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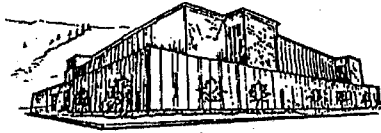
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Mechanisms for invasion by non-native trees—facilitation, ecosystem
engineering, and escape from belowground enemies

by

Kurt O. Reinhart

B.S. Appalachian State University 1996

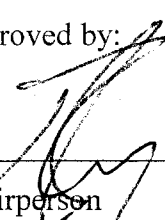
Presented in partial fulfillment of the requirements


for the degree of Doctor of Philosophy

The University of Montana

May 2003

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
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Mechanisms for invasion by non-native trees—facilitation, ecosystem engineering, and escape from belowground enemies

Director: Dr. Ragan M. Callaway 

Invasive species are one of the greatest threats to global biodiversity. Numerous hypotheses have been proposed to explain the invasive success of non-native species and the invasibility of natural systems; however, invaders are neither predictable nor mechanistically similar. My research explores facilitation and ecosystem engineering as important processes affecting the invasion of Norway maple (*Acer platanoides*). In addition, this research investigates whether the invasive success of black cherry (*Prunus serotina*), box-elder (*Acer negundo*), and *A. platanoides* in their non-native ranges relates to escape from belowground enemies found within their native ranges.

In riparian stands, correlative and experimental results suggest that *A. platanoides* has a negative effect on native species and a positive effect on conspecifics. Also, these effects are dependent upon the level of invasion suggesting that *Acer* trees experience a “lag phase” before spreading and severely impacting the native community. In coniferous forests, *A. platanoides* seedlings had higher survival and experienced less physiological stress in a conspecific patch than in an adjacent conifer forest. Substantial demographic, physiological, and environmental differences between sites appeared to be due to ecosystem engineering by mature *A. platanoides*. I propose that *Acer* trees produce a more mesic environment by modifying the structure and phenology of the forest canopy and by altering the timing of transpirational water loss. Overall, these results indicate that environmental modification by *Acer* trees may facilitate conspecifics in their understories and suppress native species.

Recent research has suggested that invasive plants may also be released from their natural soil pathogens; however, there have been no tests comparing the sensitivity of invasive plants to resident soil biota both in their native and non-native ranges. Here we provide the first evidence of invasive trees (*Acer* spp. and *Prunus*) being facilitated by the soil biota in their non-native ranges, whereas the soil biota decreases survival and growth in their native ranges. In the newly invaded forests, where the soil biota effect is slightly positive, *Acer* and *Prunus* trees are closely spaced relative to the negative soil biota effect and more widely dispersed trees in their native ranges. In total, this research suggests that several interactions (facilitation, ecosystem engineering, and plant-soil interactions) not currently considered in invasive theory may facilitate invaders and impact natural systems.

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INTRODUCTION

Invasive non-native species are the greatest anthropogenic threat to nature reserves (Usher 1988) and are one of the greatest threats to global biodiversity (Mack et al. 2000). Non-native species throughout the world cause major environmental damage and losses, which total an estimated \$137 billion per year for the U.S. alone (Pimentel et al. 2000). Unfortunately, established non-native species are rarely ever completely eradicated, and global travel and commerce constantly introduce new species to parts of the world where they are not native. Today's global economy moves "living" cargo faster, farther, and in greater quantities than during any time in human civilization. An unintended consequence of global commerce has been the accidental release of non-native species or "exotics" into natural systems. While most of these species fail to establish, a small percentage establish and become naturalized. Of the species that naturalize, an even smaller percentage spreads and dominates natural systems (Fig. 1). Thus, although it is relatively rare for an invasive species to dominate and negatively impact natural systems, when they do their impacts can be profound.

All major taxonomic groups have representatives that have become invasive (e.g. microbes, mollusks, reptiles, birds, plants, etc.). Invasive plants are especially problematic because they are difficult to control/eradicate and even more difficult to predict (Mack 1996, Kolar and Lodge 2001, Grotkopp et al. 2002). Ecologists generally accept a suite of hypotheses to explain the invasive success of individual plants (e.g. unique traits, phenotypic plasticity, evolution of increased competitive ability, etc.) and the invasibility of communities (e.g. Enemy Release Hypothesis, Empty Niche Hypothesis, Fluctuating Resources, and Anthropogenic Disturbance) (Mack et al. 2000). Unfortunately, ecologists have found invasive plants to be neither predictable nor

mechanistically similar (Mack et al. 2000). New hypotheses continue to gain support and older hypotheses are revised as our understanding of the dynamics, impacts, and control of invasive species increases (see Simberloff and Von Holle (1999) for an example of a relatively new hypothesis). However, the continued threat and unpredictability of invasive species suggests that ecologists still have much to learn about these problematic species and the communities they impact.

Plant invasions also represent a unique opportunity for ecologists to test their understanding of community assembly and how the introduction of a single species may alter the composition, diversity, productivity, and succession of plant communities. The interactions between native plants within an individual community have been shaped by generations of direct and indirect interactions with their abiotic environment, competitors, herbivores, pathogens, etc. Coexisting species are thought to often have co-evolved traits. Connell (1980) even suggested that the diversity of systems may be an artifact of “the ghost of competition past.” Thus, the interactions between species within a community have been shaped, molded, and constrained by years of evolution. These processes; however, may be particularly susceptible to biological invasions because they bring new interactions between species without a history of neither coexistence nor coevolution. These unfortunate events provide ecologists with an opportunity to better understand how species interact, how these interactions change over time, how communities respond to disturbance, and how the characteristics of some communities make them either more resistant or more vulnerable to future invasion. Thus, invasions represent both a threat to natural systems and a unique opportunity to better understand the processes that organize natural systems.

I focused on plant invasions, and concentrated on several processes that are known to be important in natural systems: facilitation (Callaway 1995), ecosystem engineering (Jones et al. 1997), and indirect effects (Levine 1999). These processes have rarely been related to plant invasions. Moreover, I revisited one of the oldest hypotheses used to explain invasive success—the Enemy Release Hypothesis. I applied a contemporary understanding of belowground interactions between plants and the soil community to reexamine this hypothesis and expand upon the historic emphasis on aboveground interactions (e.g. herbivory and disease) (Maron and Vila 2001, Wolfe 2002, Mitchell and Power 2003) to include belowground interactions.

In Chapter 1, I examine the effect of *Acer platanoides* invasion at two spatial scales on riparian forest communities in the northern Rocky Mountains. This research also attempts to identify how the invasion of *A. platanoides* impacts native species and affects conspecifics. Additionally, I determine how levels of invasion relate to lag phase, where the spread and impact of the invader early in the invasion process is temporarily stalled but accelerates over time. Moreover, I identify the effect of species-specific positive interactions on invasion dynamics.

Chapter 2 examines the recruitment patterns of *A. platanoides* along the interface between a heavily invaded patch and the neighboring coniferous forest. I focus on changes in survival and ecophysiology of seedlings along this gradient. This research also attempts to identify how the invader and native dominant trees alter the understory environment, and I relate this to the demographic and physiological responses of invasion by *Acer* seedlings in these two different environments.

Chapter 3 examines the Enemy Release Hypothesis by comparing interactions between *Acer negundo* and *A. platanoides* and soil biota in their native and non-native ranges (each species has invaded the other's country of origin) and relating these interactions to their distance to nearest conspecific neighbors in these different ranges. The Enemy Release Hypothesis predicts that invasive species will have fewer natural enemies (e.g. herbivores and pathogens) and increased vigor in their non-native than in their native ranges. However, few studies have tested the Enemy Release Hypothesis with biogeographical experiments (Maron and Vila 2001, Wolfe 2002, Mitchell and Power 2003), and of these studies, most have focused on aboveground interactions while ignoring belowground interactions.

Chapter 4 continues to examine the Enemy Release Hypothesis by comparing the plant-soil interactions for *Prunus serotina* (black cherry) in its native and non-native ranges and relating these interactions to its distance to nearest conspecific neighbors in these different ranges. In its native range, previous studies demonstrated that this species is negatively effected by soil pathogens (*Pythium* spp.) (Packer and Clay 2000). I expand on existing research by comparing the distance to nearest neighbors in its native (Indiana, U.S.) and non-native (the Netherlands) ranges. These spatial patterns provide context for experiments testing the effects of soil biota, seedling density, and soil source (near or away from conspecifics) on seedling survival and growth.

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Invasive Species Dynamics

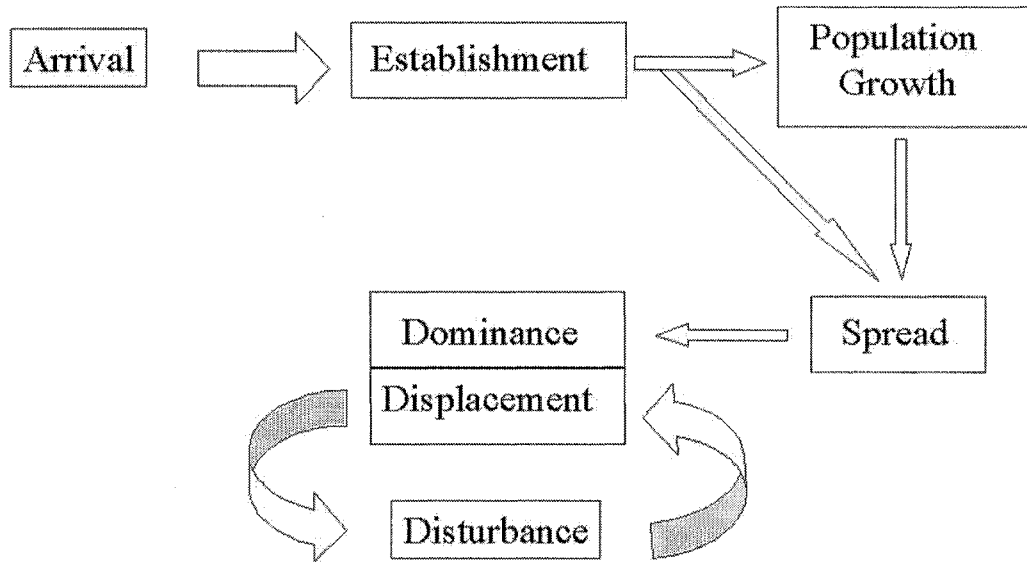


Fig. 1 Invasive species dynamics. Large numbers of species are accidentally released (Arrival stage), but only a portion of these species are able to “Establish”. Next, only a portion of the established species are able to undergo “Population Growth” and “Spread”. An even smaller fraction of the original invaders are able to dominate natural systems, displace natives, and disturb the ecosystem. This figure was modified from Mooney and Drake 1986.

Chapter 1

Effects of *Acer platanoides* invasion on understory plant communities and tree regeneration in the northern Rocky Mountains

Abstract

Many invasive plant species are thought to displace natives and reduce local biodiversity, but quantitative studies describing this pattern are rare. Here I describe the effects of invasion by Norway maple (*Acer platanoides*) on riparian plant communities and tree regeneration at two different scales (individual tree vs. stand scales) in western Montana, USA, using both descriptive and experimental approaches. Riparian stands with high levels of *Acer* invasion had lower understory species diversity (-73%), lower richness of native species (-79%), and lower richness of non-native species (-50%) than stands with low levels of *Acer*. This sharp decrease in the community richness of entire stands was paralleled by a 32-fold increase in *A. platanoides* seedlings and saplings. Community patterns under individual *Acer* trees, from a site with intermediate levels of invasion, showed similar responses to invasion. At this scale, the diversity and richness of species in the understory of solitary *Acer* trees declined as the size of the trees increased. This change in understory communities corresponded with a dramatic increase in the density of *Acer* seedlings. The effect of *Acer* at the stand scale was more dramatic than at the individual canopy scale; however, at this smaller scale I only collected data from the site with intermediate levels of invasion and not from the site with high levels of invasion. This variability between scales suggests that these effects are dependent upon the level of invasion and that the invasion experiences a “lag phase” before spreading and severely

impacting the native community. Transplant experiments with tree seedlings demonstrated that *A. platanoides* seedlings performed significantly better when grown beneath conspecific canopies than under natives, but *Populus* and *Pinus* seedlings performed better when grown beneath *Populus* canopies, the dominant native. My results indicate that *Acer* trees suppress most native species, including the regeneration of the natural canopy dominants, but facilitate conspecifics in their understories.

Keywords: invasive tree; riparian, invader; invasive non-native species feedback; facilitation; *Acer platanoides*; *Populus trichocarpa*; lag phase; resistance to invasion

Introduction

Invasive non-native plants threaten the composition and diversity of natural communities (Hobbs & Mooney 1986; Braithwaite et al. 1989; Schofield 1989; Walker & Vitousek 1991; Vivrette & Muller 1998) and pose a serious threat to the sustained conservation of natural areas (Usher 1988; Heywood 1989; Humphries et al. 1991; Luken & Thieret 1997; Schmitz et al. 1997). However, few studies have quantified the response of native communities to invasion. Non-native plants appear to invade successfully because of particular traits, such as high phenotypic plasticity, pollination by generalists, short-life spans, high fecundity, rapid growth rates (Bazzaz 1986; Rejmánek 1995), as well as the strength of their direct and indirect negative effects on native species (Vitousek et al. 1987; Gordon 1999; Marler et al. 1999; Callaway & Aschehoug 2000). Several hypotheses have been proposed to explain the remarkable vulnerability of native communities to invasion by some non-native plant species including: 1) the absence of natural enemies (Darwin 1859; Elton 1958), 2) the existence of empty niches (Elton 1958), 3) anthropogenic disturbances (Mack 1989; Hobbs & Huenneke 1992), and 4) fluctuations in resource availability (Mack et al. 2000; Davis et al. 2000).

Recently, Simberloff and Von Holle (1999) proposed that positive interactions among invasive non-native species might lead to an “invasional meltdown” in which invaders accelerate invasive success of additional non-natives causing the further demise of native species. Facilitation among plants (Hunter & Aarssen 1988; Callaway 1995) has the potential to rapidly accelerate invasion, but facilitation has received little attention in the context of biological invasion (but see Maron & Connors (1996)).

Riparian ecosystems are particularly susceptible to plant invasion because they have been disproportionately affected by human disturbance (Patten 1998). These ecosystems are especially important in arid regions because a high percentage of animal species depend on these habitats for part or all of their life cycle (Brinson et al. 1981; Kondolf & Keller 1991).

Here I focus on the impacts of invasion by a non-native tree species on riparian plant communities in western Montana. Norway maple (*Acer platanoides* Linn.), a tree native to mesic deciduous forests in Eurasia (Schmucker 1942), has invaded riparian areas in Montana. The level of invasion by *A. platanoides* in the northwestern U.S.A. as a whole appears to be low, but one study has identified virtual monocultures of the invader and modeled very rapid future spread (Greene et al. *in prep*). Currently, *Acer* is invading deciduous forests throughout the northeastern U.S. (Nowak & Rowntree 1990). *Acer* is widely used throughout northern North America as a street or shade tree (Nowak & Rowntree 1990), and as for many other invasive plants, the spread of *Acer* is partly due to its arboricultural use (Heywood 1989; Reichard & Hamilton 1997; Hodkinson & Thompson 1997; Richardson 1998). In forests of the northeastern United States, understories below *Acer* canopies have reduced species richness and increased abundances of *A. platanoides* seedlings relative to nearby non-invaded areas (Wyckoff & Webb 1996). In western Montana, Greene et al. (*in prep*) estimated that *Acer* populations are rapidly expanding and may completely exclude native tree and shrub species in some areas within decades. However, there is little quantitative or experimental data on the effects of *Acer* on understory community composition and the regeneration of native canopy dominants.

Here I describe the effects of invasion by *A. platanoides* on riparian plant communities along a stream near Missoula, Montana, USA. I studied the effect of *Acer* invasion by 1) comparing the effect of *Acer* overstories on understory species at two spatial scales: that of individual canopies and that of selected riparian stands that differed substantially in their abundance of *A. platanoides*, 2) conducting an experiment to test the effect of different overstory tree species on the growth of the non-native, *A. platanoides* seedlings and the natives ponderosa pine (*Pinus ponderosa* Dougl.) and black cottonwood (*Populus trichocarpa* T. & G.).

Methods

Study site

The study was conducted within three riparian stands along Rattlesnake Creek, Missoula, Montana, which differed substantially in their abundance of *Acer*. The high (76% *Acer* canopy coverage), intermediate (11%), and low (0%) levels of *Acer* invasion correspond with *Acer* canopy coverage estimated from transect data of aerial photographs. Because the invasion appears to be moving upstream (Green et al. *in prep*), the most heavily invaded stand was downstream of the other two stands, and the stand with the least *Acer* was upstream of the two other stands. The three stands were located along 3.4 km of the creek, and the length sampled along the creek differed between stands. The distances sampled along the creek are as follows: 520 m for the most heavily invaded stand (N46° 52.644' W113° 58.565'), 210 m for the stand with intermediate levels of invasion (N46° 53.416' W113° 58.254'), and 660 m for the stand with the lowest level of invasion (N46° 53.969' W113° 58.345'). Based on increment cores of the largest trees (R.M. Callaway, unpublished data), invasion of *Acer* into these stands probably started in the 1940's or 1950's.

The climate is semi-arid and receives on average 345 mm of precipitation per year with 140 mm falling May through August (NOAA 2002). The soils are very deep, excessively drained Totelake gravely loam (USDA 1995). All stands are below a dam established in 1905, which regulates the flow of the stream. *Populus*, *Pinus*, and Rocky Mountain maple (*Acer glabrum* Torr.) trees dominated the original native riparian community in areas now invaded by *A. platanoides* (Foote 1965) and continue to

dominate the non-invaded areas. The native understory along the Rattlesnake Creek in 1965 was dominated by grasses, forbs, and shrubs (Foote 1965).

Overstory-understory associations

Correlations between *Acer* invasion and understory community composition were made at two spatial scales: that of individual canopies and that of selected riparian stands that differed substantially in their abundance of *Acer*. Since riparian vegetation can vary naturally because of many factors (geological substrate, hydrology, disturbance history, etc.) I compared adjacent stands along the lower creek drainage to minimize potentially confounding effects.

Riparian stands

The community composition of the understory in the three stands was sampled in August and September of 1999. A total of 50 randomly located plots were sampled along ten 50-m transects which were stratified at 10 m intervals (total n= 50 per stand). Transects were distributed throughout the stands. Plots consisted of 1 m² quadrats, and each quadrat was subdivided into 100 cells with 10×10 cm dimensions for accurate quantification of species cover (Pennings & Callaway 1992). The percent cover of all species within a quadrat was estimated by summing the number of cells in a quadrat that contained each individual species. Species were identified according to Hitchcock & Cronquist (1998) and Lackschewitz (1991). The composition of the canopy (>2 m in height) directly above each plot was classified as *Acer*, native trees, or open sky.

Understory community composition was examined at the stand scale using detrended correspondence analyses as a multivariate ordination technique (DCA, Hill 1979) with PC-ORD software (McCune & Mefford 1995). Multiple DCA analyses were performed at the stand scale. The community composition of the understory was compared between stands by comparing the 2-dimensional distribution of ordinated plots for each stand. For this analysis, I incorporated all data from the transect plots into the ordination matrix. The community composition of the understory was also compared between canopy types by comparing the 2-dimensional distribution of ordinated plots for all plots including plots described below in the “*Individual canopies*” section relative to individual canopy types (*Acer*, native trees, and open sky).

