

Non-native grass invasion suppresses forest succession

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Abstract Multiple factors can affect the process of forest succession including seed dispersal patterns, seedling survival, and environmental heterogeneity. A relatively understudied factor affecting the process of succession is invasions by non-native plants. Invasions can increase competition, alter abiotic conditions, and provide refuge for consumers. Functional traits of trees such as seed size and life history stage may mediate the effects of invasions on succession. We tested the effects of the forest invader *Microstegium vimineum* on planted and naturally regenerating trees in a multi-year field experiment. We established plots containing nine species of small- and large-seeded tree species planted as seeds or saplings, and experimentally added *Microstegium* to half of all plots. Over 3 years, *Microstegium* invasion had an overall negative effect on small-seeded species driven primarily by the effect on sweetgum, the most abundant small-seeded species, but did not affect large-seeded species such as hickory and oak species, which have more stored seed resources. Natural regeneration was over 400% greater in control than invaded plots for box elder, red maple, and spicebush, and box elder seedlings were 58% smaller in invaded plots. In contrast to the effects on tree seedlings, invasion did not affect tree sapling survival or growth. *Microstegium* may be directly reducing tree regeneration through competition. Invaded plots had greater overall herbaceous biomass in 2006 and 2008 and reduced light availability late in the growing season. Indirect effects may also be important. Invaded plots

had 120% more thatch biomass, a physical barrier to seedling establishment, and significantly greater vole damage to tree saplings during 2006 and 2007. Our results show that two tree functional traits, seed size and life history stage, determined the effects of *Microstegium* on tree regeneration. Suppression of tree regeneration by *Microstegium* invasions may slow the rate of forest succession and alter tree species composition.

Keywords Life history stage · Forest composition · Tree regeneration · *Microstegium vimineum* · Japanese stiltgrass

Introduction

In many forested ecosystems, agricultural abandonment, timber harvest, and natural and anthropogenic disturbance have resulted in mosaics of old fields and forests of varying successional ages. Successional fields and young forests are a major component of the landscape in eastern North America where they provide important wildlife habitat and harbor significant biological diversity (Litvaitis 2001; Wilsey and Potvin 2000). The composition of regenerating tree species in early successional forests is affected by plant–plant competition for resources, environmental heterogeneity, seed dispersal and predation, and seedling survival (Berkowitz et al. 1995; Gill and Marks 1991; Myster 1993; Ostfeld et al. 1997; Pacala et al. 1996). A potentially major, but understudied, factor regulating the process of forest succession is invasion by non-native plants (Gorchov and Trisel 2003; Orr et al. 2005; Rudgers et al. 2007). The effects of invasive plants on tree regeneration may depend on the functional traits of species such as seed size or life history stage of trees when the invasion occurs (Lahoreau et al. 2006; Leishman et al. 2000).

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Both herbaceous and woody plant invaders have extensively colonized eastern forests where they suppress native herbaceous species (Flory and Clay 2010; Hochstedler et al. 2007; Schulz and Thelen 2000), alter nutrient cycling processes (Ehrenfeld 2003), and inhibit tree regeneration and growth (Hartman and McCarthy 2007; Stinson et al. 2006). Observational studies suggest reduced tree recruitment in invaded sites (Oswalt et al. 2007), and experimental studies have revealed that removing some invaders increases tree colonization (Flory and Clay 2009; Gorchov and Trisel 2003). Plant invaders may suppress forest succession through direct mechanisms such as competition with regenerating trees, and indirect mechanisms such as altered herbivore behavior (Meiners 2007). For example, invasion by the Eurasian shrub *Lonicera maackii* (bush honeysuckle) is associated with reduced abundance of both tree seedlings and saplings (Collier et al. 2002; Hartman and McCarthy 2008). Reduced tree recruitment in *Lonicera*-invaded areas has been hypothesized to occur because of competition for resources such as light (Hartman and McCarthy 2008), but recent evidence suggests apparent competition via small mammal seed predation may also be important (Meiners 2007). A better understanding of the effects of invasions on forest succession, and the underlying mechanisms, will allow us to more accurately predict how forest composition will be altered by invasions and to develop more effective management solutions.

The effects of plant invasions on tree regeneration may be mediated by the functional traits of regenerating tree species. Trees exhibit a seed size/number trade-off where some species produce many small seeds but establish at lower rates. By contrast, large-seeded species produce fewer seeds but seeds have more stored resources and produce larger seedlings that are less susceptible to leaf litter, disturbance, herbivory, and drought (Baraloto et al. 2005; Leishman et al. 2000; Moles and Westoby 2004). Invasive plants may provide a physical barrier to establishment (Olson and Wallander 2002), or compete for resources such as light, nutrients, or moisture (Levine et al. 2003). Accordingly, we predict that small-seeded species will be more susceptible to the inhibitory effects of invaders than large-seeded species, resulting in a shift in tree species composition during succession towards large-seeded species. Differences in the effects of plant invasions on small- and large-seeded tree species have not been studied previously, but such differences could have important implications for determining the changing composition of eastern forests subject to plant invasions.

