	3	37.011	3.18	**.0241**
Nutrient * source	1	66.565	17.14	**<.0001**
Source * proportion	3	45.431	3.90	**.0091**
Nutrient * proportion * source	3	6.801	0.58	.6260
Root/shoot (g)				
Nutrient	1	0.060	0.35	.5568
Proportion	3	1.138	2.20	.0876
Source	1	83.072	481.72	**<.0001**
Nutrient * proportion	3	0.167	0.32	.8095
Nutrient * Source	1	1.265	7.34	**.0071**
Source * proportion	3	0.411	0.79	.4980
Nutrient * proportion * source	3	0.784	1.52	.2100

Notes: Effects are *A. altissima* seed source (Chinese and USA), 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.

Chinese seed lot (Tables 1 and 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 US seed lot (Figure 1 and Table 3).

3.4. *Intraspecific and interspecific competition*

RY results for *A. altissima* (RY_A) were mostly less than 1 (11 out of 12 values), indicating interspecific competitive interference from neighboring *R. pseudoacacia* seedlings was stronger than the intraspecific competition (Table 4). RY_A was significantly different between nutrient levels (Table 5), and *A. altissima* seedlings in the lower nutrient treatment had lower RY_A, indicating relatively stronger competition from *R. pseudoacacia* than from itself (Table 4). Also, the RY_A 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 US seed lot than those from the Chinese seed lot (Tables 4 and 5). There was a significant two-way interaction between seed source and nutrient (Table 5), because the increase in RY_A from the low nutrient level to the high nutrient level for Chinese *A. altissima* was greater than that of US *A. altissima* (Figure 2).

RY results for *R. pseudoacacia* seedlings (RY_R) were mostly larger than 1 (9 out of 12 values), indicating more intraspecific competition than the negative effect from neighboring *A. altissima* seedlings (Table 4). Both nutrient and proportion treatments had a strongly significant effect, but not the seed source (Table 5). The high nutrient level group had a larger RY_R than the low nutrient level (Table 4). The greater the number of *A. altissima* seedlings in the pot, the larger was the RY_R and the stronger was intraspecific competition (Tables 4 and 5).

3.5. *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 ($p = .124$). 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 ($R^2 = .42$, $p < .0001$). There was no significant difference for this regression relationship between the two nutrient treatments ($p = .19$).

The average total numbers of nodules per plant in the high and low nutrient treatments were 81.6 ± 3.6 and 50.6 ± 3.6, respectively, and these values were significantly different ($p = .03$). *Robinia pseudoacacia* produced significantly more nodules per unit root mass at the low nutrient level ($34.36 \pm 2.01\ g^{-1}$) than that at the high nutrient level ($26.35 \pm 2.04\ g^{-1}$, $p = .05$). When grown with *A. altissima*, the number of nodules per root mass of *R. pseudoacacia* seedling was significantly lower ($27.12 \pm 1.80\ g^{-1}$) than that of *R. pseudoacacia* seedlings in monoculture ($35.31 \pm 2.36\ g^{-1}$, $p = .005$). *Robinia pseudoacacia* seedlings grown with *A. altissima* seedlings from the US seed lot had significantly fewer nodules per root weight ($18.38 \pm 0.87\ g^{-1}$), compared with *R. pseudoacacia* seedlings grown with *A. altissima* seedlings from the Chinese seed lot ($42.51 \pm 2.53\ g^{-1}$, $p < .0001$). 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 US seed lot had larger root systems.

4. Discussion

4.1. *Ailanthus altissima did not influence growth patterns of R. pseudoacacia*

In response to our first question, we found that *A. altissima* seedlings did not inhibit the growth and allocation patterns of *R. pseudoacacia* seedlings. *Robinia pseudoacacia* grew much faster and produced more above and belowground biomass than *A. altissima*. As a result, intraspecific competition was more important for *R. pseudoacacia* growth than interspecific competition with *A. altissima*. Thus, in this study, *R. pseudoacacia* was the clear winner in all the environmental conditions used. This result contradicts previous research (Call & 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. In contrast, *R. pseudoacacia* seedlings in the experiment of Call and Nilsen (2005) did not form nodules because nutrients were so high. The presence of nodules in this study suggests that these results are more applicable to early succession sites in the Mid-Appalachian regions.