The Shannon-Wiener Index of Diversity and species richness measures were calculated for each transect plot using PC-ORD software (McCune & Mefford 1995). Species dominance was estimated by calculating the frequency of species occurrence across all transect plots. I tested the effect of *Acer* invasion on the diversity and dominant species data for transect data across stands by combining the data from the stands with low and intermediate levels of invasion and comparing it with the data from the heavily invaded site. I tested the effect of *Acer* invasion on species diversity, species richness of native and non-native species, and percent cover of the 15 most dominant species with two sample *t*-tests with homogeneity of variances either assumed (pooled-variances) or not assumed (separate-variances) using SPSS software (1999).

Individual canopies

In August and September of 1999, the composition of the understory communities beneath 24 different *Acer* trees was sampled in the riparian stand intermediate in its abundance of *Acer*. Sample trees were randomly selected, but if they were located within a larger patch of conspecific trees then they were not sampled so that my measurements were as representative of one tree's effect as possible. The site with intermediate levels of invasion was sampled, because the other sites lacked a sufficient number of isolated *Acer* trees. One quadrat (1 m²), placed next to the trunk of the tree but random in aspect, was used to sample each understory. Within each quadrat, the percent cover of each species was quantified as described above, and the diameter at breast height (DBH) was recorded for each tree.

Understory community composition was examined at the canopy scale using DCA. This analysis had a data matrix including quadrats from beneath randomly selected *Acer* trees and quadrats from along transects described above. Samples from beneath *Acer* trees were compared to all other samples within the two-dimensional distribution of all ordinated plots. A second analysis was performed by selecting only the samples from beneath *Acer* trees and regressing the axis scores of these plots with the size (DBH) of the tree above the plot. The Shannon-Wiener Index of Diversity, evenness, and species richness measures were calculated for plots beneath *Acer* trees using PC-ORD software (McCune & Mefford 1995). Linear regressions were used to determine whether changes in the size (DBH) of *Acer* trees above plots explained changes in diversity measures within plots.

I also quantified recruitment under isolated *A. platanoides* trees by counting all seedlings and saplings under canopies. Seedling density was then regressed against the age of the overstory tree to estimate the rate of seedling recruitment.

Seedling Transplant Experiment

Seedlings of *A. platanoides*, *P. trichocarpa*, and *P. ponderosa* (purchased from Bitterroot Restoration Inc., Hamilton, Montana) were planted 1 m from individual trunks of *Acer* and *Populus* within the stand with intermediate levels of *Acer* invasion in June, 1998. Transplanted seedlings were initially watered and then allowed to grow until September 1999 when they were harvested. At the time of planting, the height, stem diameter, and number of leaves were measured for *Acer* and *Populus* seedlings, and height and stem diameter were measured for *Pinus* seedlings. At the harvest, all variables were re-measured. After drying at 60°C until constant weight, I measured the total biomass of *Acer* seedlings, the biomass of green leaves for *Pinus* seedlings, and the biomass of living stems for *Populus* seedlings (there were no leaves on the *Populus*). The biomass and height measurements for each species were analyzed separately with a two sample *t*-test.

Results

Riparian stands

The plant communities in the riparian stand with the greatest amount of *Acer* invasion were dramatically different in composition from stands with low or intermediate levels of invasion. There was virtually no overlap between plots from the heavily invaded stand and the stand with low levels of invasion (Fig. 1a). The plots from the stands with low or intermediate amounts of *Acer* were far more variable in ordination space than plots from the heavily invaded stand suggesting that *Acer* “homogenized” otherwise much more diverse native communities. The plots from the heavily invaded stand were closely aggregated around the species ordination point for *A. platanoides*, a pattern produced by the high abundance of *Acer* seedlings and saplings in all of these plots. Diversity was reduced by 73% for plots from the heavily invaded stand than the stand with low or intermediate levels of *Acer* (two sample *t*-test ; $t = -14.996$, $df = 147$, $P < 0.0005$). Species richness was also reduced for native (-79%) and non-native species (-50%) within plots from the heavily invaded stand relative to the stands with intermediate or low levels of *Acer* invasion (Fig. 2).

The abundance of many dominant species in the understory also differed between invaded and non-invaded stands (Table 1). The stand with the greatest level of *Acer* had far more (>30 times) *A. platanoides* seedlings and saplings in the understory, as well as trends for higher covers of two native species (*Populus* [2.1% vs. 1.0%] and *Equisetum hyemale* [10.6% vs. 4.5%]) than stands with small amounts of *Acer* in the overstory. Stands with low and intermediate levels of *Acer* in the overstory had higher covers of three non-native species (*Arctium lappa*, *Cynoglossum officinale*, and *Solanum*

dulcamara) and six native species (*Acer glabrum*, *Amelanchier alnifolia*, *Elymus glaucus*, *Galium triflorum*, *Sorbus scopulina*, and *Symphoricarpos albus*) than the stand with highest level of *Acer* invasion.

Across all riparian stands, the plant communities associated with different types of canopy cover (*Acer*, native, or open) were also substantially different in species composition (Fig. 1b). As for the ordination by canopy type, plots from beneath native canopies were much more widely dispersed in ordination space indicating a large amount of diversity in the composition of these understory communities. In contrast, understories beneath *Acer* canopies were more closely grouped in ordination space suggesting a strong homogenizing effect of *Acers* on understory species composition.

Individual Canopy Scale Patterns

Within the stand with intermediate invasion by *Acer*, the composition of understory communities under solitary *Acer* canopies was only marginally different than under other canopies (Fig. 3). Centroids for the two canopy types were in close proximity along axis 1 but deviated slightly, but significantly, along axis 2. Furthermore, I found no relationship between the size of overstory *Acer* trees and the composition of understory communities (linear regression for Axis 1 and tree DBH, $F= 0.11$, $P= 0.75$; linear regression for Axis 2 and tree DBH, $F= 0.18$, $P= 0.68$).

However, the DBH of overstory *Acer* trees was negatively correlated with the Shannon-Wiener Index of Diversity and species richness of the understory (linear regression for diversity and tree DBH, $F= 3.9$, $P= 0.06$; Fig. 4a; linear regression of richness and tree DBH, $F= 4.47$, $P< 0.05$; Fig. 4b). No relationship was observed

between species evenness and *Acer* DBH (linear regression, $F = 0.42$, $P = 0.52$). These results suggest a trend for decreasing species richness and diversity of the understory with the increasing size of neighboring *Acer* trees. Conversely, the density of *Acer* seedlings increased with the age of the overstory *Acer* trees (linear regression, $F = 35$, $P < 0.0005$; Fig. 5).

Seedling Transplant Experiment

Acer platanoides seedlings had more biomass ($t = 3.05$; $df = 1, 7$; $P = 0.014$; Fig. 6a) and were taller ($t = 4.11$; $df = 1, 24$; $P = 0.004$; Fig. 6b) when planted beneath mature *Acer* canopies than *Populus* canopies indicating a positive conspecific relationship. In contrast, *Populus* and *Pinus* seedlings were taller when planted beneath *Populus* trees than beneath *Acer* trees indicating a negative effect of the invader on native recruitment ($t_{Pinus} = 4.55$; $df = 1, 21$; $P < 0.001$; $t_{Populus} = 2.09$; $df = 1, 19$; $P = 0.044$; Fig. 6b). *Pinus* needle mass was much greater when grown beneath *Populus* trees than under *Acer* trees ($t = 11.66$; $df = 1, 18$; $P < 0.001$; Fig. 6a).

Discussion

My results indicate that *Acer platanoides* causes large shifts in the community of understory plants, suppresses the recruitment of native canopy dominants, and facilitates recruitment of conspecifics. Community shifts were much more apparent at the stand scale, which is also the scale at which the effects of *Acer* could be most confounded by physical differences between the stands. The level of *A. platanoides* invasion may have been influenced by differences between neighboring sites (e.g. geological substrate, hydrology, disturbance history, etc.), which are probably minor and do not account for the dramatic changes in the understory community that were observed.

In contrast to the strong effects at the stand scale, individual canopies had relatively minor effects on community composition of the understory. This may have resulted from the individual canopy data having been collected from a site with individual trees scattered throughout a natural matrix of vegetation with perhaps younger *Acer* trees in an initial colonization phase relative to the heavily invaded site sampled at the stand scale. However, even though *Acer* did not have a large effect on the composition of understory communities in the newly invaded stand with intermediate levels of invasion, declining understory diversity and species richness with *Acer* size indicate that *Acer*-driven shifts were beginning.

Acer canopies were also associated with large increases in the number of conspecific seedlings and saplings at both scales of the study. These correlative results suggest that as individual trees and probably patches grow and expand, they will have an increasingly positive effect on the abundance of conspecifics. These results may

illustrate a “lag phase” in invasive plant population dynamics such as described by Kowarik (1995).

The success of *Acer* at my study site may have been promoted by damming on Rattlesnake Creek. Management of rivers reduces natural disturbance within riparian areas and allows late-successional species to become more abundant. Unfortunately for the native system, riparian communities are typically dominated by early- to mid-successional species with many species adapted to hydrologic disturbance (Hansen et al. 1995). Damming the Rattlesnake Creek may have lessened the frequency and severity of floods and favored a late-successional species such as *Acer*. *Acer platanoides* is a mid- to late-successional tree species in its native communities (Schmucker 1942).

Acer canopies may suppress native vegetation by their dramatic effect on understory light quantity. In support of this, photosynthetically active radiation (PAR) was reduced by 95% beneath *A. platanoides* canopies (PAR=17.2± 4 $\mu\text{mol} \times \text{m}^{-2} \times \text{s}^{-1}$, 1 *SE*) in the heavily invaded site relative to the understory light levels from the site with the lowest level of *Acer* invasion (397.5± 47 $\mu\text{mol} \times \text{m}^{-2} \times \text{s}^{-1}$). If most native species are adapted to the much higher light levels that occur in native understories, and that are more typical of early- to mid-successional riparian communities, then deep shade may have devastating effects on mal-adapted natives (Baker 1949; Bazzaz 1979; Catovsky & Bazzaz 2000). In contrast, seedlings and saplings of *Acer* are exceptionally shade-tolerant (Niinemets 1997) which would allow them to survive in the shade of conspecific adults. However, *Acer* seedlings do not appear to just tolerate conspecific canopies. My experimental data indicate that *Acer* seedlings are facilitated by conspecific adults while *Populus* and *Pinus* seedlings are facilitated by *Populus* trees, the native dominant.

However, I do not know if there is a direct beneficial effect of conspecifics (possible canopy, soil, or humidity effects), or if seedlings benefited indirectly from the suppression of native species in the understory (see Levine (1999)). Other studies have indicated that *Acer* foliage produces water-soluble antifungal chemicals (Dix 1974), which may alter the soil mycorrhizae, pathogenic fungi, and decomposer fungi in ways that favor conspecific seedlings. Altered soilborne fungal communities also may play a role in the disruption of native riparian communities.

After initial introduction, non-native species often appear to undergo a lag phase where population growth and spread increases slowly (Kowarik 1995). After this lag phase population size may increase exponentially. The duration of these lag phases is variable, but lag periods can be very long depending on the life history traits of the individual species and the characteristics of the invaded environment. Average lag phases have been estimated at 131 years for shrubs and 170 years for trees that escaped from the Royal Botanical Gardens of the Netherlands (Kowarik 1995). Perhaps because of the long lag period, few invasions by large trees have been shown to have severe impacts (but see van Wilgen & Richardson 1985; Ewel 1986; Vitousek et al. 1987; Richardson 1998). Lag phases may result from the time required to attain sexual maturity, selection processes within the invading population, a slow breakdown in the resistance of the natural recipient community, or because invaders have slowly accelerating positive feedback effects on their environment. These mechanisms are by no means mutually exclusive. The positive relationship between the density of *Acer* seedlings and the age of *Acer* trees in the overstory suggests that a lag phase may occur for *Acer* between its establishment and rapid expansion.

The successful invasion of one species may lead to an “invasional meltdown” by facilitating the invasion of additional species (Simberloff & Von Holle 1999). This did not appear to be entirely true in my study because other non-natives, at least those that have made it to western Montana, were as highly suppressed by *Acer* as native species. My results; however, indicate that *Acer* is gradually modifying habitat in ways that favor itself and negatively impact native species, and this may open the door to gradual invasion by non-native species that have not yet arrived.

The highest level of *Acer* invasion was correlated with increased cover of *Acer*, and two native species; *Populus* and common scouring-rush (*Equisetum hyemale*). However, all of the *Populus* seedlings were actually resprouts from the roots of existing *Populus* trees (*personal observation*). Resprouts may not be a good indication of healthy recruitment. *Populus trichocarpa* naturally regenerates from seeds deposited on open alluvial bars (Reichenbacker 1984) and regeneration by resprouting may be a response to stress caused by *Acer* invasion. The greater abundance of *E. hyemale* may have been due to differences in the hydrology of the reaches on which the three stands occurred or direct facilitation by *Acer* and its effects on local hydrology.

In conclusion, I found a dramatic decline in the abundance of native species and non-native species associated with high levels of *Acer* invasion. Furthermore, *Acer* canopies enhanced the growth of experimentally transplanted conspecific seedlings and suppressed the growth of transplanted seedlings of native canopy trees. Collectively, these results are similar to those documented in the northeastern U.S. that reported a decrease in the richness of native species in the understory and an increased density of *A. platanoides* seedlings in invaded areas relative to non-invaded areas (Webb &

Kaunzinger 1993; Wyckoff & Webb 1996). The widespread cultivation of *Acer* in the northeastern U.S., eastern Canada (Dunster 1990), and northern Rocky Mountains (Nowak & Rowntree 1990) ensures that this species will continue to have many opportunities to escape and naturalize. It is likely that the threat of *Acer* will increase as invaded areas mature and alter habitats in ways that amplify both their negative effects on native species and positive effects on themselves.

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Table 1. Variation in percent cover of 15 most abundant species in relationship to levels (High [$n= 49$] or Low/Intermediate [$n= 100$]) of *Acer platanoides* invasion at different sites (mean \pm 1 SE).

	Dominant Species	High	Low and Intermediate	<i>P</i> -value
1	<i>Symphoricarpos albus</i> ¹	3.45 \pm 2.13	27.29 \pm 2.95	< 0.0005 *
2	<i>Acer platanoides</i> ²	65.86 \pm 4.37	2.04 \pm 0.64	< 0.0005 *
3	<i>Cynoglossum officinale</i> ²	0	9.30 \pm 1.64	< 0.0005 *
4	<i>Populus trichocarpa</i> ¹	2.10 \pm 0.60	1.01 \pm 0.24	0.095*
5	<i>Osmorhiza chilensis</i> ¹	2.35 \pm 2.04	2.18 \pm 0.50	0.917
6	<i>Elymus glaucus</i> ¹	0	13.46 \pm 2.83	< 0.0005 *
7	<i>Amelanchier alnifolia</i> ¹	0.04 \pm 0.04	1.47 \pm 0.36	< 0.0005 *
8	<i>Sorbus scopulina</i> ¹	0.22 \pm 0.17	0.90 \pm 0.21	0.015 *
9	<i>Galium triflorum</i> ¹	0	4.21 \pm 1.29	0.002 *
10	<i>Acer glabrum</i> ¹	0.14 \pm 0.10	1.10 \pm 0.31	0.004 *
11	<i>Rosa nutkana</i> ¹	1.27 \pm 1.27	0.95 \pm 0.27	0.147
12	<i>Prunus virginiana</i> ¹	0.16 \pm 0.13	1.20 \pm 0.61	0.097*
13	<i>Solanum dulcamara</i> ²	0.08 \pm 0.06	1.37 \pm 0.37	0.001 *
14	<i>Arctium lappa</i> ²	0	5.54 \pm 1.65	0.001 *
15	<i>Equisetum hyemale</i> ¹	10.57 \pm 3.82	4.51 \pm 1.83	0.157

Dominance based on the presence of species in each plot summing across all sites. Superscript 1= native species, 2= non-native species. Tests were performed using two sample *t*-test with homogeneity of variances either assumed (pooled-variances) or not assumed (separate-variances) with SPSS software (SPSS Inc., 1999 version 10). Asterisks (*) indicate *P*-values for *t*-tests that did not assume homogeneity of variances.

Figure 1. (A) Detrended Correspondence Analysis (DCA) comparing plots from riparian stands with three different levels of *Acer platanoides* invasion. Eigenvalues for axis 1= 0.856 and for axis 2= 0.545. Each symbol represents a single plot. Large symbols represent centroids for the smaller symbols of the same shape and show $\pm 95\%$ confidence intervals. The *Acer* label is the ordinated point for *Acer platanoides* across all quadrats. (B) Detrended Correspondence Analysis (DCA) of percent cover data from 1 m² plots relative to canopy cover above each quadrat (native vs. *Acer*). Plots with no canopy were removed from the analysis because they were too few to include (5 plots removed of 177 total plots). Eigenvalues for axis 1= 0.816 and axis 2= 0.528. Symbols, centroids, and abbreviation follow description provided in part A.

Figure 2. Non-native and native species richness in 1 m² plots sampled in stands with different levels of *Acer platanoides* invasion (low [n= 100] and high [n= 49]). *Acer* was included in these analyses as a non-native species. The stands with low and intermediate levels of *Acer* invasion were combined for presentation and analysis. There was a significant effect of the level of *A. platanoides* invasion on richness of native and non-native species ($P < 0.0005$) according to two sample *t*-test with homogeneity of variances not assumed (separate-variances) with SPSS software (SPSS Inc., 1999 version 10). Error bars represent $\pm 1 SE$.

Figure 3. Detrended Correspondence Analysis of 1 m² plots with and without *Acer platanoides* canopy cover. Plots in this analysis include 26 collected under randomly chosen *Acer* trees in the stand with an intermediate level of *Acer* invasion, and 48 plots

collected along randomly located transects in the same stand. Each symbol represents a single plot. Large symbols represent centroids for the smaller symbols of the same shape and show \pm 95% confidence intervals. Eigenvalues for axis 1= 0.6742 and for axis 2= 0.4441.

Figure 4. Regression relationships between the diameter at breast height (DBH) of *Acer platanoides* trees and (A) the Shannon-Wiener Index of Diversity and (B) species Richness in 1 m² plots beneath *Acer* trees.

Figure 5. Regression relationship between the age of nearest *Acer platanoides* tree and the density of *Acer* seedlings in 1 m² plots beneath *Acer* trees.

Figure 6. Effect of overstory canopy type on transplanted seedlings. (A) Change in leaf or needle biomass of three species (*Acer platanoides*, *Pinus ponderosa*, and *Populus trichocarpa*) of understory seedlings planted beneath either native, *Populus* or non-native canopies, *Acer*. (B) Change in height of three species of understory seedlings planted beneath either native, *Populus* or non-native canopies, *Acer*. Asterisks (*, **, and ***) indicate a significant ($P < 0.05$, $P < 0.005$, and $P < 0.0005$, respectively) difference and “ns” indicates a statistically insignificant ($P > 0.05$) difference between canopy species according to *t*-test with homogeneity of variances not assumed (separate-variances) with SPSS software (SPSS Inc., 1999 version 10). Error bars represent \pm 1 SE.

