The effects of the invasive species *Berberis thunbergii* and exotic earthworms on salamander populations in deciduous forests of the northeastern United States

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Aimee Kemp
Ecology, Evolution, and Environmental Biology
Columbia University

Thesis Advisor:
Dr. Brian Justin Mailloux
Earth and Environmental Science
Barnard College

Research Mentor:
Dr. Matthew Palmer
Ecology, Evolution, and Environmental Biology
Columbia University

ABSTRACT

Invasive species are transforming northeastern deciduous forest ecosystems. The invasive plant, *Berberis thunbergii* (Japanese Barberry) is spreading rapidly and competing with native vegetation for space and resources because of its appeal as an ornamental shrub, the dispersal of its seeds by various birds, and reduced herbivory by deer due to its unpalatable, protective spines. Leaf litter from *B. thunbergii* causes changes to the chemical and biological composition of the surrounding soil, including increases in soil pH. This increase in soil pH facilitates the spread of invasive earthworm populations, which in turn decrease the amount of organic matter in the soil and forest floor. The changes these invasive species create are expected to have pronounced effects on the leaf litter layer and subterranean components of forest ecosystems. I will conduct research from late May until mid-August 2009 on the effects that *B. thunbergii* and exotic earthworms have on salamander populations at Black Rock Forest and other deciduous forest sites in the Hudson Highlands region of New York. Using ten pairs of 10 x 10 m plots (ten dominated by native vegetation and ten dominated by *B. thunbergii*), I will estimate % cover of *B. thunbergii*, salamander populations, exotic earthworm populations, and leaf-litter arthropod density and diversity. I will also measure soil composition, including pH, organic matter, leaf litter depth, and soil depth. I will then compare the results from the plots dominated by *B. thunbergii* to plots dominated by native vegetation. I expect that salamander populations will be lower and also be smaller in size class, soil pH will be higher, organic matter will be lower, leaf litter depth will be lower, arthropod density and diversity will be lower, and earthworm density will be higher in plots dominated by *B. thunbergii* than in plots containing mostly native species. If my predictions prove to be true then these invasives could have devastating effects on the function of deciduous forests as well as the trophic interactions within them.

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INTRODUCTION

Invasive species are changing the structure, function, and composition of ecosystems around the world. Due to their ability to spread rapidly by out-competing naïve native species, invasives cause severe declines in indigenous species diversity and in some cases extinction. The threat that invasives have on native biodiversity has become one of the most important problems to ecosystems all over the world (Vitousek et. al 1996).

North eastern deciduous forests, often characterized by oak (*Quercus sp.*), maple (*Acer sp.*), birch (*Betula sp.*), and beech (*Fagus sp.*), acidic soils, and a thick layer of leaf litter serve as important regulators of biogeochemical, hydrological, and nutrient cycles (Perry et. al 2008). However, the composition and function of north eastern deciduous forests are being changed dramatically by several invasive species. Some well-established invaders include the shrub *B. thunbergii* and three species of earthworm, including *Lumbricus terrestris*, *Dendrobaena octaedra*, and *Octolasion tyrtaeum* (Eisenahuer et. al 2007). These invaders change the composition of the soil, chemistry of the leaf litter, and due to these changes, could have detrimental affects on the native flora and fauna of the forest ecosystem.

Berberis thunbergii

Japanese barberry is native to Japan and was first introduced to North America from Russia in 1875 (Steffey et. al 1985). Its brightly colored red berries made it ideal for ornamental plantings. Since being brought into Eastern North America, *B. thunbergii* has spread rapidly out of decorative gardens and into deciduous forests through the dispersal of its seeds by birds. Once established, this shrub gains a competitive advantage by avoiding deer herbivory, due to its harsh spines which are unpalatable to deer (Ehrenfeld et. al 1997). *B. thunbergii* also accelerates its invasion into northeastern deciduous forests by modifying the soil conditions through leaf litter chemistry (Ehrenfeld et. al 2001, Li et. al 2008). Berberis leaf litter increases nitrification rates in soil, creating a positive feedback cycle that allows it to outcompete native vegetation for space and soil nutrients (Ehrenfeld et. al 2001). Areas invaded by barberry have higher soil pH, less leaf litter, and reduced soil organic horizons when compared to areas with native vegetation (Kourtev et. al 1998). Aside from creating a positive feedback cycle that facilitates its own invasion, the changes caused by *B. thunbergii* also facilitate the invasion of other species throughout northeastern deciduous forests (Ehrenfeld et. al 2001, Li et. al 2008).

