THE EFFECTS OF SOIL MANIPULATIONS ON INVASION SUCCESS OF TWO EXOTIC SPECIES, JAPANESE BARBERRY (*BERBERIS THUNBERGII*) AND JAPANESE STILTGRASS (*MICROSTEGIUM VIMINEUM*)

by

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ABSTRACT OF THE DISSERTATION

THE EFFECTS OF SOIL MANIPULATIONS ON INVASION SUCCESS OF

TWO EXOTIC SPECIES, JAPANESE BARBERRY (*BERBERIS THUNBERGII*)

AND JAPANESE STILTGRASS (*MICROSTEGIUM VIMINEUM*)

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Dissertation Director:

Joan G. Ehrenfeld

Biological invasions by exotic, invasive plants are widely recognized as a threat to biodiversity and ecosystem function. Invasive plants alter ecosystem processes, specifically shifts in plant community composition and nutrient cycling. Subtle ecosystem effects of non-native species invasion are often undetected and understudied. Invasion driven changes to above- and below-ground processes and structure can create feedbacks that increase site susceptibility to further invasion. Enhancing resistance to further invasion in systems already experiencing altered community structure and nutrient cycling continues to challenge restoration ecologists. To investigate practical ecological methods designed to enhance biotic resistance of a system to invasion, I tested the effectiveness of six different soil manipulations to alter chemical, physical, and biological aspects of forest soils that influence the invasion of two invasive, exotic species, *Berberis thunbergii* and *Microstegium vimineum*. I focused on experimental manipulations of nitrogen availability, surface leaf litter, and mycorrhizal infection in greenhouse and field studies to provide a multi-pronged approach to investigations of exotic species invasion success. Nitrogen additions tested the response of two exotic and two native species to

different forms and concentrations of nitrogen. Exotic species were found to be more plastic in their growth response to either nitrogen form in both excessive or limiting concentrations. Topsoil removal showed some success in limiting inorganic nitrogen availability but trends were not consistent. Woodchip additions were not successful at immobilizing nitrogen or increasing the C:N ratio of the soil. Aluminum sulfate, added to increase soil acidity, was also not consistently effective, but did lower soil pH temporarily. The nitrification inhibitor applied to field plots proved to be ineffective in forest soils. Leaf litter addition applied to field soils in which *Microstegium* seeds were planted did not inhibit but enhanced its growth compared to soils without litter. And finally although *Microstegium* roots were found to be responsive to arbuscular mycorrhizal colonization, its growth in two different forest soils was not negatively affected by removal of mycorrhizal inoculum. This research highlights the plasticity of these two exotic species to a variety of environmental conditions and reveals the challenges of forest soil restoration.

DEDICATION

This dissertation is dedicated to my parents, Larry and Barbara Ross,

who have always encouraged my passion to study the world around me.

This dissertation would not have been possible without their

unwavering love and support.

student scientists, and they have enhanced the quality of my education. I am thankful to many faculty and staff members of the Department of Ecology, Evolution, and Natural Resources who made my time here at Rutgers easier from behind the scenes. Lastly, I would like to thank my family for their support, patience, and love.

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CHAPTER 1

General Introduction

Biological invasions into natural plant communities are widely recognized as a

threat to biodiversity and ecosystem function (Elton 1958, Mooney and Drake 1989,

Vitousek et al. 1987, Williamson 1996, Mack et al. 2000, Corbin and D'Antonio 2004).

Historically, invasions have occurred by the spread of exotic species that are introduced

into a new environment, then establish and spread across landscapes. These introductions

have often been associated with anthropogenic disturbance of habitats and ecosystems

and much research has been devoted to understanding why introduced species are so

successful outside of their native range (Fox and Fox 1986, Drake et al. 1989, Tilman

1997, Cox 1999, Mooney and Hobbs 2000). Some authors have attributed the

competitive success of invasive species to specific traits (*e.g.* fecundity, resource

utilization, adaptability) (Crawley 1987, Lodge 1993, Kolar and Lodge 2001). Others

have suggested that certain habitat characteristics such as a type of disturbance, a change

in resource availability, or habitat fragmentation can promote invasion (Levine and

D'Antonio 1999, Davis et al. 2000). Although most exotic species do not become

invasive outside of their natural range (Williamson 1996), a combination of these factors

can increase the competitive ability of exotic species leading to their invasiveness

(Dunbar and Facelli 1999). Once the introduction of an exotic species occurs, nutrient

availability or limitation of resources may influence establishment success (Fox and Fox

1986, Huenneke et al. 1990, Stohlgren et al. 1999, Davis et al. 2000).

Increasingly invasion biology research has focused on identifying factors that

increase biotic resistance of an ecosystem to invasion that limit the success of exotic,

invasive species (Elton 1958, Burke and Grime 1996, Mack 2002, Levine et al. 2004). Elton (1958) proposed that most systems demonstrate some form of ecological resistance. In other words systems possess inherent characteristics that decrease the likelihood that most introduced species will not survive in their new environment. If they do persist, then it is not likely they will drastically alter the system or become dominant. The theory that colonizing species have to overcome some form of biotic resistance in order to successfully establish into a new community has been broadly applied to explain why invaders are found in some systems and not in others. Most of the literature outlining evidence for the biotic resistance hypothesis focuses on the effects of interactions between resident species and an invader including impacts of herbivory, competition, and disease (Levine et al. 2004). Enhancing biotic resistance of a system has important conservation and restoration implications. Understanding the inherent characteristics of or interactions that exist within an ecosystem that lead to biotic resistance to invasion will inform management priorities for combating invasive species.

Resource availability plays an important role in driving invasion success (Davis et al. 2000). When an exotic plant is first introduced to a new habitat, competition for limited resources with resident species is probably the first interaction that species must overcome to establish and spread. The availability of resources fluctuates depending on climate, precipitation, disturbance, and the current resource acquisition of the resident community (Davis et al. 2000). Plant competition models predict that species that can quickly capitalize on available resources will most likely be better competitors (Tilman 1988), and that different species vary in their abilities to utilize resources efficiently (Marschner 1986). If for example, soil nitrogen availability is increased, will the

competitive hierarchy within the plant community shift to favor species that more rapidly take up nitrogen? If a particular species can manipulate resource availability to its advantage, it will increase its competitive ability (D'Antonio et al. 1998, Suding et al. 2004, Vila`and Weirner 2004).

Since many exotic species have been shown to enhance nutrient availability that can lead to further facilitation of invasion (Simberloff and Von Holle 1999, Ehrenfeld 2003), soil manipulations are commonly being used to alter resource availability (Alpert and Maron 2000, Suding et al. 2004). Soil manipulations that will decrease resource availability may be effective in limiting the competitive ability of dominant exotic species. Levine et al. (2004) suggest, instead of studying specific interactions between an exotic and a native species that are hardly generalizable to all species in all systems where they occur, it may be more useful to determine methods that will improve "abiotic" resistance of a system. Influencing abiotic factors that lead to enhancement of biotic resistance will contribute to prevention of the establishment and spread of invasive species.

A majority of the research that has attempted to alter resource availability has been done in grasslands, prairies, wet meadows, and agricultural fields where soil manipulation is not as challenging due to open canopies, short-lived vegetation, fewer sloped and rocky substrates, and less deep woody root competition (Morghan and Seastedt 1999, Blumenthal et al. 2003, Corbin and D'Antonio 2004). Few researchers have attempted to alter soil processes in forested ecosystems due to the difficulty in working with slower growing vegetation and complex woody root structures as well as the feasibility of using large-scale equipment needed to effectively administer the

manipulations. Due to the extent of invasion in forested systems, however, feasible management strategies to control exotic plants in forests are urgently needed.

Throughout natural areas in the Northeast and Mid-Atlantic, native plants are at a disadvantage due to habitat fragmentation, herbivory pressure, and increasing propagule pressure of exotic species (Kourtev et al. 1999, Ruhren and Handel 2003). Intact forest stands have undergone extensive invasion by both woody and herbaceous species such as Japanese barberry (*Berberis thunbergii*) and Japanese stiltgrass (*Microstegium vimineum*) (Ehrenfeld 1997, Kourtev et al. 1998, Ehrenfeld et al. 2001, Aronson et al. 2007). Approximately twenty years ago neither of these species was found at high densities in New Jersey forests (Dibeler and Ehrenfeld 1990). *Microstegium vimineum* (Trin.) A. Camus (Japanese stiltgrass) was introduced to the US from Japan. It was first collected in 1919 near Knoxville, TN and by 1972 it had spread to at least 14 eastern states (Barden 1987). *Microstegium* is a C_4 annual grass well adapted to low light conditions. *Microstegium* is commonly found on floodplains, disturbed areas, and intact forests competing with herbaceous and woody seedlings for light in the late spring when it germinates. Most often it is found in open areas, but also survives under closed canopy (Barden 1987).

Berberis thunbergii (Japanese barberry) a thorny horticulturally valuable shrub, was also introduced from Japan in the late 1800s (Cassidy et al. 2004). It has spread into 31 states in the US. Forming dense thickets in open areas and intact forests, it often replaces native understory species (Silander and Klepeis 1999). It spreads vegetatively, and its seeds are often dispersed by birds, small mammals, and

sometimes deer (Ehrenfeld 1999). *Berberis* seems to be a habitat generalist and is commonly found on nutrient rich soils with neutral pH.

Previous research by Kourtev et al. (1998, 1999) and Ehrenfeld et al. (2001) has shown that soil conditions found in established stands of these two exotic, invasive plants from a variety of forested sites in New Jersey have higher rates of nitrification compared to rates found in soil under native understory vegetation. They report evidence that these species are utilizing nitrate (NO_3^-) more efficiently, storing high amounts of N in leaf tissues, and contributing large amounts of N to the soil surface compared to their native counterparts (Kourtev et al. 1998). Furthermore, previous greenhouse experiments have shown that pH and nitrification rates increase when these exotics are planted into previously undisturbed forest soils with initial low pH and very low nitrification rates (Ehrenfeld et al. 2001, Kourtev et al. 2003). Their results strongly suggest that soils under these exotic species have different characteristics than soil found under native species indicating that plant mediated changes in nutrient availability or other soil properties could contribute to invasion success.

The research presented in this dissertation addresses whether soil manipulations can be effective in altering abiotic factors of a resident community already severely impacted by invasion that represent forest floor characteristics prior to invasion. I investigated the effectiveness of a series of soil manipulations to affect chemical, physical, and biological aspects of forest soils. Chapter 2 describes nitrogen additions added in two forms to both exotic and native species in a greenhouse study. I tested whether both the quantity and chemical form of available nitrogen are crucial to the invasion success of these exotic species. Chapter 3 describes the use of three types of soil

amendments in addition to topsoil removal to test whether available nitrogen in the top

5cm of the soil surface could be reduced and thereby limiting nutrient availability to these

exotics species. In Chapter 4, I tested whether the addition of native species of leaf litter

and woody debris act as a physical barrier to the invasion success of *Microstegium*

vimineum. In Chapter 5, I removed the arbuscular mycorrhizal community from two

forest soils to test whether *Microstegium vimineum* is reliant on this mutualism in order to

invade into new areas.

  If control methods can be determined as a result of this research that are

practical and affordable, park managers will be able to design system-based

management plans to enhance biotic resistance. Possibly, these methods could be

applied broadly in other parts of the country. In addition, the information gained from

this research will contribute to what little is known of the biology of *Berberis*

thunbergii and *Microstegium vimineum* and whether their ability to invade forest

communities can be altered. As managers attempt to implement best management

practices for invasive species control, further research concerning the importance of

forest soil dynamics in the establishment of these species is needed. Overall success

of restoration efforts may only be achieved if biotic factors, abiotic factors, and their

interactions are considered when making management decisions.

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CHAPTER 2

The Effects of Nitrogen Addition on the Growth of Two Exotic and Two Native

Forest Understory Plants

INTRODUCTION

Increasingly invasion biology research has focused on identifying factors that

increase biotic resistance of an ecosystem to invasion and limiting the invasion success of

exotic, invasive species (Elton 1958, Burke and Grime 1996, Mack 2002, Levine et al.

2004). The possibility of enhancing a site's ability to resist invasion based on some

intrinsic ecological dynamic or characteristic provides hope for restoration ecologists.

Most of the literature outlining evidence for the biotic resistance hypothesis focuses on

the effects of resident species interacting with an invader including impacts of herbivory,

competition, and disease that must be overcome to successfully establish and spread

(Levine et al. 2004). In their meta-analysis Levine et al. (2004) question whether these

biotic interactions alone effectively contribute to biotic resistance. Ultimately, resource

availability drives species interactions. To enhance biotic resistance, researchers must

first determine invader response to available resources. As the soil environment changes,

the competitive hierarchy within plant communities may shift. The study presented here

expands the concept of biotic resistance to include abiotic factors that may limit or

enhance invader species establishment. I propose that exotic, invasive plants with rapid

growth rates may have a differential growth response to readily available nitrogen such as

nitrate than slower-growing native species. If limiting soil nitrate alters the competitive

hierarchy, then land managers can design invasive plant control strategies that enhance biotic resistance of a system through the manipulation of abiotic resources.

Resource availability is a necessary abiotic factor that plays an important role in driving invasion success (Davis et al. 2000). When an exotic plant is first introduced to a new habitat, competition for limited resources with resident species is probably the first interaction that species must overcome to establish and spread. The availability of resources fluctuates depending on climate, precipitation, disturbance, and the current resource acquisition of the resident community (Davis et al. 2000). Plant competition models predict that species that can quickly capitalize on available resources will most likely be better competitors (Tilman 1988), and that different species vary in their abilities to utilize resources efficiently (Marschner 1986). If a particular species can manipulate resource availability to its advantage it will increase its competitive ability (D'Antonio et al. 1998, Suding et al. 2004, Vila`and Weirner 2004).

Exotic, invasive plant species richness and cover have been associated with an increase in resource availability (Stohlgren et al. 1999, Milberg et al. 1999, Davis and Pelsor 2001, Suding et al. 2004). In a review Daehler (2003) found that exotic species exhibit enhanced performance over native species in high but not in low-resource environments. Burns et al. (2007) report that the advantage invasive members of the Commelinaceae had over noninvasive congeners was reduced when grown under low nutrient conditions and when clipped. If exotic species possess characteristics that promote efficient resource acquisition, and additionally if they can out-perform native species due to release from enemies such as herbivores and pathogens in the introduced range, those exotics will eventually become invasive (Blumenthal 2006). The question

remains whether exotic invasive species actually do respond differently to nutrient enrichment than native species.

Different plant species may vary in their abilities to utilize forms of nitrogen (i.e., NO_3^- vs. NH_4^+) (Marschner 1986, Gilliam 2006, Miller et al. 2007). Miller and Bowman (2002) found that while some species tested in a greenhouse experiment preferentially took up a single form of nitrogen, other species did not differentiate in preference for a specific N form. Miller et al. (2007) described the differential uptake of nitrogen by co-occurring species in nitrogen-limited systems depended on what type of nitrogen nutrition was supplied. Species that were more plastic in their nitrogen preference were less affected by neighborhood competition. Many researchers have suggested if invasive or weedy plants respond differently than native species to nutrient enrichment (Lowe et al. 2003, Suding et al. 2004, Vila`and Weirner 2004), competitive ability and ultimately community composition may be affected (Wedin and Tilman 1993, Gilliam 2006). In general, species that thrive in high pH soils utilize nitrate preferentially whereas plants that have adapted to low pH conditions prefer ammonium nutrition (Marschner 1986). Previous research has shown that exotic species that have invaded the forest understory are commonly growing in soils of high pH whereas native species are often growing in soils of low pH (Ehrenfeld et al. 2001, Heneghan et al. 2006). The specific response of particular invasive species to various forms and quantities of nitrogen has not been well studied (but see Padgett and Allen 1999). Often researchers have either examined generalized environmental gradients of nutrients without targeting a specific resource, or have implicated nitrogen indirectly, as one component of resource availability (Davis et al. 2000, Davis and Pelsor 2001, Suding et al. 2004). The majority of the research

designed specifically to test growth response of invasive species to nitrogen addition has

been conducted in grassland or old field systems with annual and perennial herbaceous

vegetation (Huenneke et al. 1990, Milchunas and Lavenroth 1995, Wedin and Tilman

1993, Suding et al. 2005). The results from these studies are difficult to translate to more

highly biologically and structurally complex forested systems. Rarely has growth

response to nutrient availability, specifically testing different forms of nitrogen on

specific species been investigated for dominant forest understory invaders (Gilliam

2006). Determining the role that nitrogen dynamics play in the success of many plant

invaders is increasingly important particularly in areas with high rates of local and

regional nitrogen deposition and with climate change imminent (Mooney and Hobbs

2000, Howard et al. 2004).

Intact forest stands of the Northeast and Mid-Atlantic have undergone extensive

invasion by both woody and herbaceous species such as Japanese barberry (*Berberis

thunbergii*) and Japanese stiltgrass (*Microstegium vimineum*) (Ehrenfeld 1997, Kourtev et

al. 1998, Ehrenfeld et al. 2001, Aronson et al. 2007). Previous research by Kourtev et al.

(1998, 1999) and Ehrenfeld et al. (2001) has shown that soil conditions found in

established stands of these two common exotic, invasive plants from a variety of forested

sites around New Jersey have higher rates of nitrification compared to rates found in soil

under native understory vegetation. They report evidence that these species are utilizing

NO_3^- more efficiently, storing high amounts of N in leaf tissues, and contributing large

amounts of N to the soil surface compared to their native counterparts (Kourtev et al.

1998). Furthermore, previous greenhouse experiments have shown that pH and

nitrification rates increase rapidly when these exotics are planted into previously

undisturbed forest soils with initial low pH and very low nitrification rates (Ehrenfeld et al. 2001, Kourtev et al. 2003). Their results strongly suggest that both the quantity and chemical form of available nitrogen may be crucial variables determining the ability of both of these species to invade new areas and to form dense infestations. In this study we further investigate whether these two problematic species perform better when nitrogen is highly available and whether they respond differently to different forms of nitrogen (i.e., NO_3^- vs. NH_4^+) than native counterparts.

A greenhouse study was conducted to test the growth response of two exotic species and two native species to different forms and concentrations of nitrogen nutrition. Using a full-factorial design, the effects of nitrogen form (two levels: NO_3^--Hoagland's solution addition and NH_4^+-Hoagland's solution addition) and concentration (three levels: low, normal (or ambient), and high levels) were investigated by measuring the growth response and survival time of four species (two exotic and two native). Based on prior field soil analysis, the concentration level of the normal Hoagland's solution mimicked the concentration of inorganic nitrogen presently available in forest soils where these species are found (Ehrenfeld et al. 2001). The native species chosen in this experiment were once commonly found in forests where Berberis and Microstegium now dominate. They would likely compete with these exotic if herbivory pressure were curbed and native abundances could be restored. I hypothesized that (1) both Japanese barberry and stiltgrass would preferentially respond to the nitrate form of nitrogen than to the ammonium form, especially at high concentrations, and (2) that the native species would preferentially respond to the ammonium form of nitrogen, especially at ambient (or normal) concentrations. Since both of these exotic, invasive species are wide-spread

problems in the Northeast where anthropogenic sources of nitrogen are increasing

(Vitousek et al. 1997), and because nitrogen enrichment favors the spread of invasive

species (Huenneke et al. 1990, Lowe et al. 2003), knowing how problematic species

respond to specific forms of nitrogen availability will inform management efforts

designed to increase the resistance of a community to invasion.

METHODS

Characteristics of Study Species

I chose to use *Microstegium vimineum* (Trin.) A. Camus (Japanese stiltgrass) and

Berberis thunbergii (Japanese barberry) for this study because they commonly dominate

forest understories together. *Microstegium vimineum* (hereafter referred to as MV) is a

shade-tolerant grass accidentally introduced from Asia and was first collected in the US

in 1919. It has spread rapidly into disturbed and mature forests in at least 21 eastern and

southern states (Horton and Neufeld 1998, USDA 2008). It germinates in May, flowers in

September, and sheds its seed in October. Seeds have been shown to stay viable in the

seed bank for 3-5 years (Barden 1987). It has weak-stemmed tillers that root at the nodes,

providing a mechanism for local vegetative spread. The plant produces both

chasmogamous flowers in a terminal inflorescence and cleistogamous flowers in

inflorescences contained within the leaf sheaths of the upper leaves (Ehrenfeld 1999a,

Flory et al. 2007). It forms thick stands creating a monoculture that crowd out other

herbaceous and woody seedlings (Barden 1987, Horton and Neufeld 1998, Cole and

Weltzin 2004, Leicht et al. 2005). It is often observed in disturbed forests that lack multi-

layered structure, and often has a patchy distribution suggesting that some biotic or

abiotic environmental factors constrain its distribution (Cole and Weltzin 2005). MV is

commonly found on floodplains, disturbed areas, and intact forests. It has been observed in sandy loam to loamy soils from neutral to acidic pH (personal observation). Little is known about the nitrogen use of MV. Kourtev et al. (2003) found an increase in aminopeptidase activity in soils taken from under MV when compared to *Vaccinium* sp. This suggests that MV could be a nitrogen and phosphorus sink that limits nutrient availability to the microbial community.

Japanese barberry (*Berberis thunbergii*) (hereafter referred to as BT), a thorny perennial shrub, native to Japan, has spread into 31 states in the US since its introduction in the late 1800s (Cassidy et al. 2004, USDA 2008). Forming dense thickets in open areas and intact forests, it often replaces native understory species (Silander and Klepeis 1999). It spreads vegetatively, and its seeds are often dispersed by birds, small mammals, and sometimes deer (Ehrenfeld 1999a). BT is commonly found on nutrient rich soils with neutral pH and has high survival rates even in very dense shade (Silander and Klepeis 1999). Harrington et al. (2004) showed that as nitrogen availability was increased due to fertilization, foliar nitrogen content and photosynthesis at saturation increased. Cassidy et al. (2004) also report that available nitrogen, especially NO_3^- limits the relative production rate of BT. Kourtev et al. (2003) report that soils taken under BT showed increased aminopeptidase activity than *Vaccinium* sp.

To contrast the responses of the exotic species we chose two native shrubs that were once commonly found in the forests that have been invaded by MV and BT (Ehrenfeld 1999a). American witchhazel (*Hamamelis virginiana* L.) (hereafter referred to as HV) is a slow growing, multiple-stemmed tree-shrub with intermediate

fertility requirements and shade tolerance with a high tolerance for fire. It is often found on fine or medium textured soils in mesic forests. It does not spread vegetatively and has low seed abundance (USDA 2008). Nitrogen use by HV has not been well studied. Hillside blueberry (*Vaccinium pallidum* Aiton) (hereafter referred to as VP), a rhizomatous shrub is found in dryer open woods and can colonize disturbed sites. It spreads vegetatively and through berries that are dispersed by birds and mammals and is well adapted to frequent fires (USDA Forest Service 2008). As mentioned previously *Vaccinium* sp. was found to have less aminopeptidase activity in soils when compared to exotic species indicating that *Vaccinium* sp. may not take up nitrogen at the same rate as the exotic species.

Study Design

Nitrogen utilization preference of the four species was examined by growing each species in sand culture so that the amount and form of nitrogen supplied could be controlled. The two native species were purchased from local nursery stock (Pinelands Nursery, Burlington, NJ). BT seedlings were collected from field sites in New Jersey, and MV was grown from seed collected the previous fall. Preliminary analyses of sand confirmed that only negligible amounts of inorganic N and % soil organic matter were present.

Before the woody species were transplanted into sand from a potting soil mixture, the roots of each plant were thoroughly washed to remove the organic matter. Prior to transplanting each species, the sand in all pots and trays received an initial treatment of a modified Hoagland's solution (see below). Sixty individuals of each woody species were planted into 180 1gal pots while MV seed, collected the previous fall, was placed in 48

trays (18"x 8") that were filled with sand. Approximately 1-1.5 Tbs. of MV seed were gently pressed into each tray, but not buried.

Throughout the experiment, the woody species received on average 250ml of water per week in addition to the modified Hoagland's solution (see below). The flats with MV seed were placed under a mist bench and received a misting for 6 seconds every 40 minutes. All plants were grown under shade cloth to simulate forest understory conditions and rotated regularly on the bench to control for light and moisture differences. The average minimum day/night temperature in the greenhouse was between $55^{o}F$ and $60^{o}F$. Supplemental lighting was on from 6:45am to 8:45pm throughout the experiment.

The total number of pots for each species were randomly divided into two groups; one group (hereafter NO_3^- plants) received a modified Hoagland's solution in which the N form was KNO_3^- (hereafter the NO_3^- treatment) and the other group (hereafter NH_4^+ plants) received a modified Hoagland's solution with $NH_4H_2PO_4$ (hereafter the NH_4^+ treatment). The NO_3^- plants were randomly divided into thirds to receive 3 different concentrations of the NO_3^- treatment: low (0.2x; 100mmol KNO_3^-/L), normal (1.0x; 500mmol KNO_3^-/L), and high (2.0x; 1000mmol KNO_3^-/L). There were 10 replicates per woody species per concentration and 8 replicates of MV trays per concentration of the NO_3^- treatment. The NH_4^+ plants were also randomly divided into thirds to receive the same levels of $NH_4H_2PO_4$ -Hoagland's solution (low, normal, and high in the same concentrations listed for KNO_3^-) and had the same number of replicates. All species received 50ml of the modified solutions every week.

One month after establishment of the plants in the sand cultures, one sample of sand was taken from one pot of each of the concentrations (high, normal, and low) (total of 9 samples) of each of the woody species of the NH_4^+ plants to assess whether nitrification was occurring. Sand samples were not taken from the MV trays to avoid disturbing the root biomass. The sand samples were extracted in 2$_M$ KCl (4:1 KCl to soil ratio) and frozen until analyzed colorimetrically on a Lachat QuikChem Flow Injection Analyzer 8000 series (Lachat Instruments, Hach Co., Loveland, CO) (QuikChem 1986, 1987). These analyses indicated that NH_4^+ was being nitrified. To minimize the presence of NO_3^- in the NH_4^+ treatments, 1ml of N-Serve 24E™, a nitrification inhibitor (Dow AgroSciences) was added to the NH_4^+ treatment pots in addition to the Hoagland's solution twice during the experiment.

One to two months after transplanting some MV trays had to be re-seeded due to lack of germination, particularly in the medium and high concentrations of both N treatments. Many of the HV and some of the VP individuals had to be replaced due to mortality.

Measurements

Stem length and basal diameter measurements were first taken for the woody species on June 22 and 24, 2004 after all dead plants had been replaced and at least two weeks after the first application of the Hoagland's solutions. For individuals with multiple stems (BT and VP), a leader stem was marked to follow throughout the growing season. These measurements were then taken monthly until the plants were harvested for biomass measurements. Four individuals were randomly marked from each MV tray to follow through the growing season. Stem length was measured every two weeks and

averaged over the whole tray. The number of individual MV plants per tray was also counted. When the MV individuals produced ripe seeds inside the terminal inflorescence, the number and length of each terminal inflorescence was counted per tray. The length of each flower served as a proxy for seed production. Cleistogamous flowers were not counted.

Before each individual was harvested, a final stem length and diameter was taken for the woody species and a final stem length was taken for MV. The above and belowground biomass of each individual was separated, washed in water, oven-dried at 70° C, and weighed.