In addition to potential differences among small- and large-seeded tree species, the effects of invasion on forest succession may also depend on the life history stage of trees at the time of invasion. For example, invasions may differentially affect tree seeds, seedlings and saplings, and

mature trees. Tree seedling size is an important determinant of seedling success because of differences in competitive ability, predation risk, and disturbance tolerance between small and large seedlings (Armstrong and Westoby 1993; Harms and Dalling 1997; Lahoreau et al. 2006; Seiwa 2000). Larger seedlings or saplings have more developed root systems and may be tall enough to avoid the shading effects of invasive plants (Lahoreau et al. 2006). If a plant invader negatively affects trees establishing from seed but saplings are unaffected, the rate of succession may be slowed and forest composition may be restricted to species already established at the time of invasion.

We experimentally tested if invasion by the non-native grass *Microstegium vimineum* (Japanese stiltgrass) Trin. A. Camus reduced the success of tree species and inhibited forest succession based on tree seed size and life history stage. *Microstegium* is a widespread, aggressive invader that has colonized forests throughout much of the eastern US (USDA and NRCS 2005). It is a warm-season (C_4), annual species but is also highly shade tolerant (Horton and Neufeld 1998) unlike other C_4 grasses. It exhibits significant phenotypic plasticity, allowing it to colonize a wide range of soil moisture and light conditions (Claridge and Franklin 2002; Droste et al. 2010). Invasions are often first found locally in riparian areas and disturbed habitats such as along roads and trails, but *Microstegium* can also colonize deeply shaded forest interiors and old fields (Flory 2010). When *Microstegium* invades, it can reduce native species diversity and biomass, alter community composition, change soil nutrient cycling processes (Ehrenfeld et al. 2001; Flory and Clay 2010), and reduce the diversity and abundance of arthropods (Civitello et al. 2008; Simao et al. 2010). Greater cover of *Microstegium* has been associated with lower tree seedling abundance and diversity (Oswalt et al. 2007). In a removal experiment, 2 years of *Microstegium* eradication resulted in more than double the number of tree seedlings compared to intact *Microstegium* invasions (Flory and Clay 2009). These studies suggest that *Microstegium* may inhibit forest regeneration, but questions remain regarding the magnitude of effects over multiple years, differences in responses among tree species, and effects on trees at different life history stages.

In this paper, we report the results of a study designed to assess the effects of *Microstegium* on tree regeneration in early successional communities. In large, replicated field plots, we planted trees from seed, including both small- and large-seeded species, and as saplings, and quantified their survival and growth over 3 years. We also evaluated natural tree regeneration at the study site. By experimentally adding the invader, and comparing tree responses in invaded plots to *Microstegium*-free controls, we can evaluate effects due specifically to the invasion and not effects of disturbance, environmental conditions, or

other confounding factors. Specifically, we addressed the following questions:

1. Does *Microstegium* invasion reduce native tree regeneration?
2. Do the effects of invasion differ among small- and large-seeded tree species?
3. Do the effects of invasion depend on tree life history stage?

Additionally, we quantified biotic and abiotic factors that may help to elucidate the mechanisms by which invasions affect tree regeneration.

Materials and methods

Study site

This experiment was conducted at the Indiana University Research and Teaching Preserve's Bayles Road site (39°13'9"N, 86°32'29"W) north of Bloomington, Indiana, USA. The study site was previously bottomland hardwood forest, but for the past 60 years portions of the site have been maintained for agriculture or open fields for biological research. We established the experiment in a 60-m × 60-m opening surrounded by mature trees including box elder (*Acer negundo*), tulip poplar (*Liriodendron tulipifera*), and sycamore (*Platanus occidentalis*). These species are commonly found in bottomland forests in the Midwest. Prior to our study, the opening was mowed to prevent tree encroachment and was dominated by old field species.

Experimental design

We first tilled the experimental area 3 times at 2-week intervals during late summer 2005 to establish the experiment under homogeneous conditions and to reduce the resident seed bank. In September 2005, we established 32 plots (5.25 m × 5.25 m each) at 2.5-m spacing within the opening. To prevent movement of seeds among plots, we surrounded each plot with 60-cm-tall silt fence (Eco-Systems, Bloomington, Ind.) buried 10 cm deep.

