The effect of plant-soil feedback and competition on the invasion of New Hampshire thickets by non-native shrubs

Kristina Vagos
University of New Hampshire, Durham

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THE EFFECT OF PLANT-SOIL FEEDBACK AND COMPETITION ON THE INVASION OF NEW HAMPSHIRE THICKETS BY NON-NATIVE SHRUBS

BY

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B.S. in Biology, Boston College, 1998

THESIS

Submitted to the University of New Hampshire in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Natural Resources

May, 2009
This thesis has been examined and approved.

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Date
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ABSTRACT

THE EFFECT OF PLANT-SOIL FEEDBACK AND COMPETITION ON THE INVASION OF NEW HAMPSHIRE THICKETS BY NON-NATIVE SHRUBS

by

Kristina Vagos

University of New Hampshire, May 2009

Early-successional habitat is disappearing throughout the northeastern United States. Much of the remaining habitat is being invaded by non-native invasive shrubs suspected of altering native shrubland quality. To assess whether soil feedback and competition facilitate invasion, three native and three invasive shrub species were used in two greenhouse experiments. Soil feedback was examined by growing each species in soil cultured by the same species and those cultured by other species, in both tilled and non-tilled soil. Soil microbial communities were assessed using PLFA. Soil feedback effects were species specific and likely do not facilitate invasion. Additionally, cultured soil communities were different among species but were not differentiated by the culturing plant's invasive or native status. A simple pair-wise experiment showed that invasive shrubs were better competitors than natives, suggesting that competition may facilitate invasion. Differences in annual growth between native and invasive shrubs were assessed in a small field study.
GENERAL INTRODUCTION
Early-successional habitat, otherwise known as “thicket” or “shrubland” habitat, is rapidly disappearing in New England (Litvaitis, 2003). Characterized by native shrubs and saplings, the dense vegetative structure and abundant herbaceous layer of thickets (Howard and Lee, 2002) provide ideal habitat for many species (Litvaitis, 1993). In fact, due to the unique combination of thick cover and available food resources, certain species depend solely on early-successional habitat for survival. With the disappearance of this habitat, thicket-dependent species such as the New England cottontail (*Sylvilagus transitionalis*), a candidate for federal endangered designation, and birds such as the chestnut-sided warbler (*Dendroica pensylvanica*) and the least flycatcher (*Empidonax minimus*) are also in decline (Hill and Hagan, 1991; Litvaitis, 1993).

The creation and disappearance of early-successional habitat in New England can be directly linked to the land-use history of the region (Litvaitis, 1993). From the time of colonization until the mid-1800s, New England was heavily farmed and many of the forests were cleared for grazing, cropping, timber, and fuel (Litvaitis, 1993; Howard and Lee, 2002). Yet, during the latter portion of the 19th century, many New England farms were abandoned; this paved the way for an increase in shrubland habitat, as forests grew in place of fields and pasturelands (Litvaitis, 1993). Since then, these forests have matured; New Hampshire forests, which were reduced by about 48% after colonization, now cover approximately 87% of the state (Litvaitis, 1993). In addition, many of the recently neglected fields that would have become early-successional forests have been developed into housing complexes and shopping centers for the ever increasing and sprawling population in New England (Lewis, 1972; Census, 2000). Development, forest
maturation, and fire suppression are the leading causes of the decline of thicket habitat in New England.

Despite all of the factors causing the decline of early-successional habitat, there are still shrublands in existence. However, much of the remaining early-successional areas are being invaded by non-native invasive shrub species such as multiflora rose (*Rosa multiflora* Thunb.), bush honeysuckle (*Lonicera morrowii* A. Gray), and glossy buckthorn (*Frangula alnus* Mill.; Johnson et al., 2006; Searcy et al., 2006; Von Holle and Motzkin, 2007). These invasive plants thrive in disturbed areas and may alter the ecology of these areas (Fagan and Peart, 2003; Frappier et al., 2003; Frappier et al., 2004; Johnson et al. 2006). Non-native bush honeysuckle (*Lonicera tatarica* L.), for example, has been found to reduce the abundance and diversity of herbaceous vegetation and decrease native tree regeneration (Woods, 1993). Furthermore, Kourtev et al. (1998) and Ehrenfeld et al. (2001) found that Japanese barberry (*Berberis thunbergii* DC.) significantly elevates soil pH. Invasive plants can also impact native ecosystems by reducing native diversity through resource competition (Woods, 1993; Brown and Mitchell, 2000; Frappier et al., 2003; Frappier et al., 2004) and by changing the availability of resources such as nitrogen which may enable further invasions (Vitousek et al., 1987; Vitousek and Walker, 1989).