Data Analysis

Survival time, root, shoot, and total biomass, shoot:root ratio, the ratio of aboveground biomass to the total, and the ratio of belowground biomass to the total biomass were analyzed for each species with separate two-way ANOVAs (N form and concentration as the main effects with their interaction) for each species. Tukey post hoc multiple comparisons of least-squares means determined which main effect or the interaction of which treatments influenced the growth variables. In addition, separate two-way analysis of covariance tests (ANCOVAs with N form and concentration as the main effects) were analyzed for each species with the number of days surviving as a co-variate for each growth variable. Since many of the plants died within less than three months of the start of the experiment, not every individual was harvested within the same time frame. All biomass values were square-root transformed.

To examine differences in stem height and diameter over time, separate repeated-measures two-way ANOVAs were run for each woody species with the main effects and

their interaction mentioned above. Contrast statements comparing the height and

diameter at each subsequent measurement date to the initial measurement were used.

Tukey tests to compare specific least-squares means of significant treatment effects were

also used. MV was analyzed separately because stem height was measured more

frequently and no stem diameter data were taken. The number of MV inflorescences per

tray were analyzed with a two-way ANCOVA (with N form and concentration as the

main effects and the interaction) with the number of individual stems per tray as a co-

variate for the number of inflorescences produced. The average number of inflorescences

produced was analyzed with a two-way ANOVA with the main effects and their

interaction mentioned above. All data for the length and number of MV flowers

produced, stem height and diameter were ln-transformed to improve normality. All

analyses were conducted in SAS v.9.1 using PROC GLM (SSIII) (SAS Institute Inc.

2003).

<div align="center">RESULTS</div>

Nitrogen Addition Effects on Species Survival Time

The two exotic species did not respond similarly to the nitrogen additions. MV

individuals survived equally well among all treatments (Table 1A, Figure 1A). BT

survived longer in the NO_3^- than in the NH_4^+ treatments as expected, but the greatest

survival time depended on concentration. Plants receiving the lowest concentrations,

contrary to prediction, of either N form survived equally well (Table 1A, Figure 1B).

The native species also did not all respond in the same way to the nitrogen

additions. HV lived significantly longer in the low concentrations in both of the N forms.

The interaction of the nitrogen form and concentration was not significant for HV (Table

1A). The N form significantly affected VP survival time depending on the concentration. Unexpectedly, VP fared the worst in the NH_4^+-nitrogen addition treatments at all concentrations (Figure 1D). During the course of the experiment I observed that VP individuals receiving the high concentrations of NO_3^- and all of the NH_4^+ levels experienced mortality earliest (Figure 1D). Although VP plants receiving the NO_3^- low and normal concentrations did not appear healthy, they survived significantly longer than individuals in any other treatments.

Stem growth

Stem diameter of all three woody species did not respond to either N form. Stem height, however, was significantly affected by the nitrogen additions for all of the species (Table 1B). Both of the exotic species experienced continued height growth throughout the duration of the experiment. For MV the high and normal levels of the NH_4^+ additions significantly increased stem height more than any other treatments on all measurement dates (Table 1B, Figure 2A). For BT the type of N form applied was not significant, but the concentration of the N additions did change stem height. As predicted, the high and normal levels of both N forms increased height more than the low concentrations for the last two measurement dates (Figure 2B).

As expected for both of the native species the NH_4^+-nitrogen additions significantly increased stem height more than the NO_3^- additions. The amount of increase in stem height for the native species was not as pronounced after the second measuring date and growth seemed to level off later in the experiment. For HV both the form of nitrogen and the solution concentration influenced stem height over time but not their interaction (Figure 2C, Table 1B). For VP stem height was significantly affected by the

form of nitrogen, with greater stem height from the NH_4^+ addition, but there was no significant pattern with concentration (Table 1B).

Reproductive output

MV was the only species to produce reproductive structures during the experiment. Unexpectedly the lowest concentration of NO_3^- and the highest concentration of NH_4^+ additions had a significantly greater number of individual stems per tray (data not shown) and therefore higher number of inflorescences. Figure 3A shows the average number of chasmogamous inflorescences per tray, but these results reflect the average number of surviving stems in each tray (separate figure not shown) because most individuals produced a terminal inflorescence. Significant differences illustrated in Figure 3A were driven by the number of stems surviving in each tray. To approximate the average number of seeds that might be produced per treatment, the length of each inflorescence was multiplied by the number of flowers produced in each treatment. Again results reflected the number of individuals surviving in each tray as shown in Figure 3A with individuals receiving the low NO_3^- treatment producing significantly more seeds due to the greater number of individuals in these trays (p=0.005). The average flower length, a proxy for the number of seeds produced per individual (Figure 3B), was significantly shorter in the low concentrations (p<0.0001) of both N forms.

Biomass and Allocation

MV and BT responded differently to the N additions. MV had the greatest total biomass in the NH_4^+ treatments overall and allocated that biomass differently depending on the N form (Figure 4A, Table 1C). N form did not differentially affect the shoot:root ratio, but effects of concentration were significant. In the high concentrations of either N

form, MV had the highest shoot:root ratio (Figure 4B, Table 1D). The proportion of the shoot biomass to the total biomass was significantly greater in the high concentration compared to the normal concentration as well (p=0.005, F=6.20). Total biomass for BT was only affected by concentration of the treatments having significantly greater biomass in the high and normal concentrations than that in lowest concentration of either N form (Figure 4A, Table 1C). The shoot:root ratio showed a similar response to the high and normal concentrations, but N form was also significant resulting in a higher ratio in the NH_4^+ treatments (Figure 4B, Table 1D). Analyzed by itself, belowground biomass was significantly greater for BT in the NO_3^- treatments (p=0.02, F=6.08) and in the normal and high concentrations (p=0.007, F=5.39, data not shown).

For HV the effects of the N form on total biomass depended on the concentration (Table 1C). The normal concentration of NO_3^- resulted in greater total biomass than the high concentration of NO_3^- or the normal concentration of the NH_4^+ additions (Figure 4A). The shoot:root ratio was not differentially affected by concentration (Table 1D). The NO_3^- additions produced plants with significantly lower shoot:root ratios (Figure 4B) driven by greater investment in belowground biomass in the low and normal treatments (p=0.01, F=4.91 N form x concentration). VP produced the least total biomass compared to the other species across all treatments. Plants in the NH_4^+ treatments were significantly larger compared to those in the NO_3^- treatments with plants growing in the high concentrations having the smallest biomass (Figure 4A). None of the treatments affected the shoot:root ratio of VP (Table 1D). In general for all of the woody species, the NH_4^+ treatments seemed to have a greater influence on the aboveground allocation of biomass while NO_3^- treatments resulted in the greater input to belowground biomass.

Table 1. Analysis of variance and covariance results for survival time, stem height, total biomass, and shoot:root biomass ratios for all species. Survival time, total biomass, and shoot:root biomass statistics from two-way ANCOVAs with N form and concentration as the main effects and survival time as the covariate except for MV. Results shown for MV are from two-way ANOVA results since survival time did not significantly co-vary with treatment effect. Stem height statistics calculated from repeated measures MANOVA. F=F-statistic, p=p-value.* $p<0.05$, ** $p<0.01$, *** $p<0.0001$, ns = not significant.

Growth Variable	N	F	p	N form	Concentration	N form x Concentration
A. Survival Time						
MV	41	-	ns	-	-	-
BT	60	23.60	<0.0001	***	***	**
HV	60	11.92	<0.0001	***	***	ns
VP	59	39.87	<0.0001	***	***	***
B. Stem Height		time effects				
MV	41	417.84	<0.0001	***	***	***
BT	60	11.85	<0.0001	ns	**	ns
HV	60	11.69	<0.0001	***	**	ns
VP	59	8.35	0.0007	*	ns	ns
C. Total Biomass						
MV	41	32.69	<0.0001	*	***	**
BT	60	5.06	0.0004	ns	**	ns
HV	60	8.87	<0.0001	ns	ns	*
VP	59	5.52	0.0006	**	ns	ns
D. Shoot:Root						
MV	41	3.74	0.008	ns	**	ns
BT	60	5.87	<0.0001	**	**	ns
HV	60	3.96	0.002	**	ns	ns
VP	59	-	ns	-	-	-
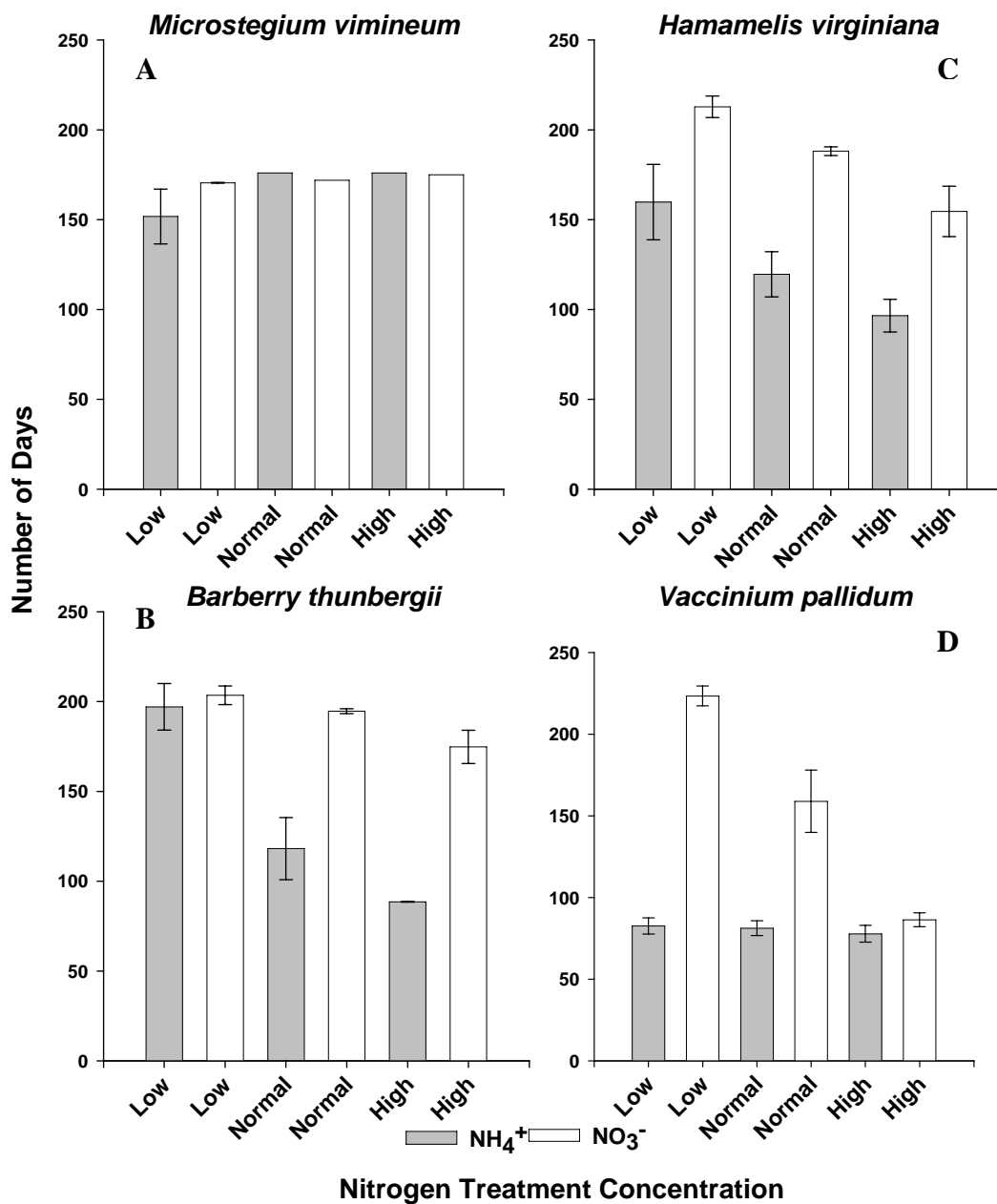
Figure 1. Average survival time (days) of each species for each N treatment. Error bars are ±1SE. A. No significant treatment effect on survival time for MV. B. Different letters denote significant differences in the interaction of the type of nitrogen added and the concentration for BT. C. Different letters denote significant differences between the N forms and asterisks signify significant concentration effects separately on survival time for HV. D. Different letters denote significant differences in the interaction of the type of nitrogen added and the concentration for VP. Shaded bars represent NH_4^+-N and open bars represent NO_3^- N treatments.

A

Microstegium vimineum

B

Berberis thunbergii

Average Stem Height (cm)

Legend: Low NO3-, Normal NO3-, High NO3-, Low NH4+, Normal NH4+, High NH4+
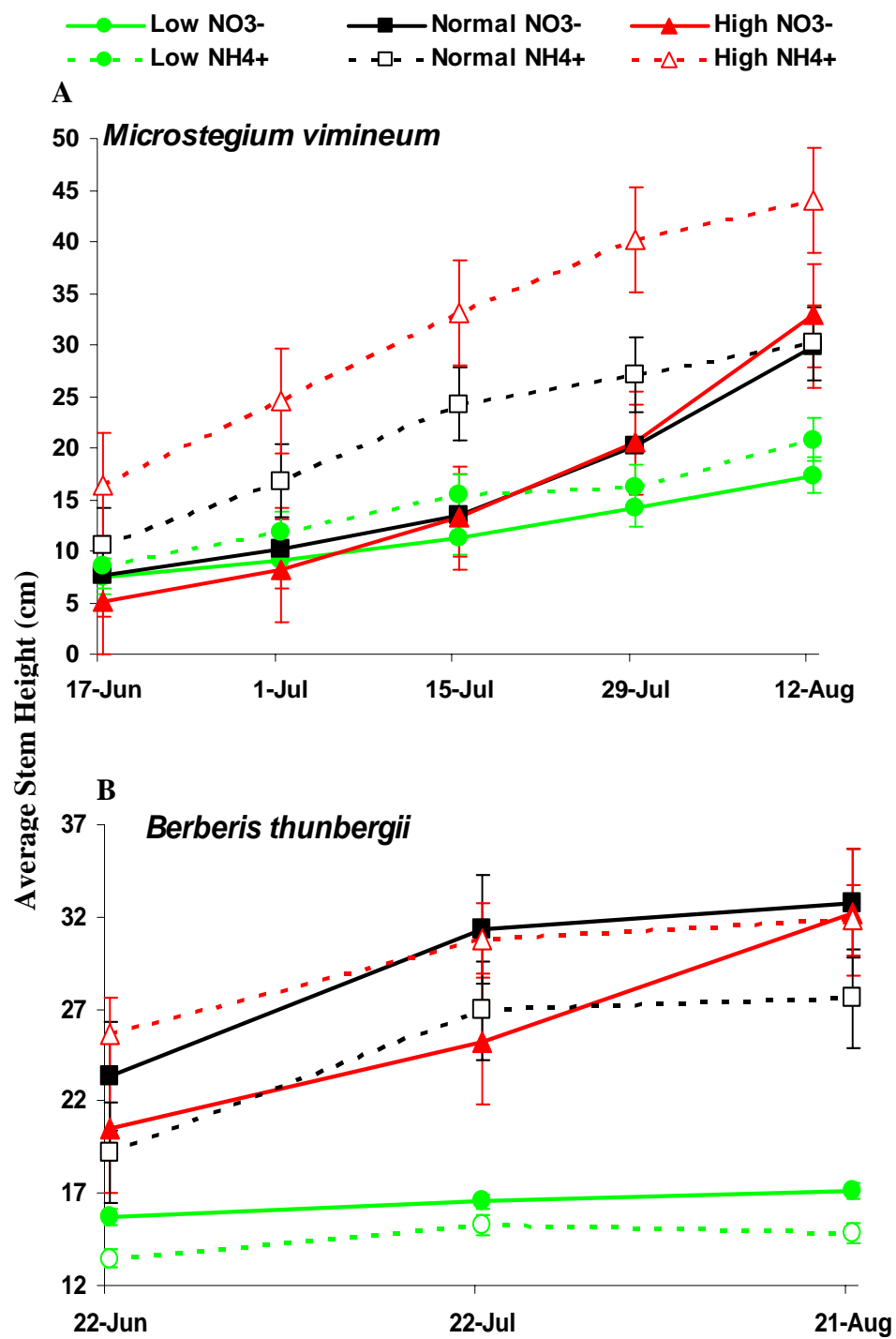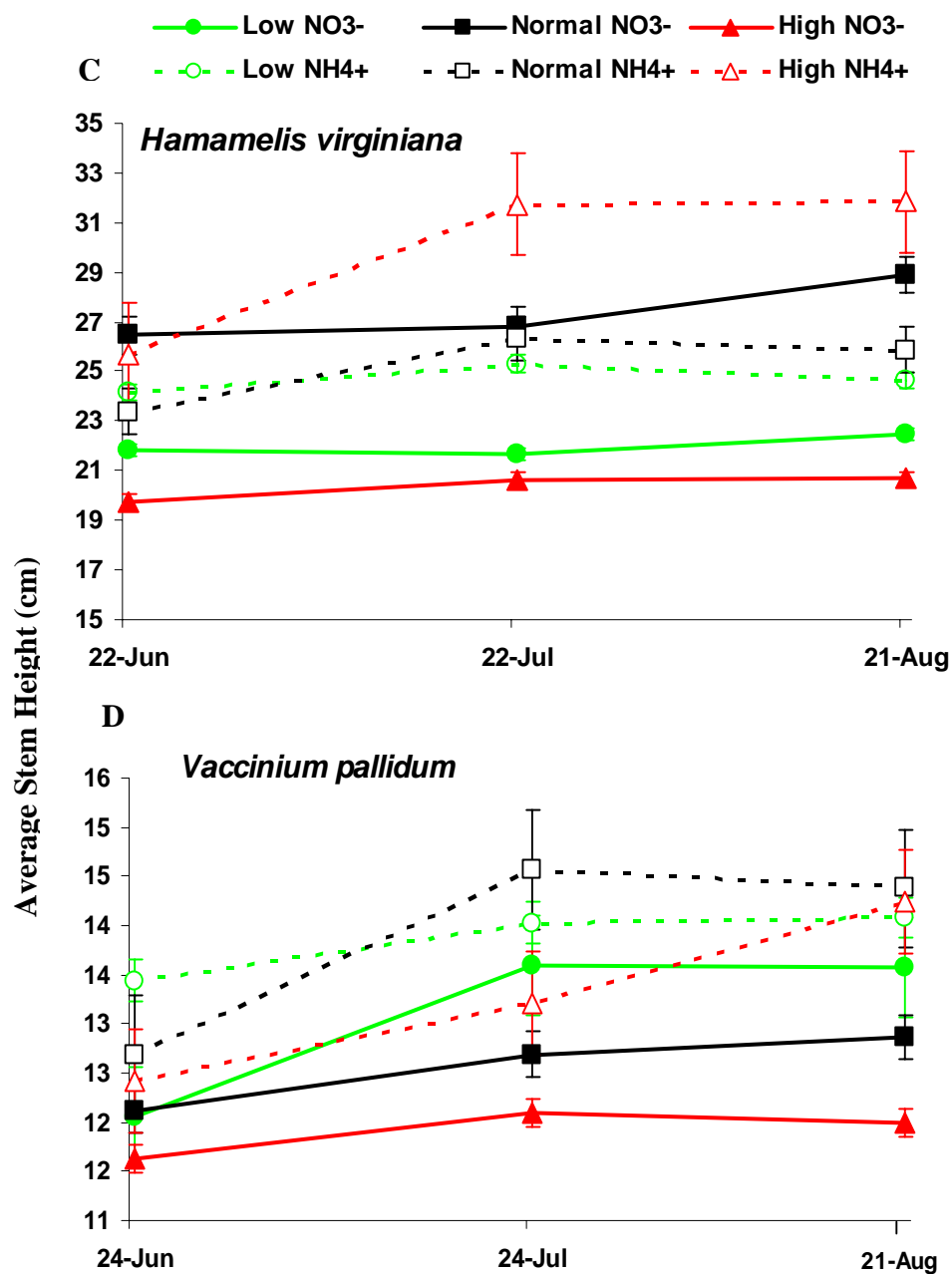
Figure 2. Change in average stem height for all species over time. A. *Microstegium vimineum*. B. *Berberis thunbergii*. C. *Hamamelis virginiana*. D. *Vaccinium pallidum*. Solid lines and solid shapes show NO_3^- N treatments. Dotted lines and open shapes show NH_4^+ N treatments. ● denotes low concentration, ■ denotes normal concentration, and ▲ denotes high concentration of the N treatments. Error bars are 1 ±SE.
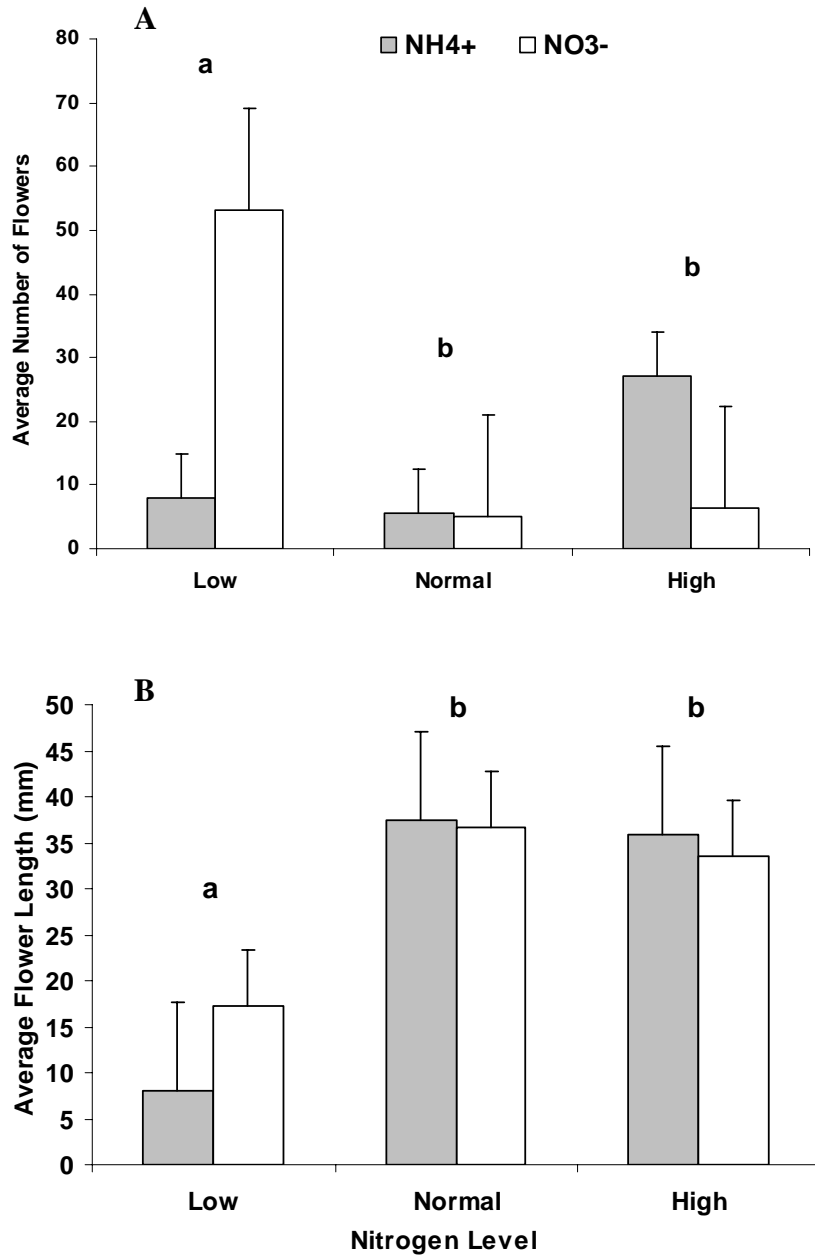
Figure 3. The reproductive output of *Microstegium vimineum*. A. The average number of terminal flowers of MV per treatment at the end of the experiment. N=32. Different letters indicate significant differences among the concentrations driven by the number of stems per tray. B. The average length of each inflorescence per treatment. N=32. Different letters above groups of bars represent significant differences among the concentrations of the treatments. Shaded bars represent NH_4^+-N treatments and open bars represent NO_3^-N treatments. Error bars are 1 ±SE.

A

B
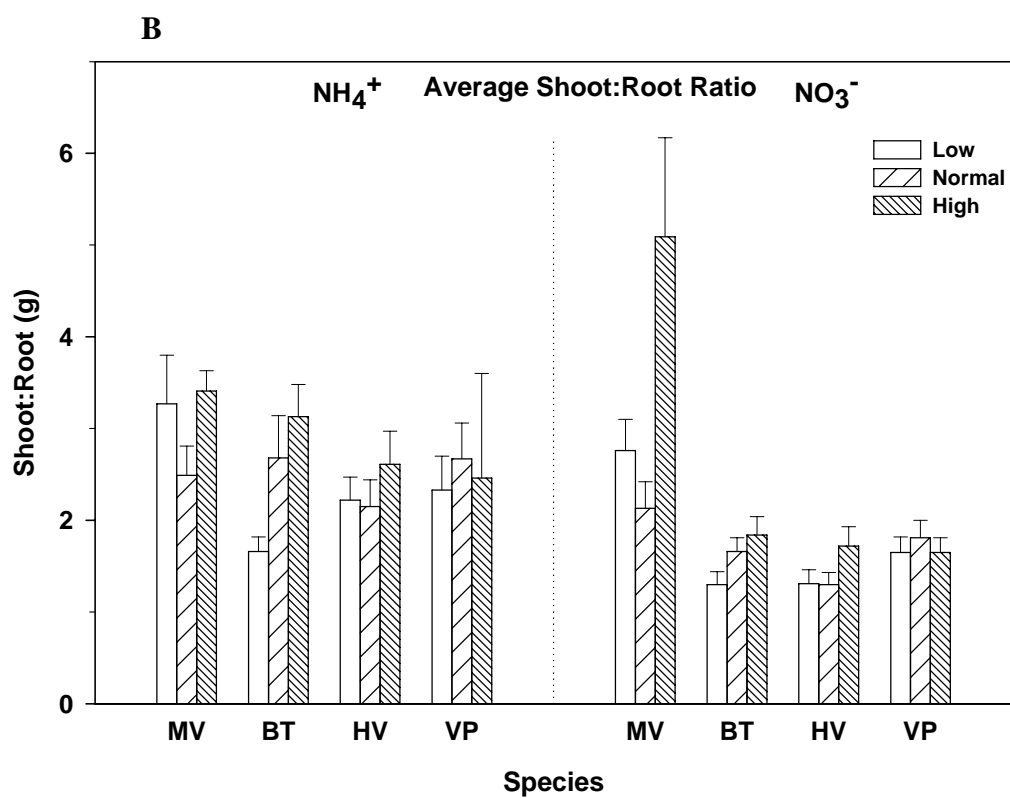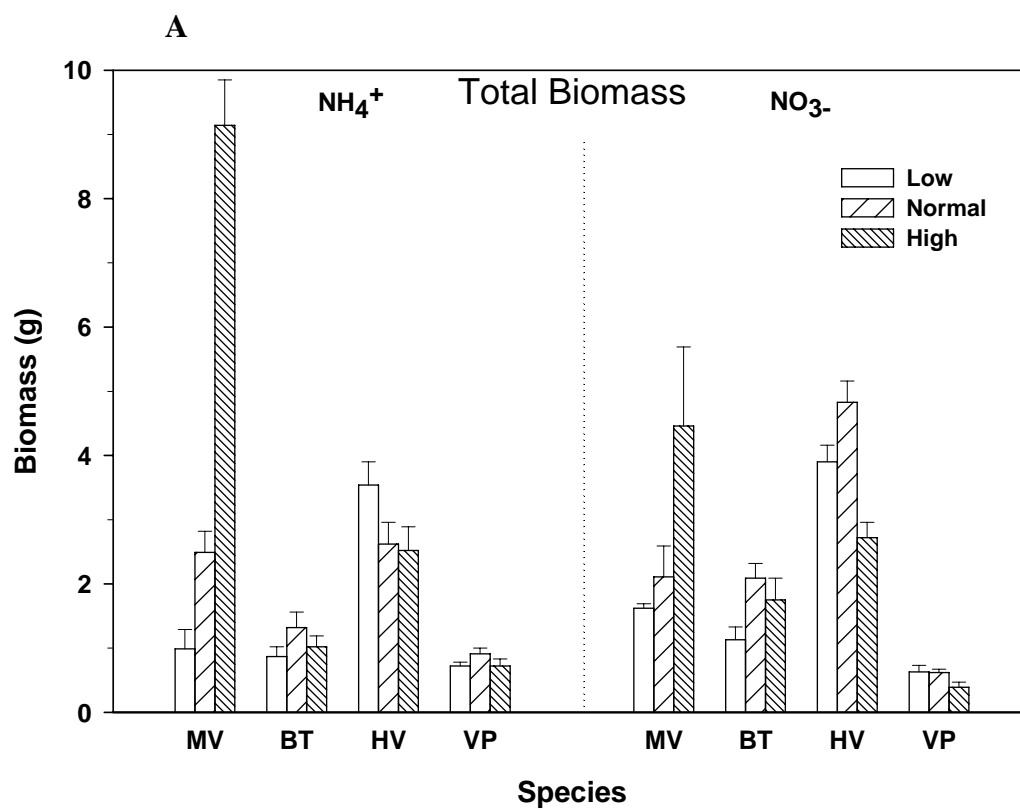
Figure 4. A. Total Biomass for all species grown in both N forms. B. Shoot:root biomass ratio for all

species grown in both N forms. Error bars are 1 ±SE. Open bars represent low concentration, coarse hashed

bars represent normal concentration, and fine hashed bars represent high concentrations of the N forms.