Earthworms

Abundance of exotic annelids has been closely tied to the invasion of *B. thunbergii*. Because barberry raises soil pH, it makes the soils in deciduous forests more suitable for earthworms which prefer to live in more basic soils (Laverack et. al 1961). Three species of earthworm have invaded northeastern deciduous forests including *L. terrestris, D. octaedra,* and *O. tyrtaeum* (Eisenhauer et. al 2007). Earthworms first arrived in North America when they were introduced by European settlers in the 16th century. Earthworms are well-known ecosystem engineers (Bohlen et. al 2004). Through burrowing and mixing soil layers they reduce the amount of organic matter in soils and also increase rates of decomposition (Holdsworth et. al 2008). In this manner, earthworms also influence nutrient cycling, mineralization, and microbial biomass in the soil and leaf litter (Eisenhauer et. al 2007). These changes affect the plant community as well as the organisms that rely on leaf litter for food and the creation of microhabitats. The presence of earthworms in deciduous forests has been correlated with less leaf litter, smaller organic horizons, and a lower density of leaf litter arthropods (Migge-Kleian et. al 2006). With fewer arthropods in the leaf litter and the alteration they cause to soil microhabitats, earthworms have been linked to declines in predators including many terrestrial salamanders (Migge-Kleian et. al 2006).

Salamanders

Salamanders are a key component of food webs in forest ecosystems. They act as the top predators in the leaf litter food web by consuming and controlling arthropod populations (Wyman et. al 1998, Walton and Maerz et. al 2006). While salamanders exhibit top-down effects on the forest floor food web, they are also an important food source for the larger predators that inhabit the forest such as skunks (Mephitidae), raccoons (*Procyon lotor*), and several species of snake (Serpentes) (Burton et. al 1974). *Plethodon cinereus* (Eastern red-backed salamander) alone accounts for the most vertebrate biomass in northeastern forests of the United States and southeastern Canada reaching densities of up to 4 individuals/m^2 (Burton et. al 1975, Wyman et. al 1998).

Salamanders, particularly lungless salamanders from the family Plethodontidae, need to have moist membranes to allow gases to exchange across their skin. Because of this they are also sensitive to soil pH levels (Sugalski et. al 1997). Terrestrial salamanders receive moisture from the leaf litter and by burrowing into the organic layer of soil or by staying in the microclimates created beneath rocks and logs. Some direct developing terrestrial salamanders also rely on leaf litter and a deep organic horizon to lay their eggs. With invasives such as *B. thunbergii* and earthworms causing changes in the composition and chemistry of deciduous forests in eastern North America, salamander populations may be in danger of decline which could cause a trophic cascade within the leaf litter and larger forest communities.

Ecosystem Interactions

With invasions of non-native species in northeastern deciduous forests becoming more prevalent, it is important to examine the effects such species have on not only native flora, but fauna as well (Figure 1). Because *B. thunbergii* changes the chemistry and composition of the soil and leaf litter layers, its expansion facilitates the invasion of exotic earthworms. In turn, this increases mixing in soils and decreases the amount of organic matter and leaf litter available for arthropods to hide in and feed on. By reducing leaf litter earthworms also change the number of microhabitats available, which are important to the survival of many terrestrial salamanders that live in the northeast. With the reduction in the abundance of leaf litter and soil arthropods it is possible that these invasives will have affects on not only salamanders but their predators as well, causing a trophic cascade that could change the function of eastern deciduous forest ecosystems. This summer I will be examining these connections to determine the affects that *B. thunbergii* has on exotic earthworm populations, how this affects arthropod densities, and ultimately how all of these changes affect salamander abundance.