Why is it that these plant species are able to invade and establish themselves in native ecosystems? The answer to this question likely involves a myriad of associated factors that contribute to a non-native plant's ability to dominate a community and displace its
native counterparts while altering the quality of the habitat. It is this ability which makes a non-native species ‘invasive’. Examples of these factors include species’ life history traits (Frappier and Eckert, 2003), landscape-level factors such as habitat fragmentation (Searcy et al., 2006), local factors such as land-use history (Von Holle and Motzkin, 2007), site history characteristics such as physical soil properties (Johnson et al., 2006), and others such as genetic variation (Lavergne, 2007) and a reduction in natural enemies (Klironomos, 2002; Wolfe, 2002; Mitchell and Power, 2003). For the purpose of managing and restoring native early-successional habitat in New England, it is essential that we identify and understand the factors that facilitate invasion. The two greenhouse experiments conducted in this study assessed whether soil feedback and competition influence invasion in shrublands, Chapter 1 and Chapter 2 respectively. To complement the greenhouse experiment on competition, a small field study was conducted to assess differences in invasive and native shrub annual growth (Chapter 3).

In Chapter 1, soil feedback, the net effect of a cultured soil community on a plant species’ growth, was assessed as a factor that may facilitate shrub invasion of early-successional areas in New England. Six shrub species, three natives and three non-native invasives, were grown in soil cultured by their own species (home culture) and soil cultured by the other shrub species (foreign culture). The direction of soil feedback was measured as the difference between the effects of the home culture and foreign culture on the shrub’s growth, and the magnitude of soil feedback was calculated as a percent change in growth between the two types of soil cultures (home vs. foreign). In addition, the effect of land-use history on soil feedback was tested by conducting the feedback study in two different
types of field soil, tilled agricultural and non-tilled forest soil. Furthermore, phospholipid fatty acid analysis was used to determine gross differences in all species’ soil community composition, as well as total microbial biomass.

The greenhouse study detailed in Chapter 2 focused on resource competition between invasive and native shrub species to assess whether competition could potentially play a role in shrubland invasion. The same six shrub species, three natives and three invasives, were used in this study as in the soil feedback experiment. The objectives of the study were 1) to evaluate whether invasive species are better competitors than their native counterparts, 2) to examine whether native species are more negatively affected by their neighbors than invasive shrubs, 3) to determine if there is a difference between invasive and native species in allocation of resources by measuring root to shoot ratios.

Chapter 3 describes a small field study conducted to evaluate differences in the annual growth of native and invasive shrubs. The objectives of this study were to compare shoot length (cm) and dry biomass (g) of annual extension growth of two invasive and four native shrub species and to measure the density of the different species in the study area. If invasive species are able to acquire more above-ground biomass annually, this may allow them to compete better for light resources than the native species and may help them to competitively exclude native flora in early-successional habitats.
CHAPTER 1

EXAMING THE ROLE OF PLANT-SOIL FEEDBACK
IN SHRUBLAND INVASION
INTRODUCTION

An invasive species is “a non-native species that causes harm, or is likely to cause harm, economically, environmentally, or to human health” (National Invasive Species Information Center 1999). Much attention has been given to invasive plants over the past few decades as their negative effects on native ecosystems have become increasingly evident. Once established in an ecosystem, populations of invasive plants may grow large enough to reduce the abundances of native species, or even eliminate them. In doing so, invasive species disrupt and alter native ecosystems, not only affecting native plant species but also indirectly affecting species that depend on the native plants (Vitousek et al., 1987; Vitousek and Walker, 1989; Hill and Hagan, 1991; Litvaitis, 1993; Woods, 1993; Brown and Mitchell, 2000; Pimentel et al., 2000; Frappier et al., 2003; Frappier et al., 2004).