DISCUSSION

The growth response of the two exotic species was in general more plastic to the

form and concentration of the nitrogen additions than the native species. The nitrogen

additions did not affect all species as predicted, but general trends were observed. The

survival time of the exotic species was significantly longer for individuals receiving the

NO_3^- additions as predicted. Individuals of all species showed increasing signs of stress

as the experiment progressed, but more replicates of MV and BT survived the course of

the experiment than either of the natives. These results reflect the growth plasticity of the

exotics to either an excess or limitation of nutrients.

The native species did not demonstrate as much plasticity in survival time. VP

especially did not live long after receiving any nitrogen nutrition except for the low and

normal concentrations of NO_3^-. These results are not surprising as other studies have

reported a decrease in *Vaccinium* species abundance in plots receiving N fertilization

(Nordin et al. 2005). Gilliam (2006) predicts that increases in nitrogen availability in

forest soils, especially NO_3^-, will induce a decrease in understory biodiversity and

possibly facilitate exotic species invasion. His hypothesis of nitrogen homogeneity

predicts that as NO_3^--N becomes the dominant form of soil nitrogen, faster growing

species that can either quickly assimilate nitrogen or that preferentially respond to NO_3^--

N will dominate the slower-growing forest herbs that preferentially utilize NH_4^+-N.

The length of time that continued growth in stem height occurred differed

between the exotics and natives. The exotics continued to increase in stem height at all

concentrations except that for BT vertical growth stagnated at low levels of either N form. The continued increase in height also demonstrates the plasticity the exotics have to nutrient addition. Although stem height increased over time for the natives as well, most vertical growth occurred between the first two measurement dates and then leveled off. Due to the law of diminishing returns, when the supply of one nutrient is increased, other nutrients may become limiting or the genetic potential for further nutrient assimilation for that species may become limiting (Marschner 1986). I observed a slight decline in stem height for many replicates of the woody species possibly due to a point of inversion in growth. A downward slope in growth can be caused by toxicity due to excess of a nutrient or due to the induced deficiency of another nutrient (Marschner 1986). The point at which the inversion occurs for native species may be different than for the exotics since they were able to continue vertical growth. A longer term study manipulating nutrient inputs to woody exotics and natives would help determine whether an inherent difference exists in the stem height growth slopes between these species. Lack of treatment effect on stem diameter response may also have been due to the short duration of the experiment.

Plant biomass is of course dependent on the species' growth form and initial fresh mass. Although we did not measure initial plant weight for every individual it is clear that the nitrogen additions significantly affected plant biomass despite differences in growth form. Somewhat unexpectedly, MV increased in biomass more than any other species. Since it is a grass that does not invest much in belowground structures, and the other species are woody, we expected that the total biomass of the woody species, especially BT to be the greatest. MV on average outperformed all of the woody species by

producing double the amount of total biomass in the highest concentrations of either N form than the largest of the woody plants (HV in the NO_3^- normal treatment) (Figure 4A). While MV preferentially responded in biomass to the NH_4^+ form of N, BT grew equally well in either N form. The total biomass of BT was driven by the concentration rather than the specific form of N.

Total biomass was not as strongly affected by the nitrogen additions as we expected for the native species. The N form treatment effect on biomass for HV was not similar to VP. HV grew larger in the NO_3^- treatments although not significantly so, and VP responded preferentially to the NH_4^+ treatments. This is in contrast to the effect of the treatments on survival time for VP. NH_4^+ treatments produced significantly larger individuals, but those individuals did not survive as long as smaller individuals in the NO_3^- treatments. Concentration level did not influence how the native species allocated biomass.

Similarities in response to the different N forms existed among all woody species regardless of origin. All species allocated significantly more biomass to aboveground structures in the NH_4^+ treatments and invested significantly more in root biomass in the NO_3^- treatments. These results suggest that for woody plants environments with high NO_3^- concentrations may play a larger role in facilitating root competition.

In summary, MV responded positively to all the different N forms in any concentration, particularly at high inputs of NH_4^+. MV invested more in total biomass than all of the other species due to life history characteristics. As a C_4 annual grass growing in the forest understory it must take advantage of light and nutrient resources quickly to grow above competing herbaceous or woody seedlings in the late spring. C_4

grasses are expected to be less competitive in moist, shady sites, however, as they are adapted to high light environments. Despite this, Oswalt et al. (2007) report that MV is able to take advantage of brief light flecks that pass through the canopy throughout the day for efficient carbon gains. In a disturbed, more open forest setting, MV is not at a disadvantage despite being a C_4 plant. Winter et al. (1982) showed that MV can be successful in as little as 5% sunlight. Due to its C_4 pathway, water demands for carbon dioxide fixation are half of what C_3 plants require. Also, C_4 plants characteristically use nitrogen efficiently. This study illustrates that MV is able to maximize its growth even when nitrogen resources are limiting (*i.e.* low N concentration solutions). Other life history characteristics of this species such as high fecundity, vegetative growth (Flory et al. 2007), seed bank longevity (Barden 1987), and low light adaptability create a super invader that is likely to invade into any environment, but the most extreme such as those in cold climates. As climate change progresses, however, it is predicted that many invasive plants such as MV will expand northward (Mooney and Hobbs 2000)

Land use history and propagule pressure seem to play a role in the invasion history of BT. The abundance of BT has been associated with abandoned agriculture and second growth forests (DeGasperis and Motzkin 2007). As forests regenerated after agricultural abandonment and more recently with the expansion of the suburbs furthering the fragmentation of the landscape, white-tailed deer populations have increased and suppressed the native forest understory vegetation. BT individuals that may have been originally planted around old farm houses and hedgerows found fertile ground on these old agricultural fields and were able to adapt to lower light conditions and compete for soil nutrients as forest grew up around them. Homeowners and landscapers began

planting deer-resistant vegetation thereby increasing propagule pressure around the fragmented successional forests. Because BT has a number of reproductive methods (i.e. seeds, root and shoot horizontal spread, genesis of clonal roots), has low seedling mortality, can survive in a wide range of light conditions, can suppress the biomass of co-occurring species (Ehrenfeld 1999b, Silander and Klepeis 1999), and as shown here, is highly plastic in its response to nutrient supply, it has become a dominant member of forest understory community structure.

Both of the exotic species investigated here pose major challenges to land managers working with already highly invaded habitats. The exotic species used in this study demonstrated a plastic growth response to any manipulation of nitrogen availability in the greenhouse. Efficient nutrient use and plastic phenotypic response to high nutrient environments indicate that these species will only become more successful as nitrogen deposition and climate change progress. Experiments that manipulate resources in the field may prove to be more effective when community dynamics such as competition and herbivory or other disturbances come into play. As Levine et al. (2004) suggest, instead of studying specific interactions between an exotic and a native species that are hardly generalizable to all species in all systems where they occur, it may be more useful to determine methods that will improve "abiotic" resistance of a system. Soil restoration techniques are commonly employed to increase nutrient availability in agricultural systems. Many researchers are using soil amendments to decrease nitrogen availability as well (Alpert and Maron 2000, Suding et al. 2004). Since many exotic species have been shown to enhance nutrient availability that can lead to further facilitation of invasion (Simberloff and Von Holle 1999, Ehrenfeld 2003), soil manipulations that will decrease

resource availability in combination with reduced herbivore pressure on native species may be effective in limiting the competitive ability of dominant exotic species in forest understories. Improving abiotic factors will ultimately lead to enhancement of biotic resistance that regulate the establishment and spread of invasive species.

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CHAPTER 3

Effects of Soil Manipulations on Nitrogen Cycling Properties and Vegetation of

Invaded Forest Understory Communities in New Jersey

INTRODUCTION

Biological invasions by exotic, invasive plants are widely recognized as a threat to biodiversity and ecosystem function (Elton 1958, Williamson 1996, Mack et al. 2000, Ehrenfeld 2003). Invasive plants alter ecosystem processes, specifically shifts in plant community composition and nutrient cycling (Vitousek et al. 1987, Stock et al. 1995, Ehrenfeld 2003, Knight et al. 2007). Elton (1958) first suggested that resident communities possess properties that promote resistance to invasion. When these properties are altered, invaders can establish and spread. Researchers have suggested that increasing the diversity of species and functional groups within plant communities will act as a mechanism to prevent invasion by amplifying the interaction effects of the resident community on the invader (Kennedy et al. 2002, Fargione et al. 2003, Levine et al. 2004). Can merely restoring the resident community structure be sufficient to enhance resistance to further invasion? Plant-soil feedbacks that leave a legacy effect can occur when dominant exotic species alter soil properties, especially nitrogen cycling (Maron and Jeffries 1999, Ehrenfeld et al. 2001, Klironomos 2002, Ehrenfeld et al. 2005). Can the resident community that is already severely impacted by invasive plants be restored through enhancement of biotic resistance despite persistent legacy effects and positive feedbacks? Many studies aimed at increasing invasion resistance have focused on biotic species interactions such as competition and herbivory (Maron and Vila 2001, Levine et

al. 2004). A few studies, however, have examined the physical or abiotic factors that potentially mediate biotic resistance such as physical habitat structure (Byers 2002) or resource availability (Suding et al. 2004a). In this study I propose enhancing "abiotic resistance" through limitation of resources, specifically nitrogen availability, will inhibit the success of two dominant exotic, invasive plants and enhance native plant competitive ability.

Nutrient availability plays an important role in invader establishment and success (Stohlgren et al. 1999, Ehrenfeld 2003, Baer et al. 2004). Exotic, invasive plant species richness and cover have been associated with an increase in resource availability (Stohlgren et al. 1999, Milberg et al. 1999, Davis and Pelsor 2001, Suding et al. 2004a). If exotic species possess characteristics that promote efficient resource acquisition, and additionally if they can out-perform native species due to release from enemies such as herbivores and pathogens in the introduced range, those exotic species will eventually become invasive (Blumenthal 2006).

Native biodiversity of forested systems in the Northeast is increasingly threatened by overabundance of invasive, exotic plants, white-tailed deer, and continued fragmentation (Aronson et al. 2007, Baiser et al. 2008). Many forests left intact are second growth recovery from old agricultural fields in suburban areas and are highly fragmented and disturbed (DeGasperis and Motzin 2007). Management focused on maintaining native biodiversity of these areas is minimal even when under the auspices of federal, state, or county governments. Natural resource managers and state biologists are overwhelmed by the quantity of land that has been invaded by exotic species. As climate

change and nitrogen deposition progress, these areas may soon protect more exotic biodiversity than native.

Several factors influence the spread and eventual dominance of exotic invasive plants in forests. An increase in forest soil nitrogen through deposition or legacy effects from past agricultural land use, may decrease the competitive ability of slower-growing native understory vegetation better adapted to low nutrient conditions or preferential use of NH_4^+, the less mobile form of inorganic nitrogen available for uptake (Vitousek et al.1997, Nordin et al. 2005, Gilliam 2006). Native plants throughout the northeastern US are at a disadvantage not only due to white-tailed deer herbivory pressure (Kourtev et al. 1999, Horsley et al. 2003, Ruhren and Handel 2003), but also due to the increased competitive ability of exotic, invasive plants (Vila` and Weirner 2004). A disturbance that opens the canopy to increased light in combination with intense herbivory pressure from white-tailed deer on native vegetation can create an opportunity for an exotic species to establish. *Berberis thunbergii* DC (Japanese barberry), a shrub introduced from Asia, has become widespread in the understory forming dense, impenetrable thickets in many large tracts of protected forests (Ehrenfeld 1999). *Microstegium vimineum* (Japanese stiltgrass), an invasive, C_4 annual grass is often found in understory communities with *B. thunbergii*. Characteristics of these species were described previously (see Chapter 2). Ehrenfeld (2003) and Kourtev et al. (1998) suggest that *B. thunbergii* creates a positive feedback (*sensu* Ehrenfeld et al. 2005) affecting nitrogen cycling that enhances its competitive ability. Ehrenfeld et al. (2001) found that *B. thunbergii* has N-rich leaf litter and a higher rate of decomposition than native counterparts (*e.g. Vaccinium pallidum*). These exotic species are thought to have greater

nitrate uptake because nitrate reductase activity measured in soil under these species was higher than for natives (Kourtev et al. 2003). Soils under *B. thunbergii* have higher pH and higher N mineralization rates compared to soils under native understory shrubs. The presence of non-native earthworm species that contribute to rapid decomposition rates are associated with the abundance of *B. thunbergii* and *M. vimineum* (Kourtev et al. 1999). Due to the evidence presented above I inferred that *B. thunbergii* and *M. vimineum* have the ability to change soil N cycling suggesting that high nitrate availability confers a competitive advantage. In the context of increasing abiotic resistance to these species, I asked whether soil properties such as pH, percent organic matter, and nitrogen cycling could be altered in such a way to immobilize nitrogen, especially nitrate-nitrogen (NO_3^--N).

Restoring soil conditions that an invader has altered to enhance native plant recovery and re-establishment is a challenge in many ecosystems. Given the association of exotic invasions with high nutrient availability, methods of limiting nutrient supply rate might be effective in inhibiting invasions. Soil amendments or treatments have historically been used in agricultural settings to increase or decrease soil fertility. Increasingly, ecologists are also employing soil manipulations as a component of multi-faceted approaches to restoring native habitats (Blumenthal et al. 2003, Corbin and D'Antonio 2004, Perry et al. 2004). D'Antonio and Chambers (2006) suggest that bottom-up approaches to restoration that focus on belowground processes or soil nutrients should compliment top-down approaches that involve planting vegetation, limiting herbivory, establishing reintroductions, or inducing other aboveground interactions. Failures to achieve restoration goals may stem from strategies that only

employ top-down methods. In cases where dominant exotic species have altered ecosystem processes, a combination of top-down and bottom-up approaches may prove successful when restoring native biodiversity and function.

In this study, I attempted several bottom-up approaches designed to inhibit invasion success of *B. thunbergii* and *M. vimineum*. Since *B. thunbergii* and *M. vimineum* are often found on soils with elevated pH compared to natives, I wanted to decrease soil pH. Many farmers and gardeners lime their soil to decrease the acidity for raising crops. Similarly, decreasing soil pH can be achieved through additions of sulfur or aluminum sulfate (S. Murphy, personal communication). Frequently, amending soil with a carbon source is used to reduce plant-available nitrogen (Magill and Aber 2000, Corbin and D'Antonio 2004, Prober et al. 2005). It is assumed that the added carbon will stimulate microbial immobilization of nitrogen (D'Antonio and Chambers 2006). Morghan and Seastedt (1999) used carbon additions on grassland plots to alter C:N ratios in attempts to inhibit the growth of several invasive forbs. They found that although N availability was indeed decreased for two years after the treatment was applied and plant size decreased, stem densities of exotic species were not affected. Alpert and Maron (2000) used sawdust additions to plots once inhabited by *Lupinus arboreus*. Once these shrubs die, patches of high nitrogen remain that become invaded by exotic, invasive grasses. In sawdust plots they observed a significant reduction in exotic grass biomass due to enhanced frequency of native and exotic forbs. Suding et al. (2004a) added sucrose, a highly labile C-source to grassland plots to significantly reduce nitrogen availability that they hypothesized positively affects *Centaruea diffusa*. Cogliastro et al. (2001) found that a combination of woodchips and sludge (recalcitrant and labile C-sources, respectively)

applied over two years immobilized available nitrogen to maintain nutrients for new seedlings planted on derelict land. Other methods to limit N availability used in an agricultural context include removal of nutrient rich surface soils (Marrs 1993) and inhibiting nitrification through the application of a bactericide specific for nitrifying bacteria (Prasad and Power 1995). Many of these studies document success with immobilizing plant-available N but have mixed results in exotic plant response. In some cases, abiotic effects improved biotic resistance by enhancing competition from resident species. In other cases growth or abundance of the exotic species was not affected.

All of these experiments utilizing carbon additions and other soil treatments have been done in grasslands, prairies, wet meadows, and agricultural fields where soil manipulation is not as challenging due to open canopies, short-lived vegetation, fewer sloped and rocky substrates, and less deep woody root competition (Morghan and Seastedt 1999, Blumenthal et al. 2003, Corbin and D'Antonio 2004). The results have varied depending on the species involved, the type of soil manipulation used, and the length of time the study was conducted (Corbin and D'Antonio 2004). Manipulating soils under intact mature forest canopy is more problematic and has not been well studied. As reviewed in Ehrenfeld (2003), there is a paucity of examples describing successful soil restoration studies conducted in upland forest habitats.

Due to the need for feasible management strategies of invasive plants in successional forests and because few researchers have attempted to alter soil nitrogen cycling in forested ecosystems, I designed a field experiment to determine whether soil conditions can be manipulated to limit nitrogen availability as a bottom-up approach to restoration. Through these manipulations, I hypothesized that plant-available nitrogen,

specifically nitrate, will be decreased. I predicted that the exotic species *B. thunbergii* and *M. vimineum* growth will be decreased and native species biomass will increase. The objectives of using soil manipulations were to (1) eliminate much of the inorganic nitrogen by removing the top 5 cm of surface soil, (2) increase C:N ratios and immobilize available nitrogen through the addition of hardwood woodchips, (3) inhibit nitrification rates with the application of a nitrification inhibitor, and (5) decrease soil pH through the application of aluminum sulfate. I hypothesized that these manipulations should alter soil properties and influence nitrogen cycling within the top 5 cm of soil. I predicted that soil properties such as C:N ratios, soil pH, and inorganic nitrogen could be altered from existing conditions underneath exotic vegetation. I also predicted that the soil treatments would negatively influence exotic plant growth within the plots. A replicated Latin square plot experiment was designed to apply the soil manipulations. Each whole plot was split into thirds to test vegetation response to the treatments. The vegetation structure in each split plot was manipulated as a top-down approach to restore native plant growth. The top-down methods involved removing a portion of the dominant *B. thunbergii* shrubs and *M. vimineum* population and replanting native species into the study plots. I hypothesized that 1) the native plants placed into the treatment plots would not be negatively affected by the soil treatments and that 2) exotic plant growth represented by the remaining *B. thunbergii* shrubs and *M. vimineum* population would be negatively affected by the soil treatments. If it is possible to alter soil properties and thus influence ecosystem processes, it is likely that the restoration of the successional trajectory toward the re-growth of native vegetation is possible.

METHODS

Study Sites

To test whether soil properties could be altered to return to pre-invasion characteristics, a replicated field experiment was established in two protected areas managed by the National Park Service in northern New Jersey. The research plots located in two national parks both contain extensive, dense, well-documented invasions of *B. thunbergii* and *M. vimineum* (Kourtev et al. 1998, Ehrenfeld et al. 2001). Morristown National Historical Park (hereafter referred to as MORR), Morristown, NJ occupies 800 ha. in the Highlands physiographic province of NJ. Soils are predominantly in the unglaciated Parker and Edneyville soil series (Typic Dystrochrept and Typic Hapludult), on Precambrian gneiss and schist (Fletcher 1979). The Delaware Water Gap National Recreation Area (hereafter referred to as DEWA) is located approximately 30 miles west of Morristown on glaciated soils (Oquaga and Steinsburg series, both Typic Dystrochrepts) of the Valley and Ridge Physiographic Province, on Ordovician sandstones. Although the soils belong to different series, they are all acidic, loamy in texture, and stony to extremely stony (Fletcher 1979). Infested areas of both parks have a closed canopy of mature hardwood trees, including oaks (*Quercus* sp.), hickories (*Carya* sp.), black birch (*Betula lenta*), tulip poplar (*Liriodendron tulipifera*), red maple (*Acer rubrum*), and others. The understories in these parks were previously composed mostly of blueberries (*Vaccinium* sp.), huckleberries (*Gaylussacia baccata*), and maple-leaved viburnum (*Viburnum acerifolium*) (Dibeler and Ehrenfeld 1990).

Experimental Design

Two replicate study sites (one in each park) were established based on similarity

in the densities of the exotic species, *Microstegium vimineum* and *Berberis thunbergii*;

similarity of canopy species and slopes; and the practicality of reaching the sites from

roads and trails for ease of equipment transport in and out of the sites. In each site 25

plots were set up in a 5x5 Latin square split-plot design so that every soil manipulation

treatment was applied across the whole plot once in each row and each column, and each

plot was split into three sections, 2.5x5m each, for the vegetation treatments (Figure 1). A

Latin square design was chosen due to the variation in slope, light, and other

environmental gradients over the study site area (approximately 90 x 90m). Each whole

plot was 5x7.5m with 5-15m between each plot depending on the density of *B.*

thunbergii.

In summer 2003, initial soil samples were taken, and then vegetation treatments

were applied. The plots were split into thirds to assess the growth response of the exotic

species versus the native species to the soil manipulations and to gauge native plant

regeneration (Figure 1). All the *B. thunbergii* shrubs were removed from one section

(split-plot R), one section had all the *B. thunbergii* shrubs removed and then replaced

with native plants (split-plot N), and in the last section, the *B. thunbergii* shrubs were left

in the plot (split-plot E). *B. thunbergii* bushes were cut at ground level and an herbicide

(Garlon 4A) was brushed onto the stumps to limit soil disturbance. In the second year,

each plot was checked to ensure that these bushes were not stump-sprouting. Five native

plants were planted into one split-plot (N) in each whole plot (1 *Hamamelis virginiana*, 1

Lindera benzoin, 1 *Vaccinium angustifolium,* 2 *Vaccinium pallidum,*) to total 125 plants

per site, and each seedling was surrounded with temporary fencing.
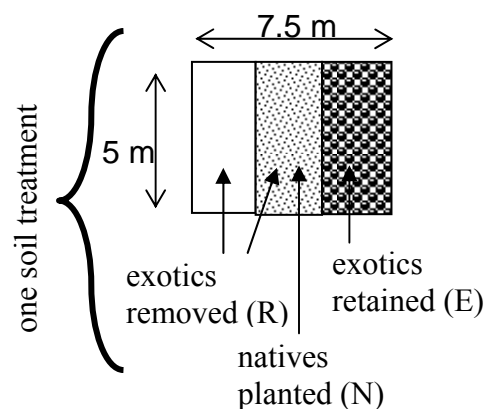
Figure 1. Experimental layout for the field plots

Soil Manipulations and Sampling

Four types of soil manipulations were applied to the whole plots to decrease the

available nitrogen (NO_3^--N, NH_4^+-N, and mineralization rates) in both sites. A fifth set of

plots did not receive a treatment to serve as a control for no manipulation (hereafter

referred to as control plots). The manipulations were 1) addition of aluminum sulfate

(6.81 kg per 37.5 m^{-2} plot, total S 15% from A.H. Hoffman Inc. Lancaster, NY) to lower

the pH by a magnitude of 1.5-2 units (hereafter pH plots); 2) addition of hardwood

woodchips (approximately 18-23kg per 37.5 m^{-2} plot) to immobilize nitrogen and

increase soil C:N (hereafter woodchip plots); 3) application of N-Serve 24E™ (Dow

AgroSciences), a nitrification inhibitor (25mL in 7.5L water per 37.5 m^{-2} plot) (hereafter

inhibitor plots); and 4) removal of 5cm of topsoil to eliminate the most active zone of

nitrification (hereafter removal plots). The same soil treatments were reapplied in spring

2004 and 2005 except for the topsoil removal which was only done the first year of the

experiment due to the disturbance to regenerating vegetation. The aluminum sulfate was

only applied in MORR (Morristown) because the average pH of the DEWA (Delaware Water Gap) was already below 5.0 and no alteration was needed.

Before the soil and vegetation manipulations were applied, a subset of soil samples was analyzed for C:N ratio to establish baseline values for both sites. To test whether the woodchip additions affected the soil C:N ratio another subset of soil samples was additionally analyzed in the last year of the manipulations.

Two soil cores were taken from each split-plot for a total of 6 cores per whole 5x7.5m plot (150 cores per site x 2 sites = 300 cores) each summer, fall, and spring for three years to capture seasonal differences and effects of the soil treatments. Each core was taken using a metal corer (Giddings Machine Company, Fort Collins, CO) inserted with an 8" acrylic liner. One core from each split-plot was put on ice and taken back to the lab for immediate analysis. The other core was left in the field to incubate in the ground for one month (Robertson et al. 1999).

The 75 cores taken back to the lab were analyzed for NO_3^--N, NH_4^+-N, percent moisture, percent soil organic matter, and pH. These response variables were chosen to capture soil nutrient changes associated with the soil manipulations. All soil analysis was done on the top 5 cm of homogenized soil from the fresh cores. Inorganic N was determined by extraction with 25ml of 2M KCl in 5g of fresh soil. Extracts were shaken for 1 hour filtered and frozen at 4° C until analyzed. Extracts were analyzed on a Lachat QuikChem FIA+ (Lachat Instruments, Hach Co. Loveland, CO) for NO_3^- and NH_4^+ (Quikchem 1986, 1987). Incubated cores were collected after one month and analyzed as described above. Results from the fresh and incubated samples were used to calculate nitrification, ammonification, and N-mineralization rates. Net nitrification is defined as

the accumulation of NO_3^- during the incubation, and net N-mineralization as the

accumulation of NO_3^- and NH_4^+ during the incubation. A UP-5 meter was used for pH

readings in a 1:5 soil:distilled water slurry (Denver Instruments, Denver, CO). Percent

moisture was determined on fresh soil (1-2g soil) gravimetrically at 105^oC for 48 hours.