To test if the effects of *Microstegium* invasion depended on tree life history stage, we randomly planted half of the plots ($n = 16$) with native tree seeds in late fall 2005 and the other half ($n = 16$) with tree saplings in spring 2006. First, we uniformly planted all plots with a mixture of 12 native grasses, sedges, and forbs to provide a constant herbaceous community and to test the effects of invasion on native herbaceous species (see Flory and Clay 2010 for species list). Additionally, many other herbaceous plant species colonized the plots from the surrounding area over the course of the experiment. To determine if tree species planted from

seed differed in their success based on seed size, we planted both large- and small-seeded species. In the plots planted with tree seeds, we planted five large-seeded tree species and adjusted the number of seeds planted per species to account for differences in germination rates with the goal of achieving similar numbers of seedlings per plot (germination tested by Vallonia State Tree Nursery, Vallonia, Ind.). All seeds were collected from natural areas throughout Indiana and obtained from Vallonia State Tree Nursery. Large-seeded species and seeding rates per plot (in parentheses) were: black walnut, *Juglans nigra* (34); bur oak, *Quercus macrocarpa* (40); pin oak, *Quercus palustris* (28); shellbark hickory, *Carya laciniosa* (20); white oak, *Quercus alba*; (22). Seeds were randomly planted in a 12 × 12 arrangement with 0.4-m spacing between seeds. After the five large-seeded species were planted, four small-seeded species were sown randomly throughout the plots: green ash, *Fraxinus pennsylvanica* (190); sweetgum, *Liquidambar styraciflua* (1,470); sycamore, *Platanus occidentalis* (1,855); and tulip poplar, *Liriodendron tulipifera* (685). The average seed size for the large-seeded species (\pm SE) was $7,438 \pm 2,565$ mg vs. 23 ± 9 mg for the small-seeded species (USDA and NRCS 2005). Finally, in late fall 2005, half of all plots planted with tree seeds ($n = 8$) and half of the plots scheduled to be planted with saplings ($n = 8$) in spring 2006 were sown with locally collected *Microstegium* ($n = 16$ total plots invaded) at a rate of ~ 690 seeds/m², corresponding to observed seedling densities in locally invaded sites.

In early spring 2006, we planted saplings of the nine tree species listed above in 16 plots (the other 16 plots were planted with tree seeds the previous fall). Four saplings of each species were randomly planted in a 6 × 6 grid with 0.75-m spacing in each plot (36 trees per plot total). Saplings were obtained from the Vallonia State Tree Nursery as bare root stock. Vallonia purchases seed from many private collectors throughout Indiana to grow saplings, thus the nursery stock we used in our experiment is genetically diverse but from local sources, similar to our experimental seeds. All species were 1 year old except shellbark hickory which was 2 years old. Average sapling height at planting ranged from 15 cm for shellbark hickory to 81 cm for tulip poplar. Although trees of this size are often referred to as seedlings, here we refer to the planted trees as saplings to clearly distinguish them from the tree seedlings grown from planted seed. Saplings were nursery grown under high resource conditions, had well-developed root systems, relatively large main stems, and multiple side limbs and so would be expected to immediately thrive. The tree species used in the experiment were selected because they represent some of the most prevalent tree species found in bottomland areas in the Midwest where *Microstegium* commonly invades (Braun 1950). We applied no other

treatments to the plots during the 3-year study period. Plots were subjected to ambient abiotic (e.g., light, precipitation, temperature) and biotic (e.g., herbivores, pathogens) conditions. At least four additional tree species not planted into the plots naturally colonized from the surrounding area during the experiment. The assignment of treatments to plots was completely random with respect to location and proximity to forest edge so the likelihood of natural tree seed dispersal among treatments was approximately equal.

Data collection

To assess tree responses to *Microstegium* invasion, we quantified the survival and growth of trees planted as seeds and as saplings over a 3-year period. For the 16 plots where trees were planted as seeds, we quantified the survival, basal diameter, and height of each individual (144 per plot; 2,304 total) in March 2007, 2008, and 2009. In March 2009, the aboveground biomass of each surviving seedling was then harvested, oven dried, and weighed.

To evaluate small-seeded species that were scattered throughout the seeded plots, we harvested all tree seedlings from nine 1-m × 1-m quadrats in each plot in March 2009. Tree harvest quadrats were systematically arranged in a 3 × 3 grid within each plot. With this method we also sampled trees that were not planted but had naturally recruited into the plots over the course of the experiment. We counted the total number of seedlings per quadrat and measured their height, basal diameter, and aboveground dry biomass.

To determine the effects of *Microstegium* on planted tree saplings, we measured total height and diameter at 10 cm from the base of each individual in March 2007, 2008, and 2009. Saplings were not destructively harvested.

Potential mechanisms

We evaluated possible direct and indirect mechanisms underlying the effects of invasion on native trees. For each possible mechanism we can correlate biotic or abiotic conditions to tree seedling and sapling responses but we are not able to assign causation. To do so, further experimental work will be needed. However, these correlational data may provide insight into potential mechanisms.

The response of trees to invasion may depend on direct competition with *Microstegium*. To quantify the effectiveness of the *Microstegium* addition treatment and the abundance of *Microstegium* and native herbaceous species, we destructively harvested 30-cm × 30-cm quadrats from each plot in September 2006 (quadrats/plot; 8), 2007 (6), and 2008 (4). Quadrat locations for each year did not overlap. All herbaceous vegetation, including *Microstegium*, in each quadrat was clipped at ground level, sorted in the lab, dried, and weighed.