4.2. *Soil nutrition affected A. altissima growth more than R. pseudoacacia*

Biomass indices for both species were very responsive to the difference in nutrient levels used in this study. Moreover, the relative effects of increasing nutrient availability caused a greater increase in biomass for *A. altissima*.

Table 4. RY for *Ailanthus altissima* (RY_A) and *Robinia pseudoacacia* (RY_R) in a full-factorial substitutive design experiment at a fixed density of six seedlings per 8 L pot.

	RY_A (CH-AA)		RY_A (US-AA)		RY_R (CH-AA)		RY_R (US-AA)	
Treatment	L	H	L	H	L	H	L	H
4A2R	0.425	1.470	0.254	0.346	1.366	3.251	1.799	3.120
3A3R	0.252	0.734	0.295	0.377	1.047	2.370	0.980	2.685
2A4R	0.308	0.727	0.306	0.320	0.776	2.018	0.895	1.782

Notes: 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 US seed lot; treatment refers to the ratio of the number of seedlings of each species in the pot (e.g. 4A2R = four *A. altissima* and two *R. pseudoacacia* seedlings in the pot).

Table 5. Results of a three-way ANOVAs on RY for *Ailanthus altissima* (RY_A) and *Robinia pseudoacacia* (RY_R) based on the final, total plant dry weight in a full-factorial substitutive design experiment at a fixed density of six seedlings per 8 L pot.

Effect	DF	Sums of squares	*F* ratio	*p*-Value
RY Ailanthus (RY_A)				
Nutrient	1	2.947	6.35	**.0137**
Proportion	2	0.882	0.95	.3908
Source	1	2.633	5.68	**.0195**
Nutrient * proportion	2	0.531	0.57	.5665
Nutrient * source	1	2.000	4.31	**.0410**
Source * proportion	2	1.106	1.19	.3087
Nutrient * proportion * source	2	0.382	0.41	.6642
RY Robinia (RY_R)				
Nutrient	1	46.120	157.27	**<.0001**
Proportion	2	16.668	28.42	**<.0001**
Seed source	1	0.172	0.59	.4465
Nutrient * source	1	0.136	0.46	.4982
Nutrient * proportion	2	1.437	2.45	.0926
Source * proportion	2	0.260	0.44	.6440
Nutrient * proportion * source	2	0.853	1.45	.2395

Notes: Effects are *A. altissima* seed source (Chinese and USA), 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.

However, the significant interactions between nutrient and proportion suggest that the interaction of species is altered in response to nutrient levels. Our second question related to 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 RY_A in low nutrient treatment). In addition, RY_R was lower under the lowest nutrient condition, which indicated weaker intraspecific competition. Thus, when nutrients are relatively scarce, *R. pseudoacacia* is an effective competitor with *A. altissima* and also competes