Loss on ignition (LOI) was determined at 500^oC in a muffle furnace for a minimum of 3

hours.

Vegetation sampling

In the final year of the experiment, the number of new shoots and their lengths

were recorded for *B. thunbergii* to assess the impact of the treatments in each of the split

plots with exotic plants remaining (E plots). To gauge growth of the native shrubs that

had been planted into the plots (N split-plots), the number of new shoots and their lengths

were also recorded for the *Vaccinium pallidum* bushes. New *B. thunbergii* seedlings that

had germinated in the plots from which the exotics had been removed were also counted

each year to gauge regeneration. To compare effects from the treatments versus no

manipulation on *M. vimineum*, aboveground biomass was collected from each plot in the

final year of the experiment, dried at 75^oC for 48 hours and weighed.

Data Analyses

Soil variables were analyzed with separate fixed factor repeated measures two-

way analysis of variance (ANOVAs) by site with main effects of soil treatment and

vegetation treatment and their interaction. All soil variable data except for pH values

were log-transformed to improve normality. In the case of the mineralization,

nitrification, and ammonification rate data for MORR only, transformation did not

improve normality so these data were analyzed with nonparametric, two-way repeated

measures Friedman's test (nonparametric ANOVA) with main effects listed above. Differences in C:N soil ratios between control and woodchip plots were determined with a t-test by site. Native plant percent survival, *B. thunbergii* seedling counts (log-transformed), *M. vimineum* biomass, and new shoot and height data were all analyzed with fixed factor one-way ANOVAs by site with soil treatment as the main effect. All analyses were conducted using SAS v.9.1 (SAS Institute 2003).

<div align="center">RESULTS</div>

Effects of Soil Manipulation on Soil Properties

The soil manipulations applied in this experiment did alter some soil properties that were measured but effects were not consistent over time or by site. Spring and summer 2004 differed greatly from spring and summer of 2005 in average amount of precipitation, especially during the months of sampling for this study. The spring and summer of 2004 received almost double the amount of rainfall between the months of April and August than 2005 (Rutgers University Office of the New Jersey State Climatologist 2008). The lack of effect from the manipulations observed in the months of 2004 may be due to the difference in precipitation.

The soil at MORR showed the most resistance of either site to change due to the manipulations. None of the soil manipulations applied to plots at MORR significantly altered soil organic matter or nitrification rates but some trends did emerge (Table 1, Figure 2A). The NO_3^--N levels were significantly affected by the soil treatments (p=0.02, F=1.82), but these effects varied by time (see below, Figure 2B). The NH_4^+-N pool at MORR was significantly decreased in spring 2004, summer 2004, and spring 2005, but not always due to the same treatment effect (Figure 2C).

At MORR the levels of nitrate and nitrification in the removal plots tended to be lower than in the other treatment plots for most of the sampling dates, but the overall effect of the treatment was not statistically significant for nitrification (Figure 2A). Removal plots seemed to consistently have low levels of NH_4^+-N, but only significantly so in spring 2004 and 2005 when compared to the pH plots in both seasons and the inhibitor plots in spring 2005 (Figure 2C). The removal treatment only decreased mineralization (p=0.005, F=4.07) and ammonification (p=0.003, F=4.47) in the very last sampling period (summer 2005) when compared to the pH plots (Figure 2D). This difference at this sampling period may be due to the lack of precipitation in the summer of 2005. The removal treatment also decreased soil moisture in summer 2004 when compared to moisture levels in the woodchip plots (p=0.04, F=1.62). The woodchip addition significantly increased soil moisture in summer 2004 when compared to the removal plots most likely from a mulching effect in a wet summer. Ammonification rates were also lower in the woodchip plots compared to the pH plots but only for summer 2005. Neither nitrification rates, NO_3^--N, nor NH_4^+-N were significantly lower with respect to the control plots, however (Figure 2A, B, C). The soil analysis of the C:N ratios showed no significant difference between the woodchip and control plots at MORR. The nitrification inhibitor seemed to maintain levels of NH_4^+-N but this was only significantly higher than control and removal plots in spring 2005 (Figure 2C). This treatment was not effective at inhibiting nitrification with respect to rates observed in control plots. The acidifier addition increased mineralization and ammonification in summer 2005 with respect to other treatments (Figure 2D), and did decrease pH in the

spring of 2004 and the summer of 2005 (Figure 2E). The acidifier treatment effects did

not last throughout the experiment, however.

Table 1. Significant differences for both sites for soil and vegetation treatments.

* p<0.05, ** p<0.01, *** p<0.0001, ns = not significant.

Soil Variable	Soil Treatment (whole plot)		Vegetation Treatment (split plot)	
	MORR	DEWA	MORR	DEWA
% Moisture	*	**	ns	ns
% Organic Matter	ns	ns	ns	ns
pH	***	n/a	ns	n/a
NO_3^-N	*	*	**	ns
NH_4^+N	*	***	ns	ns
Nitrification	ns	**	ns	ns
Ammonification	**	*	ns	ns
Mineralization	*	**	**	ns

MORR
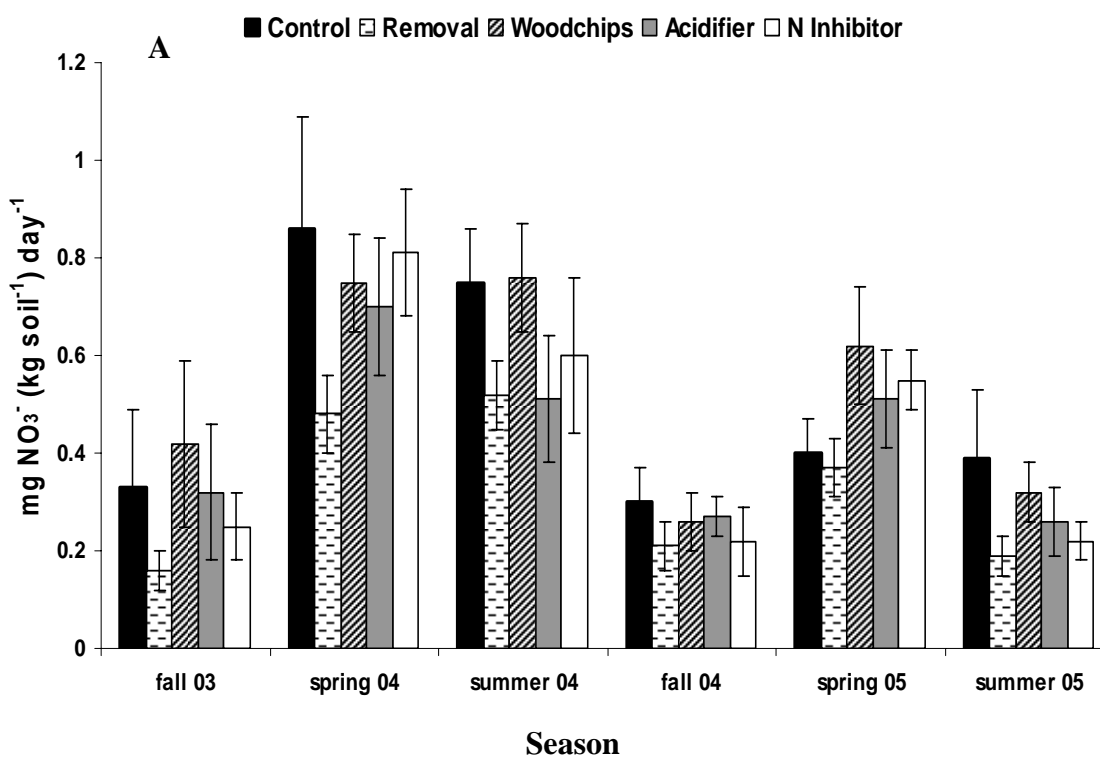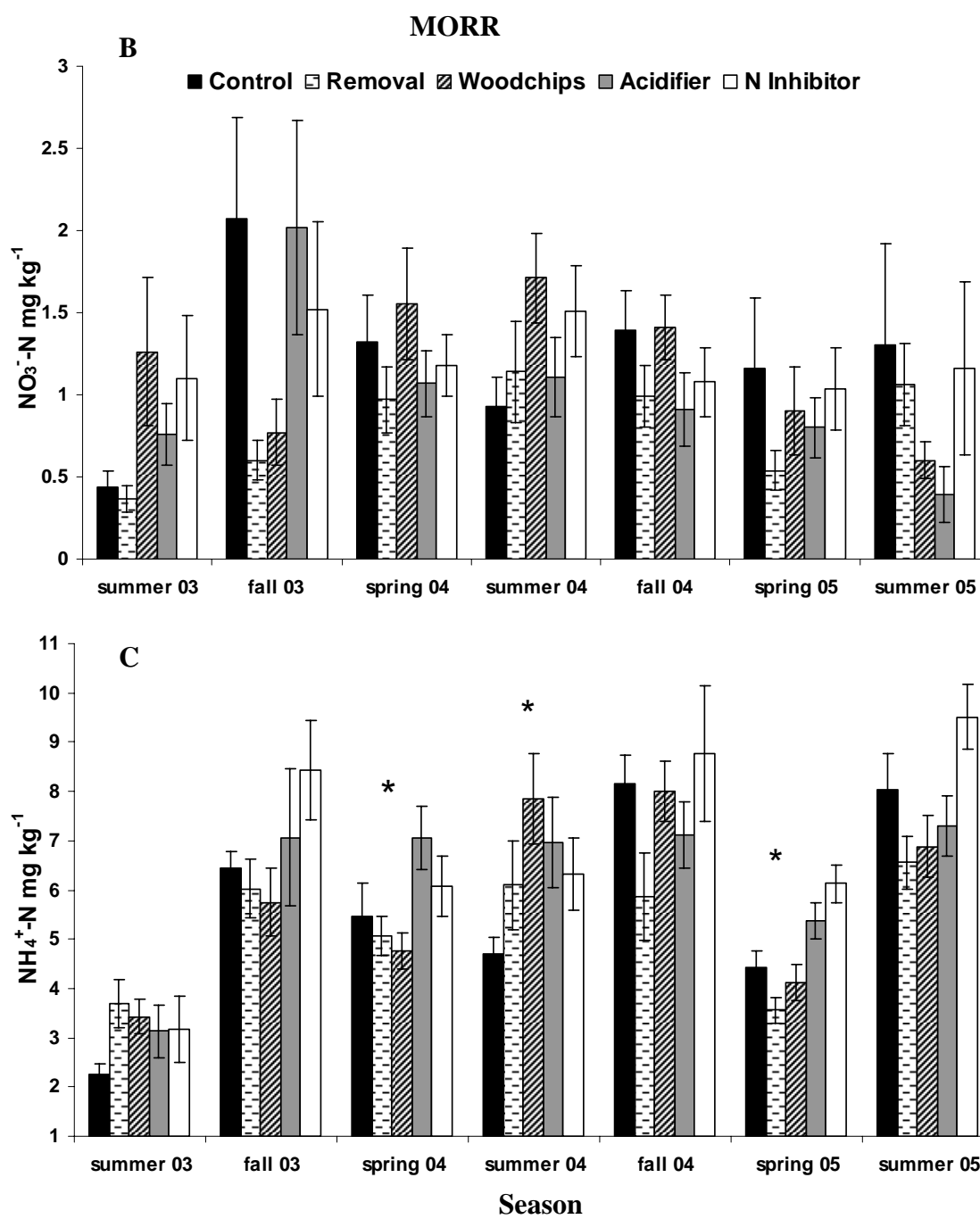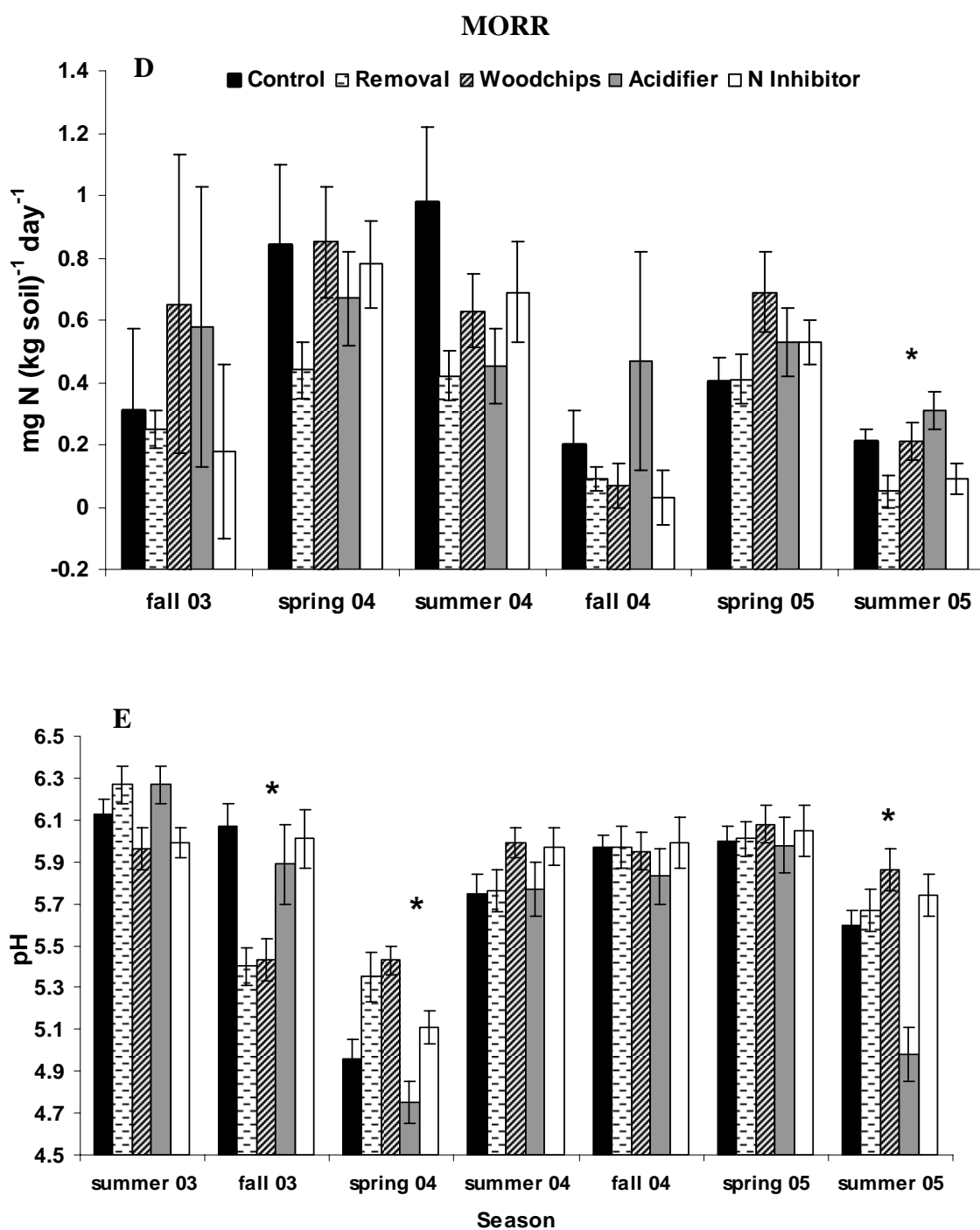
MORR

MORR

Figure 2. Results for the soil variables measured in the soil manipulation plots at MORR over time. A. Nitrification rates over time. B. NO_3^--N levels. C. NH_4^+-N levels. D. Mineralization rates. E. pH. Asterisks indicate significant differences among the plot soil manipulation treatments at MORR for that season only. Solid black bars represent control (no manipulation) plots; horizontal hashed bars represent soil removal

plots; diagonally hashed bars indicate woodchip addition plots; gray bars indicate aluminum sulfate addition plots; and open bars represent nitrification inhibitor addition plots.

At DEWA, the inorganic N pools and mineralization rates were affected by the soil manipulations, but the effects were most often significant only within the first few seasons of the experiment (Table 1). The removal plots significantly reduced NO_3^--N levels (p=0.04, F=1.75) in fall 2003, spring 2004, and summer 2004 as predicted (Figure 3A). The woodchip plots had significantly lower nitrification rates when compared to the control and inhibitor plots (p=0.0007, F=2.86) during fall 2003 and spring 2004 (Figure 3B), and significantly lowered mineralization (p=0.003, F=2.50) and ammonification rates (p=0.04, F=1.84) in spring 2004, but not during any other season (data not shown). Percent moisture was also significantly higher (p=0.0003, F=2.84) but only during the drier months of 2005 in the woodchip plots (Figure 3C). The inhibitor plots had greater levels of NH_4^+-N when compared to control plots (p<0.0001, F=3.52) as expected but only in summer 2004 and 2005 (Figure 3D). For two time periods, the nitrification inhibitor plots had the highest levels of NO_3^--N (Figure 3A). The C:N ratio of the woodchip plots was significantly higher with respect to the C:N of control plots at DEWA (p=0.05, t-stat = -1.97, data not shown).

DEWA
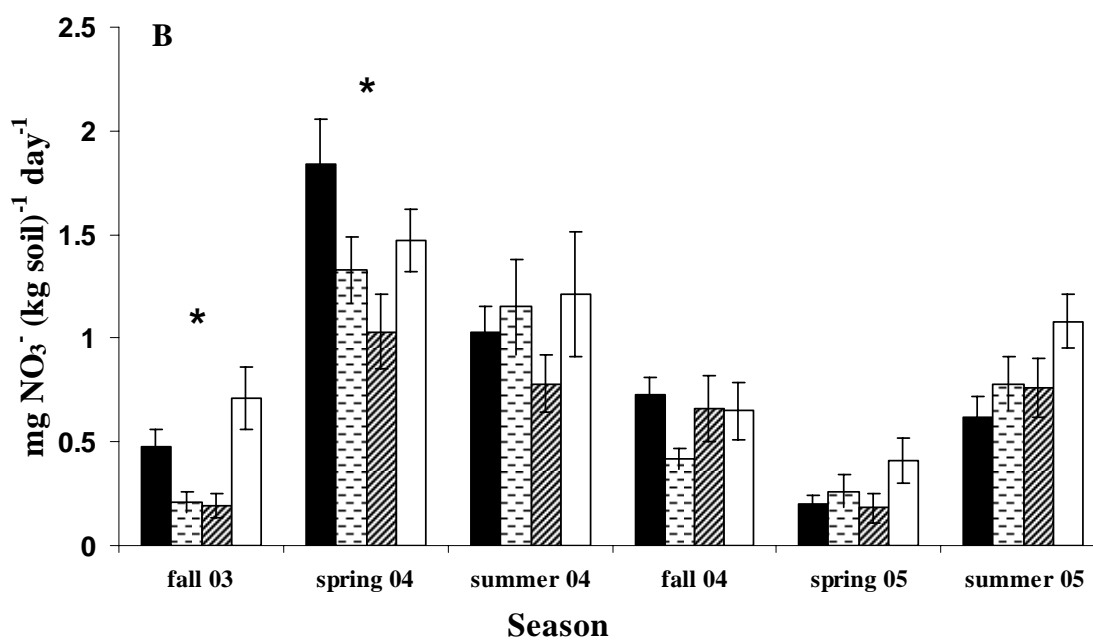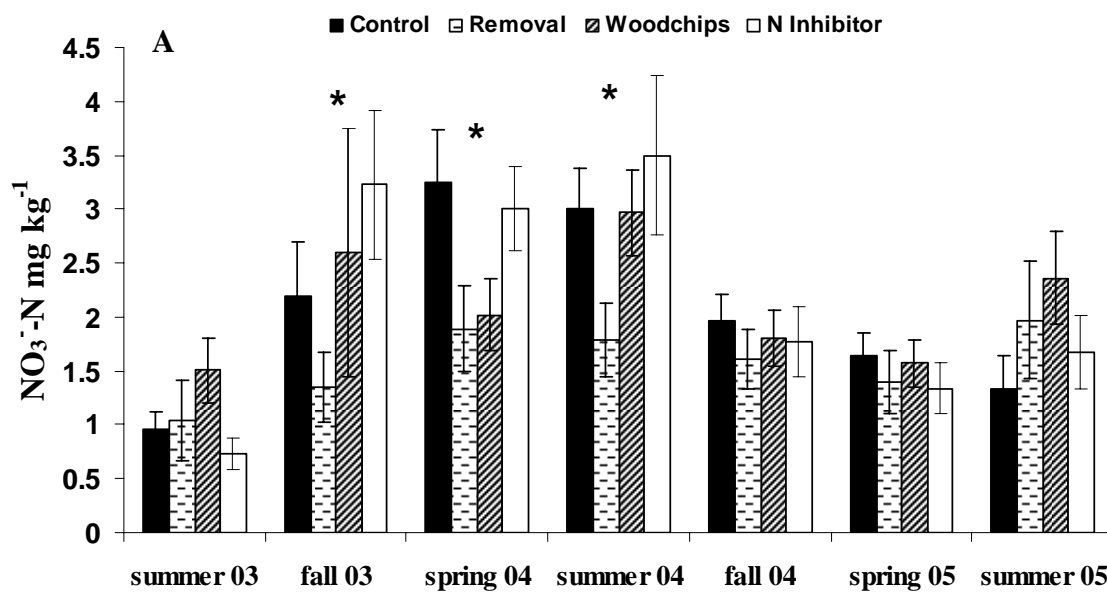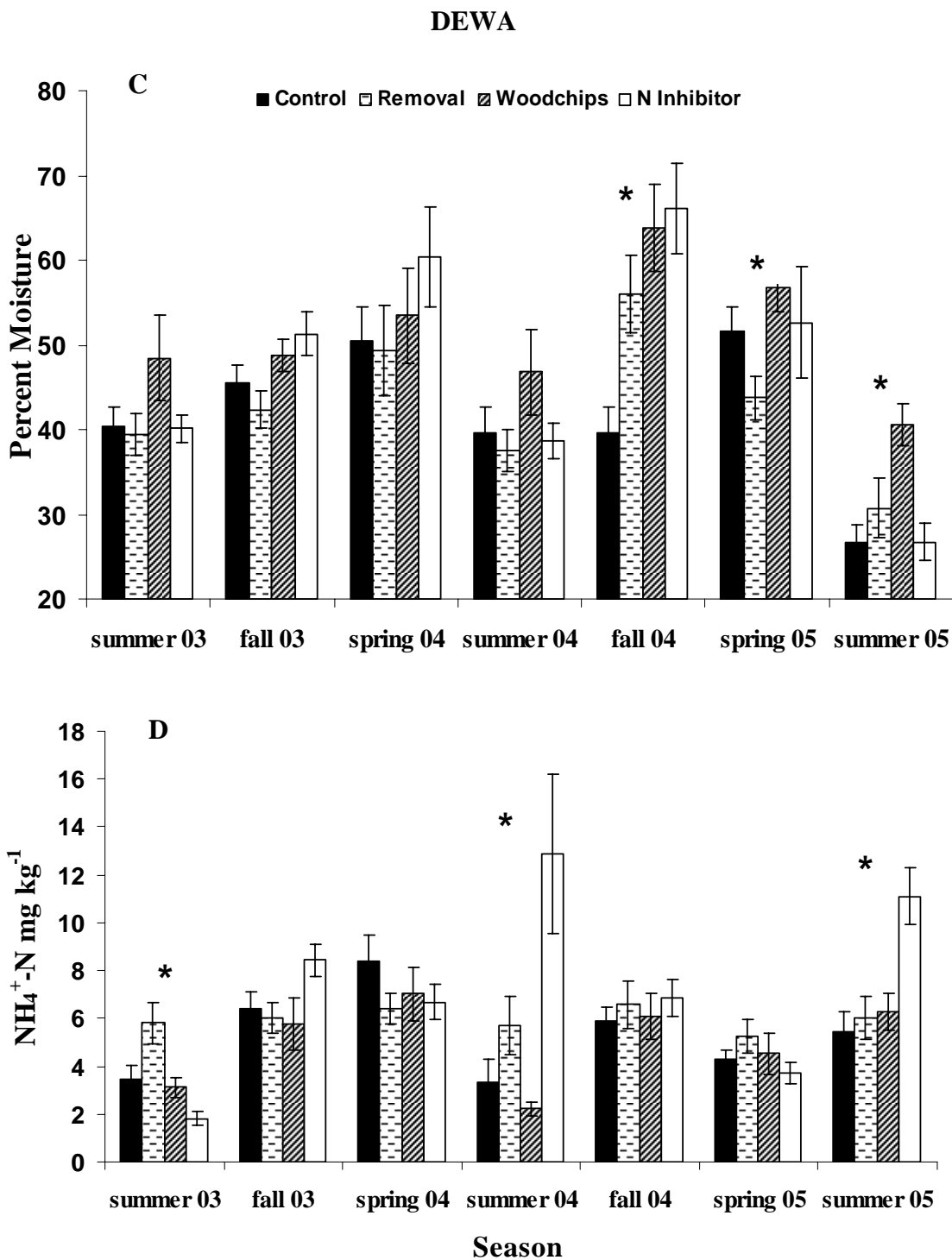
DEWA

Figure 3. Results for the soil variables measured in the soil manipulation plots at DEWA over time. A.

NO_3^--N levels. B. Nitrification rates. C. Percent moisture D. NH_4^+-N levels. Asterisks indicate significant

differences among the plot soil manipulation treatments at DEWA for that season only. Solid black bars

represent control (no manipulation) plots; horizontal hashed bars represent soil removal plots; diagonally

hashed bars indicate woodchip addition plots; and open bars represent nitrification inhibitor addition plots. Note: No aluminum sulfate was added to any plots at this site (see text).

In summary, the topsoil removal plots were somewhat effective in eliminating inorganic N in both sites, but more so at DEWA. The woodchip additions showed mixed results with lower ammonification rates in both sites but having only affected nitrification in site 2. The nitrification inhibitor did not prove to be effective at all. The acidifying treatment decreased the pH of soils at MORR but effects were not lasting.

Effects of Soil Manipulation of Plant Growth

The soil treatments did not influence the percent survival of the native species that were planted in the split-plots. Aboveground biomass of *M. vimineum* was not significantly different among the soil manipulation plots. *B. thunbergii* seedling count was only significantly greater in the woodchip plots in one sampling year (2004) in only one site (DEWA) when compared to the removal and inhibitor plots (p=0.04, F=1.90). The number of new shoots and the length of the shoots measured from *B. thunbergii* and *Vaccinium pallidum* shrubs were not significantly different for either site in any treatment plot.

Effects of Vegetation Manipulation on Soil Variables

None of the split-plot vegetation treatments significantly affected the soil variables in either site except for NO_3^--N levels (p=0.008, F=2.52) and mineralization rates (p=0.004, F=5.89) at MORR (Table 1). The treatment effect on NO_3^--N varied over time so that a specific treatment effect could not be determined. Mineralization was only affected in summer 2004. In this case the split-plots containing native species had greater

mineralization rates than the plots where the exotic shrubs remained contrary to prediction (data not shown).