In New England, these invasive species are disproportionately found in early-successional areas, also known as "shrubland" or "thicket" habitats (Lundgren et al., 2004; Johnson et al., 2006; Von Holle and Motzkin, 2007). Thicket habitats are becoming increasingly rare as forests mature, fire is suppressed, and human development pressures build (Litvaitis, 2003). The invasion of the remaining native shrublands by aggressive non-native species may affect the quality of the habitat. For example, glossy buckthorn (Frangula alnus Mill.) and bush honeysuckle (Lonicera tatarica L.), two invasive shrub species commonly found in early-successional areas of New England, have been shown to suppress native tree seedling growth and affect the abundance of herbaceous species (Woods, 1993; Fagan and Peart, 2004; Frappier et al., 2003; Frappier et al., 2004). These
effects may be detrimental to the structure and function of shrubland habitats and cause losses of native flora and fauna associated with these important ecosystems. Populations of thicket-dependent species like the New England cottontail (*Sylvilagus transitionalis*), chestnut-sided warbler (*Dendroica pensylvanica*), and least flycatcher (*Empidonax minimus*) have seen significant population declines due to the loss of native shrubland habitat (Hill and Hagan, 1991; Litvaitis, 1993).

To limit and possibly prevent plant invasions, it is essential to understand and identify the mechanisms that facilitate a non-native species' ability to invade. Much of the research on these mechanisms of invasion has focused on above-ground processes and has considered only the nutrient content and physical properties of soil as important factors influencing invasion (Silvertown et al., 1992; D’Antonio, 1993; Hughes and Vitousek, 1993; Parker et al., 1993; Luken et al., 1997; Tilman, 1997). With new approaches and the availability of new technology, there has been a growing interest in the interactions that take place below-ground such as root competition, allelopathy, and the effects of the soil community on plant species (Kourtev et al., 2002; Klironomos, 2002; Kourtev et al., 2003; Kueffer et al., 2007; Van der Putten et al., 2007; Wolfe et al., 2008).

Although the existence of soil biota was first acknowledged thousands of years ago in China, soil ecology and the idea of a “soil community” composed of organisms that have an effect on each other and on above-ground biotic and abiotic processes is relatively new (Paul, 2007). Some soil organisms have been found to interact with plants, affect plant growth, and alter ecological processes. Nitrogen fixing actinomycetes, for example, can
incorporate themselves into a plant's root system and supply the plant with needed nitrogen; the plant, in turn, supplies the symbiont with carbohydrates (Paul, 2007). In areas ordinarily devoid of nitrogen fixing plant species, introductions of such plants and their symbionts can facilitate their own invasion and that of other non-native species, and dramatically alter ecosystem structure, function, and composition (Vitousek et al., 1987; Vitousek and Walker, 1989; Asner et al., 2008). In areas of primary succession in Hawaii, invasive N-fixing plants not only facilitate invasion, they also change soil microbial composition and function, decrease native plant diversity; alter the biogeochemistry of the rainforest; and change the three-dimensional rainforest structure (Vitousek et al., 1987; Vitousek and Walker, 1989; Hughes and Denslow, 2005; Allison et al. 2006; Asner et al., 2008). These studies demonstrate how a soil microorganism can affect plant growth, ecological processes, and also contribute to the invasion of native habitats.

The soil community, however, is made up of many different types of micro and macro organisms, some of which are beneficial to plants like the nitrogen fixers, some that are benign, and some that are harmful, i.e., plant pathogens. In addition, each plant species may influence the composition of the soil community, creating a unique soil community specific to that species; this process has been termed "culturing" the soil (Bever et al., 1997). Certain beneficial and harmful associations may be specific to an individual plant species. For example, Frankia, a nitrogen fixing actinomycete, only forms a symbiosis with certain plant species and, therefore, would be present or more abundant in the associated plant species' soil community (Paul, 2007). Moreover, some diseases are
specific to one or a certain number of plant species making it possible for a plant to indirectly culture a soil community that is harmful to itself (Agrios, 2005; Sinclair and Lyon, 2005).