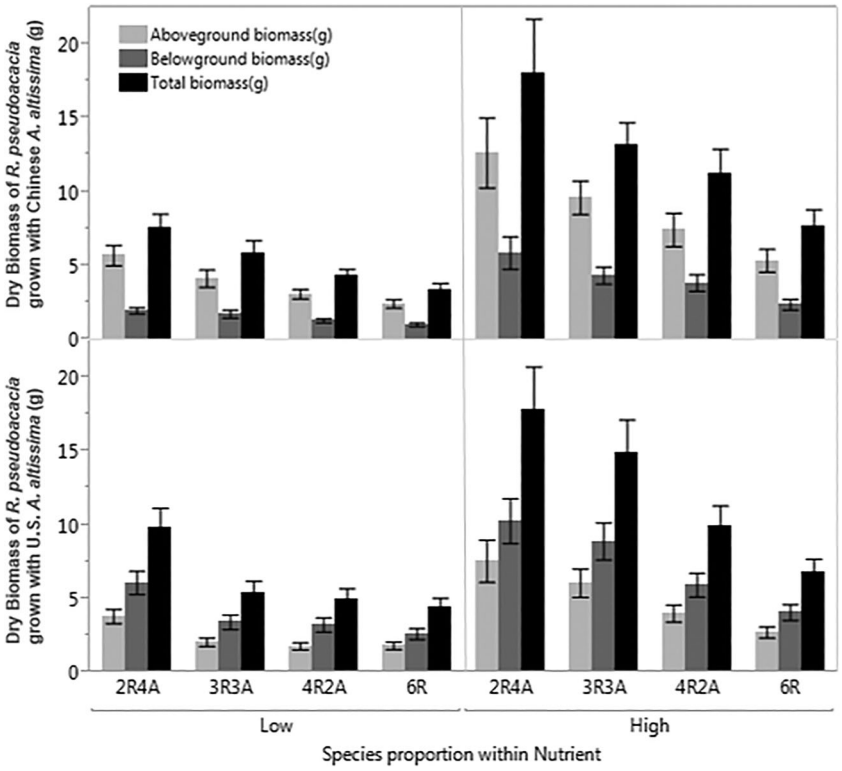

Figure 2. Aboveground, belowground and total biomass of *Robinia pseudoacacia* seedlings in a full-factorial substitutive design experiment with *Ailanthus altissima* at a constant density (six plants per 8 L pot). The solid bars and error bars represent the average biomass per *R. pseudoacacia* plant 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, US seed lot. Two nutrient levels were used for the experiment, along with four different proportions of *R. pseudoacacia* and *A. altissima* seedlings. (e.g. 2R4A = two *R. pseudoacacia* and four *A. altissima* seedlings; 3R3A = three *R. pseudoacacia* and three *A. altissima* seedlings).

less with itself. In fact, the ability to tolerate a low nitrogen environment is essential to the strong competitive ability of *R. pseudoacacia* (Boring & Swank 1984a; Gioria & 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 USA (Castro-Díez et al. 2008).

The comparatively small size of *A. altissima* seedlings in this study suggested that the nutrient levels used may have been too low for *A. altissima* to thrive in a sandy environment. For both species, the two nutrient levels 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. Nutrients were limiting because total, leaf and root biomass of both species increased from low nutrient level to high nutrient level. Therefore, our conclusion that *R. pseudoacacia* has a competitive advantage over *A. altissima* should be limited to relatively low and limiting soil nutrient conditions such as those used in this study. The results of Call and Nilsen (2005) indicated that under unlimited nutrients, *A. altissima* is a better competitor than *R. pseudoacacia*.

4.3. *A. altissima did change the growth patterns of R. pseudoacacia*

Contrary to our prediction, the presence of *A. altissima* seedlings was associated with increased biomass of *R. pseudoacacia* seedlings rather than the expected negative competitive effect. However, we think that the 'facilitation' observed in this experiment from *A. altissima* on *R. pseudoacacia* ($RY_R > 1$) was mainly due to the comparatively small size of *A. altissima* under these experimental conditions, which minimized its competitive effectiveness. The *R. pseudoacacia* seedlings had more resources available for growth when there were more *A. altissima* (and fewer *R. pseudoacacia*) in the pot because the *R. pseudoacacia* seedlings were released from intraspecific competition. *Ailanthus altissima* is known to have a substantial tap-root, therefore the constraint of the pot size may have differentially affected *A. altissima* compared with *R. pseudoacacia*. However, in a previous study (Call & Nilsen 2005) *A. altissima* seedlings produced much more root biomass in pots of similar dimension to this study.

Although *A. altissima* did not alter biomass and allocation to roots of *R. pseudoacacia*, the difference in *A. altissima* root biomass between high and low nutrient treatments became smaller as the proportion of *R. pseudoacacia* in the pot increased. 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, because RY_A did not increase as the proportion of *R. pseudoacacia* increased, this explanation seems unlikely. Therefore, this result may simply relate to the dominance of *R. pseudoacacia* in the belowground biomass under the higher nutrient condition, which led to stronger competition with *A. altissima* as the proportion of *R. pseudoacacia* increased.