Fig. 1A & B

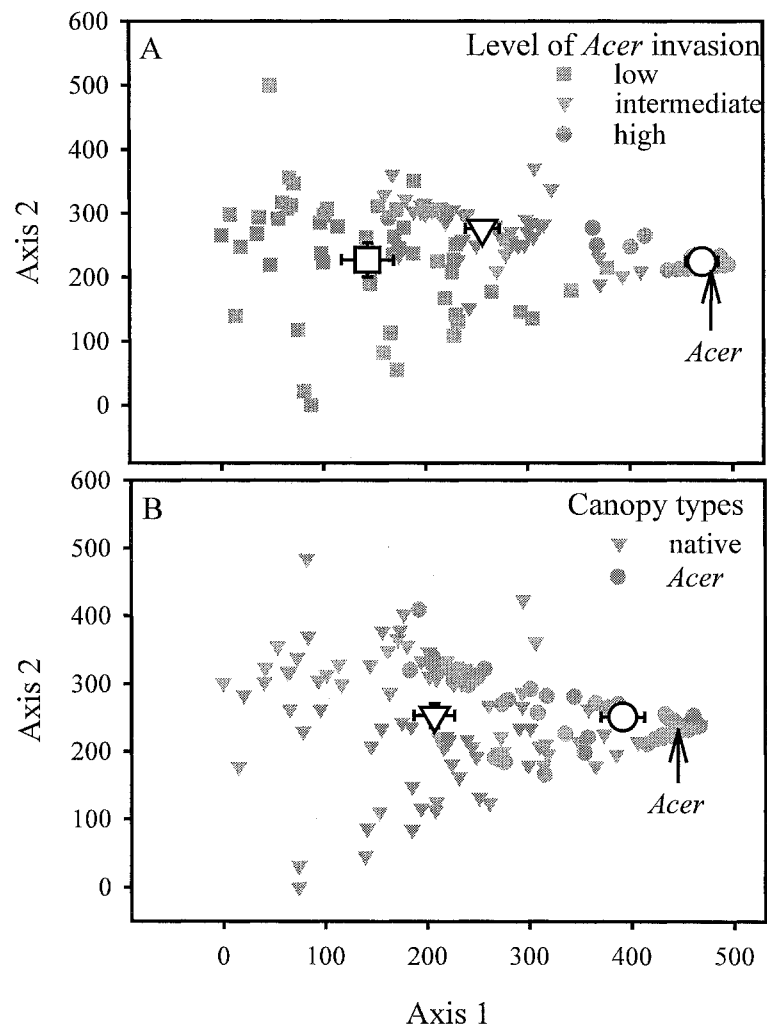


Fig. 2

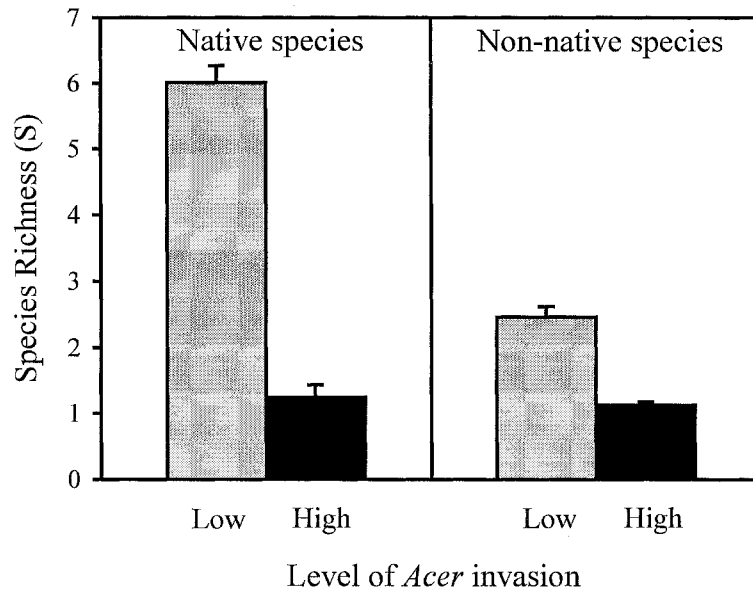


Fig. 3

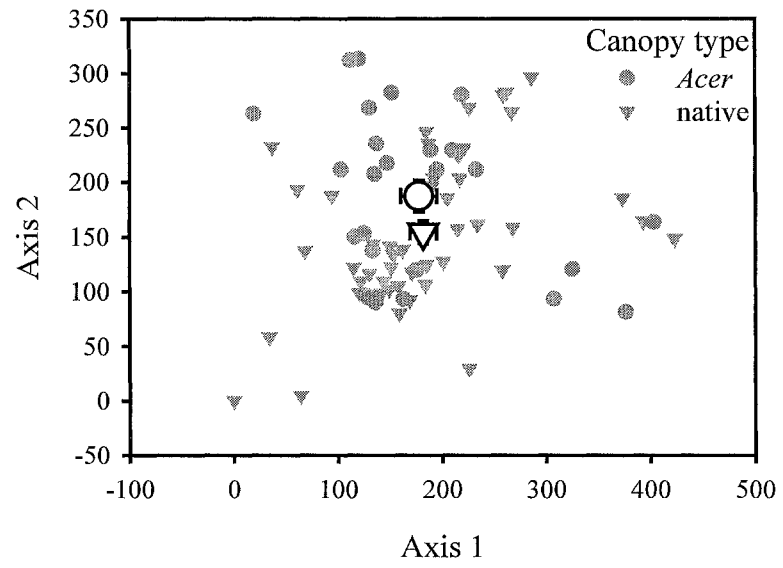


Fig. 4

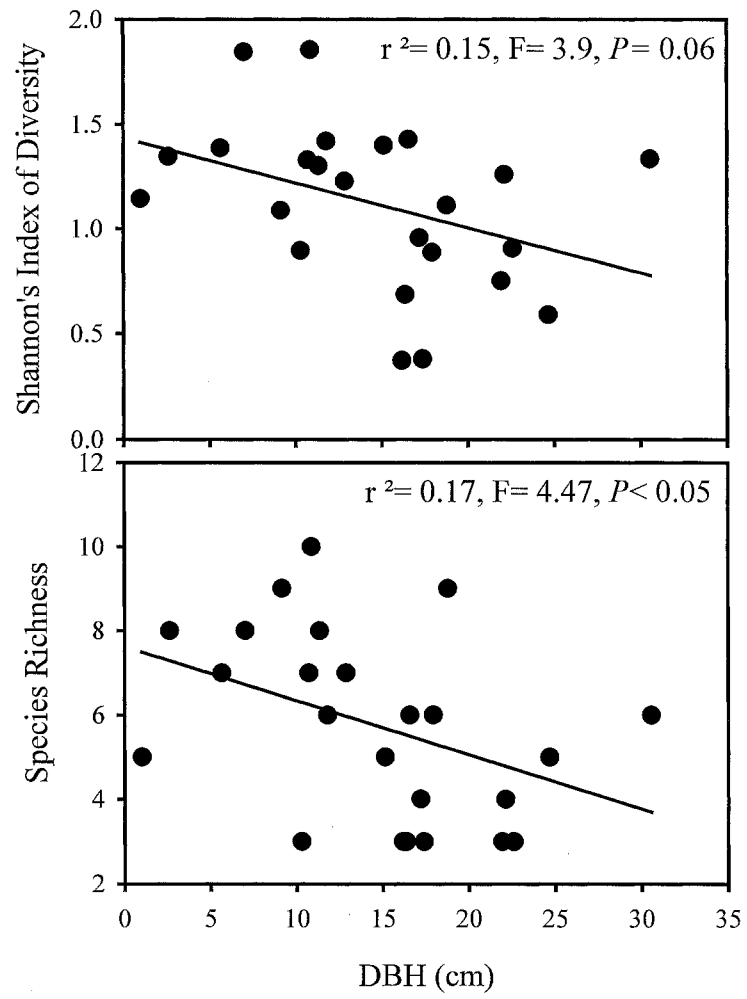


Fig. 5

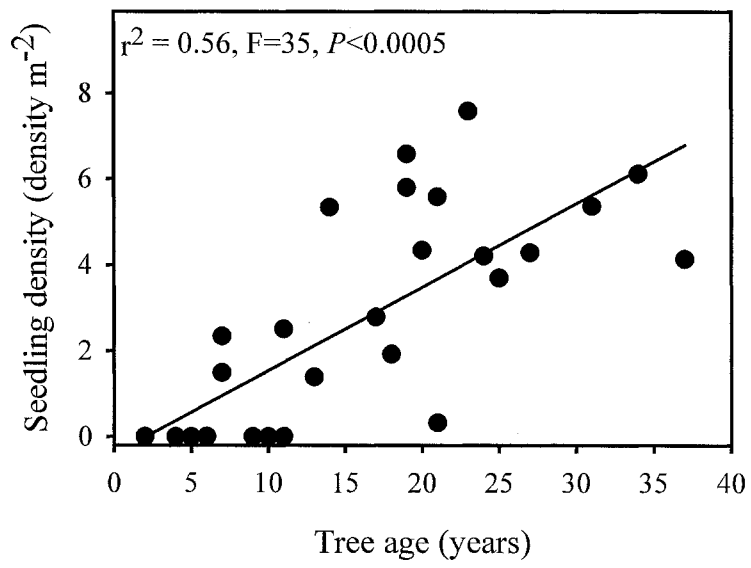
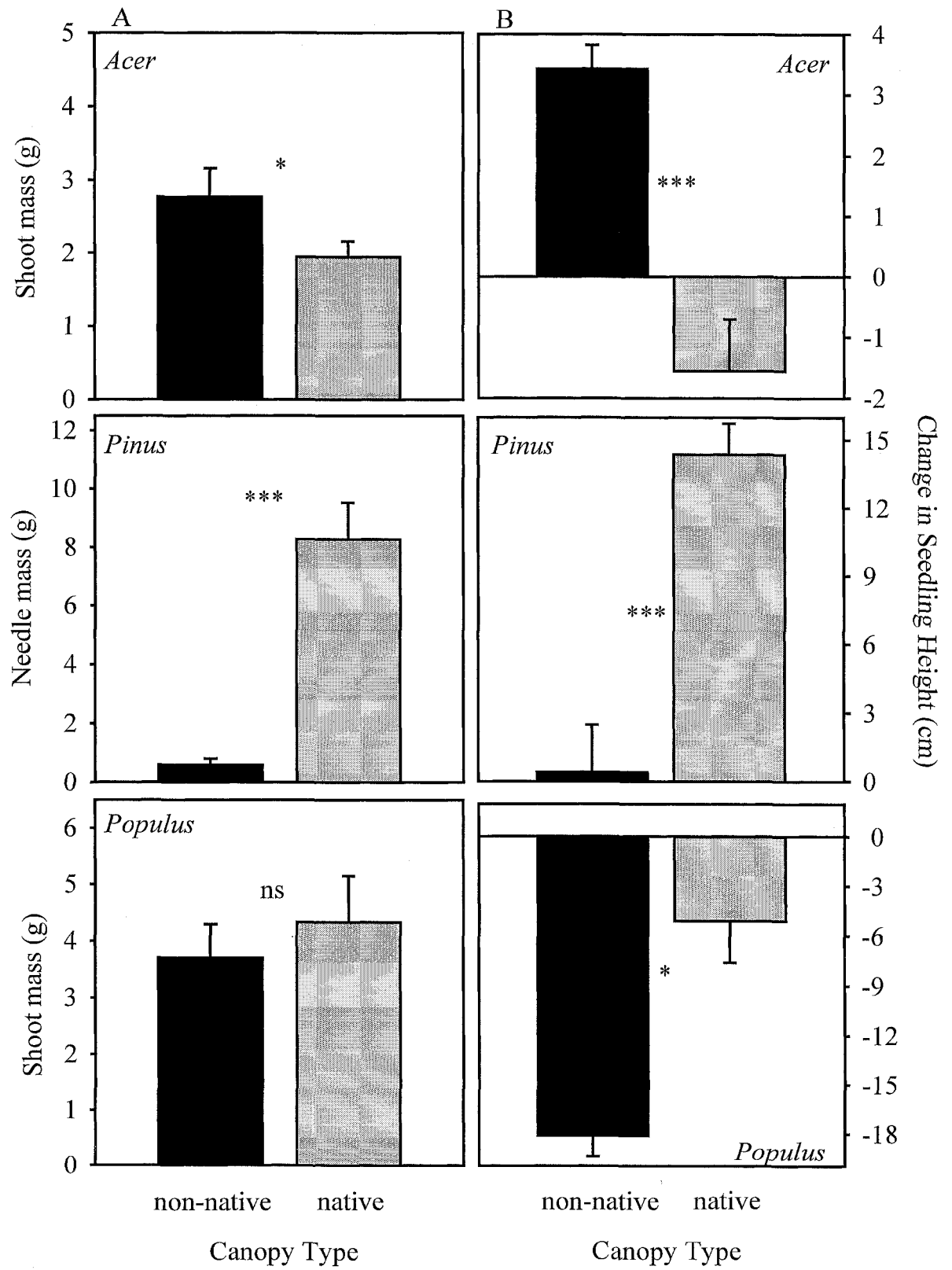


Fig. 6



App. 1 Species list of the understory community composition from Rattlesnake Creek, MT

<i>Acer glabrum</i> ¹	<i>Dryopteris expansa</i> ¹	<i>Ranunculus macounii</i> ¹
<i>Acer platanoides</i> ²	<i>Elymus glaucus</i> ¹	<i>Ranunculus uncinatus</i> ¹
<i>Achillea millefolium</i> ¹	<i>Elymus spp.</i> ¹	<i>Rhamnus cathartica</i> ²
<i>Agastache urticifolia</i> ¹	<i>Epilobium spp.</i> ¹	<i>Rhamnus spp.</i> ¹
<i>Agropyron repens</i> ²	<i>Equisetum arvense</i> ¹	<i>Ribes klamathense</i> ¹
<i>Agrostis alba</i> ²	<i>Equisetum hyemale</i> ¹	<i>Ribes oxycanthoides</i> ¹
<i>Allium cernuum</i> ¹	<i>Erigeron speciosus</i> ¹	<i>Rosa nutkana</i> ¹
<i>Alnus incana</i> ¹	<i>Fragaria virginiana</i> ¹	<i>Rubus idaeus</i> ¹
<i>Amelanchier alnifolia</i> ¹	<i>Galium boreale</i> ¹	<i>Rubus parviflorus</i> ¹
<i>Arctium lappa</i> ²	<i>Galium triflorum</i> ¹	<i>Rumex obtusifolius</i> ²
<i>Arctostaphylos uva-ursi</i> ¹	<i>Geranium robertianum</i> ²	<i>Rumex pulcher</i> ²
<i>Aster laevis</i> ¹	<i>Geum macrocarpum</i> ¹	<i>Salix spp.</i> ¹
<i>Aster occidentalis</i> ¹	<i>Heracleum lanatum</i> ¹	<i>Sambucus cerulea</i> ¹
<i>Berberis aquifolium</i> ¹	<i>Hypericum perforatum</i> ²	<i>Saxifrage arguta</i> ¹
<i>Berberis repens</i> ¹	<i>Leonurus cardiaca</i> ²	<i>Senecio triangularis</i> ¹
<i>Betula occidentalis</i> ¹	<i>Lonicera spp.</i> ¹	<i>Silene menziesii</i> ¹
<i>Bromus inermis</i> ²	<i>Lychnis alba</i> ²	<i>Solanum dulcamara</i> ²
<i>Bryonia alba</i> ²	<i>Mentha arvensis</i> ¹	<i>Solidago canadensis</i> ¹
<i>Calamagrostis inexpansa</i> ¹	<i>Mitella nuda</i> ¹	<i>Sorbus scopulina</i> ¹
<i>Calamagrostis spp.</i> ¹	<i>Myosotis scorpioides</i> ²	<i>Spiraea betulifolia</i> ¹
<i>Carex spp.</i> ¹	<i>Nepeta cataria</i> ²	<i>Streptopus spp.</i> ¹
<i>Centaurea maculosa</i> ²	<i>Osmorhiza chilensis</i> ¹	<i>Symphoricarpos albus</i> ¹
<i>Cerastium vulgatum</i> ¹	<i>Philadelphus lewisii</i> ¹	<i>Tanacetum spp.</i> ¹
<i>Chenopodium alba</i> ²	<i>Phleum pratense</i> ²	<i>Taraxacum officinale</i> ²
<i>Chenopodium spp.</i> ¹	<i>Physocarpus malvaceus</i> ¹	<i>Thalictrum occidentale</i> ¹
<i>Cirsium arvense</i> ²	<i>Pinus ponderosa</i> ¹	<i>Trifolium pratense</i> ²
<i>Cirsium vulgare</i> ²	<i>Poa spp.</i> ¹	<i>Trifolium repens</i> ²
<i>Clematis columbiana</i> ¹	<i>Populus tremuloides</i> ¹	<i>Urtica dioica</i> ¹
<i>Clematis ligusticifolia</i> ¹	<i>Populus trichocarpa</i> ¹	<i>Verbascum thapsus</i> ²
<i>Cornus stolonifera</i> ¹	<i>Prunella vulgaris</i> ²	<i>Viburnum opulus</i> ¹
<i>Crataegus douglasii</i> ¹	<i>Prunus cerasus</i> ²	<i>Viburnum spp.</i> ¹
<i>Crysanthium lycanthum</i> ¹	<i>Prunus mahaleb</i> ²	<i>Viola glabella</i> ¹
<i>Cynoglossum officinale</i> ²	<i>Prunus virginiana</i> ¹	<i>Viola spp.</i> ¹
<i>Dactylis glomerata</i> ²	<i>Pyrola asarifolia</i> ¹	
<i>Disporum trachycarpum</i> ¹	<i>Pyrus spp.</i> ²	

Superscript 1= native species, 2= nonnative species, according to the Montana Natural Heritage Association

Chapter 2

Facilitation by conspecifics as a mechanism for *Acer platanoides* invasion

Abstract

Facilitative interactions between plants are an important mechanism that organizes natural plant communities. However, the role of this process in plant invasions is less clear. I hypothesize that invading *Acer platanoides* seedlings are facilitated by overstory conspecifics. I tested this hypothesis with demographic analyses and identify facilitative mechanisms by comparing interactions in an invaded patch to an adjacent natural forest dominated by *Pseudotsuga menziesii*. *Acer platanoides* seedlings had higher densities, recruitment, and survival, and experienced less photoinhibition and water stress in the conspecific patch than in the adjacent *P. menziesii* forest. Soil moisture and canopy cover were greater within the invaded patch than the native forest. There was no difference in soil fertility or understory light levels between locations. Substantial demographic, physiological, and environmental differences between sites appeared to be due to ecosystem engineering by mature *A. platanoides*. *Acer* produces a more mesic environment by modifying the structure and phenology of the forest canopy and by altering the timing of transpirational water loss. Environmental modification by invaders that lead to positive effects on conspecifics may help us to understand the dramatic success of some invasive species.

Keywords: *Pseudotsuga menziesii*, ecosystem-level changes, ecosystem engineer, invasion resistance

Introduction

Biological invasions are a serious threat to natural systems (Vitousek *et al.* 1996; Usher 1988). Invasive non-native plants may reduce the diversity of native species, disrupt nutrient and hydrologic cycles, and modify the disturbance regimes and geomorphology of invaded ecosystems (Pimentel *et al.* 2000; Mack *et al.* 2000; Gordon 1999). Therefore, understanding the mechanisms underlying biological invasions is crucial to ecology and conservation biology and will contribute to the management and restoration of invaded and natural ecosystems (Mack *et al.* 2000).