The net effect of the cultured soil community on a plant species is called “soil feedback”; feedback can be positive, negative, or neutral. A “positive feedback” results when a plant species cultures a soil community that is, overall, more beneficial to the plant than harmful. Over time a positive feedback would cause the plant species to become more abundant in the community and, if left unchecked by other ecological processes could facilitate the formation of monocultures or invasions of the plant species. On the other hand, a “negative feedback” results when a plant cultures a soil community whose net effects on the plant are harmful; these negative soil feedbacks may still allow a plant to grow but tend to keep the population in check (Bever, 1997; Klironomos, 2002). When studying the soil feedbacks of rare native plants and invasive non-native plants from Canadian old-fields and grasslands, Klironomos (2002) found that all of the native species experienced a strong negative feedback from their soil communities and, in contrast, four out of the five invasive plants showed a positive feedback. With additional experiments, he determined that the native plants had more plant diseases associated with their soil communities. The invasive species’ growth was, therefore, less controlled by soil pathogens than native species’ growth; this resulted in a greater abundance of the invasive plants and may have contributed to their ability to invade this grassland ecosystem.
The direction and magnitude of soil feedback may be influenced by land-use history, which can alter the composition of soil communities and thus the microbes available to interact with plants. From the time of colonization (circa 1600) until the mid-1800s, New England was heavily farmed and much of the original forest was cleared for grazing, cropping, timber, and fuel (Litvaitis, 1993; Howard and Lee, 2002). Agricultural practices such as plowing the land, using chemicals for pest control, and adding nutrients to the soil have dramatically altered soil structure, soil microbial community composition, nutrient cycling, and plant community composition (Frey et al., 1999; Foster et al., 2003; Lundgren et al., 2004; Johnson et al., 2006; Von Holle and Motzkin, 2007).

Tilled soils have been found to have lower soil organic matter content and fungal biomass, both of which may affect soil feedback (Frey et al., 1999). Soil organic matter (SOM) is a source of carbon and energy for soil biota. A lack of SOM may limit the size of the soil community or alter its composition to biota that can use carbon/energy more efficiently or can access carbon/energy from other sources, i.e., through symbiosis with plants. Limited resources could reduce the effect that the community has on the plant or promote symbiotic relationships between the plant and soil microbes. Additionally, reduced fungal biomass in agricultural soils may aid in alterations of soil feedback. Fungi can be beneficial, harmful, or benign to plants; some of the functions in the soil that fungi perform are breaking down recalcitrant materials into usable resources such as nitrogen and carbon, forming soil aggregates that act as tiny microhabitats for soil biota, and also acting as predators and prey in the soil food web (Paul, 2007). In soils where the
presence of fungi is limited, these processes, as well as other fungal functions would be diminished, which may change soil-plant feedback.

Most of the studies concerning the effects of soil feedback on native and invasive species have been conducted with forbs and grasses (Klironomos, 2002; Callaway et al., 2004; Van der Putten, 2007; Kulmatiski et al., 2008). The focus of my research, however, was to examine the effects of soil feedback on plant growth in early-successional areas using native and invasive shrubs commonly found in southeastern New Hampshire’s thicket habitats. As in Hawaii’s rainforests and in Canada’s open meadows and grasslands, a positive soil feedback would facilitate a non-native species’ ability to invade. In fact in a meta-analysis of soil feedback studies, Kulmatiski et al. (2008) determined that soil feedback did affect native species more negatively than invasive species. Should positive feedback occur for invasive species in New Hampshire’s shrublands, it may pose an additional problem for the control and eradication of these species.

The primary objective of the study was to measure the direction and magnitude of soil feedback for six shrub species, three native and three non-native invasive, to determine whether feedback influences a species ability to invade. The second objective was to assess whether or not land-use history affects soil feedback by using two types of field soil, tilled agricultural and non-tilled forest soil. The final objective was to evaluate the composition of the soil community and estimate microbial biomass for the cultured and uncultured field soil. I hypothesized that the invasive shrubs would yield positive or neutral soil feedback loops and natives would show negative feedback in their soil
communities, as in the experiments conducted in grasslands. In addition, I expected that land-use history would not change the effects of soil feedback and that each plant species would culture a unique soil community.