4.4. *The presence of A. altissima inhibited nodulation of R. pseudoacacia*

The presence of *A. altissima* seedlings in the pot 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 US 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 is well known to have 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 & Ridenour 2004). Allelochemicals from some invasive species have been found to have negative impacts on microbial mutualists of native species and on nodulation of nitrogen-fixing plants (Weston & Putman 1985, 1986; Batish et al. 2007; Callaway et al. 2008; Wurst & Beersum 2009), including *A. altissima* (Lincoln 2012). In another bioassay study (Binninger and Bao, unpublished result), we found that a water extraction of *A. altissima* leaves significantly decreased both the germination rate and radicle growth of *R. pseudoacacia* seeds. These results suggest that the allelochemicals of *A. altissima* could negatively influence *R. pseudoacacia* growth and may be the reason why *A. altissima* inhibits nodulation of *R. pseudoacacia*. A direct measure of nitrogen fixation by gas chromatographic or stable isotope techniques would strengthen the proof that *A. altissima* inhibits nitrogen fixation by *R. pseudoacacia*.

4.5. *Chinese A. altissima were more competitive than US A. altissima*

Previous research found *A. altissima* had the competitive advantage over *R. pseudoacacia* primarily because of a dominant root biomass (Call & Nilsen 2005). Although *A. altissima* was not the winner of competition in this study, it did produce more roots when competing with *R. pseudoacacia*. Therefore, competition belowground is important for *A. altissima* competitive success.

The significantly higher root/shoot ratio found for the *A. altissima* seedlings from the US seed lot as compared to those from the Chinese seed lot suggests that the high root/shoot ratio might have been part of the mechanism for successful invasion for *A. altissima* in the USA. Some researchers 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 & Osborne 2014). A variety of invasive species have higher resource-use efficiency than native species, especially in low-resource environments (Funk & 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 a relatively stable environment with a reliable supply of nutrients, similar to the experimental design used in our study,

A. altissima did not show a tendency to be more competitive (Mou et al. 2013).

The two seed sources of *A. altissima* did not alter the allometric relationships between height and biomass of *R. pseudoacacia*. Yet, the two seed sources of *A. altissima* had different biomass accumulation, different responses to nutrient level and different competitive ability in their interaction with *R. pseudoacacia.* However, the only advantage of the Chinese *A. altissima* seed was the positive effect of nutrient increase on biomass production, which was a stronger effect than that of the US seedlings. This suggests that the *A. altissima* genotypes in the USA 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 US populations to low nutrition in US disturbed habitats as has occurred for other introductions (Barney et al. 2009). Post-introduction adaptation is possible because *A. altissima* was introduced to the USA more than 300 years ago and significant adaptation could occur over this duration (Prentis et al. 2008). However, the likelihood of post-introduction adaptive evolution is species specific (Harris et al. 2012). Also, it is possible that the introduced population of *A. altissima*, 300 years ago, was selected to grow well in poor nutrient conditions. As a result, the US populations would have greater nutrient use efficiency than the source population in China. Differences among seedling responses to nutrient limitation have been shown to influence competition between seedlings of other tree species (Yi et al. 2015). However, we did not find that US *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 US *A. altissima*, and no difference in RY_R, based on total biomass production, between the two seed sources. The resource allocation (root/shoot ratio and leaf biomass production) of *R. pseudoacacia* was changed significantly when grown with the 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 USA and China, and therefore our conclusions only represent the differences between these two specific seed lots. We are not aware of any studies in the literature on genetic differences between Chinese *A. altissima* populations and those in the U.S. related to nutrient use efficiency or competitive effectiveness. Thus, more studies are needed to verify these observed differences in competitive ability of *A. altissima* genotypes in the USA compared to those in China.

4.6. *Conclusion*

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. Nitrogen fixation by *R. pseudoacacia* seedlings did not facilitate the growth of *A. altissima* seedlings. However, *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. During early stages of succession, when *A. altissima* seedlings come into close proximity with *R. pseudoacacia* seedlings, *R. pseudoacacia* will have the upper hand unless the disturbed site is highly nutritious. However, if *A. altissima* seedlings survive they will inhibit nodulation of the *R. pseudoacacia* seedlings and perhaps change the trajectory of succession at the site. Longer-term research, in a more natural setting, through the sapling stage is needed to further understand the interactions between these species during forest development.

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Supplemental data

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