Successful invasion by non-native species is linked to the functional traits of invaders and the invasibility of the native plant community (Mack *et al.* 2000). The absence of natural enemies such as herbivores and pathogens (Keane & Crawley 2002), fluctuating resources (Davis *et al.* 2000), lack of resistance to particular root exudates (Callaway & Aschehoug 2000), and empty niches (Elton 1958) have been proposed to affect the invasibility of communities. Ecosystem-scale factors such as anthropogenic or stochastic forms of disturbance (Hobbs & Huenneke 1992) may also contribute to the success of invaders. Positive interactions between plants are common in natural plant communities (Callaway 1995 and 1998) and recently facilitative interactions among non-native species have been proposed as important drivers of invasion (Simberloff & Von Holle 1999). In some cases, native species may facilitate the invasion of non-native species (Maron & Connors 1996); however, there have been few empirical studies demonstrating conspecific or heterospecific facilitation by an invader as a process affecting the invasion of plant communities.

Recently, Crooks (2002) proposed that invasive species with ecosystem engineering properties may dramatically affect community- and ecosystem-level processes, especially when these properties are novel to the resident native and other non-native species. Ecosystem engineers are species that change the physical environment, produce nonfood resources (e.g. trees create habitat for epiphytes), or control the availability of abiotic resources (Jones *et al.* 1997). Trees are good examples of ecosystem engineers, because they provide habitat for arboreal and epiphytic organisms and alter the availability of water (Aston 1979), light (Seiwa 1998; Canham *et al.* 1994) and nutrients (Clark *et al.* 1998; Hoelscher *et al.* 1998) to organisms located beneath their canopy. Previous studies have emphasized ecosystem engineers within the context of natural systems (Jones *et al.* 1997), but their potentially important role in biological invasions is still poorly understood (Crooks 2002).

Acer platanoides (Linn.) is a late-successional tree naturally found in mesic deciduous forests throughout much of Eurasia (Schmucker 1942). It has invaded mixed-deciduous forests within riparian bottomland (E. Greene, unpublished data) and coniferous forest in Montana and Idaho (K.O. Reinhart, unpublished data) as well as deciduous hardwood forests throughout the northeastern U.S. (Nowak & Rowntree 1990) and eastern Canada (Dunster 1990). *Acer platanoides* invasion in the northern Rocky Mountains appears to be low at the present time. However, virtual monocultures of the invader have been described in a riparian floodplain, and models indicate that its spread will be very rapid (E. Greene, unpublished data).

Invaders with unique structural and functional attributes relative to resident native species are predicted to have a stronger impact on the composition, succession, and

productivity of the invaded community than invaders that are similar to the native plant species (Levine & D'Antonio 1999). Within deciduous hardwood forests of eastern North America, *A. platanoides* appears to be functionally similar to two native species, *Acer saccharum* (Marsh.) and *Fagus grandifolia* (Ehrb.) (Delcourt & Delcourt 2000; Lei & Lechowicz 1997). In contrast, in the northern Rocky Mountain region it represents a functionally unique species within relatively mesic evergreen coniferous forests dominated by *Pseudotsuga menziesii* (Mirbel) Franco and *Thuja plicata* (Donn.) (Peet 2000).

Here I evaluate the facilitative effects of *A. platanoides* on conspecifics through its function as an ecosystem engineer in a native *P. menziesii* forest. Based upon general observations, I hypothesized that *A. platanoides* seedlings are facilitated by a conspecific overstory, and that these seedlings will have increased survival, growth, and recruitment within their parental patch as compared to the native forest. To achieve my objective, I tested the effect of different forest types (*A. platanoides* vs. *P. menziesii*) on the demographic patterns and ecophysiology of *A. platanoides* seedlings.

Methods

Site Description

My study site is located ~600 m NE of Bonner, Montana (N46° 52.497' W113° 51.460') at an elevation of ~1030 m. The site is located near the bottom of a hillside with a north-facing slope of 25-32°. The average annual precipitation is 340 mm, with 140 mm falling from May to August, and the mean annual temperature is 6.7° C. In 2000, the initial year of this study, the region experienced a summer drought and received only 71 mm of precipitation from May to August. In the following years, the area received 153 and 144 mm of summer precipitation in 2001 and 2002, respectively. Soils are deep, well drained gravely loam (USDA 1995). The natural community is dominated by *P. menziesii*, with mixed *Acer glabrum* (Torr.) and *Betula occidentalis* (Hook.) trees, and shrub species such as *Amelanchier alnifolia* (Nutt.) and *Symphoricarpos albus* (Hook.) are common in the understory. Two patches of invading *A. platanoides* were identified at the study site with 15 and 3 reproductive *A. platanoides* individuals in the canopy. The majority of my research was conducted in the large patch of *A. platanoides* and the adjacent *P. menziesii* forest to the west, which had no *A. platanoides* trees. The “Soil experiment” described below was the only experiment that utilized both patches of *A. platanoides*. There were no obvious differences in either landscape positioning or geomorphology between the large invaded patch and the adjacent *P. menziesii* forest.

Seedling Demography

In July of 2000, I established a 42 m transect starting in the large *A. platanoides* patch and extending into the *P. menziesii* forest, this area was without any *A. platanoides*

trees. Eight 15-m parallel transects, separated by 6 m, were run perpendicular to and down slope from the 42 m transect. The first transect was placed within the large patch, the second was along the interface between the patch and the intact forest, and the remaining six transects were placed perpendicular to the patch and extended into the *P. menziesii* forest. Five 1-m² permanent plots were randomly placed along each of the 15 m transects. All *A. platanooides* seedlings within each plot were counted and tagged. In June, 2001 and 2002, I measured survival of tagged seedlings and counted and tagged new seedlings. From these data, I estimated total seedling density for all three years and quantified survival and recruitment of seedlings between the following years; 2000-2001 and 2001-2002. The effect of distance from the *A. platanooides* patch on total seedling density was analyzed with a repeated measures ANOVA, distance and time were factors. The data were transformed with a square-root function ($\sqrt{[X + 0.5]}$) to maintain equality of the covariance matrix. The effect of distance from the *A. platanooides* patch on survival and seedling density within individual years was analyzed with the nonparametric Kruskal Wallis test because of heterogeneous variances.

Seedling Physiology

I compared the physiology of *A. platanooides* seedlings within the conspecific patch to those in neighboring *P. menziesii* forest. Seven random points were selected at each location, and five *A. platanooides* seedlings were selected in close proximity to each point. I randomly selected one seedling out of five from each of seven points ($n= 7$) within each location on July 11 and August 2 and 17, 2000. On each sampling date, I recorded water status and chlorophyll fluorescence of selected seedlings. These measures

provided estimates of water stress and photosystem stress. Predawn and midday (14:00 h MST) water potential was measured in situ on small leaves using a pressure bomb (PMS Instrument Co., Corvallis, OR, USA). Chlorophyll fluorescence was measured by using a portable, pulse-modulated fluorometer (OS-100, Opti-Sciences Inc., Tyngsboro, MA, USA). Predawn and midday (14:30 h MST) measures of maximal (F_m) and minimal (F_0) fluorescence were used to calculate maximum efficiency of the photosynthetic energy conversion of PSII ($F_v/F_m = [F_m - F_0] / F_m$). Predawn measurements were performed before sunrise; midday measurements were made after 30-min dark adaptation. I tested the effect of forest type on F_v/F_m and leaf water potentials of *A. platanooides* seedlings with Two-way ANOVA (Location and Sampling date), with both factors being fixed. To maintain homogeneity of variances for these analyses, I transformed predawn F_v/F_m data with a power function (x^2), midday F_v/F_m data with a power function (x^3), and midday and predawn water potentials with a natural log function ($\ln x$). Even after transformation, the predawn water potential and the midday F_v/F_m data continued to violate the homogeneity of variances assumption, but ANOVA was still used for the analysis and was validated with separate Mann-Whitney U tests for each sampling date.

Canopy Characteristics

I accompanied the midday fluorescence measurements with measurements of photosynthetically active radiation (PAR) within the understory ($n=7$) during cloudless conditions using a light meter and quantum sensor (LI-250, LI-COR, Lincoln, NE, USA). I also characterized the canopy cover of the *A. platanooides* patch ($n=10$) and of the neighboring *P. menziesii* forest ($n=15$) by measuring leaf area index (LAI), with a LAI-

2000 Plant Canopy Analyzer (LI-COR, Lincoln, NE, USA). LAI measurements were taken from random points on September 6, 2001 during overcast conditions. Canopy measurements were compared with a reference location that was located in an open area at the edge of a cliff. Measurements were taken 1 m above the ground, and a 45° view cap was placed on the sensor head to prevent the silhouette of the handler from exaggerating the canopy measurements. I tested the effect of forest type on LAI and PAR with two sample t-tests.

Soil moisture

Gravimetric soil moisture was measured on August 2 and 17, 2000. I collected soil at midday from random points within the invaded patch and native forest ($n=10$). I cored soil to a depth of 10 cm and used the -5 to -10 cm portion of the core for moisture analysis. Differences between forest type (*A. platanooides* vs. *P. menziesii*) were tested with Two-way ANOVA (Location and Sampling date). Data were transformed with a log function ($\log [x+1]$) prior to analysis to maintain homogenous variances.

During 2001, I measured the soil moisture within the two forest types (*A. platanooides* [$n=6$] and *P. menziesii* [$n=15$]) using frequency domain reflectometry. Measurements were taken on May 16, June 19, and July 25, with the Sentry 200-AP soil moisture probe (Troxler Electronic Laboratories, Inc., Research Triangle Park, NC, USA) at three depths (-10, -20, and -30 cm from the surface). The soil moisture probe was used to sample repeatedly through permanently installed PVC pipes that were randomly located throughout the site in 2001. The effect of forest type on soil moisture was analyzed with a repeated measures ANOVA, location and time were factors. Analyses

were performed separately for each sampling depth. Prior to these analyses, the data at -20 and -30 cm were transformed with a reciprocal function ($1/[x+1]$) to maintain equality of the covariance matrix.

Soil Experiment

In this experiment, I evaluated the effect of soil from the two forest types (*A. platanoides* and *P. menziesii*) and two sites (two *A. platanoides* patches and adjacent *P. menziesii* forest) on the growth of *A. platanoides* seedlings. On August 27, 2001, I collected field soil (0–10 cm in depth) from 6 and 4 randomly selected points within the large and small *A. platanoides* patches, respectively. Soil was also collected from 6 and 4 randomly selected points within the *P. menziesii* forest adjacent to the large and small *A. platanoides* patches, respectively. The soil was then mixed to a 1:1 ratio with commercial silica sand averaging 0.60–0.85 mm in diameter (20/30 grit, Lane, MT, USA), and this mixture was used to fill individual plastic pots (16.5 cm wide and 17.5 cm tall). *Acer platanoides* seedlings of the same approximate size, which germinated in 2001, were collected from the large patch on August 30, 2001. Immediately after field collection, seedling roots were surface sterilized to eliminate any preexisting soil biota on the outside of the roots. However, I expect that surface sterilization only killed organisms growing on the outside of the roots and probably did not eliminate arbuscular mycorrhiza, which were present throughout the root cortical cells. This sterilization treatment, although incomplete, has identified soil biota effects in other experiments with *A. platanoides* (Reinhart & Callaway in review). Surface sterilization was accomplished by placing the seedling roots in a 5% solution of commercial NaOCl for 10 minutes

followed by rinsing with deionized water. After root sterilization, one *A. platanoides* seedling was planted into each pot. The initial basal diameter, leaf number, and stem height of each seedling was measured. Plants were kept in the University of Montana greenhouse and watered 3-4 times a week. Supplemental light was added to prevent senescence in the autumn and winter. After nine months in the greenhouse, each seedling was measured for final basal diameter, leaf number, and height, and the plants were harvested thereafter and divided into roots, stems, and leaves. All harvested material was dried at 65°C until constant weight. The effect of soil location on the total biomass, root mass ratio (root biomass: total biomass), and the relative change in seedling height ($[(\text{final height} - \text{initial height}) / \text{initial height}] * 100$) were measured with Two-way ANOVA (soil type and site).

Results

The density of *A. platanoides* seedlings was ≥ 3 times higher within or near the parental patch than away from the patch (Fig. 1a; repeated measures ANOVA [ANOVAR], Location $F= 83.2$, $df= 7$, $P < 0.0005$). There was a significant effect of sampling year on total seedling density (ANOVAR, Time, $F= 60.3$, $df= 2$, $P < 0.0005$). I also found ≥ 3.3 times higher seedling recruitment within the patch than away from the patch (Fig. 1b; Kruskal Wallis Test, Distance₂₀₀₁, $\chi^2= 32.6$, $df= 3$, $P < 0.0005$; Distance₂₀₀₂, $\chi^2= 23.3$, $df= 3$, $P= 0.002$). Seedling survival was ≥ 2.8 times higher within the conspecific patch than away from it (Fig. 1c; Kruskal Wallis Test, Distance₂₀₀₀₋₂₀₀₁, $\chi^2= 26.3$, $df= 7$, $P < 0.0005$; Distance₂₀₀₁₋₂₀₀₂, $\chi^2= 20.8$, $df= 6$, $P= 0.002$).

I found a strong effect of the forest type on the physiological performance of *A. platanoides* seedlings (Fig. 2). Seedlings within the conspecific patch had greater water potentials (-0.75 vs. -1.85 MPa predawn mean values [$P < 0.0005$] and -1.11 vs. -2.22 midday [$P < 0.0005$] and F_v/F_m (0.74 vs. 0.61 predawn [$P < 0.0005$] and 0.71 vs. 0.49 midday mean values [$P < 0.0005$]) than seedlings within the *P. menziesii* forest at both predawn and midday.

Canopy cover (LAI) was 55% greater in the invaded patch than in the adjacent native forest (Fig. 3; two sample t-test, $t= 6.44$, $df= 23$, $P < 0.0005$). These structural differences did not correspond with a difference in understory light quantity ($t= 1.07$, $df= 40$, $P= 0.292$).

Soil moisture was also different between forest types. Soil moisture was higher in the *A. platanoides* patch (12.7 ± 1.3 and 11.0 ± 0.7 ; ± 1 SE) than in the *P. menziesii* forest (5.1 ± 0.4 and 6.7 ± 0.9) on August 2 and 17, 2000, respectively (ANOVAR, location, $F=$

59.2, $df= 1$, $P < 0.0005$). In 2001, soil moisture was also greater in the *A. platanoides* patch at various depths within the soil profile than in the *P. menziesii* forest (Table 1 and Fig. 4). Soil moisture varied over time and increased at all depths and locations after the site received several cm of snow (Fig. 4).

Soil from the different forest types did not differ in their effect on *A. platanoides* seedling growth. There was no effect of forest type on total biomass, root mass ratio, or relative change in seedling height (Table 2).

Discussion

Acer platanoides seedlings had higher survival and experienced less photoinhibition and water stress within the conspecific patch than in the adjacent *Pseudotsuga menziesii* forest. Thus, the patch of *A. platanoides* trees appeared to facilitate conspecific seedlings within the natural coniferous community. *Acer platanoides* also had greater density (Fig. 1a) and recruitment (Fig. 1b) of seedlings within the *Acer* patch than the adjacent *Pseudotsuga* forest suggesting that recruitment limitation is slowing the spread of *Acer*. These results suggest that facilitation and recruitment limitation may affect the invasion of *Acer*. My results agree with a study suggesting adult *A. platanoides* facilitate the recruitment of conspecifics in deciduous forests in the northeastern U.S. (Wyckoff & Webb 1996), and provide a mechanistic understanding of this facilitation.

Increased performance of *A. platanoides* seedlings was closely related to changes in forest structure and environmental conditions promoted by conspecific adults, which were primarily increased soil moisture in the *A. platanoides* patch relative to the *P. menziesii*-dominated forest. These differences may be confounded by subtle differences in geomorphology and landscape position and the lack of replication; however, my general observation of a homogeneous landscape suggests that environmental changes were due to modification by the invading *A. platanoides*.

Pseudotsuga menziesii is physiologically active earlier in the spring (Lewis *et al.* 1999) than *A. platanoides*, which must produce leaves before transpirational water loss is possible. Full-size leaves are not developed until mid- to late-May in Montana. Thus, the initial differences in soil moisture between locations in mid-May of 2001 appear to be

due to early transpiration by *P. menziesii*. Invasive plants with different phenologies than natives have been shown to impact hydrology in other ecosystems (Mueller-Dombois 1973). Differences in soil moisture may also have been due to the reduction of water infiltration into the soil by evergreen canopy of *P. menziesii* intercepting greater amounts of winter and spring precipitation than the deciduous *A. platanoides* canopy (see Iroumé & Huder 2002). *Pseudotsuga menziesii* is also known to negatively effect the water use and growth of other native conifer species by aggressively using water resources and reducing the moisture content of the soil (A. Sala unpublished data). Additional research is necessary to identify functional differences in water use by *Acer* and *Pseudotsuga* where they co-occur.

During the growing season, *A. platanoides* produced greater canopy cover than *P. menziesii*. In contrast, I did not detect a difference in understory light quantity between forest types, which may have resulted from low sample size and high variability in PAR measurements associated with the mixture of sun flecks and deep shade. Increased shade may reduce leaf temperatures, transpiration, and light damage (see Shumway 2000; Egerton *et al.* 2000), and shade produced by the overstory may benefit other plants by reducing evaporation rates. Canopies may intercept precipitation and reduce the amount of water reaching the understory; however, many understories are often more moist than neighboring open areas (McLeod & Murphy 1977; Ko & Reich 1993; Belsky & Amundson 1989). This canopy effect may result from decreased evaporation rates or through hydraulic lift through the roots of plants (Horton & Hart 1998). Hydraulic lift is the passive movement of water via the roots into dry soil, while other parts of the root system in moist soil and often at depth absorb water (Caldwell & Richards 1989). *Acer*

saccharum facilitates neighbors during drought periods by increasing the soil moisture by hydraulically lifting water (Dawson 1993); however, *P. menziesii* also hydraulically lifts water (Brooks *et al.* 2002).

My results indicate that the effect of the different overstory trees on soil neither facilitates nor inhibits invasion of *A. platanooides*. Research in the northeastern U.S. also found no effect of soil from invaded and uninvaded sites on the growth of conspecifics (T.G. Howard unpublished data). Thus, hydrologic processes, rather than soil fertility, appear to be the primary factors affecting the invasion of *A. platanooides* in upland ecosystems in the northern Rocky Mountains.

In conclusion, my results suggest that *A. platanooides* facilitates conspecific seedlings by creating a more mesic environment within upland coniferous forest. *Pseudotsuga menziesii* forests appear to be relatively resistant to invasion by *A. platanooides*, and the primary limiting resource for *A. platanooides* invasion is water. Natural and anthropogenic gaps, or unusually wet years, may initiate colonization of *A. platanooides* in conifer forests and modification of the physical environment by colonists may start a form of “invasional meltdown” (Simberloff and Von Holle 1999) in which positive feedback between invader and environment drives exponential population increase as described for many successful exotic species.

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Table 1. Summary of repeated measures ANOVA with the effect of forest location (*Acer platanoides* vs. *Pseudotsuga menziesii* forest) and sampling times on the soil moisture at three different depths.