Native tree recruitment from seed may be inhibited by the persistent layer of thatch created by senesced *Microstegium* (Ehrenfeld et al. 2001). To quantify thatch abundance in the invaded and control plots, we destructively harvested three 30-cm × 30-cm quadrats of thatch from each plot planted with tree seeds on 5 April 2008. Samples were oven dried to constant mass and weighed.

Microstegium may also alter abiotic conditions such as light availability. We measured the percent of ambient light reaching ground level at a single time point in June and September 2008 at four locations in each plot. At each location on each date we recorded ambient light above the vegetation canopy and the amount of light at ground level under any existing thatch (AccuPAR Linear PAR/LAI ceptometer; Decagon Devices, Pullman, Wash.). We then calculated the percent of ambient light reaching ground level.

Finally, to determine if *Microstegium* invasion indirectly impacted tree saplings by altering small mammal behavior, we also measured the amount of vole damage for each tree sapling in March 2007, 2008, and 2009. Voles leave characteristic 45° bite marks on tree seedlings (Rudgers et al. 2007). Damage was quantified on a scale from 0 to 3 with: 0 = no damage, 1 = few small bite marks, 2 = bark removed from up to half the circumference of the sapling, and 3 = sapling completely girdled or clipped off.

Data analysis

Tree responses

We calculated the proportion of survival and final height, diameter, and biomass of surviving individuals per plot for each large-seeded tree species. For small-seeded species, we determined the total number of seedlings found in each plot. Too few planted small-seeded species were recovered at the end of the experiment to evaluate tree size responses. We used mixed-model ANOVAs with invasion treatment and species as fixed effects and plot as a random effect to analyze large-seeded species survival and size and survival of small-seeded species (Proc Mixed; SAS Institute 2002).

We calculated the total number of trees that naturally recruited into each plot (i.e., volunteer species that were not experimentally planted) and analyzed values with the fixed effect of treatment (Proc GLM; SAS Institute 2002). The same model was also used to analyze recruitment of the four naturally regenerating species separately, but only box elder (*Acer negundo*) was found in sufficient numbers to evaluate size responses.

We calculated survival and average size responses of surviving tree saplings per plot and analyzed these data using the same mixed-model ANOVA described above. Plot was the level of replication for all analyses.

Potential mechanisms

To test for differences in *Microstegium* and native species biomass between the control and invaded plots over the 3 years of the study, we averaged quadrat values within plots for each year and applied ANOVA with invasion treatment as a fixed effect and year repeated (Proc GLM; SAS Institute 2002). Thatch biomass was also averaged across quadrats within plots and compared between control and invaded plots (Proc GLM; SAS Institute 2002). We analyzed light availability between control and invaded plots across the two sampling dates using ANOVA with treatment as a fixed effect and date repeated. To determine if the amount of vole damage to tree saplings was greater in invaded plots, we used mixed-model ANOVAs with invasion treatment and species as fixed effects, plot as a random effect, and year repeated (Proc Mixed; SAS Institute 2002). We used autoregressive covariance structure, determined by comparing multiple covariance structures using corrected Akaike's information criteria. Plot was the level of replication for all analyses. Tukey post hoc tests were used to test for differences in responses across treatments.

Results

Tree responses

The response of trees to *Microstegium* invasion depended on both tree seed size and life history stage. *Microstegium* invasion significantly reduced the abundance of both experimentally planted small-seeded tree species and species naturally recruited into the plots, but there were no effects on

large-seeded species or planted tree saplings. Average survival of large-seeded species varied strongly among species ($F_{4,48} = 40.85$, $P < 0.0001$) but was unaffected by the invasion treatment ($F_{1,12} = 1.12$, $P = 0.31$). There was a marginal interaction between invasion and tree species ($F_{4,48} = 2.32$, $P = 0.07$) with trends for reductions in bur, pin, and white oak in invaded plots, but no differences for shellbark hickory or black walnut (Fig. 1a). There were no overall differences in the height, diameter, or biomass of large-seeded tree species due to the invasion treatment and no interactions for size responses between large-seeded species and treatment (all responses $P > 0.05$).

From the sampling quadrats, we harvested a total of 203 individuals of small-seeded tree species that were planted in the plots including sweetgum (88% of individuals), tulip poplar (7%), and green ash (5%). Very few sycamores planted as seed survived to the end of the experiment so sycamore was not included in the analysis. Some of the sampled tulip poplar and green ash may have been naturally dispersed into the plots but such dispersal would have been random since the arrangement of the plots across the field was completely random. Overall, *Microstegium* invasion reduced small-seeded tree species recruitment by 77% ($F_{1,14} = 6.14$, $P = 0.026$), but the reduction in recruitment due to the invasion varied by tree species (treatment \times species: $F_{2,28} = 6.38$, $P = 0.005$). On average, there were 10 times more sweetgum in control plots than in invaded plots (Fig. 1b). There were similar trends for tulip poplar and green ash, but the differences were not significant (Fig. 1b). Thus the much lower rates of recruitment for small-seeded species in invaded compared to control plots were primarily driven by the large differences in recruitment by sweetgum, which comprised the majority of all small-seeded species recruitment.