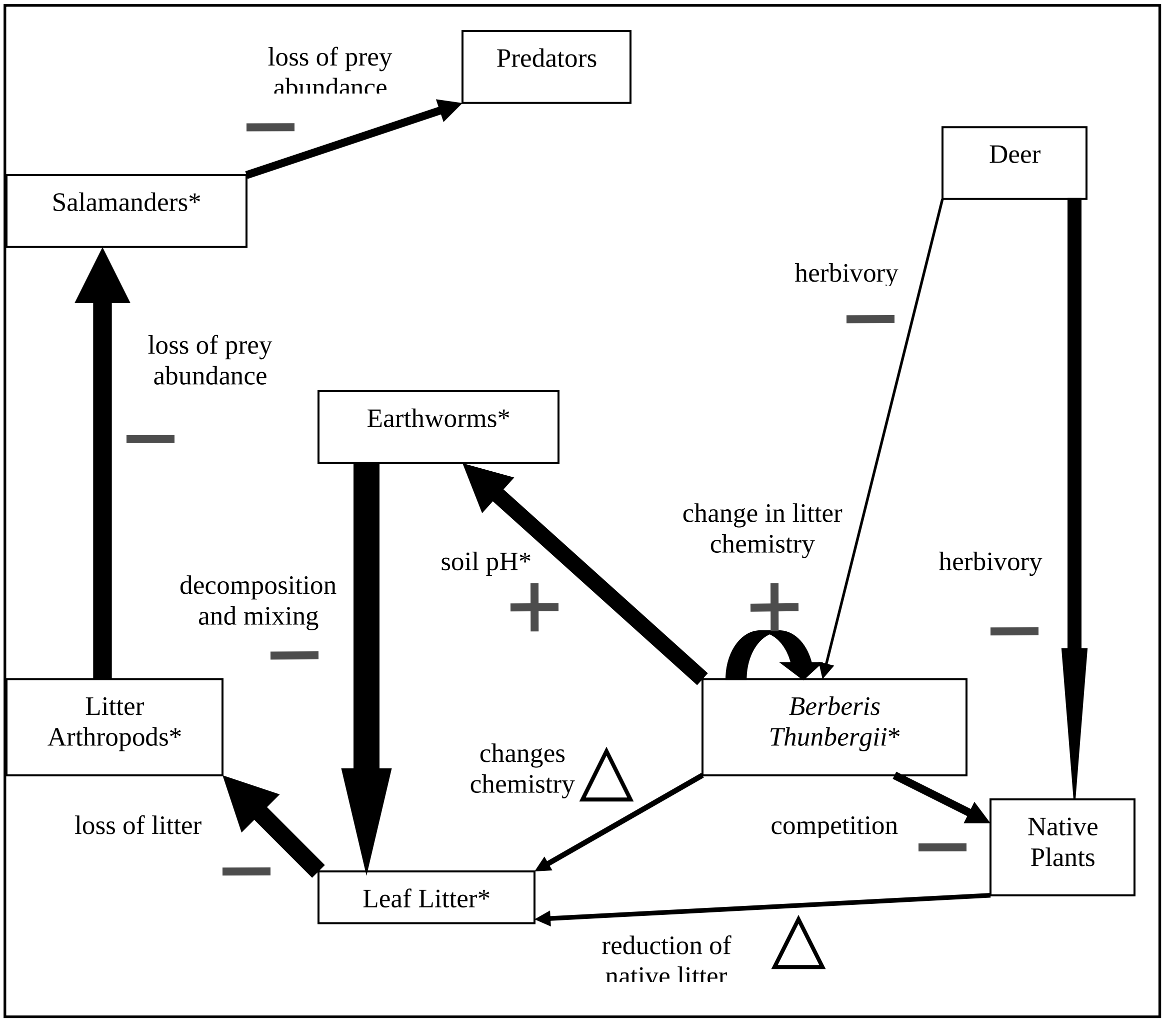

Figure 1: The interactions between native and non-native organisms in northeastern deciduous forests as well as the abiotic factors that affect the structure of the forest ecosystem can be seen here. Thick arrows indicate that there is a strong relationship between one factor and the next. Minus signs indicate a decrease in an ecosystem component caused by interactions while plus signs indicate an increase. A triangle indicates a change in an ecosystem component. The boxes containing stars are the ones that will be measured and studied from May to August 2009.

THESIS STATEMENT

Based on the connections between invasive and native species in northeastern deciduous forests I predict that:

-Plots dominated by *B. thunbergii* will have higher soil pH, less leaf litter and organic matter on the forest floor and in the soil, and lower densities of arthropods than in plots dominated by native vegetation.

-Due to an increase in soil pH, plots dominated by *B. thunbergii* will have higher densities of earthworms than in plots dominated by native vegetation.

-Plots dominated by *B. thunbergii* will have lower salamander densities, due to their sensitivity to changes in pH and the decrease in litter arthropods as their main source of prey, than in plots dominated by native vegetation. Salamanders will also be smaller in plots dominated by *B. thunbergii* because of the reduced availability of their arthropod prey.

METHODS

Study Site

Research will be completed at Black Rock Forest (BRF; 41º24'N, 74º01'W) with some plots in the surrounding forests. BRF is a 1530-hectare deciduous research and educational forest located in the Hudson Highlands of southeastern New York (Figure 2) characterized by shallow, medium-textured loamy soils with glacial granite gneiss as its base (Li et. al 2008). Black Rock is also characterized by a mixed deciduous forest, dominated mostly by oak, beech, maple, and birch (Schuster et. al 2008). Its average rainfall is about 1190 mm/year (Schuster et. al 2008).