DISCUSSION

Soil manipulations applied in this field study did not have consistently strong effects on soil properties is either study site. The inconsistent results demonstrate the difficulties in manipulating soil conditions within intact forests. Although general conclusions can be difficult to glean from such studies, these results do provide insight into bottom-up approaches to restoration and plant-soil feedbacks. The impacts of dominant plant invaders on soil and plant community structure may leave persistent legacy effects. Soils that have a longer history of invasion may be more difficult to alter without drastic manipulations. For example, the length of time that the feedback cycle between these invasive plants and soil properties has had to develop differs between the sites. MORR has been more densely invaded by both exotic species studied here longer than DEWA. Soil properties at MORR were less affected by the soil treatments over all than soil from DEWA. The native plant communities of MORR have withstood a higher intensity of deer browse than DEWA because hunting is not permitted. DEWA is a National Recreation Area with regular hunting seasons. Over time this release from resident plant community competition in MORR has contributed to the long term dominance of these exotic species possibly allowing more time to develop persistent legacy effects in the soil. The feedback cycle between these exotic plants and soil nutrient cycling can complicate efforts to increase biotic resistance of the resident community. Suding et al. (2004b) refer to system resistance to restoration efforts due to feedback mechanisms as the ecological resilience of degraded systems. MORR has a land use

history of human disturbance through agricultural practices and deforestation since before

designation as a National Park. Although now a protected area, the successional

trajectories of forested areas in MORR may have been so drastically altered through a

historical combination of disturbances resulting in a system with an alternative degraded

state (Suding et al. 2004b). These forms of intensive, long-term human disturbances,

along with a more recent increase in propagule pressure from exotic, horticulturally

valuable, aggressive species have promoted the establishment of many exotic species in

the forest understory. Both study areas are public lands preserved to protect high quality

habitat for native flora and fauna. The spread of exotic, invasive plants and herbivory

pressure continue to reduce native plant biodiversity in these protected areas, however.

These results demonstrate the resilience of forest soils to manipulation. Even

dramatic change to the soil surface demonstrated with the removal of topsoil was not

consistently effective in eliminating available nitrogen as predicted, although a trend

toward decreased levels of inorganic N were observed. Mineralization rates showed a

significant decrease in the driest sampling period (summer 2005), but nitrification was

not affected. At DEWA the woodchip addition showed more of a negative effect on

nitrification than the soil removal treatment. Several possibilities exist to explain the lack

of effectiveness of the topsoil removal treatment. Plots that had the topsoil removed,

especially at MORR, also lacked a well-developed organic matter layer that normally

prevents rainwater from scouring the soil surface carrying away more mobile nutrients.

Another possibility is that the quantity of inorganic N in the mineral soil below the top 5

cm did not differ from the soil that was removed. Soil analyses were run to examine

differences in topsoil removed versus mineral soil layers for NO_3^--N and NH_4^+-N. The

amount of NO_3^--N was significantly different between the layers for both sites analyzed together (p=0.02, F=6.19), but NH_4^+-N was not different (p=0.07, F=3.53). Alternatively, N deposition inevitably could be a constant N source in both of these sites that would mask the differences between all treatment plots despite our efforts to limit N availability.

The addition of hardwood woodchips also did not seem to effectively immobilize nitrogen as I had predicted even after 3 years. Other studies utilizing carbon additions have had mixed results either in affecting C:N ratios or in decreasing N availability. Groffman (1999) did not observe an increase in C:N ratios when sodium acetate was added to forest soils. Nitrogen availability actually increased as well. Prober et al. (2005) found that the addition of sucrose to open woodland plots did lower NO_3^--N but only in dry conditions. The more recalcitrant forms of carbon tested in forest soils by Magill and Aber (2000) did not stimulate net immobilization or net mineralization. The use of a very recalcitrant form of carbon in this study prevented a pulse in microbial activity that would lead to increased mineralization and possibly favor a shift in the fungal:bacterial biomass. The intent was to apply sufficient carbon to slowly decompose, increase the C:N ratio, and enhance the organic matter in the soil, but it is possible that insufficient carbon was added to effectively cover the whole plot in depth. After the first growing season of the experiment, evidence of the woodchips remained but fungal hyphae were clearly present. It is possible that the fungi respired or converted the carbon into fungal biomass resulting in less incorporation into the soil in the short term. The rate of woodchip decomposition may have been too slow to capture differences in percent organic matter or C:N ratio between the woodchip and control plots during the time scale of this experiment. In some

cases the woodchips were washed out of the plots due to heavy rains, especially in DEWA.

The vegetation manipulations in the split-plot design were ineffective at altering soil properties. The exotic bushes left in the plots were many decade-old adults with well-established root systems. The native seedlings planted in the split-plots were slow-growing species that only had two years to establish. It is unlikely that the soil manipulations affected the adult exotic bushes to a great extent. *B. thunbergii* has a plastic growth response to disturbance. It stump sprouts easily when cut or will send out shoots from rhizomes several feet away from the main stem (Ehrenfeld 1999). The native species chosen for this experiment were very sensitive to disturbance from transplanting, fencing, or some other environmental condition. Many of the initial plants had to be replaced due to high mortality especially at DEWA.

Plant growth was not responsive to the soil manipulations. The treatments were not drastic enough to affect well-established exotic shrubs. Observationally, the removal treatments did limit the amount of *M. vimineum* growing in the plots in the short term, but since local propagules were readily available, it had re-established in the next year. The only significant differences seen in the *B. thunbergii* seedling count was probably due to the mulch effect of the woodchips in the wetter of the two years. The *B. thunbergii* counts were extremely variable by plot.

I have demonstrated that forest soils can be altered, but a high level of intensive management over a longer period of time is required. The manipulations tried in this study might be more effective if applied more frequently and at higher rates. Aluminum sulfate and nitrification inhibitor application rates were based on best estimates for

agricultural soils where these amendments are commonly used. Application estimates for forest soils were not available for these amendments. Therefore, the application rate used in this study may need to be increased in order to detect a response. Normally most soil amendments are tilled into the soil, but this is not possible on a large scale in rocky forest soils filled with large roots. In addition, since a heavy precipitation event could have washed away any treatment effects before soil sampling occurred, more frequent sampling intervals might have captured more of a treatment response in soil properties. Therefore, large scale soil manipulation in the understory of intact forests might not be feasible due to the heterogeneity of forest soils, the complexities of nutrient cycling, and the intensive amount of management required. Based on the results of this study I recommend that land managers promote a combination of approaches that take advantage of normal forest successional processes to restore native biodiversity to invaded forests. Preventing intensive herbivory, eliminating vines that choke out the canopy layer and increase forest gaps, and selectively removing invasive species while re-planting native vegetation will eventually re-establish the multi-layered canopy structure often missing in invaded forests. Increasing abundances of native vegetation that provide food and habitat for wildlife will become a local propagule source. Targeting source individuals or populations of exotic species if the invasion has not become widespread will help to prevent further establishment. Rapid response to new invaders also requires intensive vigilance on the part of land managers. Creating or improving volunteer networks trained in constant vigilance for new populations of recognizable exotics and new-comers can contribute to the labor force needed for such vigilance.

      The goal of this study was to enhance site resistance to invasion through soil manipulations aimed at limiting nutrient availability. Although our results were not consistent over time, restoring ecosystem processes through limiting nutrient availability may still improve the successional trajectory of the native community toward a desired state. Understanding the effects abiotic factors such as soil properties or other aspects of the soil interface have on exotic, invasive plants and how those factors might be manipulated to represent pre-invasion conditions will inform restoration practices. Most likely a combination of top-down and bottom-up measures that alter links in the feedback cycle are necessary to enhance biotic resistance of a system. Overall success of restoration efforts may only be achieved if biotic factors, abiotic factors, and their interactions are considered when making management decisions.

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CHAPTER 4

The Effects of Leaf Litter on the Growth Response of *Microstegium vimineum* (Trin)

A. Camus, an invasive C$_4$ annual grass.

INTRODUCTION

Invasive plants have been shown to alter ecosystem processes and plant

community structure (Vitousek et al. 1987, Mack et al. 2001, Ehrenfeld 2003, Mack and

D'Antonio 2003, Lindsay and French 2005). Domination by a single plant species of

what was once a diverse community can result in changes in soil fertility, in the

replenishment of soil organic matter, and in the physical structure of the forest floor

(Vitousek 1987, Kourtev et al.1998, Ehrenfeld et al. 2001, Downs and Cavers 2005,

Lindsay and French 2005). In forested systems in the Northeastern US, establishment and

spread of introduced understory shrubs, vines, and herbs are an increasing threat to native

herbaceous biodiversity and forest regeneration (Kourtev et al. 1998, Meekins and

McCarthy 1999, Ehrenfeld et al. 2001, Forseth and Innis 2004). Although factors such as

nutrient availability, shade, and competition may limit the spread of invasive plants, some

species that are able to establish a vigorous population from just a few individuals might

have an advantage when disturbance occurs within an intact forest (Knight et al. 2007).

Although, many studies examined the relationship between invasive, exotic plants

and their ecosystems to devise management strategies aimed at restoring native

community structure and function (Kourtev et al. 2002, Kourtev et al. 2003, Mack and

D'Antonio 2003, Lindsay and French 2005, Pritekel et al. 2006), enhancing biotic

resistance to invasion remains challenging (Levine et al. 2004). Few studies have focused

on utilizing the physical layer of un-decomposed organic matter of the forest floor as a restoration strategy to maintain nutrient sinks and inhibit the spread of invasive plants. The goal of this study was to determine whether *Microstegium vimineum*, a shade tolerant, C_4 annual grass introduced to the US from Asia is negatively affected by different types of leaf litter and woody debris found commonly in the forest understory. If so, it is worth exploring ways to increase native species leaf litter within invaded forests.

The presence of leaf litter has been shown to be one of the main factors controlling the composition of plant communities (Sydes and Grime 1981, Facelli and Picket 1991a, Xiong and Nilsson 1999, Donath and Eckstein 2008). Litter can directly influence plant communities through effects on seed and seedling habitat or indirectly through altered species interactions (Facelli and Picket 1991b, Peterson and Facelli 1992, Facelli 1994, Molofsky et al. 2000). Different quantities and types of leaf litter are likely to differentially affect the plant community composition depending on individual species characteristics and performance under site-specific environmental conditions (Facelli and Pickett 1991a, b, Peterson and Facelli 1992, Donath and Eckstein 2008). Quested and Eriksson (2006) suggest that the identity of the plant litter matters in controlling growth response of seedlings. In some cases, leaf litter plays an important role in the germination and establishment of certain species by creating an important structural component of the physical environment of emerging vegetation (Facelli and Pickett 1991a, Smith and Capelle 1992, Xiong and Nilsson 1999, Molofsky et al. 2000). Litter provides a protective microhabitat that promotes shading and prevents soil desiccation (Molofsky et al. 2000, Donath and Eckstein 2008).

Leaf litter can also create environmental conditions that prevent plant establishment and growth (Xiong and Nilsson 1999, Molofsky et al. 2000, Downs and Cavers 2002). Some litter types can be an inhibitory physical component by limiting light availability and providing habitat for seedling herbivores (Facelli 1994). Regeneration of understory vegetation, especially species with small seed sizes such as grasses can be negatively affected by large quantities of leaf litter (Sydes and Grime 1981, Peterson and Facelli 1992, Vellend et al. 2000, Kostel-Hughes et al. 2005). Facelli and Pickett (1991a) found that oak litter significantly suppressed biomass, percent cover, and mean number of seeds per study plot for an old-field annual grass (*Setaria faberii*). Vellend et al. (2000) tested whether forest tree litter affected different sedges (*Carex* sp.). As with other forest herbs, they determined that litter acted as a physical barrier to light, inhibiting seedling emergence. Stinchcombe and Schmitt (2006) tested whether oak litter had the ability to alter the evolutionary dynamics of *Impatiens capensis*. Although not a grass, *I. capensis* is an annual whose phenology and growth form was significantly affected by oak litter. Downs and Cavers (2002) found that when testing three different species of litter cover on the seedling emergence of bull thistle (*Cirsium vulgare*), the type of leaf litter made a difference in its germination rate. Oak and maple litter that covered more surface area were more effective at inhibiting seedling emergence than black walnut litter which withered and left more soil surface exposed.

Litter presence can also inhibit plant growth if the particular species is not adapted to deep layers of litter. Donath and Eckstein (2008) found that woodland seedlings were more inhibited by litter from grassland species than by woodland litter, while grass species were not differentially affected by either litter source. Some non-

native species may be less likely to succeed in areas with recalcitrant litter layers because they have not evolved in habitats with thick litter depths. The leaf litter of native canopy species has been shown to inhibit the seedling emergence of weedy species that colonize disturbed habitats (Facelli 1994, Smith and Capelle 1992, Downs and Cavers 2002, Bartuszevige et al. 2007). Facelli (1994) tested the direct and indirect effects of oak litter on seedling emergence of *Ailanthus altissima*. He found that the presence of litter indirectly negatively affected *Ailanthus* due to seedling predation by herbivores that found refuge in the litter. However, litter presence created an indirect effect that positively enhanced *Ailanthus* seedling growth due to reduced competition from other herbaceous species that were inhibited by the presence of oak litter.

Some studies have shown that the species origin of the native leaf litter has differential effects on invasive plants. Smith and Capelle (1992) found that the native leaf litter cover inhibited the germination and growth of chicory (*Cichorium intybus*) in a greenhouse study. In a field study Bartuszevige et al. (2007) tested two exotic, invasive species with and without native canopy litter in forested plots. They found that in the litter removal plots the number of *Lonicera maackii* and *Alliaria petiolata* seedlings increased when compared to plots with double the amount of litter. In another greenhouse study Ellsworth et al. (2004) found that the quantity of intact litter from oak-dominated forests was more effective in decreasing *Celastrus orbiculatus* seedling emergence when compared to treatments with fragmented litter or no litter.

Several studies have examined the effects of litter on exotic, invasive plant growth, but none have specifically addressed whether *Microstegium vimineum* (Trin) A. Camus, (Japanese stiltgrass) is susceptible to the structural barrier of native canopy leaf

litter. *Microstegium vimineum* (hereafter referred to by the generic name), one of the most noxious understory dominants in the eastern United States (Judge et al. 2005) is an annual, C_4 grass that did not evolve in closed-canopy forests. It must rely on establishment through seed germination in the late spring when much of the canopy has flushed. *Microstegium* forms thick stands creating a monoculture (shown in Figure 1A) that crowds out other herbaceous and woody seedlings (Barden 1987, Horton and Neufeld 1998, Cole and Weltzin 2004, Leicht et al. 2005). It is often observed in disturbed forests with limited understory or seedling regeneration but can have a patchy distribution suggesting that some biotic or abiotic environmental factors constrain its distribution (Cole and Weltzin 2005). Patches where it is dominant in the understory often lack thick native leaf litter mats of recalcitrant quality (personal observation).

Microstegium is a shade-tolerant grass accidentally introduced from Asia and was first collected in the US in 1919. It has spread rapidly into disturbed and mature forests in at least 21 eastern and southern states (Horton and Neufeld 1998, USDA 2008). It germinates in May, flowers in September, and sheds its seed in October. Seeds have been shown to stay viable in the seed bank for 3-5 years (Barden 1987, Gibson et al. 2002). Stems can root at the nodes as they have a decumbent growth form and send out tillers into newly disturbed areas (Ehrenfeld 1999, Flory et al. 2007) thereby creating dense lawns in the understory of intact forests (Barden 1987, Fairbrothers and Gray 1972). When *Microstegium* dies, the litter covers the ground like straw (Figure 1B) (Gibson et al. 2002, personal observation). This thatch remains on the ground through early spring and can serve as a positive feedback to itself due to the seed source from the cleistogamous flowers along auxiliary stems that fall to the ground when the plants

senesce. The thatch may also have an inhibitory effect on native plant seedlings (personal observation).

Figure 1. A. Monoculture of *Microstegium vimineum* in forest stand. B. *Microstegium* thatch left on ground in early spring.

A

B
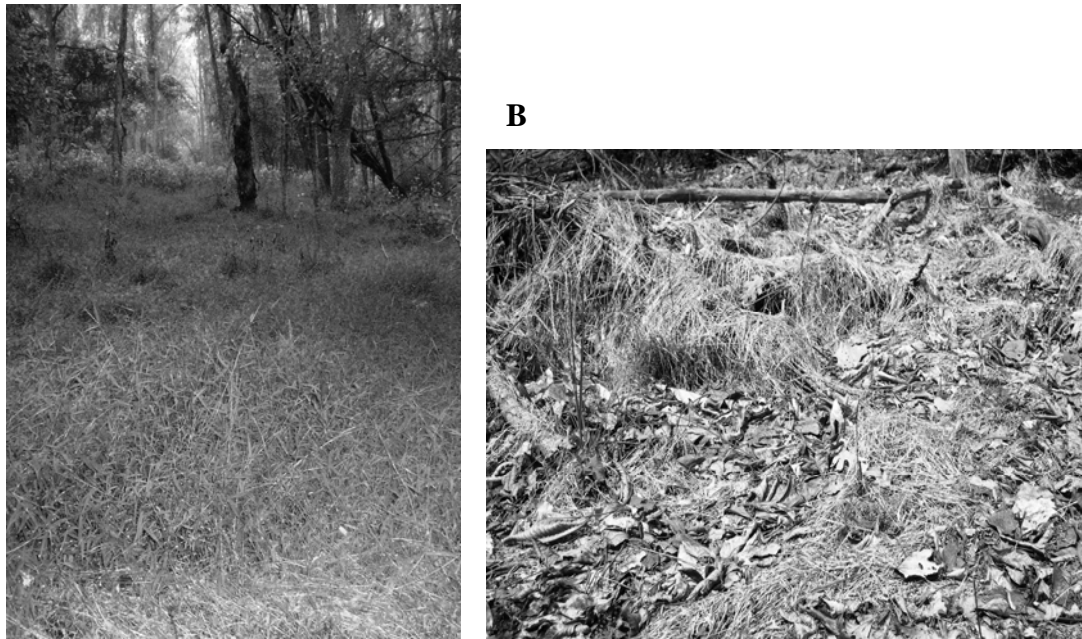
Much of the research involving *Microstegium* has not addressed the forest floor's role in its establishment. *Microstegium* is known to invade many forest types including oak-dominated and maple-dominated stands. Observationally, however, *Microstegium* is not as commonly found in undisturbed oak-dominated forests with closed canopies and an intact understory layer (Cole and Weltzin 2005). Drier, rockier soils on which oak forests tend to be found may be less hospitable to seeds than mesic, maple-dominated forests. Soil properties as well as microhabitat conditions influence seed germination and growth. Cole and Weltzin (2005) tested several environmental conditions such as light, moisture, and soil texture and nutrients in greenhouse and field studies to determine understory constraints on *Microstegium* distribution. They found that low light levels conditions, most negatively affected *Microstegium* when a mid-canopy level was present

in the understory. They did not, however, take into account the physical effects of the leaf litter produced by canopy or mid-layer species.

It is possible that *Microstegium*, a novel life form as an annual, exotic, invasive grass establishing in mature forests with a small seed size (*c*. <5mm), is susceptible to the presence of native canopy litter. Kostel-Hughes et al. (2005) examined the impact of forest leaf litter on tree seedlings with a range in seed size. Generally they found that smaller-seeded species (*Betula lenta* and *Liquidambar styraciflua*) experienced reduced seedling emergence and modified growth forms in the presence of leaf litter. Studies involving other invasive species have had some success in inhibiting growth with litter treatments or have observed greater invasive plant establishment in areas with reduced litter layers (Downs and Cavers 2002, Ellsworth et al. 2004, Kostel-Hughes et al. 2005, Bartuszevige et al. 2007). Restoration projects aimed to control invasive plants often utilize more recalcitrant forms of carbon such as woody debris or mulch which is readily available and affordable to enhance soil organic layers (Cogliastro et al. 2001). Woodchips and leaf mulch are often used to prevent weed growth in lawns and gardens.

To investigate whether leaf litter dynamics inhibit *Microstegium* seedling establishment, a factorial greenhouse experiment was designed to grow *Microstegium* from seed in two soil types under four litter treatments. Since *Microstegium* is often observed along trails, roadsides, or other disturbed sites, I grew seeds in a no-litter treatment to simply test whether the presence of litter facilitates or inhibits seedling establishment. To test whether the growth and survival of *Microstegium* is affected by litter types of different physical qualities, I used woodchips and oak litter to represent more recalcitrant forms of litter and maple leaves as a native species litter that

decomposes more quickly. To test whether *Microstegium*'s own litter would create a positive feedback for growth since seeds have to be able to germinate underneath thick layers of thatch where it has already invaded, *Microstegium* litter was also chosen as a litter treatment. I hypothesized that the presence of leaf litter or woodchips would inhibit the growth of *Microstegium*. I also hypothesized that the quality of the litter would differentially affect the growth response of *Microstegium* with the more recalcitrant litter treatments resulting in a greater decrease in growth. Of the four litter treatments it was predicted that *Microstegium* growth would be more inhibited by the effects of woodchips and oak litter (more recalcitrant types) when compared to the maple litter, which decomposes more quickly. Because *Microstegium* is less often dominant in oak forest understories, I wanted to test different soil types to assess whether growth was influenced by inherent differences in soil quality as well. To test whether soil conditions or the interaction of soil conditions with litter type influenced *Microstegium* growth differently, two types of field soil from forests into which *Microstegium* had not yet invaded were used.

METHODS

Litter effects were tested in greenhouse microcosms, using field-collected soils. A factorial experimental design allowed testing for variation in growth response of *Microstegium* and soil properties between two soil types, among five litter treatments (oak, maple, *Microstegium*, woodchips, no litter), and any interactions between soil type and litter treatment.

Soil Collection and Analysis

Field soil was collected from two forest types: an oak/hickory-dominated forest (*Quercus* sp., *Carya* sp.) with *Vaccinium pallidum* and *Gaylussacia baccata* as the main species in the understory (from Allamuchy Mountain State Park in Warren County, NJ Rockaway series, coarse-loamy mesic Typic Fragiudults derived from glacial till from Pre-Cambrian schists) and from a red maple-dominated forest (*Acer rubrum*) primarily with *Viburnum dentatum*, *Lindera benzoin*, and *Podophyllum peltatum* dominating the understory (from Helyar Woods, Middlesex County, NJ Fallsington series, fine-loamy mesic Typic Endoaquult, derived from Cretaceous Coastal Plain sediments) (USDA-NRCS 2008). These sites had not been invaded by *Microstegium* at the time of soil collection. Soil was sieved in the field on a 0.5 cm sieve to eliminate large debris and homogenized. Seven samples of the homogenized soil from each source were taken for soil property analysis (percent moisture content, percent organic matter loss-on-ignition, pH, and 2M KCl extractions (4:1 KCl to soil ratio) for inorganic nitrogen. KCl extractions were frozen until analyzed colorimetrically on a Lachat QuikChem Flow Injection Analyzer 8000 series (Lachat Instruments, Hach Co., Loveland, CO) for NO_3^--N and NH_4^+-N concentrations (QuikChem 1986, 1987).

Fifty greenhouse trays (16.7cm x 12.3cm each) were filled with the maple forest soil (hereafter maple soil) and 50 were filled with the oak/hickory forest soil (hereafter oak soil). Each tray was filled with approximately 325g of soil to a depth of 3cm. All trays received approximately 0.5 g (*c.* 600 seeds) of *Microstegium* seed collected the previous year from dried litter. Seeds were placed on the soil surface, and all trays in each

treatment were gently watered twice a week to prevent seeds from splashing out of the tray.

Litter Treatments

Newly fallen oak and maple litter was collected from the ground under mature trees growing in open areas during the previous fall, air dried, and bagged. Species were sorted and clipped into smaller pieces. *Microstegium* culms and leaves were collected from the field the previous fall after the first frost and bagged. Culms were gently crushed and clipped into smaller pieces to release seed before being applied to trays. Newly chipped woodchips were collected from local piles of hardwood mulch near the maple forest. All litter types were dried in an oven for 3 hours at $75^{o}C$ to eliminate insects and reduce microbial growth. To determine the amount of maple and oak litter to be used in the treatments, the litter cover was measured every 10m along one 220m transect in each forest type (oak/hickory and maple). Litter depths were recorded for the litter layer left on the forest floor in the spring when *Microstegium* is normally germinating. Litter depths were averaged for each transect and used as a model for the litter applications in the greenhouse. The average litter depth in the maple forest was 4.4cm (±0.29) and in the oak forest was 4.0cm (±0.50). The thickness of the *Microstegium* litter and woodchips placed in the trays was approximately equal to the depth of the oak and maple litter.

The four types of litter were applied on top of the seed and one set of trays did not receive any litter to serve as the "no litter" treatment. Ten trays from each soil type received each litter type resulting in 10 replicates of each litter treatment for each of the two soil types (to total 100 trays). *Microstegium* was grown on a greenhouse bench covered with shadecloth to simulate the shade of the canopy under light (supplemental

HID lights 14h day/10h night) and kept at a constant temperature (12-15°C) for a maximum of 12 weeks. All treatments were watered as needed. Every two weeks all trays were rotated on the bench to avoid effects of variation in light and air circulation.

Growth and Soil Measurements

After the first 4 weeks of the experiment, the number of seeds that had germinated was counted in each tray for the no-litter treatment only to determine whether something inherent about the soil types without litter affected germination. At the end of the experiment, 10 individuals were chosen at random from each tray (total of 100 individuals per treatment) for stem height measurements. The total number of individuals per tray surviving at harvest (hereafter termed survival) was counted and aboveground biomass was harvested before plants set seed. Aboveground biomass was oven-dried for 48h at 75°C and weighed. At the time of harvest five trays from each treatment were randomly chosen from which to take one soil sample to analyze for percent moisture content, percent organic matter (loss-on-ignition), pH, and 2M KCl extractions for NO_3^--N and NH_4^+-N using the method described above. Soil samples collected before the experiment were compared with soil properties measured after the experiment to see whether changes occurred among treatments and to see if *Microstegium* itself had an effect on soil after the growth experiment. The belowground biomass was pooled for each tray and was rinsed, dried, cleaned using forceps, dried again for 48h at 75°C, and weighed.

Data Analysis

Two-sample t-tests were used to analyze the difference in *Microstegium* germination between the two soil types, to measure the differences in soil properties

between the two soil types from initial soil samples collected from the field (prior to the experiment), and to measure the differences in soil properties between the two soil types from the no-litter trays when the experiment was terminated. A fixed effect two-factor analysis of variance (ANOVA) was used to test for differences in the growth response variables for *Microstegium* among the litter treatments (main effect) and each soil type (main effect). Percent survival was calculated from the total number of individuals remaining in each tray. Orthogonal contrast statements determined from *a priori* comparisons between the no-litter treatment and all of the litter types, between the *Microstegium* litter and the other litter types, and between oak versus maple litter were performed to test for significant differences among means (Quinn and Keough 2002). A non-orthogonal contrast to compare oak litter versus the woodchips was also performed. Additional *a posteriori* multiple comparisons of means were performed using Tukey tests to show whether any litter treatments were significantly different from the no-litter treatment for all main effects. A two-way analysis of covariance (ANCOVA) determined whether the number of surviving individuals per tray was affecting the results of the litter treatment effects on total biomass and stem height. The same two-way ANOVAs and post-hoc tests were conducted to test for differences in soil properties between the two soil types at the end of the experiment. The data for percent organic matter, NO_3^--N and NH_4^+-N were log transformed to improve normality (Quinn and Keough 2002). All analyses were run in SAS v.9.1 (SAS Institute Inc. 2003).