**METHODS**

*Experimental Design*

The experiment took place at the University of New Hampshire greenhouses, Durham, New Hampshire. The experiment was run for approximately six months from September 2007 to March 2008. Six of New England’s common early-successional shrub species, three native and three invasive, were used to evaluate soil feedback. Native species were arrowwood (*Viburnum dentatum* L.), common elderberry (*Sambucus canadensis* L.), and meadowsweet (*Spiraea alba* Du Roe) and invasive species were glossy buckthorn (*Frangula alnus* Mill.), winged euonymus (*Euonymus alatus* (Thunb.) Siebold), and bush honeysuckle (referred to here as *Lonicera* sp.). My *Lonicera* material, was of the *Lonicera morrowii* – *L. tartarica* complex, i.e., *L. morrowii* A.Gray., *L. tatarica* L., or their hybrid *L. x bella* Zabel. In SE New Hampshire, the two species are commonly intermixed and appear to hybridize freely. Additionally, two types of field soil differing in land-use history, plowed agricultural soil and non-tilled forest soil, were used to test the effects of land-use history on soil feedback (see additional information below).

The study consisted of two rounds; each round ran for three months (Figure 1.1). First, individuals of each of the six plant species were grown in separate pots in both the agricultural soil and the forest soil in order to culture the soil to that particular species.
At the end of this round, these individuals were removed from the pots, and the soil was shaken from the roots, bulked by treatment, and used again in round two. The purpose of the second round was to subject each plant species to soil cultured by an individual of the same species as well as soil cultured by other species so that soil feedback could be assessed. At the beginning of the second round, new individuals were transplanted into clean pots. Some individuals were planted in soil that was cultured by the same species, the “home” treatment, and some were planted into soil that was cultured by another species, the “foreign” treatment. This was done separately for each of the soil types, “agricultural” and “forest”. Thus in addition to the six different plant species, the experiment included two other experimental treatments each having two levels; two soil types, “agricultural” and “forest”, and two types of soil culture, “home” and “foreign”. Soil feedback was determined at the end of the second round.

**Round 1: Culturing the Soil**

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**Round 2: Soil Feedback**

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<tr>
<td>FRST</td>
<td>A/A</td>
<td>B/B</td>
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**FIGURE 1.1.** A simplified version of the experimental design. Each square represents a pot filled with soil. A, B, and C are the plant species. "AG" represents tilled agricultural soil; "FRST" represents non-tilled forest soil. "H" represents the home soil treatment; plants planted in soil "cultured" with a history of the same plant. For example, A/A depicts plant A grown in soil conditioned by plant A. "F" represents the foreign soil treatment; plants planted in soil conditioned by a different plant species. For example, B/A depicts plant B grown in soil with a history of plant
The experiment had a randomized block design. Each of the twenty blocks set up on the greenhouse benches contained twenty-four individually potted plants resulting in a sum of 480 pots. Pots were plastic square jumbo pots (14 cm; Dillen Products, Middlefield, OH). Both soil types and all six plant species were represented in each block, resulting in four pots per plant species: two individuals culturing agricultural soil and two individuals culturing forest soil. In the second round, new plants were grown in new pots of the same size used in round 1. Individuals of each of the six species were grown in both of the soil types and in both of the soil cultures, which amounted to 20 individuals of each species in “agricultural home” soil, 20 individuals in “forest home” soil, 20 individuals in “agricultural foreign” soil and 20 individuals in “forest foreign” soil. The foreign treatments were made up of 4 individuals grown in each of the other 5 species’ soil cultures (Klironomos 2002, Bever et al. 1997). At the end of the second round, the plants were harvested and the soil was washed from the roots. Each plant was separated into three parts: roots, shoots, and foliage/fruit. The harvested biomass was dried at 60°F for 72+ hours then weighed to determine dry biomass. Dry biomass was used as the measurement of plant growth. Soil feedback was then determined by comparing a plant species growth in its own soil culture ("home" biomass) versus the growth of the species in other plants' soil cultures ("foreign" biomass). If a species had a significantly higher home biomass, soil feedback was positive. However, if a species’ foreign biomass exceeded its home biomass, soil feedback was negative.

Throughout the experiment, all plants were watered as needed. As natural day length shortened and outdoor temperatures decreased, grow lights (400W High Pressure Sodium
fixtures/bulbs; PL Light Systems, Inc., Beamsville, ON) and heat were used to simulate growing season conditions. Plants were not fertilized during either round of the experiment. Greenhouse pests, such as powdery mildew and spider mites, were treated; however, no systemic products were used (Appendix A). The soil was covered with a protective layer of clear plastic wrap (Hannaford Brothers, Co., Scarborough, ME) before each spray to ensure that the treatments did not penetrate the soil.