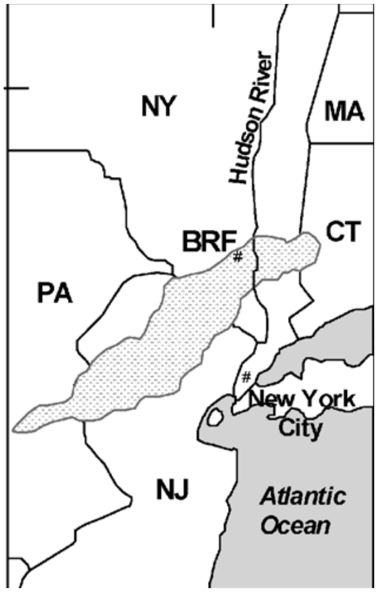

Figure 2: This is a map of Black Rock Forest showing its location within the Hudson Highlands and its location in relation to the states around it as well as New York City (Schuster et. al 2008).

Plots

There will be ten 10m x 10m pairs of plots throughout Black Rock Forest and the surrounding region. Within each pair, one plot will be dominated by native vegetation and the other plot will be dominated by *B. thunbergii*. Plots will be separated by 5-20 m while pairs will be separated by 0.5 km. All of the plots will be evaluated to make sure that the only difference between them is the presence or absence of *B. thunbergii*. Each plot will be characterized as a suitable habitat for salamanders depending on the number of rocks and logs within the plot that would create a good microclimate. The plots will be matched to have similar amounts of woody debris and rocks, as well as a similar slope, gradient, etc. so as not to bias the results of the study. In order to reduce bias in the study, plots will be established such that they have similar abundances of rocks and logs available for hiding (rock diameter of 30-80 cm; log diameter of 15 cm).

Biotic Measurements

Salamander Population Estimates

To estimate the size of salamander populations, a photographic record will be made of all individual salamanders that are found in each plot. In order to find salamanders, cover objects (i.e. rocks 30-80 cm and logs 15 cm in diameter) and leaf litter will be searched when rain has fallen in the last 24 hours (Williams et. al 2004). Once a salamander has been captured it will be identified to species, measured from snout to vent (SVL) to the nearest millimeter (for an estimate of size-class structure), labeled as a juvenile or adult (an adult will be considered any individual >34mm; Dodd et. al 2004), then photographed to make it identifiable as a previously captured individual. Salamanders have unique patterns on their bellies which make them easy to identify. Twenty four hours after the next rain, salamanders will be searched for again and a note of how many previously photographed individuals are recaptured will be taken. Any newly discovered individuals will also be captured, identified, measured, and photographed. Using the total number of previously photographed individuals that are found while searching again, population estimates will be extrapolated based on the formula:

$N = [(n_1 + 1)(n_2 + 1)/(m_2 + 1)] - 1$ (Equation 1)

where N = population size, n_x = the number of individuals obtained in a single sampling period, and m_2 = the number of previously photographed individuals (Chapman et. al 1951).

B. thunbergii

The cover of *B. thunbergii* will be estimated for each plot. Plots containing <5% cover by *B. thunbergii* will be considered plots dominated by native vegetation while plots containing >50% cover by *B. thunbergii* will be considered dominated by the invasive. Percent cover will be determined using a 10 x 10 sampling grid where percent cover will be equal to the number of boxes covered by *B. thunbergii* will equal its cover out of 100.

Earthworm Population Estimates

Population estimates of earthworms will be determined using the 'hot mustard method' (Lawrence et. al 2001). Since pouring a dilute hot mustard solution onto the soil may disturb salamanders in the plots, all earthworm sampling will be done in randomly located 1 m^2 plots outside of the 10 x 10 m salamander sampling plots, but in areas with similar vegetation and cover objects. The size of the earthworm populations in each smaller plot will be extrapolated to determine an estimate of the earthworm populations per each 100 m^2 plot. All earthworms collected will be identified to species (Kricher et. al 1998).

Arthropod Diversity and Population Estimates

Arthropod diversity and population estimates will be conducted at five randomly determined locations per plot using the Berlese funnel method (Wyman et. al 1998). Leaf litter will be collected at each of the five locations, mixed, and placed into a funnel. The funnel will be placed below a 40-watt light bulb and above a jar of 20% ethanol. As the light bulb dries the leaf litter, any arthropods that have been collected will move toward the base of the funnel where the leaf litter is still moist and will eventually fall into the jar of ethanol below. Arthropods will be extracted from the leaf litter for 48 hours (Wyman et. al 1998). Each jar will be emptied with its contents sorted into two categories: 1) salamander prey (including Oribatid mites, Non-oribatid mites, Collembola, Enchytraeids, Psuedoscorpions, Isopods, Diplopods, Coleoptera, Curculionidae, Gastropods and Annelids; Maerz et. al 2005, Walton et. al 2006) and 2) non-salamander prey.