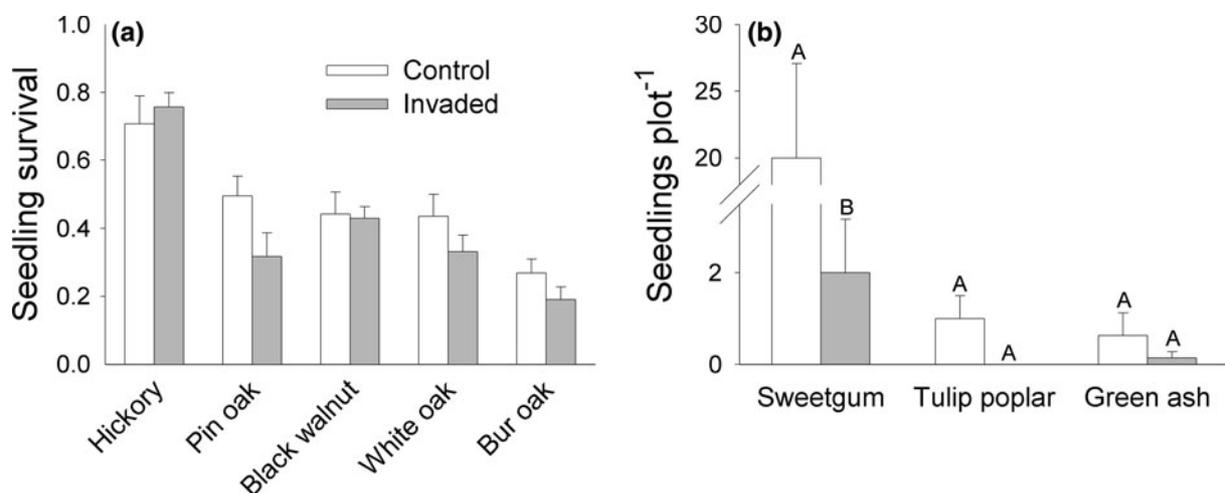


Fig. 1 Proportion of individuals per plot (mean \pm SE, $n = 8$) that **a** survived the 3-year experiment for the five large-seeded tree species in control and invaded plots and **b** number of tree seedlings (mean \pm SE, $n = 8$) found in control and invaded plots 3 years after

planting for three small-seeded species. Different letters indicate significant differences within species due to the invasion treatment. There were no significant differences between treatments for large-seeded species

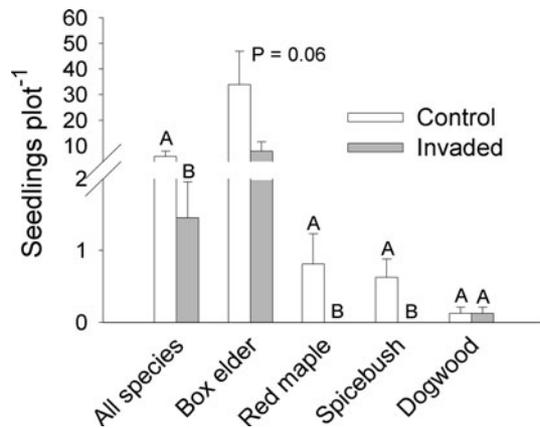


Fig. 2 Number of tree seedlings (mean \pm SE, $n = 16$) that naturally colonized control and invaded plots. Values are shown for all species combined and for each of the four species separately. Different letters indicate significant differences due to the invasion treatment. The P -value shown is for box elder only

From the sampling quadrats, we found 729 individuals of four woody species that naturally colonized the plots including box elder (96% of individuals), red maple (*Acer rubrum*, 2%), spicebush (*Lindera benzoin*, 1%), and red osier dogwood (*Cornus sericea*, 1%). All of these are relatively small-seeded species (approximate average seed weights: 39.9, 19.9, 100.8, and 24.6 mg, respectively; USDA and NRCS 2005). Invasion reduced natural recruitment by an average of 80% across all species ($F_{1,30} = 4.40$, $P = 0.04$; Fig. 2). There were significantly fewer spicebush ($F_{1,30} = 5.95$, $P = 0.02$) and marginally fewer box elder ($F_{1,30} = 3.70$, $P = 0.06$) and red maple ($F_{1,30} = 3.73$, $P = 0.06$) in invaded plots (Fig. 2). The average biomass of box elder individuals, the predominant natural colonist, was 58% lower in invaded than control plots ($F_{1,19} = 3.79$, $P = 0.06$; mean \pm SE; 2.4 ± 0.5 g vs. 5.5 ± 1.6 g).

There were significant differences among tree species for height and diameter ($P < 0.001$) of saplings but the invasion treatment caused no differences in sapling survival, height, or diameter ($P > 0.05$; Fig. 3).