Soil depth	Dependent variables	df	F	P
-10 cm	Location	1	30.85	< 0.0005
	Location × Time	2	0.27	0.765
-20 cm	Location	1	16.40	0.001
	Location × Time	2	5.16	0.011
-30 cm	Location	1	6.87	0.019
	Location × Time	2	3.04	0.062

Tests were performed using the Type three sums of squares from SPSS version 10 (SPSS Inc., Chicago, IL, USA). Between-subject effects of the model tested Location, and within-subject effects of the model tested Location × Time. Data were transformed to maintain equality of covariance matrix according to Mauchly's Test of Sphericity.

Table 2. Summary of Two-way ANOVA of effects of soil source (*Acer platanoides* vs. *Pseudotsuga menziesii*) and site on the total biomass, root mass ratio (root mass \times total biomass⁻¹), and relative change in height of *A. platanoides* seedlings.

Dependent variable	Factor	df	MS	F	P
Total biomass	Soil	1	2.86	0.16	0.69
	Site	1	2.97	0.17	0.69
	Soil \times Site	1	20.50	1.18	0.29
	Error	16	17.42		
Root mass ratio	Soil	1	0.0005	0.11	0.74
	Site	1	0.0087	1.91	0.19
	Soil \times Site	1	0.0029	0.63	0.44
	Error	16	0.0046		
Relative change in height	Soil	1	10,113.0	0.97	0.34
	Site	1	54.0	0.01	0.94
	Soil \times Site	1	9,440.0	0.90	0.36
	Error	16	10,476.0		

Tests were performed using the Type three sums of squares from SPSS version 10 (SPSS Inc., Chicago, IL, USA).

Figure 1. Total density (A), recruitment (B), and survival (C) of *Acer platanoides* seedlings with increasing distance from the parental patch. The effect of distance from the patch on seedling densities per plot over three years was tested using repeated measures ANOVA with sphericity assumed. Effect of distance from the *Acer* patch on the recruitment and survival for separate years was tested with Kruskal Wallis test. Error bars represent 1 *SE* ($n= 5$ at each distance).

Figure 2. Effect of location on leaf water potential and maximum efficiency of photosystem II (F_v/F_m) of *Acer platanoides* seedlings ($n= 42$) during summer 2000. Seedlings were located either within a patch invaded by *Acer* (gray circles) or within the neighboring *Pseudotsuga menziesii* forest (black circles). Error bars represent 1 *SE* ($n= 7$ per treatment). Predawn water potentials and midday F_v/F_m data were transformed to maintain homogeneity of variances. Transformed data continued to violate the homogeneity of variances assumption, but ANOVA was still used for the analysis and was validated with separate Mann-Whitney U tests for each sampling date. Predawn water potential (Mann-Whitney U tests, $P \leq 0.005$) and midday F_v/F_m ($P \leq 0.01$) data differed significantly between forest types.

Figure 3. Effect of forest type (invasive *Acer platanoides* vs. native *Pseudotsuga menziesii*) on photosynthetically active radiation (PAR) in the understory and leaf area index (LAI). PAR did not differ between forest types (two sample t-test, $t= 1.07$, $df= 40$, $P= 0.292$), but LAI was greater in the invaded patch than the native forest ($t= 6.44$, $df= 23$, $P < 0.0005$). Error bars represent 1 *SE*.

Figure 4. Effect of forest type (invaded patch of *Acer platanoides* [$n= 6$] vs. native forest of *Pseudotsuga menziesii* [$n= 15$]) on soil moisture on different days and at different depths below the soil surface. Error bars represent 1 *SE*.

Fig. 1

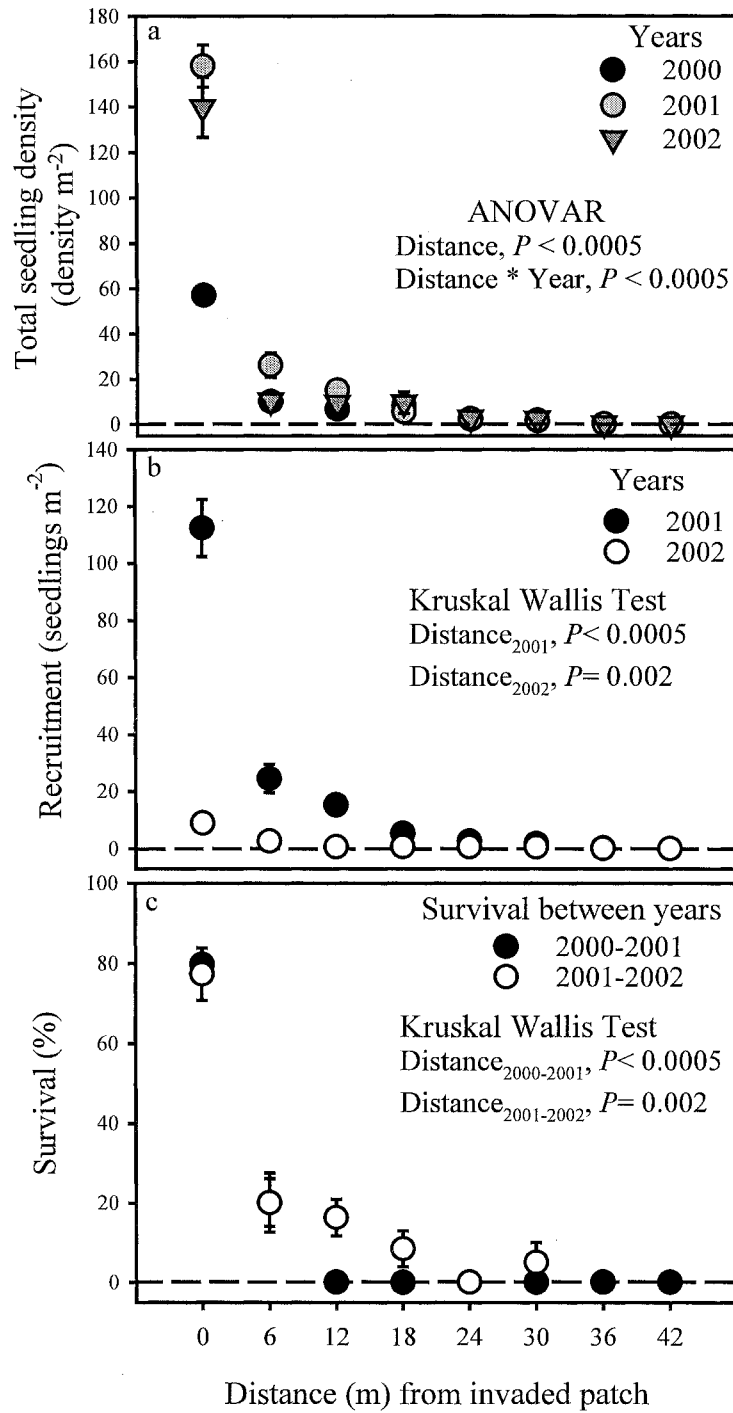


Fig. 2

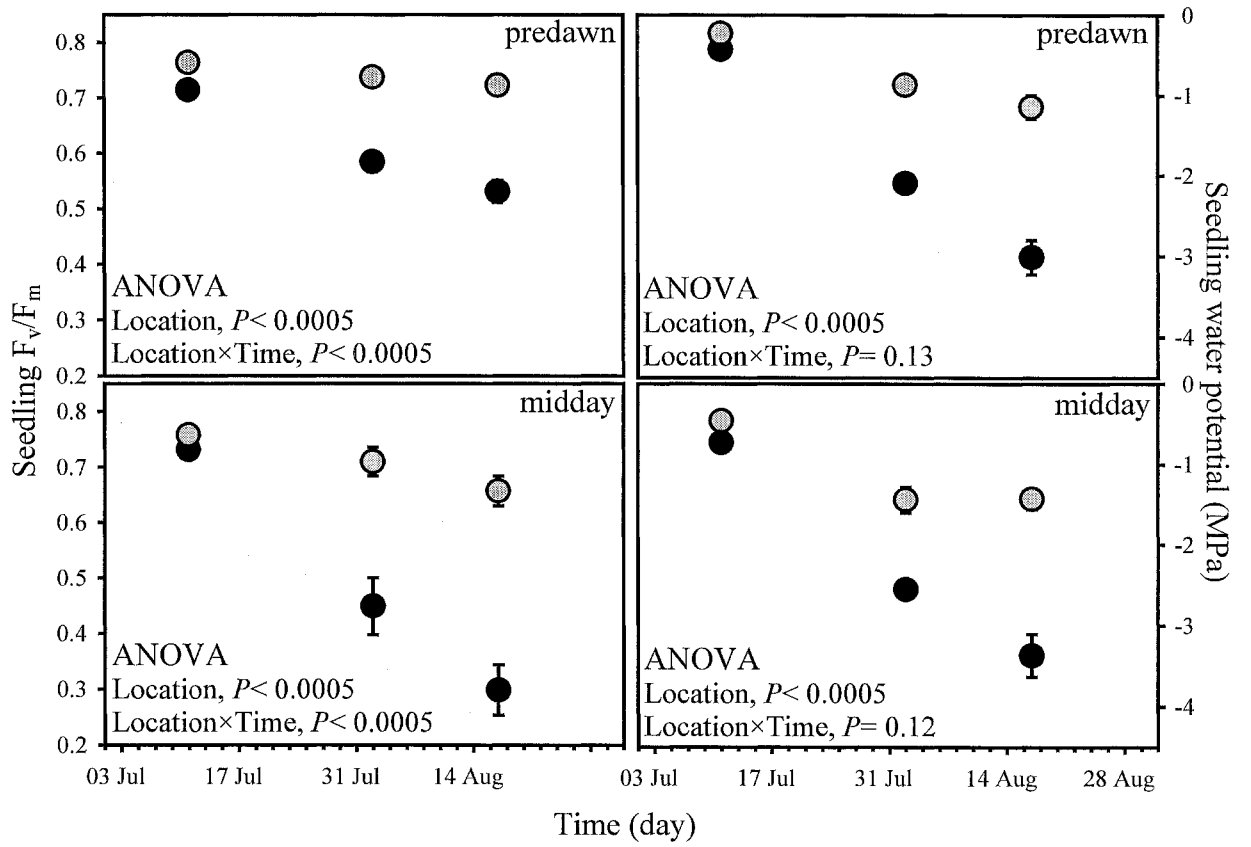


Fig. 3

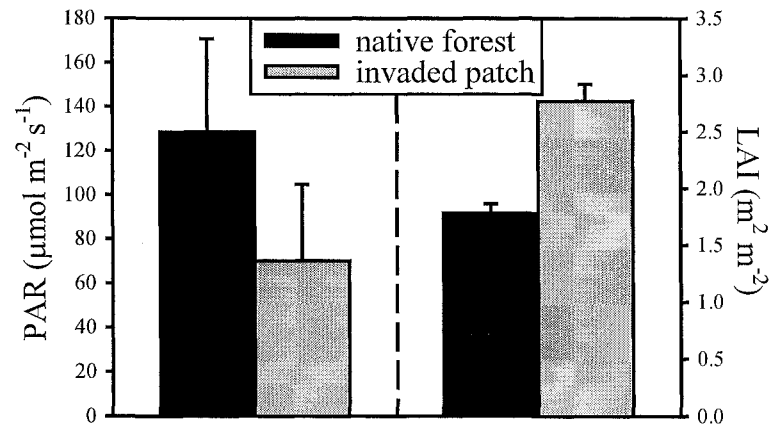
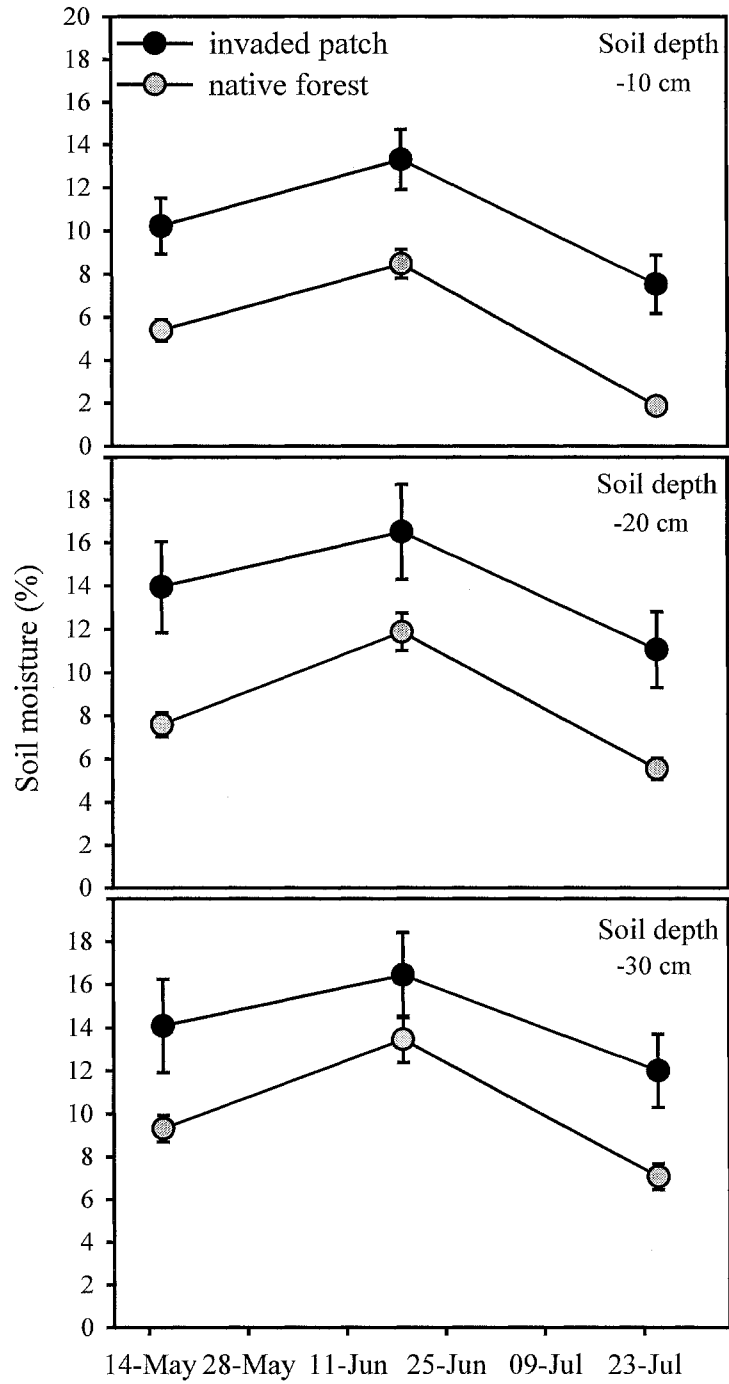


Fig. 4



Chapter 3

Regional effects of soil biota facilitate *Acer* invasion in Europe and North America

Abstract

Invasive species often experience less herbivory and disease and exhibit more vigor in their non-native ranges than in their native ranges. Few studies have tested the Enemy Release Hypothesis with biogeographical experiments, and of these studies, most have focused on aboveground interactions rather than belowground interactions. I tested the Enemy Release Hypothesis focusing on these poorly understood belowground interactions. I compared plant-soil interactions between *Acer negundo* and *A. platanoides* in their native and non-native ranges and related these interactions to distances between established trees and their nearest conspecific neighbors in native and invaded ranges.

Distances from established trees to the nearest *Acer* conspecifics were 56-77% less in the non-native ranges of these *Acers* than in their native ranges, suggesting stronger density-dependent regulation in native regions. Soil biota effects also differed between native and non-native ranges. In the native ranges, soil biota associated with the rhizospheres of conspecifics and heterospecifics decreased the growth of *Acer* seedlings by 35% and 40%, respectively. In the non-native ranges, soil biota associated with conspecifics decreased the growth of *Acer* seedlings by 52%, but the soil biota associated with heterospecifics increased growth of *Acer* seedlings by 59%.

Our results suggest the invasion of *Acers* was enhanced by the soil communities that developed in association with the native dominant species relative to the plant-soil

interactions associated with *Acers* in their native ranges. In the native ranges, regional negative effects of the soil biota predominated instead of either positive or negative plant-soil feedbacks where host-specific communities affect their hosts differently than the soil communities associated with other plant species. Understanding plant-soil interactions in the native ranges of invaders and potential invaders may help to predict future invasions and provide insight into the mechanisms driving current invasions.

Keywords: Enemy Release Hypothesis, soil biota effects, plant-soil feedback, invasive species, facilitation, *Acer negundo*, *Acer platanoides*

Introduction

One of the most intriguing phenomena in ecology is the astounding proliferation of some species that are introduced into new regions of the world. Some invasive species pose a great threat to global biodiversity (Usher 1988, Pimentel et al. 2000, Mack et al. 2000), but the relative success of particular species is neither predictable nor mechanistically similar (Mack et al. 2000). One of the leading hypotheses for successful invasion is that they have escaped the natural enemies that control them; in other words, they experience fewer herbivores and pathogens in their non-native ranges than in their native ranges (Maron and Vila 2001, Keane and Crawley 2002, Wolfe 2002, Mitchell and Power 2003). This “Enemy Release Hypothesis” is the central underpinning of biological control theory (Lawton 1990, Murdoch and Briggs 1996) and release from natural enemies may allow selection for reduced constitutive defense and increased growth (Siemann and Rogers 2001).

The Enemy Release Hypothesis is one of the oldest hypotheses explaining the success of non-native species (Darwin 1859, Elton 1958). However, to my knowledge the only examinations of biogeographical differences have been correlative, and these generally suggest that fewer natural enemies are present in invaded ranges than in natural ranges (Maron and Vila 2001, Keane and Crawley 2002, Wolfe 2002, Mitchell and Power 2003). There have been no explicit comparisons of the effects of competitors, pathogens, parasites, or herbivores on the same plant in their native and non-native ranges. However, one study has demonstrated stronger allelopathic effects of the invasive *Centaurea diffusa* on new neighbors than neighbors in its natural range (Callaway and Aschehoug 2000). Moreover, previous studies have focused on

aboveground plant-plant and plant-insect interactions aboveground and have not addressed the effect of soil communities - potentially a powerful determinant of plant growth and survival (Van der Putten 2001).

Recent studies have demonstrated dynamic feedbacks between plants and the soil biota in their rhizospheres (Augsburger 1984, Van der Putten, Van Dijk, and Peters 1993, Mills and Bever 1998, Packer and Clay 2000, Klironomos 2002, Bever 2002, Bever 2003). The direction of these feedbacks can be positive, negative, or neutral. The direction of the feedback depends on the relative negative effects of accumulating soilborne pathogens, herbivores, and parasites (Van der Putten 2001) versus the positive effects of accumulating mycorrhizal fungi, nitrogen fixing bacteria, and helper bacteria (Garbaye 1994), and the indirect effects of these plant-soil biota interactions on plant-plant interactions (Bever 2003). Negative soil community feedbacks appear to predominate in natural systems, and they provide density-dependent regulation of expanding populations and maintain plant species diversity (Florence 1965, Augspurger and Kelly 1984, Van der Putten, Van Dijk, and Peters 1993, Mills and Bever 1998, Packer and Clay 2000, Klironomos 2002, Bever 2003). Positive feedbacks between soil biota and plants have been demonstrated but appear less common than negative feedbacks (Bever et al. 1997, Klironomos 2002). However, in a study with major implications for exotic invasions, Klironomos (2002) found that non-native plants, and some dominant native species, experienced positive soil feedbacks while rare native species experienced negative soil feedbacks (Klironomos 2002). Klironomos found that rare native species appeared to accumulate species-specific pathogens while dominant invasive herbs did not.