Abiotic Measurements

pH

Soil pH of the soil will be measured by collecting five 50 gram samples from randomly determined locations in each plot. Next, after crushing any large clumps, 50mL of deionized water will be added to the soil jars. Then each jar will be shaken for 45 seconds and left to sit for eight minutes in order to let the soil settle. Finally, the pH of each soil sample will be taken using a pH meter (Eckert et. al 1995).

Soil Organic Matter

To measure the amount of organic matter in the soil, five samples will be taken from randomly determined locations in each plot. Each sample will undergo a loss on ignition (LOI) analysis. Each sample will be weighed and then placed in a crucible and dried for 12-24 hours in a muffle furnace at 550◦C. After drying is complete the sample will be weighed again to determine the mass lost. The difference between the first weight and the weight after drying will equal the amount of organic matter present in the soil (Santisteban et. al 2004).

Soil Depth

In order to measure the depth of the soil, particularly the organic (O) horizon, a soil corer will be used to take five soil core samples from randomly determined locations within each plot. The depth of each horizon will be measured to the nearest 0.5 cm. The texture and color of each soil layer will be described. To characterize soil depth across the plot, a narrow steel probe will be driven into the soil and the depth to rock recorded for each of 100 points in a 10 x 10 sampling grid.

Leaf Litter Depth

Leaf litter depth will be measured using the same 10 x 10 sampling grid as for soil depth characterization. Each measurement will be taken to the nearest millimeter. The measurement will be taken based on where the top-most leaf in the litter touches the metric ruler for each sample.

Statistical Analyses

In order to compare the plots with native vegetation and the plots with *B. thunbergii* a paired t-test will be used to determine if there is a significant difference in salamander populations between them. Linear regressions will also be used to test the relationships of 1) litter depth vs. % *B. thunbergii*, 2) litter depth vs. earthworm density, 3) arthropod abundance vs. litter depth, and 4) salamander abundance vs. arthropod abundance.

Time Table

May 5: Turn in Thesis Proposal
May 12: Go to BRF with Matt and set up plots, characterize % *B. thunbergii* per plot
May 24: Move in at BRF
May 25: Gather soil samples and leaf litter depth data
June 1: Set up Berlese funnels and get leaf litter samples for arthropods
June 2: Mix leaf litter and put into funnels and get soil depth samples
June 3: Sample salamanders (if 24hr after rain)
June 4: Empty Berlese funnels and identify arthropods
June 5: Sample earthworms
June 8: find soil pH
June 9: Begin LOI
June 10: Sample salamanders (if 24hr after rain)
June 11: Finish LOI
End of June-July: Continue sampling salamanders
August 21: Fly back to CA
September to November ’09: Analyze data and begin to write Thesis
December ’09: Poster Session and turn in Thesis

DISCUSSION

Salamanders are key components of forest ecosystems. They create a top-down effect on forest floor trophic levels by consuming large amounts of arthropods, including many detritivores which break down leaf litter and woody debris and release carbon into the atmosphere (Wyman et. al 1998). If the plots invaded by *B. thunbergii* and earthworms are shown to have lower populations of salamanders, it will be an indication that these invasive species have the capacity to indirectly influence trophic interactions. If *B. thunbergii* continues to change the composition of forest ecosystems then there will be fewer salamanders in forest ecosystems which will lead to less control over arthropod abundance and also less prey for the larger predators in deciduous forest ecosystems such as raccoons (*Procyon lotor*), skunks (Mephitidae), and various species of snakes (Serpentes).

CONCLUSIONS

With the changes that *B. thunbergii* and European and Japanese earthworms are causing in northeastern deciduous forest composition, it is important to determine how these changes affect native organisms as well. Because salamanders are such a key component of forest food webs (Wyman et. al 1998), if their populations are in fact lower in patches that are dominated by these invasives, then there could be a change in trophic interactions as well. As *B. thunbergii* and exotic earthworms spread throughout northeastern deciduous forests, if they cause salamander populations to decrease then, as a result, they could also contribute to a decline in predator populations that rely on salamanders as a food source causing a trophic cascade that could prove detrimental to the health of the entire forest ecosystem.

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