Possible mechanisms

All experimentally invaded plots had dense populations of *Microstegium* during the course of the experiment. No control plots contained *Microstegium* during 2006 or 2007, but a small amount of *Microstegium* was found in two of the 16 control plots in 2008. Native herbaceous biomass was significantly lower in invaded plots than in control plots during all 3 years ($F_{1,30} = 49.49$, $P < 0.001$; Fig. 4). There was significantly more total community biomass in invaded than control plots in 2006 and 2008, but no difference in 2007 (Fig. 4). Thatch biomass was over 120% greater in invaded plots than in control plots (mean \pm SE; 19.7 ± 1.8

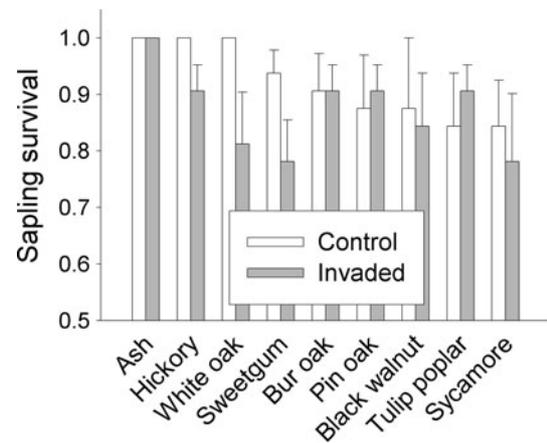


Fig. 3 Proportion of tree saplings surviving (mean \pm SE, $n = 8$) in the control and invaded plots for the nine experimentally planted tree species. There were no significant differences between treatments for any species

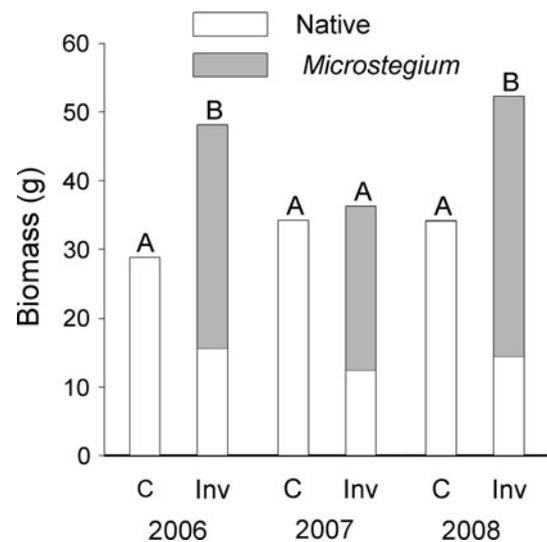


Fig. 4 Native herbaceous plant community and *Microstegium* biomass (mean \pm SE, $n = 16$, 30-cm \times 30-cm quadrats) in control (C) and invaded (Inv) plots for 2006, 2007, and 2008. Different letters indicate significant differences in total community biomass between C and Inv plots within years

vs. 9.0 ± 0.9 ; $P = 0.003$) when measured in early spring 2008. Significantly more light reached ground level in the invaded plots than in control plots in June, but this pattern was reversed in September with more light reaching ground level in control plots than in invaded plots (treatment \times date: $F_{1,30} = 23.40$, $P < 0.0001$; Fig. 5).

Vole damage to tree saplings was significantly greater in invaded than in control plots when averaged across the 3 years of the study ($F_{1,14,1} = 8.29$, $P = 0.01$). This effect was consistent among tree species (treatment \times species: $P > 0.05$). However, there were strong differences in vole damage among the 3 years of the study (treatment \times year: $F_{2,104} = 5.74$, $P = 0.004$) with significantly more damage in

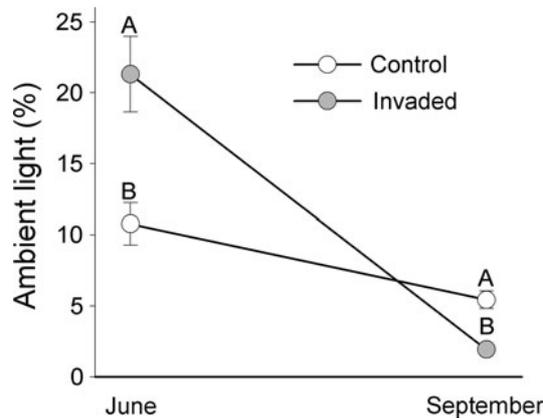


Fig. 5 Percent of ambient light reaching ground level (mean \pm SE, $n = 16$) in control and invaded plots for June and September 2008. Different letters signify significant differences between treatments within dates