Our objective here is to test the Enemy Release Hypothesis by comparing plant-soil feedback interactions between two *Acer* species, North American box elder (*Acer negundo* L.) and European Norway maple (*A. platanoides* L.), and soil communities from their native ranges and from the ranges they have invaded (each species has invaded the other's continent). I relate these interactions to their nearest neighbor distances in the different ranges. In natural systems, tree species can be negatively effected by the soil biota associated with conspecifics (Florence 1965, Augspurger and Kelly 1984, Mazzola 1998, Packer and Clay 2000). Therefore, I predicted that *Acer negundo* and *A. platanoides* would experience negative effects of the soil biota in their native ranges, especially the biota associated with conspecific rhizospheres, but would experience neutral to positive soil biota effects in their non-native ranges as a result of escaping from their natural belowground enemies. I also predicted that negative soil community feedbacks would correspond with dispersed aggregations of conspecifics, and positive soil community feedbacks would be associated with dense aggregations of conspecifics.

Methods

Site Description

We sampled *Acer negundo* soils and spatial patterns near the center of its natural distribution in Madison, Wisconsin (N43° 3.047' W89° 22.241') (Schmucker 1942). This site was dominated by *Fraxinus pennsylvanica* and *A. saccharum*. I sampled *A. negundo* in its invaded range in mesic deciduous riparian forests of the Rhône River near Sablons, France (N45° 19.0' E4° 46.0') where *Populus* species were the dominant natives.

Acer platanoides naturally occurs in late-seral riparian communities and mesic deciduous forests of Europe (Schmucker 1942). I sampled this species in its native range, France. I collected subsamples from two areas within France. I sampled a riparian forest of the Rhône River, near Sablons (N43° 3.047' W89° 22.241'), and from a mesic deciduous forests near Séchillienne, France (N45° 4.0' E5° 49.6'). These forests were dominated by *Fraxinus excelsior*. I sampled *A. platanoides* in two sites in its invaded range in the U.S. The first site was a 3-5 km corridor of riparian forest of the Rattlesnake Creek, Missoula, Montana. I collected an equal number of subsamples from three preserves along the Rattlesnake Creek including: Greenough Park (N46° 52.644' W113° 58.565'), Tom Greene Memorial Park (N46° 53.416' W113° 58.254'), and Bugbee Park (N46° 52.998' W113° 58.273'). These riparian areas were composed of mixed deciduous forests dominated by the natives *Populus trichocarpa* and *Pinus ponderosa*. The second site where I sampled *A. platanoides* in its non-native range was in late successional forests in New Jersey. In New Jersey, I divided my sampling between two areas. I sampled the Drew Forest Preserve, Drew University (N40° 45.0' W74° 25.0') and the

Hutcheson Memorial Forest (N40° 30.157' W74° 34.310'). Each of these forests was dominated by the natives *Fagus grandifolia* and *A. saccharum*.

Nearest neighbor measurements

At all sites (France, Montana, New Jersey, and Wisconsin), I measured the distances from randomly chosen *A. negundo* and *A. platanoides* trees in the canopy to the three nearest conspecific trees ≥ 2 m in height. If neighboring conspecific trees were not found within 50-55 m of the focal tree then the distances to the nearest neighbors were conservatively (because distances in native sites were often much longer than 50 m) recorded at 50 m. The distances to the nearest three neighbors were averaged, and I tested the effect of range (native vs. non-native) and species of *Acer* on average distance to the nearest conspecific using Two-way ANOVA with SPSS version 10 (SPSS Inc., Chicago, IL, USA).

Effect of soil biota

A greenhouse experiment was conducted to test the effect of soil from different ranges (native vs. non-native), soil biota (non-sterile vs. sterile), and soil from the rhizospheres of different tree species (conspecific vs. heterospecific) on the growth of *Acer negundo* and *Acer platanoides* seedlings (n= 12 per treatment combination). This experiment utilized soil from the native (Wisconsin) and non-native (France) ranges of *A. negundo* and from the native (France) and non-native (Montana and New Jersey) ranges of *A. platanoides*. The soil for the *A. negundo* portion of the experiment was collected from the native range in Wisconsin on August 5, 2001. In the non-native range, soil was

collected from Sablons, France from June 26-29, 2001. In the native range of *A. platanoides*, soil was collected near Sablons and Séchilienne, France between June 26-29 (n= 6 per site). In the invaded range of *A. platanoides*, soil was collected from Drew Forest Preserve and Hutcheson Memorial Forest, New Jersey (n= 6 per site) on July 2, 2001 and from the three sites in Missoula, Montana (n= 4 per site) on July 17, 2001. General site descriptions are given above.

To compare the effects of soil from *Acer* rhizospheres on conspecific *Acer* seedlings to the effects of soil from the rhizospheres of other neighbor species within a site, soil was collected beneath randomly selected target *Acer* trees and also beneath the other dominant tree species at the site ≥ 20 m from the target or any other *A. negundo* or *A. platanoides*. These other dominant species were *Fraxinus pennsylvanica* in the native range of *A. negundo* and *F. excelsior* in its invaded range, *F. excelsior* in the native range of *A. platanoides* in France, *Populus trichocarpa* in the invaded range of *A. platanoides* in Montana, and *Fagus grandifolia* in the invaded range of *A. platanoides* in New Jersey. I did not mix soil collected from different individual trees so I could use individual trees as experimental replicates (n=12 per each site [France, Montana, New Jersey, and Wisconsin]). All soil was transported according to USDA specifications (Animal and Plant Health Inspection Service 1983) and guidelines established for R.M. Callaway's "Application for Permit to Receive Soil" by APHIS, USDA. Soil was shipped to Missoula, Montana and stored for a maximum of two weeks at room temperature in a growth chamber at the University of Montana. All tools, materials, and surfaces coming in contact with non-sterile soil were sterilized to avoid cross contamination by one of five methods: autoclaving for 60-180 minutes, flame sterilization, surfaces sprayed or material

soaked in $\geq 10\%$ bleach solution (itself 5.25% aqueous NaOCl), surfaces sprayed with 70% ETOH solution, or material heated at $\geq 110^\circ\text{C}$ for $\geq 16\text{ hr}$ in a drying oven. All of the field soil was prepared by dicing the roots and crumbling the soil until it passed through a 1 cm^2 opening in a mesh sieve. Soil aliquots of 125 and 25 ml were removed from each replicate within a region and then double autoclaved to kill all soil biota. Then 12 125-ml aliquots of the sterile soil from each region were combined into conspecific and heterospecific composites. The sterile soil composites were then added to each pot as a sterile background soil to help control for fertility differences between conspecific and heterospecific soils and help dilute the fertility effect associated with soil sterilization (see Troelstra et al. (2001) for discussion of fertilization effects). Twelve replicate pots with a capacity of ca. 2.5 L were filled with a background soil mixture and either sterile or non-sterile soil from beneath individual *Acer* or non-*Acer* dominant trees. The background soil consisted of ca. 2.5 L of sterile (once autoclaved) commercial silica sand averaging 0.60-0.85 mm in diameter (20/30 grit, Lane, MT, USA) and 50 ml of sterilized soil with half from bulked conspecific and half from bulked heterospecific sources. Thus, the potting medium consisted of $\sim 97\%$ sterile sand, 25 ml ($\sim 1\%$) of either sterile or non-sterile soil inoculum, and 50 ml ($\sim 2\%$) of bulked sterile soil, with half from conspecifics ($\sim 1\%$) and half from heterospecifics ($\sim 1\%$) within each region (i.e. France, Montana, New Jersey, and Wisconsin). The experimental pots for the *A. negundo* portion of the experiment were planted with *A. negundo* seeds (purchased from Lawyers Nursery Inc., Plains, MT) that were surface sterilized by placing the seeds into 5% bleach solution for 10 minutes and then rinsing the seeds with deionized water. The seedlings were thinned to one plant per pot after 30-60 days, depending on individual experiment,

following the sowing of seed. The *A. platanoides* portion of the experiment could not be planted with seeds because of problems with germination. Instead, I planted *A. platanoides* seedlings of equal size, which germinated in 2001, collected on the University of Montana campus. I surface sterilized the roots in order to avoid transplanting rhizosphere biota from the field soil into the experimental units. The roots of each seedling were surface sterilized by submerging the roots in a solution of 5% bleach for 10 minutes and then thoroughly rinsing with dionized water. These experiments were started between July and August, 2001. Seedlings that died within a couple of weeks following planting were replaced. The initial basal diameter and stem height of each seedling was measured at 30-60 days after the initiation of each regional experiment. Plants were watered manually and with an automated misting system. Supplemental light was added in the autumn and winter, and shading was provided in the summer to ameliorate extreme temperatures and light intensities. To encourage the development of mycorrhizal symbioses I applied low concentrations of nutrients (particularly phosphorus) to the soils (Johnson et al. 1992, Smith and Read 1997). During 2002, plants were fertilized approximately once every two weeks with 100 to 110 ml per plant of Scott's Miracle-Grow Excel (NPK ratio of 15-2-20, Scotts-Sierra Horticultural Products Company, Marysville, OH) mixed at 0.171g L^{-1} . In spring and summer 2002, the plants were also provided with 0.61 ml of Osmocote, Outdoor & Indoor Slow Release Plant Food at monthly intervals (19-6-12, Scotts-Sierra Horticultural Products Company, Marysville, OH) to provide a continuous supply of nutrients. All of the regional experiments were harvested from August 24-31, 2002, each seedling was measured for final basal diameter and height, and the plants were harvested

and divided into roots, stems, and leaves. All harvested material was dried at 65° C until constant weight.

We tested the effect of non-native sites (Montana and New Jersey) for *A. platanoides* on the total biomass and relative change in seedling height ($[(\text{final height} - \text{initial height}) / \text{initial height}] \times 100$) with a Three-way ANOVA (sites, soil biota, and rhizosphere sources). I found no effect of site and combined the *A. platanoides* data from Montana and New Jersey. Next, I used MANOVA to test the effect of species (*Acer negundo* and *A. platanoides*), soil sterilization (sterile vs. non-sterile), and rhizosphere source (conspecific vs. heterospecific) on the total biomass and relative change in height for each range (native and non-native) using SPSS version 10 (SPSS Inc., Chicago, IL, USA). Two-way ANOVAs were also used to identify effects of soil sterilization and rhizosphere source on the growth of *Acer negundo* and *A. platanoides* seedlings in separate ranges. To maintain homogeneity of variances for these analyses, I transformed data with either a square root function ($\sqrt{[x+ 0.5]}$) or log function ($\log [x+ 1]$). The data for relative change in height continued to violate the homogeneity of variance assumption following transformation (Levene's Test; $F= 2.27$, $df=7, 76$, $P= 0.037$), but MANOVA was still used for the analysis.

Results

Overall, neighboring conspecifics were 56-77% more closely spaced in non-native than in native ranges (ANOVA; $F_{\text{range}} = 10.60$, $df = 1,32$, $P = 0.003$; Fig. 1). For *Acer negundo*, there was a trend for greater dispersion of conspecifics in native forests than in non-native forests (Fig. 1a; two-sample t-test; $t = 1.72$, $df = 7,9$, $P = 0.129$). Conspecifics of *A. platanoides* were almost six times more widely dispersed from conspecific trees in native forests than in non-native forests (Fig. 1b; $t = 3.76$, $df = 21,23$, $P < 0.0005$).

Sterilization of soil from native communities had a more positive effect (negative effect of soil biota) on the growth of *Acer* seedlings than sterilization of the soil from the communities invaded by *Acer* species. In the native ranges of *Acers*, the MANOVA between-subjects analysis revealed a significant effect of *Acer* species on growth (Table 1; ANOVA, $F = 57.49$, $df = 2,75$, $P < 0.0005$) and a marginally significant positive effect of soil sterilization ($F = 2.87$, $df = 2,75$, $P = 0.063$) on growth. The within-subjects analysis identified an effect of species and a positive effect of soil sterilization (negative effect of the soil biota) on total biomass (Table 1; ANOVA, $F_{\text{species}} = 49.74$, $df = 1,84$, $P < 0.0005$; $F_{\text{sterilization}} = 5.44$, $df = 1,84$, $P = 0.022$) and relative change in height ($F_{\text{species}} = 107.91$, $df = 1,84$, $P < 0.0005$; $F_{\text{sterilization}} = 5.23$, $df = 1,84$, $P = 0.025$) of *Acer* seedlings (Figures 2-3). Other between subject tests did not approach statistical significance (Table 1, $P \geq 0.38$). In contrast, in the non-native ranges, the between-subjects analysis demonstrated a significant interactive effect of soil sterilization and rhizosphere source (Table 2; MANOVA, $F = 3.00$, $df = 2,111$, $P = 0.054$) and an effect of species ($F = 33.17$, $df = 2,111$, $P < 0.0005$) on the dependent variables, and the within-subjects analysis

identified an interactive effect of soil sterilization and rhizosphere source and an effect of species on the total biomass (ANOVA, $F_{\text{sterilization} \times \text{rhizosphere}} = 4.53$, $df = 1, 120$, $P = 0.036$; $F_{\text{species}} = 189.00$, $df = 1, 120$, $P < 0.0005$) and relative change in height ($F_{\text{sterilization} \times \text{rhizosphere}} = 5.90$, $df = 1, 120$, $P = 0.017$; $F_{\text{species}} = 3,180.00$, $df = 1, 120$, $P < 0.0005$) of seedlings (Figures 2-3). Other between subject tests did not approach statistical significance (Table 2, $P \geq 0.16$). Thus, in the non-native ranges, sterilization of soil associated with conspecifics had a positive effect on seedling growth (negative soil biota effect); but unlike in the native ranges of *Acer*, sterilization of soil associated with heterospecifics had a neutral to negative effect on seedling growth (neutral to positive soil biota effects).

In the native range of *Acer negundo*, there was a trend towards a positive effect of soil sterilization on the seedling growth with a 26% increase in biomass (Fig. 2a; ANOVA, $F = 3.22$, $df = 1, 48$, $P = 0.079$) and a 19% increase in relative height (Fig. 2b; $F = 2.39$, $df = 1, 48$, $P = 0.129$) of seedlings grown in sterilized soil. In the non-native range of *A. negundo*, seedling biomass (Fig. 2c) and relative height (Fig. 2d) were not affected by soil sterilization (Two-way ANOVA, $F = 1.09$, $df = 1, 48$, $P = 0.30$ and $F = 0.37$, $df = 1, 48$, $P = 0.55$, respectively), rhizosphere source ($F = 0.39$, $df = 1, 48$, $P = 0.53$ and $F = 0.42$, $df = 1, 48$, $P = 0.52$, respectively), or the interaction between these factors ($F = 0.60$, $df = 1, 48$, $P = 0.44$ and $F = 1.12$, $df = 1, 48$, $P = 0.30$, respectively).

Acer platanoides seedlings were also affected by the sterilization of soil from different biogeographical ranges. Within its native range, there was no effect of soil sterilization on seedling biomass (Fig. 3a; Two-way ANOVA, $F = 1.34$, $df = 1, 36$, $P = 0.26$), but the sterilization of soil increased the relative change in height by 55% (Fig. 3b;

F= 20.8, df= 1,36 $P < 0.0005$). In the non-native range of *A. platanoides*, biomass (Fig. 3b) and relative change in height (Fig. 3d) of *A. platanoides* seedlings were affected by soil sterilization and differently by soil from different rhizospheres (conspecifics vs. heterospecifics). This was shown by a significant interaction between soil sterilization and rhizosphere source (Three-way ANOVA for biomass, $F_{\text{sterilization} \times \text{source}} = 6.74$, df= 1,72, $P = 0.012$; and for relative height, $F_{\text{sterilization} \times \text{source}} = 4.9$, df= 1,72, $P = 0.030$). Sterilization of soil associated with conspecifics increased growth by 54%; whereas, sterilization of soil associated with heterospecifics decreased growth by 80%.

Discussion

Our results show that *Acer negundo* and *A. platanoides* are more negatively affected by the resident soil biota in their native ranges than in recently invaded forests. Furthermore, although *A. platanoides* appears to accumulate suppressive soil biota even in its invaded range, the strong positive effect of soil biota from beneath North American heterospecifics suggests that *A. platanoides* benefits substantially from the general soil biota present in its invaded region. These experimental results correspond with substantially denser aggregations of conspecifics around focal trees in non-native than native ranges. Together, these results suggest that the resident soil biota facilitate the spread of species in their non-native ranges and suppress their spread in their native ranges. Alternatively, other biogeographical differences in the abiotic environment (e.g. climate, precipitation, temperature, nutrient availability, etc.) and additional biological interactions between regions may affect the invasiveness of species and the abundance patterns of species between regions.

Because host-specific soil pathogens often accumulate in native ranges, I hypothesized that *Acer negundo* and *A. platanoides* would experience negative effects of the soil biota in their native ranges, especially from the biota associated with conspecific rhizospheres. Supporting this, in the native ranges of *Acers* I identified a net negative effect of resident soil biota on growth regardless of rhizosphere source. I also predicted that the soil communities associated with conspecifics and heterospecifics would differ because they were being “trained” by different plant species (see Bever (2003) for discussion of soil training). Previous studies have shown that the soil biota that develops in the rhizospheres of plants often has a negative effect on conspecifics (Klironomos

2002). In contrast, the effect of the soil biota that develops in the rhizospheres of heterospecifics is often neutral to positive (Packer and Clay 2000, Klironomos 2002, Bever 2003). The contrasting effects of soil trained by conspecific *versus* heterospecifics may play a role in the reciprocal replacement of tree species in forests (Horn 1971, Forcier 1975, Fox 1977, Woods 1979). The replacement dynamic that has been described in native forests is consistent with negative soil community feedbacks (Bever 2003) although other density dependent regulation (Janzen 1970, Connell 1971), autoinhibition, and species-specific mutualisms may also cause directional succession (Fox 1977, Woods 1979).

We predicted that *Acer* species would experience neutral to positive soil biota effects in their non-native ranges as a result of escaping from their natural belowground enemies and benefiting from generalist arbuscular mycorrhizal fungi (Richardson et al. 2000). My results supported this prediction in general, but the effects of soil biota were more complicated than expected. In the non-native ranges, soil biota associated with conspecifics generally had a more negative effect on *Acer* growth than the soil biota from heterospecifics with significant interactions between soil sterilization and rhizosphere source. The positive or neutral soil biota effects associated with the rhizospheres of heterospecifics in the non-native ranges may contribute to the invasiveness of *Acer negundo* and *A. platanoides* by facilitating their establishment in native forests and afterwards their spread away from established conspecifics. The contrasting effects between conspecific and heterospecific rhizosphere sources indicate that negative soil feedbacks eventually develop beneath *Acers* even in invaded regions.

We predicted that the soil biota associated with conspecific rhizospheres would differ between ranges and that these differences would correspond with changes in nearest neighbor distances in the native and non-native ranges. This prediction was based on studies showing that native plant species often experience negative soil community feedbacks resulting in density-dependent control. However, I found that the soil biota in the native ranges of the two *Acer* species was consistently negative regardless of rhizosphere source suggesting that regionally distributed soil biota inhibited *Acer* growth rather than accumulation of host-specific pathogens. In fact, although the regional soil biota in invaded ranges had strong positive effects on invading *Acers*, actual feedback effects were negative. This contrasts with positive feedbacks reported for exotics by others (Klironomos 2002, Reinhart et al. *in review*, Callaway et al. *in review*). However, my unexpected finding of a negative feedback in *invaded* ranges conflicts with the closer spacing of *Acer* conspecifics in the same communities. Closer spacing suggests *Acers* were less inhibited by intraspecific mechanisms of density dependent regulation where they have invaded. I suggest that either soil feedback processes are not operating at the scale of my spatial measurements or interactions not accounted for in my experiment are also affecting the spatial distribution of *A. negundo* and *A. platanoides*.