RESULTS

Microstegium Growth Response to Litter Treatments

Several of the litter treatments positively affected plant growth while the no-litter

treatment negatively affected growth. After four weeks of growth, significantly more

seedlings had germinated in the oak soil (t-value 8.07, p<0.0001) than in the maple soil.

The average number of seedlings that germinated in the maple soil was 65.4 per tray (±SE

= 20.9) and for the oak soil was 255.6 seedlings per tray (±SE = 10.9).

At the end of the experiment (12 weeks), however, the opposite was true for

percent survival. When taken together percent survival was significantly greatest in the

maple soil ($\bar{x}$ = 27%, ±SE = 2%, F=13.71, p=0.0004) compared to the oak soil ($\bar{x}$=18%,

±SE=2%) across all the treatments as predicted. When analyzed by litter treatment,

percent survival was enhanced by most of the litter types, but the effect of the each litter

type depended on soil type (Table 1A). When grown in the oak soil, all litter treatments

except for the maple litter significantly increased percent survival compared to the no

litter treatment (F= 4.04, p=0.05) (Figure 2A). Contrary to prediction, when grown in the

oak soil, the oak litter treatment greatly benefited survival compared to the other litter

types, especially the maple litter. Significantly more plants survived when under oak litter

than when under maple litter (p<0.0001). *Microstegium* litter significantly enhanced

survival when compared to maple litter but did not increase survival as much as the oak

litter effect when grown in the oak soil (p<0.001). When grown in the maple soil, all the

litter treatments significantly, especially the woodchips increased percent survival

compared to the no litter treatment (p<0.0001). Therefore, at least for the maple soil type,

percent survival was only decreased in the absence of a litter treatment.

When analyzed for all treatments together, the average stem height for plants in the maple soil was significantly shorter than that in the oak soil (p=0.05, $\bar{x}$=33cm, ±SE=1.33, $\bar{x}$=41cm, ±SE=1.2, respectively). The effect of the litter treatment depended on the soil type (Table 1B), but overall the litter treatments increased stem height, except for the woodchips (Figure 2B). In the maple soil, the woodchip treatment was the only litter type to significantly reduce stem height (p<0.0001, Figure 2B).

Total biomass was significantly different between the two soil types (Table 1C). *Microstegium* had greater biomass when grown in the oak soil ($\bar{x}$=7.6g, ±SE=3.42) than in the maple soil ($\bar{x}$=6.8g, ±SE=3.5) contrary to prediction. Plant biomass in the no-litter treatments was less than for plants grown in the litter treatment in both soil types (maple soil p=0.01, oak soil p=0.03, Figure 2C). In the oak soil, *Microstegium* litter had the greatest positive effect on total biomass than the other litter types which was mostly driven by the differences in belowground biomass (Figure 2C). The results for the maple soil differed, however. Although total biomass was significantly different among the litter treatments (p=0.01) in the maple soil, the *Microstegium* litter did not differ significantly compared to the other treatments.

Root and shoot biomass were analyzed separately to examine the influence of each component on total biomass. The litter treatments and soil types separately influenced biomass allocation to shoots (Table 1D). Only in the maple soil did the no-litter treatment produce significantly less shoot biomass than all of the other treatments (p=0.02). In the oak soil, the no litter and woodchip treatments decreased shoot biomass, but not significantly so (Figure 2C). The litter treatment effect on root biomass depended on the soil type (Table 1E). In the oak soil the no litter treatment had plants with smaller

root biomass compared to the other treatments (p=0.002), and the *Microstegium* litter enhanced root biomass compared to the other litter treatments (p=0.01).

The average number of individual stems per tray (survival) did not covary with total biomass, but did affect the stem height results (Table 2). A linear regression analysis determined a weak relationship between stem height and number of stems per tray R^2=0.38, p<0.0001). After accounting for the effect of number of stems per tray on stem height, the litter treatment effects were still significant (p<0.0001).

Effects of Litter Treatments on Soil Properties

Prior to the experiment the initial soil properties of the two soil types (maple vs. oak) did significantly differ (data not shown). Initial maple soil had significantly higher percent moisture and NO_3^--N, and lower pH and NH_4^+-N levels than the initial oak soil (all p-values <0.05). There was no difference in percent organic matter. Then, at the termination of the experiment, soil samples were taken from the no-litter treatments from each soil type (initial maple soil vs. no-litter treatments in maple soil and initial oak soil vs. no-litter treatments in oak soil). When post-experiment soils were compared to the initial soil properties from the no-litter treatments only, percent moisture had decreased in the maple soil. In the oak soil, percent organic matter, NO_3^--N, and NH_4^+-N levels decreased by the end of the experiment (all p-values <0.05, data not shown). Finally, all post-experiment soils were compared among all litter treatments. At the end of the experiment the soil moisture was affected separately by the treatment and soil type, but not their interaction (Table 3A). The maple and oak soils did not differ in NH_4^+-N, but maple soil had higher percent organic matter than the oak soil. Maple soil maintained lower pH and higher NO_3^--N during the experiment.

Some soil properties were affected by the litter treatments as well except for percent organic matter (Table 3). The woodchip treatment had the highest percent moisture but not significantly so for either soil type (Table 4). The pH of the oak soil under the no-litter (p=0.006) and the *Microstegium* litter (p=0.001) treatments was significantly less than the other treatments, and soil under the oak litter (p-0.03) had significantly higher pH than the soil under the maple litter. In the maple soil, the no-litter treatment had significantly lower pH (p=0.01) than the other litter types mostly driven by the pH of the woodchip treatment (Table 4). Nitrate and ammonium levels in both soil types were significantly higher under the oak litter (all p-values <0.05) when compared to the maple litter treatments. The woodchip layer had the lowest levels of nitrate and ammonium in both soil types (Table 4).

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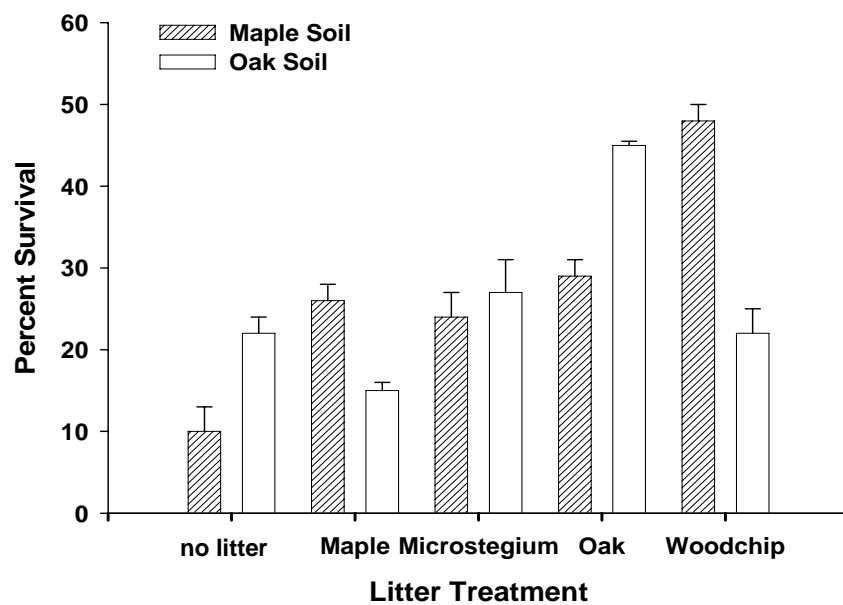
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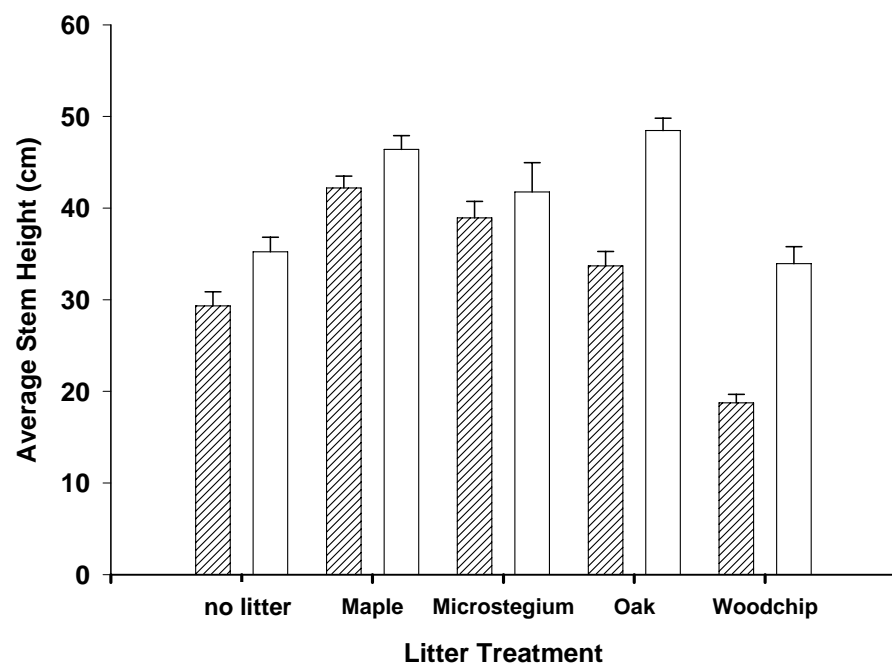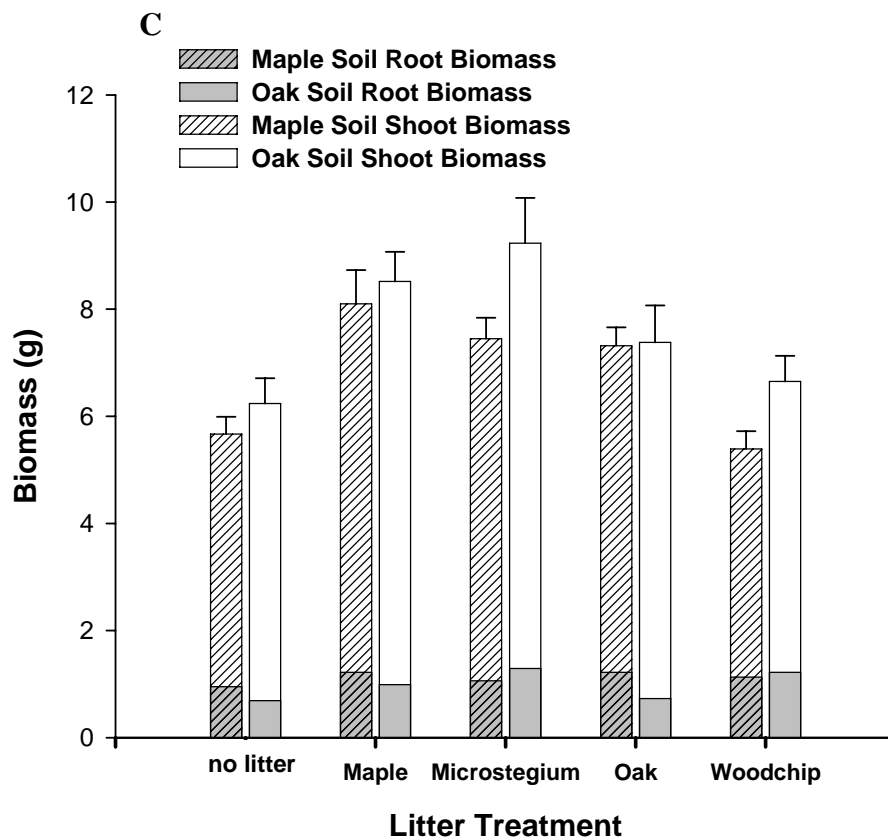
Figure 2. A. Average percent survival per treatment at 12 weeks for both soils. B. Average stem height for 10 randomly selected individuals per tray for both soils. C. Average total biomass for *Microstegium* plants harvested at the end of the experiment for both soils. See Table 1 for ANOVA results.

Table 1. Analysis of variance of percent survival, stem height, total biomass, aboveground biomass, and

belowground biomass; N=100; df=degrees of freedom, F=F-statistic, p=p-value, ns = not significant.

Growth Variables	df	F	p
A. Percent Survival			
Soil type	1	37.87	<0.0001
Litter treatment	4	20.48	<0.0001
Soil type X Litter treatment	4	24.71	<0.0001
Error	90		
B. Stem Height			
Soil type	1	59.67	<0.0001
Litter treatment	4	35.10	<0.0001
Soil type X Litter treatment	4	5.73	0.0004
Error	90		
C. Total Biomass			
Soil type	1	4.91	0.03
Litter treatment	4	8.00	<0.0001
Soil type X Litter treatment	4	0.70	ns
Error	90		
D. Shoot Biomass			
Soil type	1	8.07	0.006
Litter treatment	4	8.75	<0.0001
Soil type X Litter treatment	4	0.30	ns
Error	90		
E. Root Biomass			
Soil type	1	3.51	0.06
Litter treatment	4	3.71	0.008
Soil type X Litter treatment	4	3.37	0.01
Error	90		

Table 2. Analysis of covariance of total biomass, stem height with survival

as a covariate; df=degrees of freedom, F=F-statistic, p=p-value.

Source of Variation	df	F	p
A. Total Biomass			
Soil type	1	0.47	ns
Litter treatment	4	0.46	ns
Survival	1	2.52	ns
Survival X Litter treatment	4	0.41	ns
B. Stem Height			
Soil type	1	0.42	ns
Litter treatment	4	85.87	<0.0001
Survival	1	16512.4	<0.0001
Survival X Litter treatment	4	81.57	<0.0001

Table 3. Analysis of variance of percent moisture, percent organic matter, pH, NO_3^--N, and NH_4^+-N from soil samples taken from greenhouse trays after 12 weeks of *Microstegium* growth. Percent organic matter, NO_3^--N, and NH_4^+-N data were log-transformed; N=5; df=degrees of freedom, F=F-statistic, *p*=p-value, ns=not significant.

Source of Variation	df	F	*p*
A. Percent Moisture			
Soil type	1	29.54	<0.0001
Litter treatment	4	10.72	<0.0001
Soil type X Litter treatment	4	1.74	ns
Error	49		
B. Percent Organic Matter			
Soil type	1	415.38	<0.0001
Litter treatment	4	1.46	ns
Soil type X Litter treatment	4	2.86	0.04
Error	49		
C. pH			
Soil type	1	310.58	<0.0001
Litter treatment	4	11.06	<0.0001
Soil type X Litter treatment	4	5.08	ns
Error	49		
D. NO_3-N			
Soil type	1	12.28	0.001
Litter treatment	4	7.87	<0.0001
Soil type X Litter treatment	4	4.26	0.006
Error	49		
E. NH_4-N			
Soil type	1	0.00	ns
Litter treatment	4	28.41	<0.0001
Soil type X Litter treatment	4	4.07	0.007
Error	49		

Table 4. Mean (±standard error) soil properties: percent moisture, percent organic matter, pH, NO_3^--N (mgkg^{-1} dry soil), and NH_4^+-N (mgkg^{-1} dry soil) for soil samples taken from 12-week growth experiment of *Microstegium vimineum* plants. Soil type indicates original source of field soil (oak-dominated and maple-dominated forest soils). N=5 for all treatments.

Soil Property	Soil Type	Litter Treatment	Mean (±SE)	Soil Type	Litter Treatment	Mean (±SE)
% Moisture	Oak	No Litter	56.83 (10.76)	Maple	No Litter	73.75 (14.52)
		Maple	19.44 (2.25)		Maple	59.15 (14.53)
		Microstegium	24.45 (10.45)		Microstegium	61.51 (13.88)
		Oak	16.63 (1.17)		Oak	45.77 (16.66)
		Woodchips	59.77 (11.65)		Woodchips	133.99 (7.42)
% Organic Matter	Oak	No Litter	23.13 (1.67)	Maple	No Litter	43.22 (1.64)
		Maple	19.44 (2.25)		Maple	52.48 (3.22)
		Microstegium	17.55 (1.78)		Microstegium	46.68 (1.06)
		Oak	16.63 (1.17)		Oak	46.60 (4.38)
		Woodchips	18.27 (1.13)		Woodchips	52.33 (2.06)
pH	Oak	No Litter	4.67 (0.06)	Maple	No Litter	4.22 (0.04)
		Maple	4.80 (0.04)		Maple	4.28 (0.03)
		Microstegium	4.67 (0.04)		Microstegium	4.28 (0.04)
		Oak	4.94 (0.03)		Oak	4.28 (0.04)
		Woodchips	4.84 (0.04)		Woodchips	4.53 (0.05)
NO_3-N	Oak	No Litter	0.22 (0.09)	Maple	No Litter	6.58 (2.11)
		Maple	0.07 (0.05)		Maple	0.74 (0.51)
		Microstegium	0.10 (0.02)		Microstegium	1.65 (1.24)
		Oak	1.00 (0.23)		Oak	8.10 (5.24)
		Woodchips	0.06 (0.01)		Woodchips	-0.57 (0.05)
NH_4-N	Oak	No Litter	23.49 (1.39)	Maple	No Litter	19.05 (3.10)
		Maple	14.52 (3.19)		Maple	10.37 (2.16)
		Microstegium	5.74 (0.86)		Microstegium	11.43 (3.56)
		Oak	48.08 (4.94)		Oak	27.61 (7.66)
		Woodchips	3.14 (0.73)		Woodchips	6.25 (0.92)

DISCUSSION

The results show that the ability of *Microstegium* to survive and grow is enhanced by the presence of a litter layer on the soil surface. Moreover, the effects of different

kinds of litter vary with the type of soil in which this plant is growing. The effects on different aspects of growth also varied with litter and soil type. Contrary to expectation, neither survival nor growth was uniformly enhanced by the presence of *Microstegium* litter, and oak litter proved to be no more inhibiting to growth than the maple litter. Soil conditions of the maple soil that might otherwise promote growth (higher moisture, organic matter, and nitrate levels) did not enhance growth more so than the oak soil. In general, plants were taller and had greater biomass in the oak soil type. The overall effects of the leaf litter treatments in this greenhouse experiment indicate that *Microstegium* is more successful when grown under a litter layer. The hypotheses that *Microstegium* would be more successful when grown in maple soil and when grown in the absence of litter were not supported in this study.

In a previous study, I have shown that *Microstegium* produces higher total biomass and stem height after treatment with high NH_4^+-N nutrient solution (see Chapter 2). The initial soil samples collected from the oak forest indicated that this soil was higher in NH_4^+-N than the maple soil. Apparent preference for NH_4^+-N might have contributed to the overall success of *Microstegium* in the oak soil. As observed in another greenhouse study, *Microstegium* is able to access sufficient resources to reproduce even when stem height is greatly reduced (see Chapter 2). If this invasive plant can produce enough total biomass to produce seed even in adverse environmental conditions, it will effectively outcompete its neighbors through reproduction.

None of the litter types served as an inhibiting physical structure in this study. There were no consistent effects of one litter type over another. The more recalcitrant litter types (oak leaves and woodchips) did not differentially inhibit plant growth

compared to *Microstegium* litter or maple leaves as expected (Figure 2). One exception was seen in the woodchip treatment. Unlike the other litter types, the woodchips in the maple soil resulted in shorter individuals on average. It is possible that the hypocotyls of each seedling were longer in this treatment, thereby affecting final allocation to the internodes of the stem (Stinchcombe and Schmitt 2006). Also, due to the weight of the woodchips, it may have taken longer for the hypocotyls to reach above the woodchip cover. Stinchcombe and Schmitt (2006) observed that seedlings of *I. capensis* grown under leaf litter emerged later and had longer hypocotyls than those grown in bare soil which resulted in shorter first internodes and fewer branches. Overall, however, the results of this study indicate that this invasive grass benefits from litter cover.

Gibson et al. (2002) suggested that *Microstegium*'s own litter that forms a thatch might serve as a negative feedback inhibiting germination of the subsequent year's seed crop. The results of this experiment do not support this hypothesis. The absence of litter for both soil types more effectively hampered growth compared to the presence of litter, including the *Microstegium* litter. Total biomass and stem height were especially greatest under *Microstegium* litter in the oak soil (Figure 2C). These findings are consistent with previous studies on graminoids or grassland species. For example, Facelli and Pickett (1991a) found that *Setaria faberii*, an annual grass produced larger individuals and twice as many seeds under oak litter than under other litter types. Donath and Eckstein (2008) found that seeds of grassland species responded more positively to litter types than woodland species.

Several other types of manipulations could be tried in future studies. The litter depth placed on the trays in this experiment was chosen to mimic conditions on the forest

floor in stands where *Microstegium* has invaded. It is possible under greater depths of litter or woody debris, *Microstegium* growth would be hindered. Other types of litters could also have been used such as *Lindera benzoin* or *Vaccinium pallidum* that are common understory shrubs that once dominated invaded forests. Fenced areas in forests where *Microstegium* completely dominates contain regenerating *Lindera* bushes that in response to alleviation of deer browse produce stump sprouts. Either due to the dense shade under these bushes or due to the chemistry of the leaf fall, *Microstegium* does not grow directly underneath these shrubs (personal observation). Further research could examine the leachate effects from different litter types. The environment of the greenhouse may also have affected the response of plant growth to litter treatments. The shallow trays used in this study may have been prone to desiccation and therefore the litter cover acted as a mulch. Further research in the field is needed to confirm the results presented here.

In a companion study I examined other factors that may enhance or inhibit the growth of *Microstegium* because soil properties alone do not influence the establishment of this plant. Other abiotic or biotic interactions with the belowground community components such as mycorrhizal mutualisms might influence *Microstegium* success. A complementary experiment designed to investigate the mycorrhizal responsiveness of *Microstegium* was conducted and is presented in Chapter 5.

Research implications

Generally, C_4 plants are expected to be more successful in high-light environments. *Microstegium,* however, is a shade-tolerant C_4 grass that is able to take advantage of sunflecks and does a majority of its growth once the forest canopy has

closed (Horton and Neufeld 1998, Morrison et al. 2007). Horton and Neufeld (1998) suggest that *Microstegium* may be an example of reverse evolution of a C_4 plant because of its success in low light environments. *Microstegium* is able to tolerate low light environments, but maintains advantageous C_4-plant traits that allow it to take advantage of sunflecks in the understory. If *Microstegium* can rapidly gain more carbon during sunflecks compared to carbon gain by native species, this C_4 characteristic will allow it to be competitively superior in low light environments (Horton and Neufeld 1998). The successful germination and survival of hundreds of individuals in some of the greenhouse trays even when covered with litter layers also indicates that the seeds do not need light exposure to germinate. As Gibson et al. (2002) suggested, the availability of moisture near the soil surface may play a more important role than light in the success of *Microstegium* establishment. Belote et al. (2003) also found that productivity and percent cover of *Microstegium* increased when precipitation increased under elevated CO_2 conditions. Since climate change models predict that the northeastern region of the US will experience increased precipitation (Hayhoe et al. 2007), invasive species that benefit from increased soil moisture may spread more rapidly.

As managers attempt to implement best management practices for invasive species control, further research concerning the importance of forest floor dynamics in the establishment of these species is needed. Whole system approaches to native biodiversity restoration should include the management of overabundant herbivores and improvements to nutrient cycling. Since the leaf litter layer plays an integral role in nutrient storage in forested systems, invaded forests with decreased litter inputs due to rapid decomposition and other disturbances may experience a decrease in ecosystem

function. Encouraging the build up of leaf litter, the regeneration of native canopy species, and re-introducing mid-canopy and shrub layers to disturbed and degraded forests will be crucial to managing exotic, invasive plants and instrumental to increasing nutrient storage.

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CHAPTER 5

Mycorrhizal Responsiveness of *Microstegium vimineum* (Trin) A. Camus, in Two

Forest Soil Types of the Northeastern US

INTRODUCTION

The impacts of invasive plants on whole ecosystems can be obvious when

changes in community structure are directly apparent (Vitousek 1990). Subtle ecosystem

effects of exotic species invasion are often undetected and understudied. Recent research

has begun to uncover some of the less conspicuous aspects of biological invasion

(Kourtev et al. 1999, Belnap and Philips 2001, Ehrenfeld et al. 2001, Klironomos 2002,

Sigüenza et al. 2006, Niu et al. 2007). Invasion-driven changes to above- and below-

ground processes and structure can create feedbacks that increase site susceptibility to

further invasion (Vitousek et al. 1987, Ehrenfeld et al. 2001, Kourtev et al. 2003,

Callaway et al. 2004, Hawkes et al. 2006). The role of mycorrhizae in either facilitating

or inhibiting invasion is not well understood. Some non-native species have been shown

to benefit from mutualisms with mycorrhizal fungi (Klironomos 2002, Sigüenza et al.

2006, Niu et al. 2007). *Microstegium vimineum*, an invasive, annual C_4 grass (hereafter

referred to by generic name) represents an ecologically novel life form as it spreads in

forests throughout the eastern US. Most often *Microstegium* is observed invading young

successional or disturbed forests dominated with woody species that associate with

arbuscular mycorrhizal fungi. Dominance of *Microstegium* in forests with canopy species

that form ectomycorrhizal fungal relationships is less common. Soil communities

dominated by ectomycorrhizal fungi may be more resistant to invasion by a non-native

weed that relies on arbuscular mycorrhizal associations for success. The purpose of this study was to test whether the ability of *Microstegium* to develop symbioses that enhance growth is greater when growing in soils from communities dominated by vesicular arbuscular mycorrhizal species than in soils from communities dominated by ectomycorrhizal species. I aim to discover mycorrhizal responsiveness and test plant success of *Microstegium* in two soil types with dissimilar mycorrhizal communities. Therefore, I hypothesized that forest soils with ectomycorrhizal-dominant communities may be less likely to enhance *Microstegium* growth if in fact this species is greatly responsive to arbuscular mycorrhizal colonization.

Mutualistic relationships linking below- and above-ground community structure may play a particularly important role in biological invasions. When a non-native species becomes a superior competitor in a system, the role mycorrhizal relationships play in the interactions among invasive and native species is not well understood (Marler et al. 1999). Arbuscular mycorrhizal fungi (AMF) have been shown to contribute to a decrease in species diversity if they favor a superior competitor. In some cases AMF associations increase invasion success by enhancing the competitive ability of non-native species after establishment (Marler et al. 1999, Klironomos 2002, Bray et al. 2003, Callaway et al. 2003, Carey et al. 2004, Sigüenza et al. 2006). The role that mycorrhizal symbiosis plays in the biotic resistance of a system to invasion has been recently reviewed in Levine et al. (2004). In the experiments they reviewed, mycorrhizal fungi did not significantly enhance invasive seedling performance. This suggests that in some systems mycorrhizae may contribute to the biotic resistance of a site.

Little is known about changes that may occur in the mycorrhizal fungal community when ruderal or early successional species invade soils largely inhabited by late successional canopy species associated with ectomycorrhizal fungi (EMF) (Rowe et al. 2007). Soil type, plant species, growth stage, and the successional stage of the plant community influence the type of mycorrhizal fungi that will dominate in the soil community (Allen 1991). Early successional species and weedy species tend to associate with different mycorrhizal fungi than late successional species (Allen 1991). Forests of late successional canopy species such as *Quercus* sp., *Carya* sp., and *Vaccinium* sp. that grow successfully on acidic, well-drained, nutrient-limited soils and contain accumulations of surface litter are dominated by ectomycorrhizal and ericoid mycorrhizal fungal communities. Forests of canopy species that compete well on less acidic, mesic to less well-drained, nutrient-rich mineral soils such as *Acer* sp., *Liquidambar* sp., and *Viburnum* sp. are more likely to contain a diverse arbuscular mycorrhizal fungi community (Allen 1991, Miller and Jastrow 1994, Smith and Read 1997).