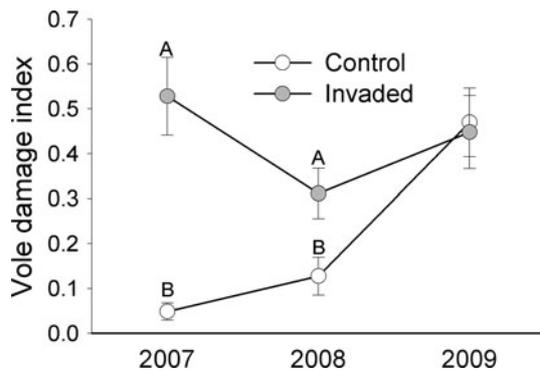


Fig. 6 Vole damage to tree saplings (mean \pm SE, $n = 8$) in control and invaded plots for 2007, 2008, and 2009. Different letters indicate significant differences between treatments within year

invaded plots in 2006 and 2007 but no difference between control and invaded plots in 2008 (Fig. 6). Many planted and naturally recruited tree seeds or seedlings may also have been killed by voles but we were not able to quantify this potential effect.

Discussion

Our results demonstrate that two functional traits, seed size and life history stage, determine the effects of a plant invasion on tree regeneration in eastern US forests. *Microstegium* invasion inhibited the success of planted small-seeded species, primarily due to dramatic reductions in recruitment of the most abundant small-seeded species (sweetgum), and reduced natural colonization rates for three common early successional tree species. However, *Microstegium* invasion did not affect the success of large-seeded tree species or trees that were planted as saplings prior to the invasion. Over the course of the experiment, invaded plots had higher

overall community biomass, reduced light availability in fall, and greater vole damage to saplings, possibly contributing to the negative effects of invasion, but these effects varied considerably within and among growing seasons. The reduced success of some planted and naturally regenerating tree species in invaded plots shows that the invasive grass *Microstegium* can inhibit succession and may alter the species composition of successional forests.

Tree responses

As we predicted, *Microstegium* reduced the establishment and survival of small-seeded but not large-seeded species. The effects on small-seeded species were largely driven by the strong effects of invasion on sweetgum recruitment, but there were similar non-significant trends with other less abundant, small-seeded species. Large-seeded species had seeds that were, on average, over 300 times larger than small-seeded species ($7,438 \pm 2,565$ mg vs. 23 ± 9 mg). Because large seeds have relatively more stored reserves that produce bigger seedlings, saplings from large-seeded species often have better access to light and water and greater tolerance to competition (Armstrong and Westoby 1993; Harms and Dalling 1997). Additionally, seedlings with more reserves may grow faster and emerge above competing vegetation quicker, a key factor in determining seedling establishment in successional fields (Hill et al. 1995). The relative advantage of large-seeded species may be enhanced when tree seeds are competing for resources with herbaceous plants (Lahoreau et al. 2006). Furthermore, this effect may be especially pronounced when trees are in competition with a highly abundant invasive plant such as *Microstegium*. A similar pattern of greater seedling survival for large-seeded tree species was shown for 23 savanna tree species from Australia, Brazil, and West Africa, where larger seedlings were better able to compete with grasses and tolerate drought years (Lahoreau et al. 2006). In our system, larger seedlings from large-seeded species would likely suffer fewer consequences of increased herbaceous community biomass due to the invasion, and may be more tolerant of accumulated *Microstegium* litter than small-seeded species. Reductions in the colonization rates of small-seeded species (e.g., sweetgum) due to *Microstegium* invasion may have significant effects on the rate and direction of succession in early successional communities. Such species are among the first woody species to colonize old fields and disturbed areas where they may facilitate the recruitment of other successional species (Berkowitz et al. 1995).

By planting both small- and large-seeded tree species and *Microstegium* into the plots at the same time, we simulated the scenario where trees and *Microstegium* are simultaneously colonizing the same successional habitat.

A second and possibly more common situation occurs when trees colonize areas with existing invasions. Many plant invasions may persist for years in the same location, creating a sustained barrier to tree regeneration. We found that after three growing seasons over 400% more seedlings of woody species had naturally colonized control plots compared to invaded plots. *Microstegium* significantly reduced the natural regeneration of box elder, red maple, and spicebush, which can each be significant components of early successional Midwestern bottomland forests (Braun 1950). Although we tested this phenomenon at a single experimental site, a previous study found that removing *Microstegium* with a grass-specific herbicide at eight forested sites with a range of light availability increased natural tree regeneration by more than 120% (Flory and Clay 2009). Likewise, Oswalt et al. (2007) showed that there was a negative relationship between *Microstegium* cover and woody seedlings density, and Marshall et al. (2009) reported reduced growth of red maple and tulip poplar when grown in competition with *Microstegium*. Our results, and those of others, demonstrate that invasions in successional forests inhibit tree regeneration and suggest that efforts to control *Microstegium* invasions in eastern US forests will result in greater tree recruitment, especially of small-seeded species.