Other research indicates that *A. platanoides* trees in riparian forests of the northern Rocky Mountains indirectly facilitate conspecific seedlings by producing much deeper shade than native species. Shade from established *A. platanoides* suppresses recruitment of native trees and the growth of native understory species; reducing the competitive effects of natives on exceptionally shade tolerant *A. platanoides* seedlings (Reinhart et al. *unpublished data*). Furthermore, established *A. platanoides* appear to

increase soil moisture in the understory which may facilitate conspecific seedlings (Reinhart et al. *unpublished data*). These effects of established adults may contribute substantially to the aggregation patterns observed in mixed deciduous and coniferous forests in Montana, but other processes are probably operating in late-successional deciduous forests in northeastern U.S. and Europe.

Our results are complicated by the use of infertile silica sand for a background soil. Others have used sterile potting soil (Packer and Clay 2000) or sterile field soil (Mills and Bever 1998) which more closely match field soils and potentially provide a more biotically active substrate for the inoculum to act on. I did not use potting soil because it is unrealistically fertile, and transport of large amounts of soil from Europe to use as a sterile background is logistically unrealistic. However, overcoming these logistical problems in future biogeographical comparisons of soil biota effects by using large amounts field soils to develop a sterile background to control for soil texture and chemistry would likely yield even stronger results.

Plants in their non-native ranges are often facilitated by soil biota (Vitousek et al. 1987, Marler et al. 1999, Callaway et al. 2001, Klironomos 2002) while native plant species are often negatively effected by the accumulation of host-specific portions of the soil biota (Klironomos 2002). Native plant species often experience a negative soil community feedback (Packer and Clay 2000, Klironomos 2002, Bever 2003) which functions as a form of density dependent control. In contrast, a recent study suggests that non-native species experience positive soil community feedbacks, which help these plants attain local dominance (Klironomos 2002). My results did not identify a switch from negative feedback in native ranges to positive feedback in non-native ranges as

predicted. Instead, I found a more complicated pattern with a general regional negative effect of the soil biota on the growth of *Acers* in their native ranges and no plant-soil feedback as expected. In the non-native range to my surprise, I observed a negative plant-soil feedback; however, the positive effect of soil communities associated with the native dominant trees relative to *Acers*' soil communities suggests a general regional positive effect of the soil community on *Acers*. The major effect of the soil community in the non-native range was not the development of feedbacks, but a general regional effect that may influence the invasive spread of *A. negundo* and *A. platanoides*. The general negative effects of the soil community on plants in their native ranges relative to the positive effects of the soil community on plants in their non-native ranges suggests that invasive species may be facilitated at a regional scale by the escape from their natural belowground enemies. Improved understanding of plant-soil interactions in non-native ranges relative to native ranges will help identify the processes contributing to plant invasions. The Enemy Release Hypothesis has historically been emphasized from an aboveground perspective (Darwin 1859, Maron and Vila 2001, Wolfe 2002, Mitchell and Power 2003), but my results suggest that relating this hypothesis to belowground and aboveground interactions will improve the generality of this hypothesis and our general understanding of plant invasions.

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Table 1. Summary of MANOVA of effects of tree species (*Acer negundo* and *A. platanoides*), soil sterilization (non-sterile vs. sterile soil), and rhizosphere source (conspecific vs. heterospecific) on total biomass and relative change in height of seedlings grown in soils from native ranges.

Source of variation	Dependent variable	df	Wilks' lambda	ms	F	P
a) Between-subjects effects						
Species	...	2, 75	0.395	...	57.49	<0.0005
Sterilization	...	2, 75	0.929	...	2.87	0.063
Rhizosphere	...	2, 75	0.989	...	0.41	0.664
Species × Sterilization	...	2, 75	0.999	...	0.05	0.956
Species × Rhizosphere	...	2, 75	0.996	...	0.15	0.862
Sterilization × Rhizosphere	...	2, 75	0.989	...	0.42	0.658
Species × Sterilization × Rhizosphere	...	2, 75	1.000	...	0.01	0.989
b) Within-subjects effects						
Species	biomass	1, 84	...	93.96	49.74	<0.0005
	height	1, 84	...	7,843.09	107.91	<0.0005
Sterilization	biomass	1, 84	...	10.27	5.44	0.022
	height	1, 84	...	380.33	5.23	0.025
Rhizosphere	biomass	1, 84	...	1.46	0.77	0.382
	height	1, 84	...	55.04	0.76	0.387
Species × Sterilization	biomass	1, 84	...	<0.01	<0.01	0.949
	height	1, 84	...	0.87	0.01	0.913
Species × Rhizosphere	biomass	1, 84	...	0.53	0.28	0.599
	height	1, 84	...	9.43	0.13	0.720
Sterilization × Rhizosphere	biomass	1, 84	...	0.02	0.01	0.910
	height	1, 84	...	26.10	0.36	0.551
Species × Sterilization × Rhizosphere	biomass	1, 84	...	0.03	0.01	0.906
	height	1, 84	...	0.16	<0.01	0.963
Error	biomass	1, 84	...	1.89		
	height	1, 84	...	72.68		

Tests were performed using the Type three sums of squares from SPSS version 10 (SPSS Inc., Chicago, IL, USA). Biomass and relative change in height data were transformed with a square root function ($\sqrt{[x+0.5]}$). The data for *Acer negundo* were collected in Wisconsin, U.S. and for *A. platanoides* were collected in France.

Table 2. Summary of MANOVA of effects of tree species (*Acer negundo* and *A. platanoides*), soil sterilization (non-sterile vs. sterile soil), and rhizosphere source (conspecific vs. heterospecific) on total biomass and relative change in height of seedlings grown in soils from non-native ranges.

Source of variation	Dependent variable	df	Wilks' lambda	ms	F	P
a) Between-subjects effects						
Species	...	2, 111	0.626	...	33.17	<0.0005
Sterilization	...	2, 111	0.968	...	1.81	0.169
Rhizosphere	...	2, 111	0.997	...	0.16	0.853
Species × Sterilization	...	2, 111	0.972	...	1.60	0.206
Species × Rhizosphere	...	2, 111	0.998	...	0.12	0.885
Sterilization × Rhizosphere	...	2, 111	0.949	...	3.00	0.054
Species × Sterilization × Rhizosphere	...	2, 111	0.991	...	0.51	0.603
b) Within-subjects effects						
Species	biomass	1, 120	...	189.00	65.35	<0.0005
	height	1, 120	...	3,180.00	49.90	<0.0005
Sterilization	biomass	1, 120	...	5.41	1.87	0.175
	height	1, 120	...	3.01	0.05	0.828
Rhizosphere	biomass	1, 120	...	0.89	0.31	0.580
	height	1, 120	...	15.99	0.25	0.617
Species × Sterilization	biomass	1, 120	...	0.06	0.02	0.885
	height	1, 120	...	66.17	1.04	0.310
Species × Rhizosphere	biomass	1, 120	...	0.62	0.22	0.644
	height	1, 120	...	14.12	0.22	0.639
Sterilization × Rhizosphere	biomass	1, 120	...	13.11	4.53	0.036
	height	1, 120	...	376.25	5.90	0.017
Species × Sterilization × Rhizosphere	biomass	1, 120	...	2.74	0.95	0.333
	height	1, 120	...	21.13	0.33	0.566
Error	biomass	112, 120	...	2.90		
	height	112, 120	...	63.73		

Tests were performed using the Type three sums of squares from SPSS version 10 (SPSS Inc., Chicago, IL, USA). Biomass and relative change in height data were transformed with a square root function ($\sqrt{[x+0.5]}$). The data for *Acer negundo* were collected in France and for *A. platanoides* were collected in Montana and New Jersey, U.S..

Figure 1. Distances between conspecifics of **a)** *Acer negundo* and **b)** *Acer platanoides* in their native ranges (black bars) and their non-native ranges (gray bars). The native range for *A. negundo* is Wisconsin (n= 5 trees) and for *A. platanoides* is France (n= 8). The non-native site for *A. negundo* was in France (n= 4) and for *A. platanoides* were Montana (n= 9) and New Jersey (n= 6). Bars represent the average distance from randomly selected *A. negundo* and *A. platanoides* trees to the three nearest trees (>2 m tall) \pm 1 s.e. We tested the effect of region (native vs. non-native) and species (*Acer negundo* and *A. platanoides*) on nearest neighbor distances for *Acer* with a Two-way ANOVA ($F_{\text{region}}=10.60$, $df=1,32$, $P=0.003$; $F_{\text{species}}=0.08$ $df=1,32$, $P=0.781$; $F_{\text{r}\times\text{s}}=1.37$, $df=1,32$, $P=0.252$). The data were transformed with a log function ($\log [x+1]$) to maintain homogeneity of variances.

Figure 2. The effect of soil sterilization and rhizosphere source on total biomass and relative change in height of *Acer negundo* seedlings grown in soil from their native (**a** and **b**, Wisconsin) and non-native (**c** and **d**, France) ranges. In the native range, there was a strong trend towards a positive effect of soil sterilization (negative soil biota effect) on biomass (Fig. 2a; ANOVA, $F=3.22$, $df=1,48$, $P=0.079$) and relative change in height (Fig. 2b; ANOVA, $F=2.39$, $df=1,48$, $P=0.129$) of seedlings. The soil was collected either near (ca. 1 m) conspecifics or near heterospecifics (ca. 1 m) and away from conspecifics (≥ 20 m).

Figure 3. The effect of soil sterilization and rhizosphere source on total biomass and relative change in height of *Acer platanoides* seedlings grown in soil from their native (**a**

and **b**, France) and non-native (**c** and **d**, Montana and New Jersey) ranges. Figure legend follows the description provided for Figure 2. In the native range, there was a positive effect of soil sterilization (negative soil biota effect) on the relative change in height (Fig. 3b; Two-way ANOVA, $F= 20.8$, $df= 1,36$, $P<0.0005$) of seedlings. In the non-native range, seedling biomass and the relative change in height were affected by the interaction between soil sterilization and rhizosphere source (Fig. 3c-d; Three-way ANOVA for biomass, $F= 6.74$, $df= 1,72$, $P= 0.012$; and for relative change in height, $F= 4.9$, $df= 1,72$, $P= 0.030$).

Fig. 1

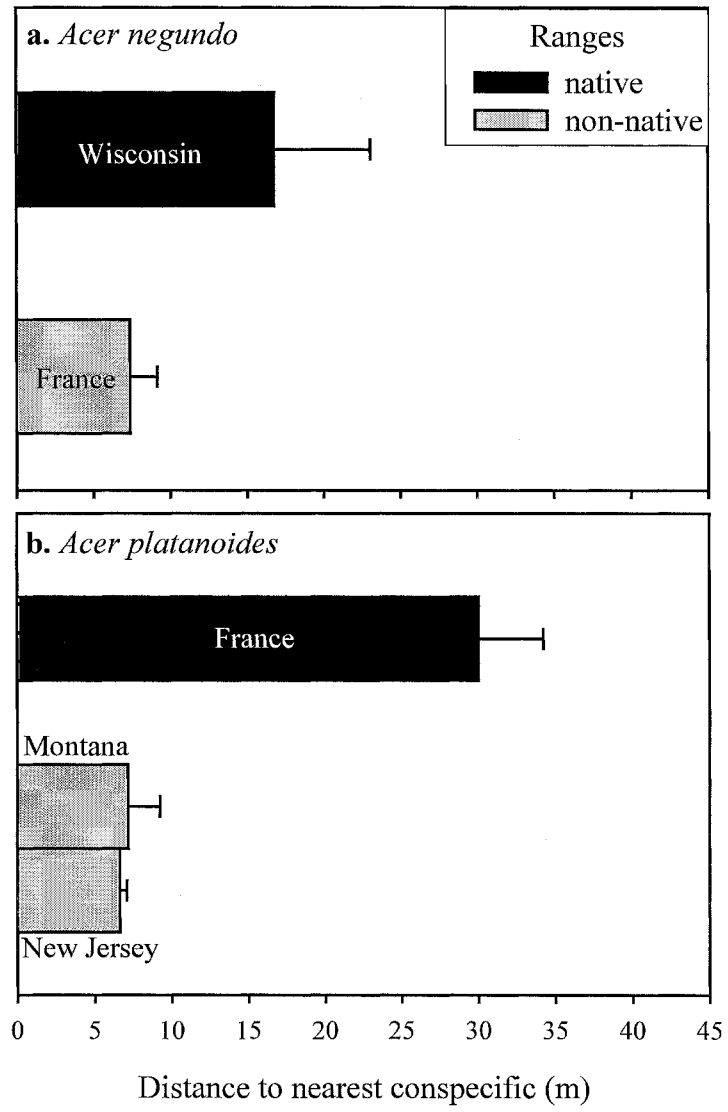


Fig. 2

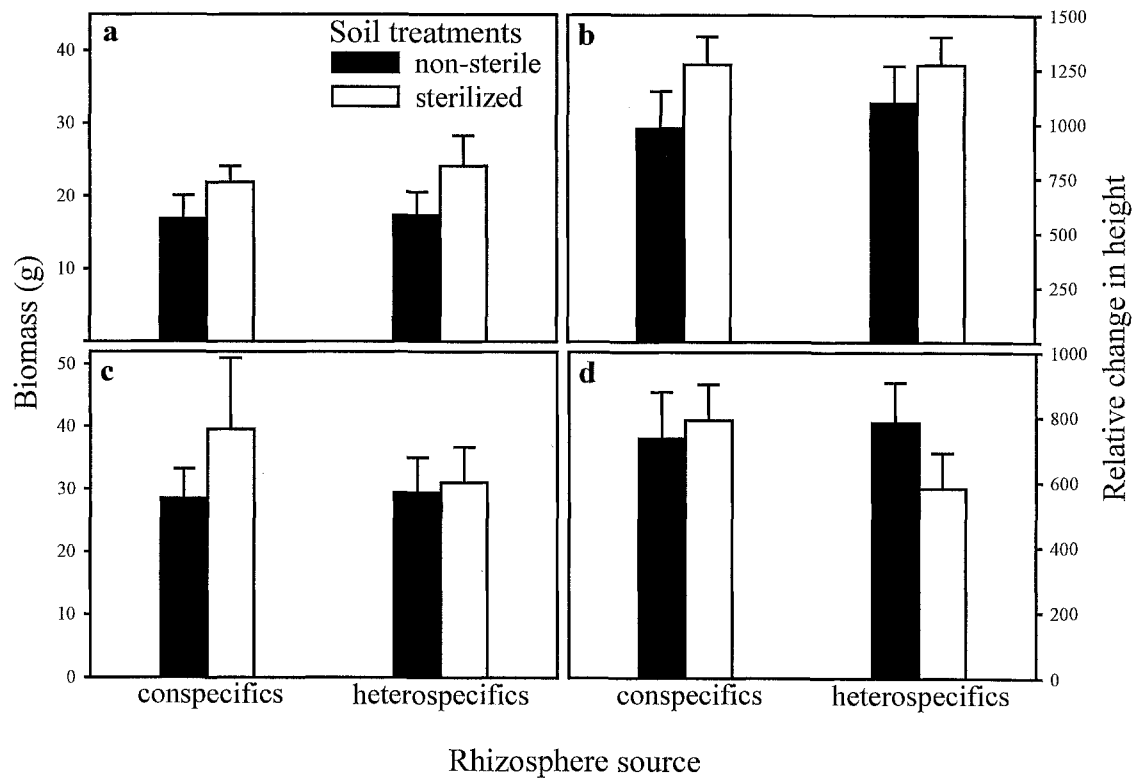
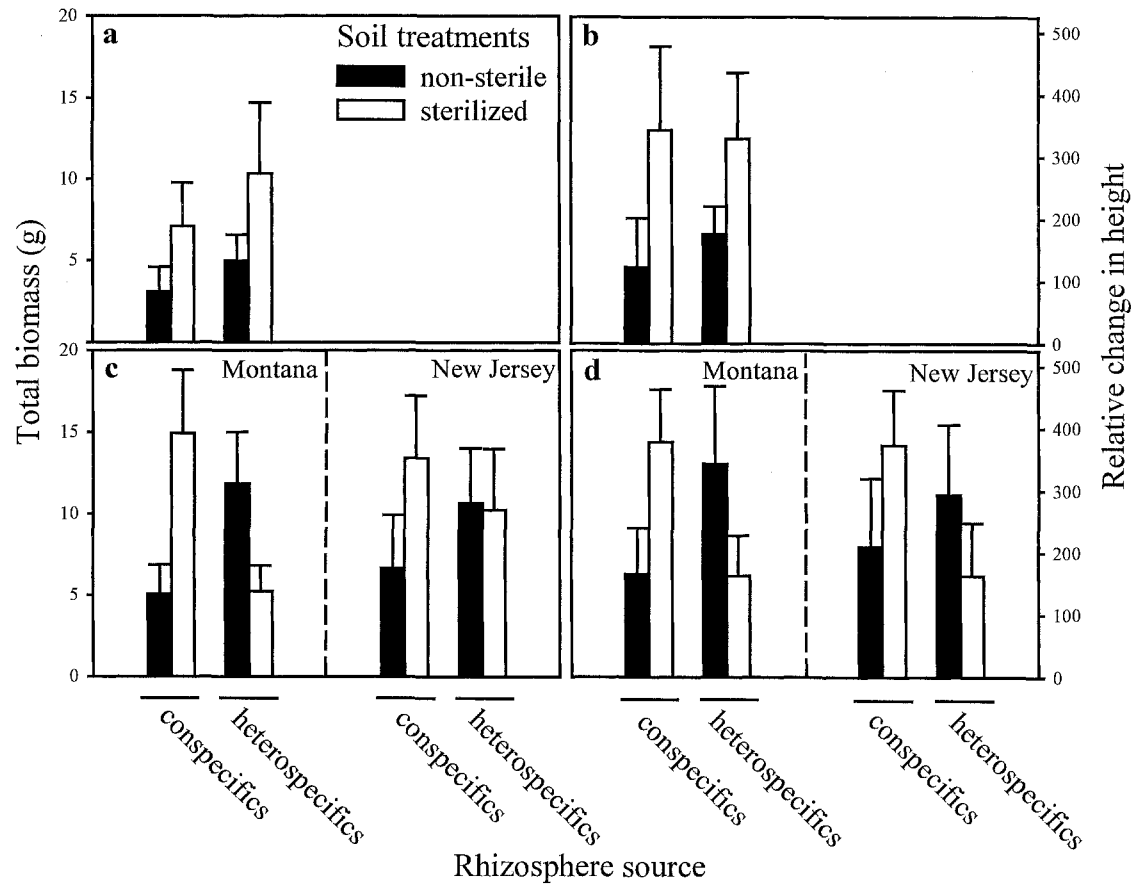


Fig. 3



Chapter 4

Escape from natural soil pathogens enables a North American tree to invade Europe

Abstract

Invasive plants are a major threat to global biodiversity (1) and invasiveness is often enhanced by the absence of aboveground enemies (2-5). Here I present evidence showing that the invasive spread of a North American tree species, black cherry (*Prunus serotina*), is due to the absence of soil pathogens or reduction in pathogenic activity. In the U.S., the abundance of black cherry is inhibited by soil pathogens (6). In contrast, I found that organisms in European soils enhanced growth of black cherry trees. Understanding the effects of pathogenic and beneficial soil organisms on plant abundance will improve our ability to predict and counteract plant invasions.