Within the past 20 years, *Microstegium* has been successfully invading intact forest stands (Kourtev et al. 1998). As it invades, *Microstegium* may encounter mycorrhizal communities associated with late-successional plant species (e.g. *Viburnum* sp, *Vaccinium* sp., *Quercus* sp.) that associate with AMF, ericoid, and/or ectomycorrhizal communities. Studies of the below-ground biology of a few invasive plants have examined their responsiveness to AMF colonization and their effects on interspecific competition or ecosystem processes (Rowe et al. 2007). Most of these studies have focused on grassland species and whole soil communities instead of specifically addressing the AMF community or whether roots of these invaders are actually colonized

by mycorrhizal fungi. Little is understood of the changes in soil biology, specifically in the mycorrhizal communities that may occur in forested ecosystems when soils are disturbed and new species not native or naïve to these respective soil communities become dominant. The mycorrhizal responsiveness, or the response of root cells to fungal colonization, of many plant species, especially invasives has not been studied (Rowe et al. 2007). To date no studies have examined whether *Microstegium*, an exotic, C_4 annual, invasive grass, dominating the forest understory as an ecologically novel life form is responsive to mycorrhizal colonization (USDA 2008). Phospholipid fatty acid markers for AMF have been found in rhizosphere soil under *Microstegium* (Kourtev et al. 2003). Root colonization by fungi has been observed in *Microstegium* inhabiting old growth forests in New Jersey (M. Aronson, personal communication). If *Microstegium*, a weedy species likely to only associate with AMF, if at all, encounters a soil community dominated by ectomycorrhizal fungi, establishment may be inhibited. Examining the specific mycorrhizal relationships of new, highly invasive, non-native plants is one way to characterize and understand potential competitive interactions and community resistance to invasion (Belnap and Phillips 2001). The goal of this study was to determine whether *Microstegium vimineum* forms mycorrhizal relationships and whether different soil types containing different mycorrhizal communities differentially affect its growth response. If so, it may help to explain why in some cases this problematic invader commonly forms monospecific lawns across entire forest understories dominated with AMF-associated vegetation and in other cases it has a patchy distribution in communities with ecto- or ericoid mycorrhizal-associated species.

Plant performance has been shown to differentiate in the presence of AMF communities of distinct composition harvested from dissimilar natural habitats (Moora et al. 2004). If plant response changes due to a difference in the AMF community composition, it follows that plant establishment and growth may be affected when roots are introduced to inoculum containing completely dissimilar dominant mycorrhizal community types (*e.g.* ectomycorrhizal vs. arbuscular mycorrhizal inoculum). To investigate whether different mycorrhizal fungal communities contribute to differential establishment and growth of *Microstegium*, I utilized inoculum from two sites with distinct vegetation and soil characteristics in a greenhouse seedling-establishment experiment of factorial design. The two soil types, one with a dominant ectomycorrhizal fungal community and one with a dominant arbuscular mycorrhizal community represent vegetation communities into which *Microstegium* is known to invade. The soil collected for this experiment had not yet been invaded by this plant, however, and is therefore "naïve" to specific plant host effects. The ectomycorrhizal-dominated community is represented by soil collected from an oak/hickory forest community on rocky, well-drained soils (referred to as oak soil). The arbuscular mycorrhizal-dominated community is represented by soil collected from a red maple forest type on mesic, less well-drained soils (referred to as maple soil).

I hypothesized that if *Microstegium* is responsive or receptive to mycorrhizal fungi, the quantity of colonized roots and the growth response of *Microstegium* would be more positive in the maple soil than in the oak soil. To test whether *Microstegium* growth depends on AMF colonization, I treated the soil in two ways to reduce inoculum. I used a fungicide known to reduce AMF. To control for other community interactions that might

influence growth once AMF were removed, I also sterilized the soil. I measured plant growth with and without fungal inoculum. I predicted that plants grown in greenhouse trays containing the maple soil applied with fungicide and trays with soil subjected to sterilization would experience reduced growth compared to plants grown in the oak soil under similar treatments.

METHODS

A greenhouse study was conducted to test the role of soil type in the establishment and effectiveness of mycorrhizal infection in *Microstegium*. Using a full-factorial design, the effects of inoculum (three levels: normal soil, steam-sterilized soil, and fungicide-treated soil) and soil source (two levels: oak forest soil and maple forest soil) were assayed by measuring *Microstegium* growth and root colonization.

Soil Collection, Analysis, and Treatments

Field soil was collected from two forest types: an oak/hickory-dominated forest (*Quercus* spp., *Carya* spp.) with *Vaccinium pallidum* and *Gaylussacia baccata* as the main understory species (from Allamuchy Mountain State Park in Warren County, NJ) ('oak soil' below) and from a red maple-dominated forest (*Acer rubrum*) primarily with *Viburnum dentatum*, *Lindera benzoin*, and *Podophyllum peltatum* dominating the understory (from Helyar Woods, Middlesex County, NJ) ('maple soil' below). Soils from the oak forest are in the Rockaway series and are coarse-loamy mesic Typic Fragiudults derived from glacial till from Pre-Cambrian schists. Soils from the maple forest are in the Fallsington series and are fine-loamy mesic Typic Endoaquult, derived from Cretaceous Coastal Plain sediments (USDA-NRCS 2008). Neither site was invaded by *Microstegium* at the time of soil collection. Soil was sieved in the field on a 0.5 cm sieve to eliminate

large debris and homogenized. To compare soil properties between the forest types prior to the experiment, seven samples from each source were taken for soil property analysis, including percent moisture content, percent organic matter (loss-on-ignition), pH, and 2M KCl extractions (4:1 KCl to soil ratio) for inorganic nitrogen. KCl extracts were frozen until analyzed colorimetrically on a Lachat QuikChem Flow Injection Analyzer 8000 series (Lachat Instruments, Hach Co., Loveland, CO) for NO_3^--N and NH_4^+-N concentrations (QuikChem 1986, 1987).

Thirty greenhouse trays (16.7cm x 12.3cm each) were filled with each soil. Each tray was filled with approximately 325g (0.325kg) of soil to a depth of 3cm. Soil in 10 of the 30 trays for each soil type containing mycorrhizal inoculum served as the unmodified soil replicates and did not receive any soil treatment. Another set of 10 trays from both soil types was each treated with 50ml of Benomyl®, a commonly used fungicide in aqueous suspension (2.5g powder per L of water) (Pedersen and Sylvia 1997, Callaway et al. 2003). The first treatment of fungicide was applied to the soil surface before seeds were added so that soil was drenched with solution. The second application occurred 14 days later after seeds had germinated. The last 10 trays received sterilized soil. A subset of soil from both forests for this treatment was autoclaved 4 times for 60 minutes with 2-3 days between sterilization each at 121°C before being added to the trays. All trays received approximately 0.5 g (*c*. 600 seeds) of *Microstegium* seed collected the previous year from dried litter. Seed was placed on the soil surface, and all trays in each treatment were gently watered twice a week to prevent seed from splashing out of the tray.

The two soil manipulations using the fungicide and sterilization methods were designed to limit the AMF inoculum present in the field soils. The fungicide targets AMF

but does not necessarily reduce other types of fungi. Once AMF are suppressed, other fungal or microbial components of the soil community can potentially affect plant growth. To avoid this bias, a sterilization treatment was used to eliminate all microbial components of the soil.

At the end of the experiment biomass was harvested from each tray. Five trays from each treatment were randomly chosen from which to take one soil sample to analyze for percent moisture content, percent organic matter (loss-on-ignition), pH, and 2M KCl extractions for NO_3^--N and NH_4^+-N using the technique described above. Soil samples collected before the experiment were compared with soil properties measured after the experiment from the untreated soil only to determine whether *Microstegium* itself had an effect on soil after the growth experiment without the effect of litter.

Arbuscular mycorrhizal analysis

Before roots were processed for belowground biomass analysis, one 5cm wide x 3cm deep soil core containing belowground biomass was randomly collected from each tray to sample for mycorrhizal root infection. Roots were thoroughly cleaned for mycorrhizal analysis. Roots were stored in 50% ethanol until they were processed. Root pieces were cleared for 10 minutes at 90^oC in 10% KOH, and stained using trypan blue for 10 minutes at 100^oC (Koske and Gemma 1989). The root fragments were stored in acidified glycerol until they were cut into 1cm lengths and mounted on slides with Aquamount® mounting media.

Root pieces were observed for arbuscular mycorrhizal root infection using a modified magnified intersection method (McGonigle et al. 1990). One-cm root pieces were aligned perpendicular to the long axis of the slide and observed at x200 with a

differential interference contrast scanning microscope (Nikon Eclipse 80i). An ocular grid defined the field of view which was moved using the stage graticule to make 15 complete passes across each piece of root perpendicular to its long axis. The distance between passes (the width of the ocular grid) was constant for all samples. The position where the edge of the grid crossed the root was taken as the point of intersection. No pass of the grid was counted within four grid squares from either end of the root piece. Due to the thin root tissue of *Microstegium*, the plane of focus only had to be slightly adjusted to move completely through the root piece. For each intersection with the edge of the ocular grid, presence/absence of AMF was recorded, and then if present, the type of fungal structure (arbuscule, vesicle, hyphae only) was recorded. Once the presence of infection was noted the edge of the root piece (top vs. bottom) was randomly chosen from where to begin counting the number of intersections. A minimum of 135 intersections for each sample were scanned. A total of 504cm of root was examined and used in the final data analysis. A minimum of 9cm of root from each sample was observed for infection.

Arbuscular, vesicular, and hyphal colonization were calculated according to McGonigle et al. (1990). The width of each root piece based on the ocular grid (1 square = 50μm) and the presence/absence of root hairs were recorded as well. A total of 220 and 284 root pieces were examined from the maple soil and the oak soil, respectively. The difference in the quantity of root pieces between soil types was due to the fact that fewer treatment replicates in the sterilized treatment of the maple soil had any surviving plants. Very little belowground biomass was harvested from this treatment as only 6 of the 10 replicates had any germination, and very few individuals germinated within those 6 trays. Only 4 of 10 replicates were viewed for mycorrhizal infection from this treatment.

Growth Measurements

After the first 4 weeks of the experiment, the number of seeds that had germinated was counted for all treatments to determine whether something inherent about the soil types with and without inoculum affected germination. At the end of the experiment (12 weeks), 10 individuals were chosen at random from each tray (total of 100 individuals per treatment) for stem height measurements. The total number of individuals per tray surviving at harvest (hereafter termed "survival") was counted, percent survival was calculated based on original input of 600 seeds per tray, and aboveground biomass was harvested before plants set seed. Aboveground biomass was oven-dried for 48h at 75°C and weighed. The belowground biomass remaining in the trays after samples were removed for mycorrhizal analysis was pooled for each tray and was rinsed, dried, cleaned using forceps, dried again for 48h at 75°C, and weighed. After all necessary mycorrhizal slides were created, the remaining unused root biomass collected for mycorrhizal analysis was dried as described above and pooled with the initial root biomass harvest. Root biomass used for slides was negligible and was therefore not included in the final belowground biomass weight for each tray.

Data Analysis

To first test whether the soil manipulations made a difference in mycorrhizal colonization regardless of soil type, a one-way analysis of variance (ANOVA) test was used. Then, to separate differences in percent root colonization, percent arbuscular colonization (%AC), percent hyphal colonization (%HC), and all growth variables between soil types, separate two-factor ANOVAs were used with soil type and soil manipulation as the main effects. The values for percent arbuscular colonization (%AC),

and percent hyphal colonization (%HC) were arcsine square-root transformed to improve

normality (Quinn and Keough 2002). Since the results in the ANOVAs using the

transformed survival data and percent survival did not differ, only results based on

percent survival are presented here. Post hoc multiple comparisons of least squares means

with Tukey tests were used to test comparisons among soil types, soil treatments, and

their interaction (Quinn and Keough 2002). To determine the effect that survival might

have on total biomass and average stem height an analysis of covariance (ANCOVA)

tested the differences between factor level means that were adjusted for survival as the

covariate.

A one-way ANOVA was used with soil type as the main effect to determine if

initial differences existed between the soil properties of the two soil types before

treatments were applied. A second one-way ANOVA was used to compare the soil

properties between the two soil types in the trays of untreated soil at the termination of

the experiment to determine soil differences without effects of either the fungicide or

sterilization. Then, fixed effects two-factor ANOVAs were performed to determine the

difference in soil properties among the soil treatments (main effect) for each soil type

(main effect) and their interaction. Again, Tukey tests were applied to the multiple

comparisons of least squares means. The data for percent organic matter, NO_3^--N and

NH_4^+-N were log transformed to improve normality (Quinn and Keough 2002). All

analyses were run using PROC GLM in SAS v.9.1 (SAS Institute Inc. 2003).

RESULTS

Mycorrhizal Colonization of Microstegium Roots

Inspection of the stained root segments clearly showed that *Microstegium* supported AMF colonization, and that the soil treatments had a significant effect on root colonization (p<0.0001, F=21.02). The overall average percent root length colonized for *Microstegium* was 73% (±SE 6%) in the untreated soil across both soil types. Figure 1 shows examples of root cells from *Microstegium* grown in each soil type.
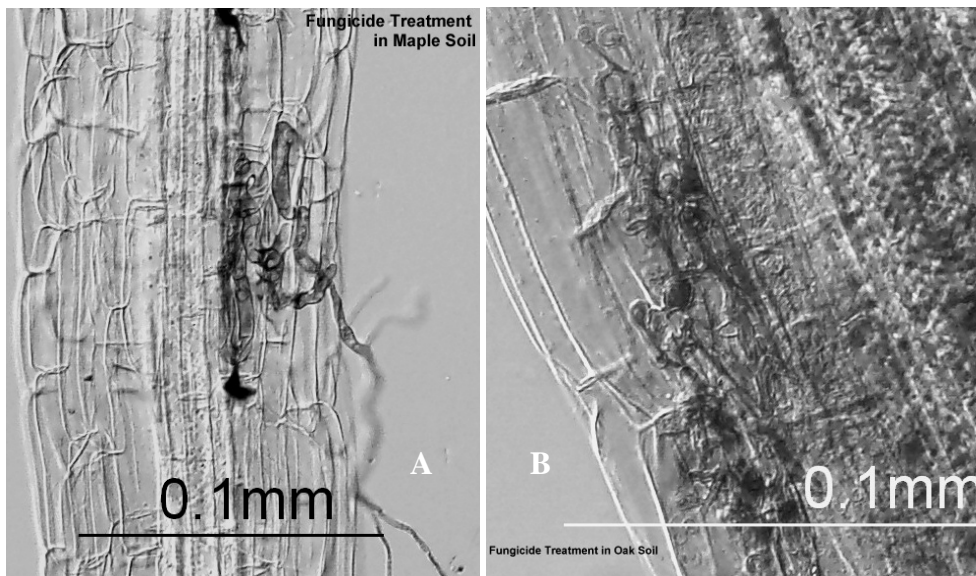
Figure 1. A. Mycorrhizal colonization in the maple soil. B. Mycorrhizal colonization in the oak soil.

The fungicide treatment with Benomyl® reduced percent colonization ($\bar{x}$ = 35 ± 7%), but not as effectively as soil sterilization (13 ± 6 % colonization) (Figure 2). When analyzed separately, the percent root length colonization in each soil type depended on the soil treatments (Figure 3, Table 1). *Microstegium* roots grown in the maple soil containing a dominant AMF community had significantly greater percent colonization than the oak soil ($\bar{x}$ = 93%, $\bar{x}$ = 54 %, respectively) as predicted (Figure 3). The effectiveness of the fungicide treatment depended on the soil type as well (Table 1). The fungicide treatment

was effective at limiting root colonization for roots in the maple soil, but seemed to have no effect at all in limiting colonization in the oak soil (Figure 3). Sterilization of soil was the most effective treatment for limiting colonization in roots for both soils.

In both soil types, only arbuscules and hyphae were observed. No vesicles were observed except for within 1 root piece grown in the maple soil and harvested from a control treatment tray. The percent hyphal colonization (%HC) and arbuscular colonization (%AC) depended on the soil type and the treatments (Table 1). A similar pattern appeared for %AC and %HC as for percent total root length colonization in the different soil types and soil treatments. Greater %HC and %AC was observed in the roots grown in the maple soil due to greater overall percent colonization in this soil type. Again, the effectiveness of the fungicide was limited in the oak soil and sterilization greatly decreased both %HC and %AC in both soil types.

The average root width of each root piece examined for colonization did not affect whether the root was colonized by mycorrhizae or not. The presence/absence of fine root hairs was not significantly different (p=0.09, $\chi^2 = 2.93$) for the number of root pieces colonized versus not colonized by mycorrhizae.

Microstegium Survival and Growth Response to Mycorrhizal Colonization

Contrary to my prediction, *Microstegium* showed greater initial (4 weeks) and final survival (12 weeks) in the oak soil than in the maple soil (Figure 4, Table 2A, B). After 4 weeks of the experiment, *Microstegium* seedlings survived with and without inoculum (Figure 4A). The soil treatments also affected survival more in the oak soil than in the maple soil, as the fungicide treatment had little effect on survival in the maple soil,

but did in the oak soil. The sterilization treatment reduced survival throughout the experiment in both soils, but more strongly in the maple soil.

Initial survival was highest in the oak soil across all treatments particularly in the control treatment. The highest average number of seedlings per tray, 255.6 ($\pm$1 SE = 10.9), was counted in the control treatment of the oak soil and was significantly different than the number in each of the reduced-inoculum treatments. A similar pattern was exhibited in the maple soil although was not significant between the control and fungicided treatments (Figure 4A). Some of the replicates of the sterilized soil treatment containing maple soil did not germinate. Only 6 out of the 10 trays contained any individuals (between 1-3 plants) most likely due to physical changes in the soil from repeated high heat ($121^{o}C$) autoclaving (Endlweber and Scheu 2006). The findings for percent survival calculated at the end of the experiment (12 weeks) mirror the results observed for germination (Figure 4B).

Average stem height differed from the pattern for initial and final survival (Figure 5A). The response in height of plants grown in each soil treatment depended on soil type (Table 2C). The maple soil trays applied with the fungicide produced the tallest plants on average. Stem height was not different for individuals among the soil treatments in the oak soil, however. Total biomass results reflect the pattern observed in that of stem height. Again, plants harvested from the maple soil trays that received the fungicide were significantly larger on average than those in the other treatments (Figure 5B). Both shoot and root biomass increased in the fungicided soil compared to the other treatments in both soil types. Overall growth was greatest for plants raised in the oak soil even though roots cultivated in the maple soil had the highest percent colonization of total root length

(see above). In the oak soil, the fungicide treatment did not affect growth (height or biomass). In contrast, in the maple soil, the large decrease in root colonization (Figure 3) resulted in increased height and biomass (Figure 5).

Certain growth responses may be functions of survival since the number of survivors at harvest would affect competition among individuals. I tested this possibility using analysis of covariance to discover whether significant differences observed in two-factor ANOVAs were maintained once factor level means for total biomass and average stem height were adjusted for survival (square-root transformed) as the covariate (Table 3). Survival acted as a covariate for both total biomass and average stem height for soil treatment effects but not for soil type effects on these growth variables. The relationship between survival and both total biomass and stem height varied with soil treatment. While total biomass and stem height did vary with stem density, reflecting intraspecific competitive interactions, these relationships did not alter the overall response to the soil treatments.

Soil property analysis

The pre-experiment soil from the maple forest contained higher percent moisture and NO_3^--N concentration but had lower pH and NH_4^+-N levels than soil collected from the oak forest (Table 4). At the conclusion of the experiment, only untreated soils significantly differed in pH and NO_3^--N levels (all p-values <0.05). The maple soil maintained a higher NO_3^--N concentration and lower pH and NH_4^+-N levels than the oak soil through the duration of the experiment (Table 4). These results suggest that the maple soil might have been the most hospitable environment for plant growth, but this was not reflected in the response of the plants grown in the control soils (Figures 4 and

5). The fungicide treatment in the maple soil, however, contained significantly higher percent moisture, organic matter, and pH than in the oak soil which could have influenced the *Microstegium* stem height and biomass in this treatment (Table 5, 6).

The soil properties measured at the termination of the experiment were significantly different among the treatments depending on the soil type (Table 6). For the oak soil, the untreated soil was only significantly different from the fungicided soil in percent organic matter. The untreated soil of the maple soil overall was not significantly different from the fungicided treatment for any of the soil properties (Table 6). The sterile soil for both treatments was drastically different from both the control and fungicided treatments due to the sterilization process.
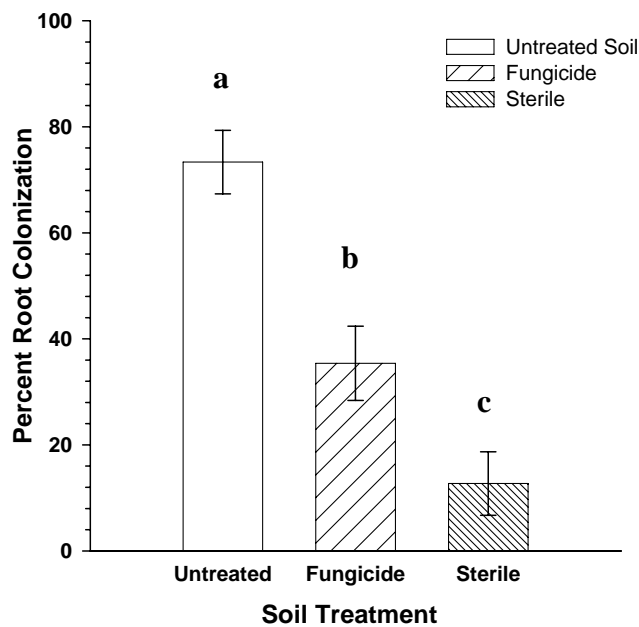
Figure 2. The average percent of the total length of roots colonized by arbuscular mycorrhizae in *Microstegium vimineum*. Bars represent average percent colonization for both soil types with error bars ±1 SE. N=20 for all treatments (except for the sterilized treatment, N=14). Open bar is the untreated soil.

Coarse hashed bar is the fungicide treatment. Fine hashed bar is the sterilized treatment. Different letters indicate significant differences between soil types and among treatments.
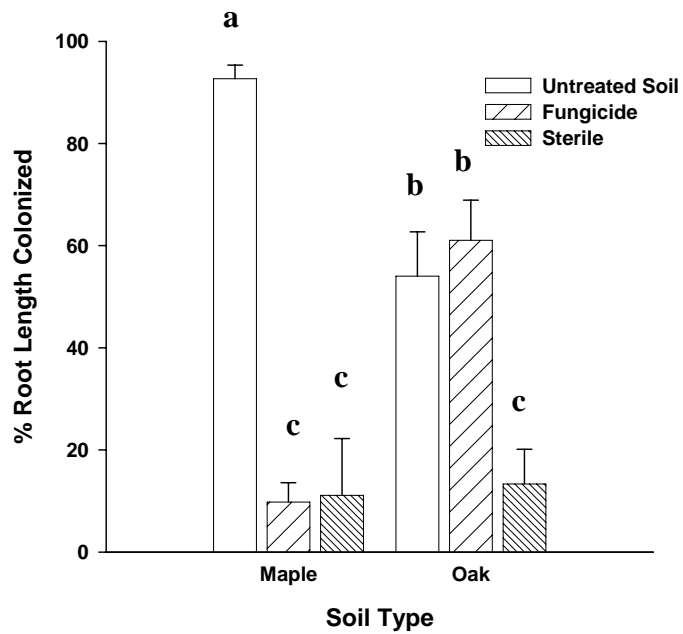
Figure 3. Average percent of the total length of root colonized by AMF. N=10 for all treatments (except for maple soil for the sterilized treatment, N=4). All error bars are ±1 SE. Open bars represent the untreated soil. Coarse hashed bar represent the fungicide treatment. Fine hashed bar represent the sterilized treatment. Different letters denote significant differences between soil types and among soil treatments.
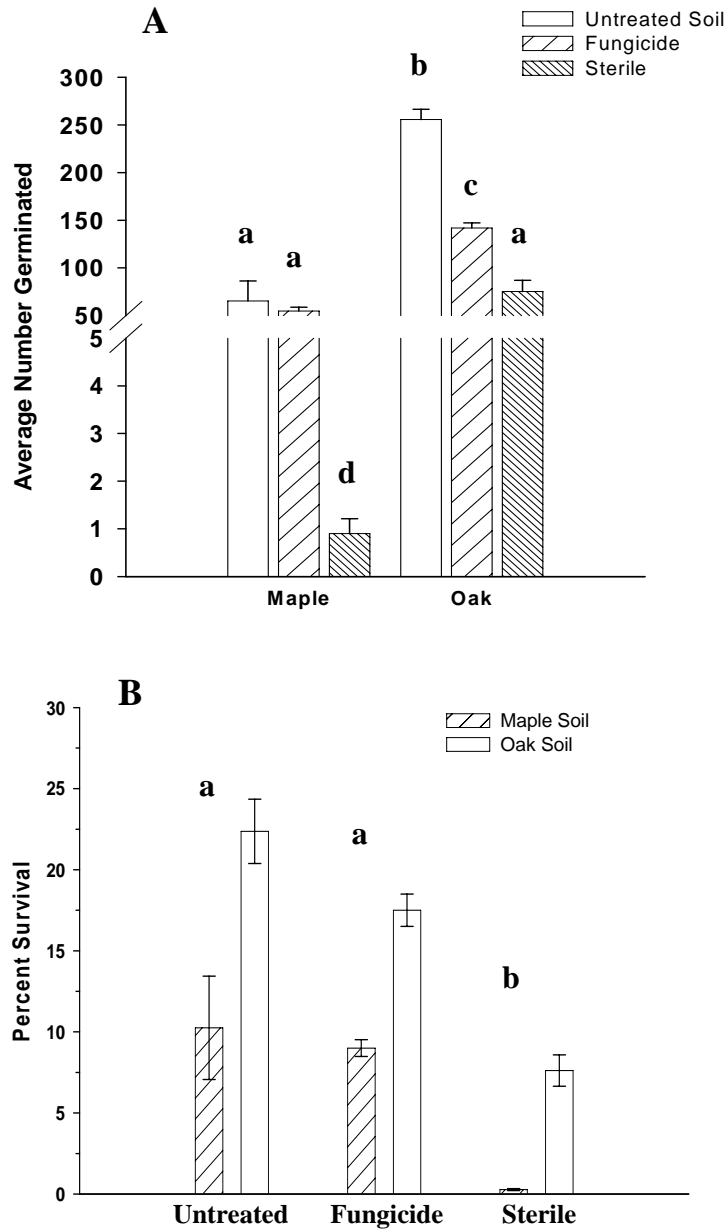
Figure 4. A. Average number of germinated *Microstegium vimineum* seedlings after 4 weeks, N=10. Different letters indicate significant differences between soil types and among treatments. Open bars represent the untreated soil. Coarse hashed bars represent the fungicide treatment. Fine hashed bars represent the sterilized treatment. B. Average percent survival at harvest, N=10 (except N=6 for the sterilized treatment of the maple soil). Different letters indicate differences among the three treatments only. Hashed bars represent maple soil, open bars represent oak soil. All error bars are ±1 SE.