In contrast to the effects of invasion on tree species regenerating from seed, *Microstegium* invasion did not affect the survival or size of trees planted as saplings. This result was generally expected because planted tree saplings already had a well-developed root system for accessing water and nutrients and were often tall enough to avoid shading by *Microstegium*. We obtained tree saplings as bare root stock from a local state tree nursery where trees were grown under high-resource conditions. At the time of planting, tree saplings were 0.25–1.25 m tall with robust root systems, allowing them to establish and grow rapidly. Thus, our measure of the effects of invasion on tree saplings may be conservative and invasions may more effectively suppress survival and growth of tree saplings under natural field conditions. Although there were no main effects of invasion or interactive invasion \times species effects, it is worth noting that there were trends for reduced survival of hickory, white oak, and sweetgum saplings. Further experimental work is needed to more precisely evaluate the effects of *Microstegium* on established tree saplings under field conditions.

Possible mechanisms

The reductions in tree recruitment we observed for some small-seeded and naturally regenerating species may be due to greater herbaceous community biomass, more thatch biomass, differences in light availability, or other factors we did not quantify such as changes in the soil microbial

community or soil nutrient availability. *Microstegium* invasion decreased native community biomass during each year of the experiment but total herbaceous biomass was higher in invaded plots. There was 71% greater total community biomass in invaded than control plots in 2006 and 53% more biomass in 2008, suggesting more intense competition. The extreme drought conditions in the region in 2007 reduced the productivity of all species (NOAA and NCDC 2008). Herbaceous plants can provide a significant competitive barrier to establishing tree seedlings (Berkowitz et al. 1995; Desteven 1991; Hill et al. 1995), so greater community biomass with *Microstegium* invasion could account for reductions in recruitment and survival. For example, Berkowitz et al. (1995) found that removing herbaceous competitors increased the survival and growth of experimentally planted tree saplings across 23 sites in upstate New York. In the same system, Hill et al. (1995) reported that the communities most resistant to tree establishment were dominated by little bluestem grass (*Schizachyrium scoparium*), a perennial, deep-rooted rhizomatous bunchgrass. By contrast, *Microstegium* produces a dense layer of fine roots near the soil surface and is an annual. *Microstegium* may compete with trees by capturing water or nutrients before they are leached deeper into the soil and accessed by tree saplings. The dense growth of *Microstegium* at the peak of the growing season in late summer also reduced the amount of light available to establishing seedlings, possibly reducing their ability to store overwintering reserves. The reversal of high light availability in spring and fall likely reflected reduced herbaceous plant cover and juvenile *Microstegium* in invaded plots in spring and dense *Microstegium* in fall compared to control plots. Furthermore, invaded plots had over twice as much thatch biomass as control plots in the spring, possibly providing a significant physical barrier to small, emerging tree seedlings despite the higher light conditions. Further experiments directly manipulating light availability, soil fertility, and thatch cover are required to better elucidate the specific mechanisms responsible for the inhibitory effects of *Microstegium* on tree regeneration.

Predators and herbivores can reduce survival of tree seedlings and saplings, and alter succession in old fields and young forests (Desteven 1991; Gill and Marks 1991; Ostfeld et al. 1997). There is also evidence that the interaction between invasive plants and rodents can alter the establishment and growth of native species via apparent competition (Meiners 2007; Orrock et al. 2008; Rudgers et al. 2007). *Microstegium* creates a dense layer of persistent thatch that may provide refuge for small mammals such as voles (*Microtus* spp.) and mice (*Peromyscus* spp.), allowing them to feed more effectively on the seeds and saplings of native trees. We do not know whether predation caused the negative effects on experimentally planted and naturally regenerating seedlings, but we found dramatic

differences in vole damage to tree saplings in the first 2 years of the study. These results suggest that voles, and possibly other small mammals, preferentially inhabited invaded plots or more actively fed in invaded plots due to greater cover. Further experimental work is needed to elucidate the role of small mammal predation as a mechanism for the negative effects of *Microstegium* invasion on tree seed and seedling survival.

Conclusion

Understanding the consequences of biological invasions is critical for determining how invasions might change the diversity and distribution of species and alter ecosystem functions. Our results show that a widespread, aggressive forest invader can inhibit native tree regeneration, and that inhibition depended on tree seed size and life history stage. Reduced rates of tree seedling establishment in invaded communities may slow the rate of forest succession. Furthermore, because *Microstegium* invasion only reduced the success of a subset of tree species, the species composition of successional forests may be altered by invasion. These results are consistent with previous correlational (Oswalt et al. 2007), removal (Flory and Clay 2009), and common garden (Marshall et al. 2009) studies, but longer term studies in natural areas and experimental plots are needed to determine the long-term consequences of *Microstegium* invasion for eastern US forests. The suppression of tree regeneration due to *Microstegium* invasion provides further evidence that dominance of this invasive species has significant community and ecosystem consequences (Ehrenfeld et al. 2001; Flory and Clay 2010; Simao et al. 2010). Our results highlight the relatively underappreciated role of invasive herbaceous species, particularly grasses, in altering forest community dynamics (Martin et al. 2009), and suggest multiple possible mechanisms underlying the effects of invasions on tree regeneration. Based on these results, future models of eastern deciduous forest succession should incorporate the potential effects of plant invasions.

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