Body

Host-specific natural enemies are thought to play a major role in regulating both the abundance of individuals and the diversity of species in natural ecosystems (7,8). Many invasive exotic plant species have fewer natural enemies in their invaded habitats than in their native habitats suggesting that release from natural enemies may contribute to invasiveness (2-5). This Enemy Release Hypothesis (9) has been supported by correlative studies comparing the number of enemies and the relative damage caused by enemies on plants in their native and invaded habitats (4,5). However, these studies have focused on natural enemies above ground (2-5), and there have been no comparative experiments conducted in native and exotic ranges to determine the relative effect of enemies in the native and invaded habitats (2).

The interactions between plants and the community of soil organisms that develops in their rhizospheres can result in a dynamic feedback (6,10-16). The direction of this plant-soil feedback depends on the negative effects of accumulating soil-borne pathogens, parasites, and herbivores (17,18) *versus* the positive effects of accumulating mycorrhizal fungi (19), nitrogen fixing bacteria (20), and helper bacteria (21). The net feedback effect of the soil community affects plant establishment, vigor, and plant-plant interactions (14,17). Several recent studies suggest that negative soil feedbacks are common in natural systems and function as a form of density dependent control helping to maintain plant diversity (6,10-14,16).

We studied the effects of the soil community on black cherry (*Prunus serotina*) in its newly invaded habitat and native habitat. First, I compared the spatial distribution of black cherry in its native and invaded habitats to test whether it had escaped from its natural enemies and attained greater densities in its invaded habitat than in its native habitat (22). Last, I identified the effect of the soil

community in its newly invaded habitat in north-western Europe (23), and these results were compared with data collected from the native habitat in the eastern U.S., where tree abundance is controlled by soil-borne fungal pathogens (*Pythium* spp., that belong to the oomycete fungi) (6,24). In its native habitat, black cherry trees produce sparse stands and co-exist with other tree species, but in its newly invaded habitat black cherry has become a dominant species producing dense stands. This trans-Atlantic comparison enabled us to rigorously test the Enemy Release Hypothesis for soil pathogens.

Black cherry seedlings, saplings, and trees were 251% to 1,112% farther from randomly selected conspecific trees in forests in the native habitat than in the invaded habitat (Fig. 1; Two-way MANOVA, habitat, $F_{3, 39} = 303.72$, $P < 0.0005$). The distance between black cherry seedlings, saplings, and trees to the nearest randomly selected conspecific tree differed between sites (Two-way MANOVA, site, $F_{3, 39} = 3.00$, $P = 0.04$). The variability in neighbor distances between sites also varied between habitat types (Two-way MANOVA, habitat×site, $F_{3, 39} = 11.11$, $P < 0.0005$) with distance to nearest sapling varying more between sites in the native habitat than the non-native habitat (Two-way ANOVA, habitat×site, $F_{1, 45} = 23.21$, $P < 0.0005$). The failure of tree seedlings to establish underneath the canopy of black cherry trees in the native habitat is due to the pathogenic activity of soil-borne *Pythium* fungi (6). The relatively small distance between black cherry trees, as well as the presence of establishing seedlings and saplings underneath mature trees in Europe suggests that the soil feedback effect on black cherry was not as negative as in North America. Therefore, I tested the hypothesis that the transfer of black cherry to north-western Europe may have enabled the tree to escape from its native soil pathogens, as predicted by the Enemy Release Hypothesis (9).

We tested the Enemy Release Hypothesis by quantifying the response of black cherry seedlings to soil biota associated with mature black cherry trees in forests from its invaded habitat in north-western Europe (the Netherlands) (21). I also used data from a previous experiment, conducted in 1998 in the native habitat (Indiana) of black cherry (6), in new analyses. Within the native and invaded habitats, I tested the effect of soil sterilization by growing plants in non-sterile *versus* sterilized soil. In addition, I tested the effects of density of black cherry seedlings (1 *versus* 3 seedlings planted per pot) and distance from mature black cherry trees (collected near *versus* far from black cherry trees (21)) on seedling growth and survival. Thus, I tested the effect of soil sterilization, seedling density, and the distance at which the soil was collected from the nearest black cherry trees on survival and biomass of the experimentally planted black cherry seedlings.

We predicted neutral to positive soil feedbacks (for soil feedback equation see 25) for seedling survival and biomass in its invaded habitat, which would indicate the invasive black cherry has escaped from soil pathogen effects in the U.S. (6). Overall, there was no effect of soil sterilization on the survival of seedlings in either their native or newly invaded habitats (Fig. 2a; Log linear analyses for the native habitat, $\chi^2 = 1.28$, $P = 0.258$ and for the newly invaded habitat, $\chi^2 = 0.36$, $P = 0.551$). However, there was an interaction between soil sterilization, seedling density, and soil distance on seedling survival in the native habitat (Fig. 2a; Log linear analysis, $\chi^2 = 4.85$, $P = 0.028$). In the native habitat, seedling survival ranged from 39% to 92%, whereas 75% to 100% of the seedlings survived in soils from the invaded habitat where there were no treatment effects on seedling survival ($\chi^2 \leq 1.4$, $P \geq 0.236$). In the native habitat, on the other hand, survival of seedlings was reduced by $\geq 44\%$ when grown at high densities, in non-sterile soil collected near mature black cherry trees relative to other treatments (Fig. 2a).

In both the native and invaded habitats, soil sterilization had a significant effect on seedling biomass (Table 1). However, the directions of the soil feedback effects differed between native and invaded habitats. In the invaded habitat, there was an overall positive effect of the soil community on the growth of black cherry seedlings with the biomass of seedlings increasing by 25% in non-sterile soils compared to sterilized soils (Fig. 2b; Table 1). This is stronger support for the Enemy Release Hypothesis than I predicted, since the soil community in the invaded habitat enhanced the growth of black cherry seedlings (26). These positive effects of the soil community in the invaded habitat may be due to arbuscular mycorrhizal fungi (27); however, the effectiveness of these, or possibly other beneficial soil organisms, has not been tested. In contrast, in the native habitat there was an overall negative effect of the soil community on growth of black cherry seedlings with the biomass of seedlings decreasing by 25% in non-sterile soils compared to sterilized soils (Fig. 2b; Table 1). In the native habitat, a positive soil feedback effect was only observed when seedlings were planted at high densities and in soil collected under mature black cherry trees (Fig. 2b). This positive effect also contributed to the interaction between soil sterilization and distance, and soil sterilization, distance from the mature tree, and seedling density in the pots (Table 1). However, this treatment also experienced the greatest seedling mortality (Fig. 2a) which dramatically reduced the initial planting density from three seedlings per pot to an average density of 1.2 seedlings per pot (11% of pots maintained high density) in the non-sterile soil relative to the 2.7 seedlings per pot (67% of pots maintained high density) in the sterilized soil treatment. This suggests that the release from intraspecific competition following severe seedling mortality may have obscured the soil feedback effect for biomass of seedlings planted at high densities and in soil collected under mature black cherry trees. Such intraspecific competition appeared across all treatments in the native and non-native habitats (Table 1) and in pots with sterilized soil collected near black cherry in the native range. In sterilized soil,

seedling biomass varied between density treatments with one seedling per pot producing 0.45 g of biomass and three seedlings per pot producing 0.20 g of biomass per seedling. The 0.45 g of biomass per seedling grown at low densities and in sterilized soil was greater than the 0.31 g of biomass per seedling reported for pots with nonsterile soil and starting densities of three seedlings per pot, but the average density was reduced to 1.2 per pot due to high seedling mortality. However, the biomass for these two treatments was greater than for seedlings grown in sterilized soil and planted at high densities. Therefore, the positive feedback effect on seedling total biomass in the native habitat with seedlings planted at high densities and in soil collected under mature black cherry trees (Fig. 2b) is mainly the result of calculating a proportion while the density of the survived plants varied between sterilization treatments, which interacted with total biomass production.

Within the invaded habitats, there was no effect of soil collected away from black cherry trees on seedling survival relative to soil collected under mature black cherry trees (Log linear analysis, $\chi^2 = 1.40$, $P = 0.236$). The same pattern was observed for biomass (Table 1). The distance treatments may have been less extreme in the invaded habitat where the high density of mature black cherry trees prevented us from collecting soil as far away from mature trees as in the native habitat. This may have resulted in greater similarity between my near and away treatments in the invaded habitat than the native habitat. However, if host-damaging soil pathogens would have accumulated in the invaded habitat, the direction of the soil biota effect should have been negative in both cases, rather than neutral to positive as observed in my experiment. Within the native habitat, there was no effect of distance on either survival (Log linear analysis, $\chi^2 = 1.91$, $P = 0.167$) or biomass of seedlings (Table 1).

Our results strongly suggest that invasion and far more aggressive growth of black cherry in Europe, compared to in North America where it is native, is driven

by differences in the resident soil communities, and in particular the absence of soil pathogens or reduced pathogenic activity. My study provides evidence that a tree species, whose distribution is limited by soil pathogens in its native habitat (6), may become invasive in an exotic habitat where native soil pathogens are not controlling its abundance. I propose that contrasting plant-soil feedbacks between native and invaded habitats are driven by escape from soil pathogens. However, my results are based on soil samples from one area in the U.S. and one area in Europe. There may be additional mechanisms affecting black cherry across its native and invaded habitats, and the importance of these different mechanisms may vary between sites.

Together with Callaway *et al.*'s (26) study of an invasive forb, our results provide strong evidence that escape of invasive plant species from their natural soil pathogens or pathogenic activity may contribute to their invasiveness in new habitats. Understanding the profound effect of soil communities on the abundance of native (23) and exotic species will improve predictions of both the invasiveness of plant species and the invasibility of natural communities. Future strategies for managing invasive exotic plants need to consider the important role soil organisms play in controlling plant abundance and maintaining plant species diversity in natural and invaded communities.

Methods

Nearest neighbor measurements

Nearest neighbor measurements were collected from two sites in each habitat type. In the native habitat, I sampled two sites in Indiana with mesic deciduous forests and moist well-drained soils. The forest composition was previously described (6). In the invaded habitat, the two sites in the Netherlands were in pine-oak forests with dry sandy soils. The sites were heavily invaded by black cherry, and *Pinus sylvestris* and *Quercus* spp. were the native dominants. Nearest neighbor distances were measured for seedlings (> 1 growing season of age and 0-1 m in height), saplings (1.1-3 m), and trees (≥ 3.1 m) using a modified pointed-centered quarter method (28) with randomly selected mature black cherry trees (n=10 or 11 focal trees per site) as center points instead of random locations on the landscape. Nearest neighbor estimates were measured from 0 to 50 m away from focal trees. If a black cherry neighbor was not found within an individual quarter, then the distance for this sample was conservatively estimated at 50 m since the actual distance to nearest neighbor was >50 m. The distance from focal trees to the nearest seedling, sapling, and tree was analysed using Two-way MANOVA, in which the main effects of habitat (i.e., native *versus* invaded) and sites were tested. I tested the effect of habitat and site on each life history stage using Two-way ANOVAs. Data were transformed to meet assumptions of homogeneity of variance. Data for distance to nearest seedlings were transformed with a reciprocal function ($1/(X+1)$) and distance to nearest saplings and trees were transformed with a log function ($\log[X+1]$) to make the variances more homogenous. The data for distance to the nearest saplings continued to violate the homogeneity of variance assumption following transformation but were still used in the analysis.

Soil biota experiments

Greenhouse experiments were conducted to test the effect of habitat (native *versus* invaded), soil sterilization (non-sterile vs. sterile soil), density of cherry seedlings (1 vs. 3 seedlings planted per pot), and soil distance (collected near [ca. 1 m] vs. away [4-30 m] from black cherry trees). Methods for the greenhouse experiment conducted in the native habitat (Indiana) of black cherry were previously reported for the 1998 experiment (6). The greenhouse experiment conducted in the invaded habitat (the Netherlands) used resident soil and seedlings and followed the experimental design utilized in the previous study (6). The Netherlands experiment deviated from the previous design in five minor ways. First, I did not mix soil collected from different trees, so that the individual trees were the replicates. Second, the away soil distance was ≥ 4 m away from the nearest conspecific instead of 25-30 m. The soil distance was intended to compare the effect of soil associated with roots of heterospecific trees on black cherry seedlings relative to soil associated with roots of conspecific trees. I was forced to sample at closer distances in the invaded habitat because the site was heavily invaded by black cherry. Third, seedlings were collected from one of the two field sites previously described, and the roots were surface sterilized in order to avoid the transplanting of rhizosphere biota from the field soil into the experimental units. Fourth, treatment soils were diluted with a 2:1 v/v instead of 1:1 v/v sterile potting soil to treatment soil mix. Autoclaved potting soil was added to reduce the fertilization effect associated with sterilization of field soils (29). Lastly, a foliar contact fungicide (Euparen-M, active ingredient Tolyfluanid, Bayer Inc.) was carefully applied directly onto leaves. This helped reduce a powdery mildew occurring on the foliage, while not impacting the soil biota.

For treatments with high seedling density, survival of one randomly selected target seedling per pot was analysed. Similarly, biomass of one randomly selected living seedling was analysed in high density pots. The effect of soil sterilization,

seedling density, and soil distance on seedling survival was analysed with a log linear analysis (PROC CATMOD, SAS version 8.0, SAS Institute Inc., Cary NC, USA). Three-way ANOVAs were used to examine the effects of soil sterilization, seedling density, and soil distance on seedling biomass (SPSS, version 10, SPSS Inc., Chicago, IL, USA). I estimated the mean soil feedback effect ($1 - [\text{sterile soil} / \text{non-sterile soil}]$) for each density \times soil distance treatment in each range using nonparametric bootstrapping techniques (JACKBOOT, BC_a, 3,000 resamples, SAS). Within each density \times soil source treatment, soil feedback effect estimates were calculated for percent survival and for least squares means of biomass were determined similarly.

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Table 1. Summary of ANOVA of effects of soil sterilization (non-sterile vs. sterile soil), initial seedling density, and soil distance (close to vs. away from) relative to black cherry trees (*Prunus serotina*) on the seedling biomass of black cherry in their native and invaded habitats.

Native habitat				
Effect	df	MS	F	P
Sterilization	1	0.128	11.17	0.001
Density	1	0.415	36.09	<0.0005
Distance	1	0.0003	0.03	0.87
Sterilization × Density	1	0.101	8.80	0.004
Sterilization × Distance	1	0.123	10.71	0.001
Density × Distance	1	0.0101	0.88	0.35
Sterilization × Density × Distance	1	0.0883	7.67	0.007
Error	108	0.1151		
Invaded habitat				
Effect	df	MS	F	P
Sterilization	1	1.657	11.35	0.001
Density	1	4.910	33.62	<0.0005
Distance	1	0.136	0.93	0.34
Sterilization × Density	1	0.004	0.03	0.87
Sterilization × Distance	1	0.015	0.10	0.75
Density × Distance	1	0.035	0.24	0.63
Sterilization × Density × Distance	1	0.111	0.76	0.39
Error	85	0.146		

Tests were performed using the Type three sums of squares from SPSS version 10 (SPSS Inc., Chicago, IL, USA).

Figure 1 Distances between conspecifics of black cherry (*Prunus serotina*) in their native habitat (gray bars) are much greater than in their newly invaded habitat (black bars). Bars represent the distance from randomly selected mature trees (n= 20-22) to the nearest seedlings (<1 m in height and >1 yr in age), saplings (1-3 m), and trees (>3 m) \pm 95% C.I. Asterisks (** and ***) represent significant effects of habitat ($P < 0.01$ and $P < 0.0005$, respectively) on distance between conspecifics after individual Two-way ANOVAs with habitat and site as factors.

Figure 2 Net positive effect of soil biota on black cherry (*Prunus serotina*) seedlings in their invaded habitat and net negative effect of soil biota on cherry seedlings in their native habitat. Effect of initial seedling density and soil distance on the soil feedback effect of **a**, survival and **b**, biomass data for black cherry (*Prunus serotina*) seedlings in their native (gray bars, Indiana) and invaded (black bars, the Netherlands) habitats. The estimated mean soil feedback effect (25) of survival (**a**) and least squares means of biomass data (**b**) was calculated using bootstrap resampling. Estimated means near zero indicate no effect of soil feedback effects on seedlings, estimated means >0 represent positive soil feedback effects, and estimated means <0 represent negative soil feedback effects. The data for the native range were reported previously (6). Arrows identify data with estimated means equal to or close to zero. Soil distance was either close (ca. 1 m) to or away (25-30 m in native habitat and >4 m in newly invaded habitat) from mature black cherry trees. In high density pots, survival was determined for one randomly selected seedling per pot, and total biomass was determined for one randomly selected living seedling per pot. The positive effect of soil sterilization on seedling survival for seedlings in their native habitat and planted at high densities and in soil collected near cherry (Fig. 2a)

affected the total biomass results for this treatment (Fig. 2b). Overall, seedling density had a negative effect on individual seedling biomass (Table 1), which I believe impacted the total biomass results of seedlings in their native habitat and grown in soil collected near black cherry and planted at high densities. Moreover, the large relative size of seedlings planted in non-sterile soil relative to sterilized soil resulted from unequal densities between sterile and non-sterile soil treatments (2.7 *versus* 1.2 seedlings per pot, respectively). Thus, release from intraspecific competition rather than soil sterilization effects caused the observed positive soil feedback effect in the native habitat.

Fig. 1

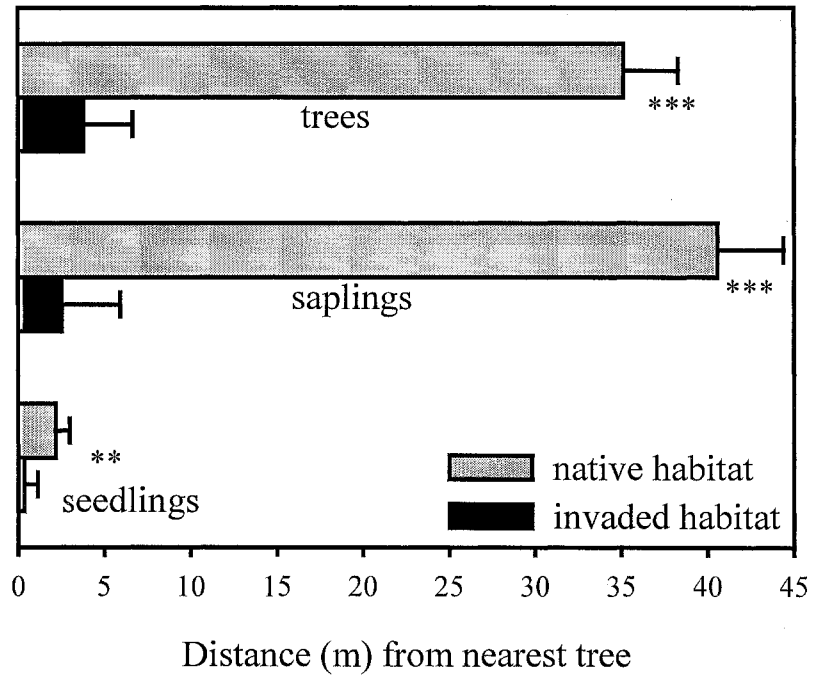


Fig. 2