Figure 5. A. Average stem height (cm) at harvest. B. Average total biomass (g dry weight tray^{-1}) with ±1 SE error bars for *Microstegium vimineum* at harvest. Hashed gray bars represent data for root biomass in maple soil. Hashed open bars are shoot biomass in maple soil. Open gray bars represent root biomass in oak soil. Open bars represent shoot biomass in oak soil. N=10 for all treatments except N=6 for the

sterilized treatment of the maple soil. Letters denote significant differences in total biomass only between soil types and among treatments.

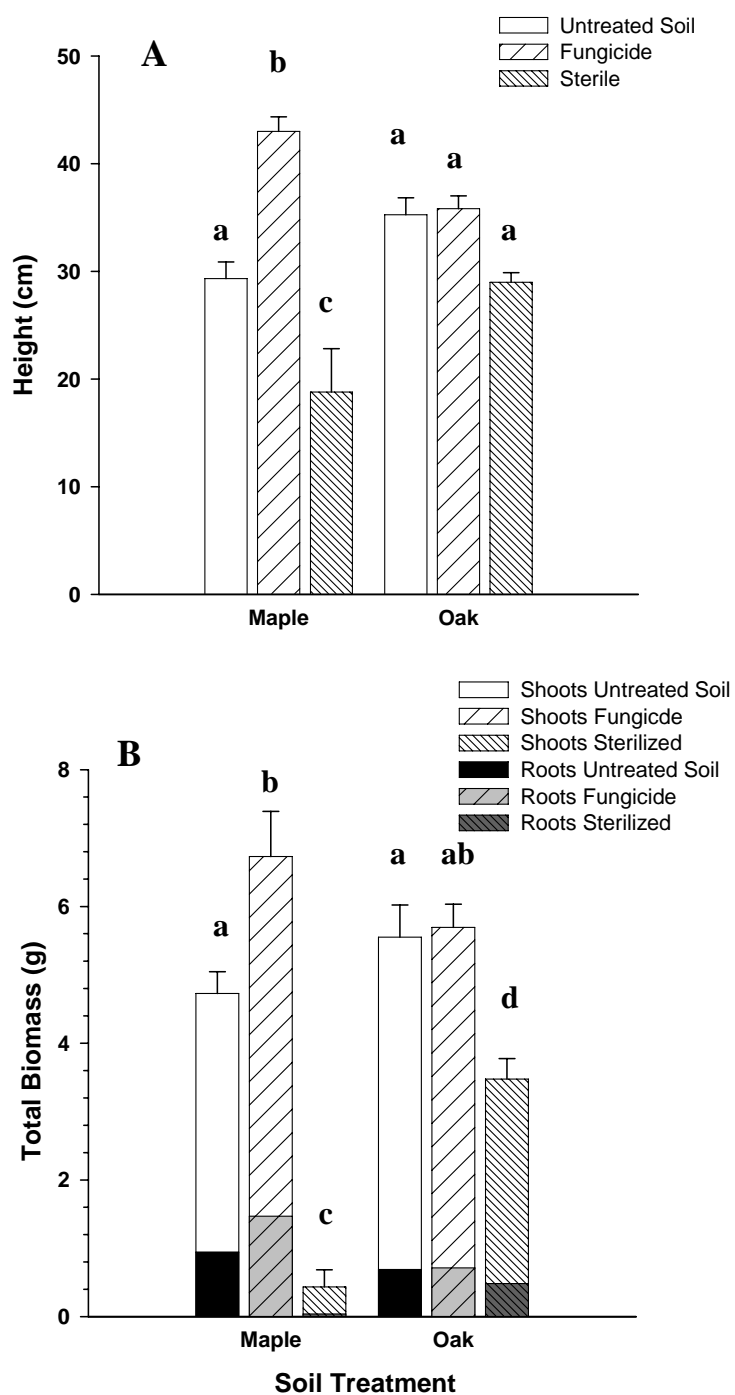Table 1. Analysis of variance and comparison of means results for average percent root length colonization, percent hyphal colonization (%HC), and percent arbuscular colonization (%AC) for *Microstegium vimineum*. Soil type indicates original source of field soil (Oak-dominated and Maple-dominated forest soils). N=10 for all treatments (except N=4 for the sterilized treatment of the maple soil), df=degrees of freedom, F=F-statistic, *p*=p-value.* p<0.05, ** p<0.01, *** p<0.0001, ns = not significant.

Mycorrhizal Colonization		df	F	*p*
A. % Total Root Length Colonization				
Soil type		1	0.86	ns
Soil treatment		2	36.74	<0.0001
Soil type X Soil treatment		2	22.66	<0.0001
Error		48		

Differences between Soil Types		Differences within Soil Type		
Treatment	**Maple vs. Oak**	**Treatment**	**Maple**	**Oak**
Control	**	Control vs. Fungicide	**	ns
Fungicide	***	Control vs. Sterile	**	**
Sterile	ns	Fungicide vs. Sterile	ns	***

B. % Hyphal Colonization		df	F	*p*
Soil type		1	0.65	ns
Soil treatment		2	9.67	0.001
Soil type X Soil treatment		2	7.86	0.003
Error		26		

Differences between Soil Types		Differences within Soil Type		
Treatment	**Maple vs. Oak**	**Treatment**	**Maple**	**Oak**
Control	ns	Control vs. Fungicide	***	ns
Fungicide	**	Control vs. Sterile	**	**
Sterile	ns	Fungicide vs. Sterile	ns	**

C. % Arbuscular Colonization		df	F	*p*
Soil type		1	0.01	ns
Soil treatment		2	15.07	<0.0001
Soil type X Soil treatment		2	11.29	0.0005
Error		26		

Differences between Soil Types		Differences within Soil Type		
Treatment	**Maple vs. Oak**	**Treatment**	**Maple**	**Oak**
Control	ns	Control vs. Fungicide	***	ns
Fungicide	**	Control vs. Sterile	**	**
Sterile	ns	Fungicide vs. Sterile	ns	**

Table 2. Analysis of variance of germination, percent survival, stem height, total biomass, shoot biomass, and root biomass. N=10 for germination data for all treatments. N=10 for all other variables for all treatments except N=6 for the sterilized treatment of the maple soil; df=degrees of freedom, F=F-statistic, *p*=p-value, ns = not significant.

Source of Variation	df	F	*p*
A. Germination			
Soil type	1	166.70	<0.0001
Soil treatment	2	60.59	<0.0001
Soil type X Soil treatment	2	16.29	<0.0001
Error	54		
B. % Survival			
Soil type	1	40.03	<0.0001
Soil treatment	2	23.45	<0.0001
Soil type X Soil treatment	2	0.97	ns
Error	50		
C. Stem Height			
Soil type	1	4.65	0.04
Soil treatment	2	40.17	<0.0001
Soil type X Soil treatment	2	14.50	<0.0001
Error	50		
D. Total Biomass			
Soil type	1	3.12	ns
Soil treatment	2	46.82	<0.0001
Soil type X Soil treatment	2	12.14	<0.0001
Error	50		
E. Aboveground Biomass			
Soil type	1	6.72	0.01
Soil treatment	2	45.36	<0.0001
Soil type X Soil treatment	2	9.96	0.0002
Error	50		
F. Belowground Biomass			
Soil type	1	2.97	ns
Soil treatment	2	18.33	<0.0001
Soil type X Soil treatment	2	9.49	0.003
Error	50		

Table 3. Analysis of covariance of stem height, total biomass with survival as a covariate; df=degrees of freedom, F=F-statistic, p=p-value, ns = not significant.

Source of Variation	df	F	p
A. Stem Height			
Soil type	1	0.81	ns
Soil treatment	2	17.89	<0.0001
Survival	1	2.86	ns
Survival X Soil type	2	10.00	0.0002
Survival X Soil treatment	1	0.01	ns
B. Total Biomass			
Soil type	1	0.26	ns
Soil treatment	2	5.37	0.008
Survival	1	8.63	0.005
Survival X Soil type	1	3.66	ns
Survival X Soil treatment	2	0.18	0.03

Table 4. Mean (±standard error) soil properties: percent moisture, percent organic matter, pH, nitrate-nitrogen (mgkg^{-1} dry soil), and ammonium-nitrogen (mgkg^{-1} dry soil) for soil samples taken from 12-week growth experiment of *Microstegium vimineum* plants. Soil Type indicates original source of field soil (Oak-dominated and Maple-dominated forest soils). The before soil treatment represents soil samples taken before experiment was begun, N=7. For all other soil treatments, N=5.

Soil Property	Soil Type	Soil Treatment	Mean (±SE)	Soil Type	Soil Treatment	Mean (±SE)
A. % Moisture	Oak	before	80.17 (8.41)	Maple	before	120.16 (12.78)
		Control	56.83 (10.76)		Control	73.75 (14.52)
		Fungicide	18.13 (3.77)		Fungicide	93.11 (5.17)
		Sterile	36.13 (10.00)		Sterile	14.97 (1.92)
B. % Organic Matter	Oak	before	61.72 (15.06)	Maple	before	61.76 (16.21)
		Control	23.13 (1.67)		Control	43.22 (1.64)
		Fungicide	16.51 (1.22)		Fungicide	53.30 (1.57)
		Sterile	20.32 (1.21)		Sterile	48.29 (4.80)
C. pH	Oak	before	4.56 (0.11)	Maple	before	4.11 (0.09)
		Control	4.67 (0.06)		Control	4.22 (0.04)
		Fungicide	4.67 (0.07)		Fungicide	4.27 (0.03)
		Sterile	4.85 (0.06)		Sterile	4.73 (0.07)
D. NO$_3$-N	Oak	before	0.69 (0.12)	Maple	before	6.73 (1.85)
		Control	0.22 (0.09)		Control	6.58 (2.11)
		Fungicide	0.46 (0.41)		Fungicide	0.77 (0.24)
		Sterile	1.96 (0.52)		Sterile	99.20 (54.52)
E. NH$_4$-N	Oak	before	51.49 (7.73)	Maple	before	30.73 (4.80)
		Control	23.49 (1.39)		Control	19.05 (3.10)
		Fungicide	22.22 (6.59)		Fungicide	17.87 (1.67)
		Sterile	62.06 (14.86)		Sterile	1129.36 (265.83)

Table 5. Analysis of variance of percent moisture, percent organic matter, pH, nitrate-nitrogen, and ammonium-nitrogen from soil samples taken from greenhouse trays after 12 weeks of *Microstegium* growth. Percent organic matter, NO_3-N, and NH_4-N data are log-transformed; df=degrees of freedom, MS=mean squares, F=F-statistic, *p*=p-value. N=5 for all variables, ns = not significant.

Source of Variation	df	F	*p*
A. % Moisture			
Soil type	1	10.63	0.003
Soil treatment	3	10.94	0.004
Soil type X Soil treatment	3	14.93	<0.0001
Error	24		
B. % Organic Matter			
Soil type	1	282.12	<0.0001
Soil treatment	4	0.52	ns
Soil type X Soil treatment	4	9.02	0.001
Error	24		
C. pH			
Soil type	1	47.68	<0.0001
Soil treatment	3	23.58	<0.0001
Soil type X Soil treatment	3	4.87	0.02
Error	24		
D. NO_3-N			
Soil type	1	13.09	0.002
Soil treatment	3	7.55	0.003
Soil type X Soil treatment	3	3.35	0.05
Error	24		
E. NH_4-N			
Soil type	1	22.82	<0.0001
Soil treatment	3	89.54	<0.0001
Soil type X Soil treatment	3	34.38	<0.0001
Error	24		

Table 6. Comparison of least squares means with Tukey tests for all soil properties by soil type. N=5 for all variables, * p<0.05, ** p<0.01, *** p<0.0001, ns = not significant.

Soil Type and Treatment Combinations

Differences between Soil Types		Differences within Soil Type		
A. % Moisture				
Treatment	**Maple vs. Oak**	**Treatment**	**Maple**	**Oak**
Control	ns	Control vs. Fungicide	ns	ns
Fungicide	***	Control vs. Sterile	**	ns
Sterile	ns	Fungicide vs. Sterile	***	ns
B. % Organic Matter				
Treatment	**Maple vs. Oak**	**Treatment**	**Maple**	**Oak**
Control	***	Control vs. Fungicide	ns	*
Fungicide	***	Control vs. Sterile	ns	ns
Sterile	***	Fungicide vs. Sterile	ns	ns
C. pH				
Treatment	**Maple vs. Oak**	**Treatment**	**Maple**	**Oak**
Control	**	Control vs. Fungicide	ns	ns
Fungicide	**	Control vs. Sterile	***	ns
Sterile	ns	Fungicide vs. Sterile	***	ns
D. NO_3-N				
Treatment	**Maple vs. Oak**	**Treatment**	**Maple**	**Oak**
Control	ns	Control vs. Fungicide	ns	ns
Fungicide	ns	Control vs. Sterile	ns	ns
Sterile	*	Fungicide vs. Sterile	**	ns
E. NH_4-N				
Treatment	**Maple vs. Oak**	**Treatment**	**Maple**	**Oak**
Control	ns	Control vs. Fungicide	ns	ns
Fungicide	ns	Control vs. Sterile	***	ns
Sterile	***	Fungicide vs. Sterile	***	*

DISCUSSION

Microstegium vimineum is colonized with AMF equally well in soils from communities dominated by AMF and in soils dominated by ectomycorrhizal communities. Furthermore, the results suggest that in these soils, mycorrhizal colonization does not contribute strongly to plant growth. Indeed, in the maple soil, growth was better with less root colonization as seen in the fungicide-treated soils.

Colonization does differ, however, depending on the kind of soil that *Microstegium* inhabits. In this case *Microstegium* had not yet invaded the forests from which either soil type was collected, yet *Microstegium* was able to successfully germinate and survive in both soils. Therefore, these results do suggest that an ectomycorrhizal-dominated soil (oak soil type) will not be any more resistant to invasion by *Microstegium* than a soil with a dominant AMF community. In fact, *Microstegium* survival was enhanced in the oak soil, although plant height and biomass were not necessarily positively affected by mycorrhizal colonization (Figure 5). These results suggest that root colonization may negatively affect plants growing in soils where nutrients and moisture are less limiting (maple soil). In AMF-dominated soils, *Microstegium* roots may be parasitized by these fungi. In ectomycorrhizal-dominated soil, *Microstegium* may benefit at least at first by AMF colonization to aid in establishment when soils are more well-drained.

The percent of the total root length colonized was twice as great in the AMF-dominated soil than in the ectomycorrhizal dominated soil, as predicted. The pattern of colonization in either soil was not very intense, however. In most of the root pieces scanned, colonization was sparsely distributed throughout the cortex cells. The percent of the colonized roots that contained arbuscules was very low (<20%) and a vesicle was only observed in one root piece. Observing colonization at only one point in the experiment may not have captured the full responsiveness of the roots. Arbuscules are known to develop and be most active a short time after initial root colonization in faster growing species. Mycorrhizal activity declines with plant age (Smith and Read 1997). Root processing for slide preparation could have also affected the arbuscules (Brundrett et al. 1996). The need for vesicles that serve as storage organs for the fungus in the roots

of an annual plant like *Microstegium*, may be reduced in short-lived species. Quantifying mycorrhizal responsiveness using percent colonization of total root length is limited due to the static nature of this measurement. Colonization is only evaluated at one point in time and is dependent on both the fungal and root growth rates (Brundrett and Kendrick 1990, Schwab et al. 1991). Destructive sampling of below-ground biomass throughout the duration of the experiment was not undertaken in this study due to the sensitivity of *Microstegium* to soil disturbance and desiccation once it is established, and due to the high density of individuals per tray in most replicates. In future below-ground studies of this species, multiple harvests of samples should be collected and analyzed for evidence of percent colonization, specifically arbuscular colonization over time and at different stages of plant development.

Percent colonization was not reflected in the growth response of the plant. This is not inconsistent with other studies that have found no relationship between percent root colonization and plant growth response (Klironomos 2003, Rowe et al. 2007). Hodge et al. (2001) report that plant growth was not correlated with colonization or uptake of nutrients by AMF. Complex root physiological dynamics involving the host root environment and the specific colonizer may create plant responses other than what is measured by above- or below-ground growth parameters.

The presence of root hairs was not correlated with mycorrhizal colonization in this study which is not entirely inconsistent with other research. In some cases plants with limited root hair development rely more heavily on AMF colonization, but it is more likely that the co-evolutionary history of AMF-plant relationships and the immediate

environment of the rhizosphere are better predictors for plant response to colonization (Allen 1991).

This greenhouse study suggests that *Microstegium* is not obligately mycorrhizal. Plants raised in the sterilized oak soil were able to successfully germinate and survive (Figure 4), although in reduced numbers, indicating that it does not rely on any component of the soil microbial community for establishment. This invasive species is known to germinate in extremely reduced soil layers such as accumulation found in cracks of pavement and in crevices of the bark of fallen trees. Only a few individuals have to survive to establish a population, since even short-stemmed plants are reproductively successful (personal observation). *Microstegium* may not be inhibited by "naïve" soil communities and may even be parasitized by AMF in certain soil types.

Several possibilities exist to explain the differences seen in the effectiveness of the fungicide used here. The AMF communities differed in composition between the two soil types used in this study. Some fungal species may be resistant to Benomyl® and therefore no significant differences were found in the oak soil between treatments. The AMF in the maple soil may have been less resistant to the fungicide. Benomyl® has been shown to inhibit nematodes and other fungi that do not form mycorrhizal relationships (Pedersen and Sylvia 1997). If those species of AMF in the oak soil were not inhibited by Benomyl® (germination and percent survival were greater in the oak soil), then indirect effects may have inhibited other fungi that compete with AMF and an increase in colonization may have been possible. Another explanation could be that if *Microstegium* is not obligately mycorrhizal, that when colonized, the fungi could negatively affect growth. The costs of colonization for the plant may have been greater or exacerbated in

greenhouse conditions (soil desiccation and nutrient run-off) as seen in the significantly greater stem height and biomass in the fungicide treatment of the maple soil (Figures 4). A third possibility is that the application rate and frequency of the fungicide was not sufficient to diminish inoculum; however, a significant decrease in percent colonization was observed in the maple soil (Figure 3).

The poor growth in the sterilized treatment of the maple soil was most likely due to over-heating that altered the physical structure of the soil and created a high nutrient flush. This was more evident in the maple soil than the oak soil properties. Possibly because the maple soil was collected from a forested wetland, the structural changes involving water holding capacity due to autoclaving were more dramatic. Sterilization at lower temperatures has been shown to be effective at eliminating microbial activity, yet not produce large structural or chemical changes in soil properties (Endlweber and Scheu 2006).

Inoculum potential was not tested for either soil in this study due to the large number of replicates needed and time constraints. Establishing the presence of specific community types and inoculum potential will be valuable for future studies testing differences between soil types. Further research is needed to identify the particular AMF community or species colonizing *Microstegium* roots through examination of spore morphology and/or utilizing molecular techniques. Complementary field experiments sampling *Microstegium* roots in different soil conditions and within diverse plant communities will also increase the body of knowledge regarding the invasion success of this species. Different fungal species may be more likely to colonize *Microstegium* roots than other native plants. Research has shown that AMF can be selective of host plants

suggesting certain plants will be colonized with a certain suite of AMF. Also, individual

plant response to colonization by one AMF species or to a suite of species is not uniform

for all plants. AMF may be more species specific than has been previously thought (van

der Heijden et al. 1998, Helgason et al. 2002). The difference in percent colonization in

the roots observed from the two soil types used here suggest that AMF communities can

be quite different and therefore plant performance will vary accordingly. Early

successional and weedy species tend to associate with different mycorrhizal fungi than

late successional species (Allen 1991). Therefore, the invasion of *Microstegium* might

increase the biomass of the AMF community or promote a shift in the mycorrhizal

community composition.

Shifts in mycorrhizal community composition can lead to differences in root

exudates, nutrient uptake, changes in carbon turnover or other forms of nutrient cycling

that ultimately lead to changes in ecosystem function (Allen 1991). Since previous

research has shown an increase in the fatty acid biomarker for AMF and a difference in

the bacterial:fungal fatty acid ratio in soils under *Microstegium*, this C_4 annual grass may

be contributing to a shift in the dominant fungal community in forest soils where it

invades. Understanding below-ground dynamics of invasive, non-native plants will

inform management practices aimed at improving biotic resistance of non-invaded

ecosystems.

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CHAPTER 6

Synthesis

This research provided several important findings about the impacts of soil

manipulations on the invasion success of two invasive, exotic plants. Several types of soil

manipulations were attempted to inhibit the growth and spread of two common exotic

invasive species, *Berberis thunbergii* (Japanese barberry) and *Microstegium vimineum*

(Japanese stiltgrass). I focused on soil manipulations as a way to restore the forest soil

community and dynamics in areas where these exotic species and many others have

almost completely replaced native understory vegetation. In fragmented, highly

suburbanized areas of New Jersey, the control of white-tailed deer has not been possible

or has not been prioritized to protect native biodiversity. Therefore, forest managers have

few alternatives to hunting except for installing acres of fencing. As the status of native

forest biodiversity decreases, more deer exclosures are being installed within or around

forested areas. I wanted to investigate whether merely installing a fence and planting

native vegetation is sufficient for restoring understory communities. In some cases exotic

species have dominated for many years resulting in persistent positive feedback cycles

between soil and plants that reinforce the survival of the exotic species (Suding et al.

2004). These feedbacks may contribute to an altered degraded state of the ecosystem that

is stable. I wanted to investigate some of the mechanisms behind feedback cycles in order

to enhance biotic resistance of the system to invasion.

The first soil manipulation I studied was nitrogen nutrition. Few studies have

investigated the response of specific invasive plants to nitrogen nutrition. Most studies

have focused on correlating increased nutrient availability with the presence of exotic

species (Stohlgren et al. 1999). Since many native plants in forested systems in the Northeast have been replaced by the two dominant exotic species described here, it is possible that exotic and native species respond differently to nitrogen availability. The response of the exotic to nitrogen additions was more plastic than the native species in this study. *Berberis* and *Microstegium* were able to survive and continue growth longer than the native species even when nitrogen levels were extremely low. Yet, the two exotic species differed in their response to the different forms of N. The total biomass of *Microstegium* was many times greater than all other species tested in the NH_4^+-N treatments, especially at the higher concentration. It did, however, produce seed in every treatment regardless of the type of N or concentration that was added. *Berberis* was equally successful in either N form, but its greatest shoot:root ratios were observed in the NH_4^+-N treatments. The native species also differed from each other in performance in each type of N addition. The performance of *Hamamelis* varied by growth variable. Plants were taller in the NH_4^+-N treatments, but survival time and total biomass were greater in the NO_3^--N treatments. The survival time for *Vaccinium* was also much shorter in the NH_4^+-N treatments, but biomass was not affected by the N additions. Interestingly, the woody species all responded similarly to the N additions in the allocation of biomass. Plants grown in the NO_3^--N treatments had higher overall root biomass whereas those grown in the NH_4^+-N treatments invested more in shoot biomass. The form of N addition affected how the woody plants allocated resources.

Second, I manipulated soils in a field study with the aim of limiting nitrogen availability. Since invasive plants have been associated with high nutrient availability, especially high nitrogen, and since previous research on *Berberis* and *Microstegium*

suggests that these species benefit from high NO_3^- availability (Ehrenfeld 2003), several soil amendments and topsoil removal were attempted to remove or immobilize nitrogen. I cleared the top 5cm of soil from study plots, added woodchips as a carbon source, added aluminum sulfate to acidify the soil, and used a nitrification inhibitor to minimize nitrifiers in the soil. Results were not consistent for all manipulations over time, but trends did emerge. Both forms of nitrogen and mineralization rates seemed to be decreased by removing topsoil, but results were not consistently significant over time. Woodchips were not very effective at immobilizing nitrogen or increasing soil C:N ratios. The aluminum sulfate used to lower pH was not consistently effective but seemed to have some effect in drier months. Finally, the nitrification inhibitor was not effective at all. The challenge for land managers interested in using soil amendments is knowing the quantity and frequency of application. The feasibility of manipulating soil in forested systems is also more difficult than grasslands or open areas because often times amendments cannot be mixed into the soils and vegetation re-growth from disturbance is a longer term process. Soil amendments may have been more effective if they had been tilled into the earth or applied in higher quantities with more frequency.

Another soil manipulation technique I measured was using leaf litter as a barrier to seedling establishment and growth of *Microstegium*. Contrary to my hypothesis, leaf litter on the soil surface served to enhance the growth of *Microstegium*. Overall growth and height was greater in many of the litter additions. Moreover, the effects of different kinds of litter vary with the type of soil in which this plant is growing. The effects on different aspects of growth also varied with litter and soil type. Contrary to expectation, neither survival nor growth was uniformly enhanced by the presence of *Microstegium*

litter, and oak litter proved to be no more inhibiting to growth than the maple litter. Soil conditions of the maple soil that might otherwise promote growth (higher moisture, organic matter, and nitrate levels) did not enhance growth more so than the oak soil. Overall plants were taller and had greater biomass in the oak soil type.

The last soil manipulation I investigated tested whether *Microstegium* responds to mycorrhizal colonization and whether *Microstegium* relies on mycorrhizae for growth. In a greenhouse study I grew *Microstegium* in field soil with and without arbuscular mycorrhizae. I used a fungicide and a sterilization treatment on both soil types to minimize mycorrhizal inoculum. The responsiveness of *Microstegium* roots to colonization by arbuscular mycorrhizae was previously unknown. I quantified the total percent colonization of arbuscular mycorrhizae (AMF) and its components in *Microstegium* roots in two different soils and measured growth response. I discovered that in the greenhouse *Microstegium* is not reliant on mycorrhizal colonization as it was successful in the soil treatments that reduced AMF inoculum. *Microstegium* was able to successfully germinate and survive in both soils. Growth response was different depending on the soil type, however. Plants were more successful when grown in soil taken from oak forests with a dominant ectomycorrhizal community which was unexpected. I had predicted that growth would be more enhanced in the soil collected from a maple forest dominated by AMF. In the maple soil, growth was better with less root colonization as seen in the fungicide-treated soils. Therefore, these results suggest that an ectomycorrhizal-dominated soil (oak soil type) will not be any more resistant to invasion by *Microstegium* than a soil with a dominant AMF community.

The soil manipulations tested in this research had limited success but the results provide useful insight into enhancing biotic resistance to invasion. Soil properties in forested systems are incredibly complex and resistant to change. Manipulation of soil nutrient availability in addition to the re-introduction of native species will be necessary to enhance resident community biotic resistance. The reduction of native understory vegetation has provided invader species with an opportunity to compete and capture under-utilized resources. Disturbances in the forest understory due to human activity, intensive deer browse, or other causes that increase the availability of under-utilized resources may provide suitable invasion sites for exotic species that can capitalize on available resources more efficiently that the resident plant community (Marshall and Buckley 2008). More field studies are needed to increase our understanding of how different invasive species respond to resource availability and competition. Identifying the susceptibility of a site to further invasion will help land managers with limited human and financial resources prioritize restoration efforts, and restoration priorities can be determined. Multi-faceted techniques that improve biotic resistant such as limiting nutrient availability while increasing the abundance and diversity of native species and functional groups will allow degraded systems to be more robust to newly establishing exotic species.

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Curriculum Vitae

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