In addition to the dry biomass measurements, soil nutrient analyses were performed on the original soil collected from the field prior to planting, and phospholipid fatty acid analysis (PLFA) was also completed for the initial soil (pre-planting) and for the cultured soil from all six species (collected after the first round; for more details see below). The microbial biomass and community composition of the two base soils (pre-planting) were compared using PLFA to distinguish any differences that could be attributed to land-use history, as well as to set a baseline for the cultured soils from each species. Moreover, the biomass and composition of the soil communities cultured by each of the six species during round one were also assessed. Comparisons were made among the six species and base soil for the two soil types, forest and agricultural, separately.

Plant Species Selection & Propagation

The six species used in this experiment were chosen based on a review of candidate species and preliminary attempts at propagation. A list of native and invasive early-successional shrub species common to southeastern New Hampshire was initially created using field observations, botanical texts, and the current literature (Gleason and
Cronquist, 1991; Haines and Vining, 1999; Howard and Lee, 2002; Johnson et al., 2006). From that list, five native species and five invasives were collected as softwood cuttings in June-July of 2007 at several locations (to allow for genotypic diversity) in the vicinity of Durham, NH; softwood cuttings are clippings taken from new green growth. Due to space constraints in the greenhouse, the experiment was designed to test the soil feedback of three native and three invasive shrub species. However, to ensure propagation success of at least three native and three invasive species for use in the experiment, all five native and invasive shrub species were collected and their propagation was attempted. The native species used in the experiment were arrowwood, common elderberry, and meadowsweet. Choke cherry (*Prunus virginiana* L.) and gray dogwood (*Cornus racemosa* Lam.) were collected but did not root during propagation attempts. The invasives used in the experiment were glossy buckthorn, winged euonymus, and bush honeysuckle. Japanese barberry (*Berberis thunbergii* DC.) and autumn olive (*Elaeagnus umbellata* Thunb.) did not root.

Softwood cuttings of all species were typically taken in the field in the morning before the plants became water stressed. After clipping 12-20 cm cuttings from the field plants, the cuttings were placed in plastic bags inside a cooler and were promptly brought back to the greenhouse for propagation. Once at the greenhouse in the mist/fog room, the leaves from the lower third of each cutting were removed, and the stem was sliced just below a leaf node at a 45 degree angle leaving at least 3-5 nodes remaining on the cutting. They were then dipped in the appropriate concentration of rooting hormone, Dip
N’ Grow (Dip ‘N Grow, Inc., Clackamas, OR), to encourage root growth (Cullina, 2002; Dirr and Henser, 1987; MacDonald, 1986).

After being dipped in rooting hormone, the cuttings for the first round were put into a nursery flat with 100% perlite medium (Whittemore Company, Inc., Lawrence, MA) to discourage rot and limit the amount of exposure to microbes. The cuttings for the second round of plants, which were taken a few weeks after the round one cuttings, were also propagated from softwood cuttings; this type of cutting can only be taken in early to mid-summer. As these second round cuttings were not to be potted up until after the first round ended in late November, they were rooted in sterilized peat:perlite medium (Conrad Fafard, Inc., Agawam, MA; Whittemore Company, Inc., Lawrence, MA) and grown there while the soil was being cultured in the first round. Prior to use, the medium was sterilized in an autoclave at 120 °C on the dry cycle for three consecutive days.

All of the cuttings remained under mist until they started to root, at which time they were removed from mist but kept in the humid, moisture-rich fog room. The plants for the first round were transplanted into the experimental pots with the field soil when their roots were 2.5-7.5 cm long and held the rooting medium when pulled. All plants were in their pots at the end of August 2007. Second round cuttings remained in their rooting flats in the sterile peat:perlite medium until they were needed for transplanting at the end of November 2007. These cuttings were given a small amount of fertilizer so that they would not perish before being used in the experiment (Jack’s Professional Water Soluble Fertilizer; J.R. Peters, Inc., Allenstown, PA).
**Initial Biomass Measurements**

The dry biomass measurements taken at the end of the second round of the experiment represented the performance of plants in conditioned soil. However, some of the variation in the final biomass may have been due to variation in initial plant biomass as individual cuttings did vary in size, if only slightly. I used dimension analysis to estimate the initial biomass of the rooted cuttings. Twenty additional individuals of each species were grown at the same time as the rooted cuttings that were to be used in the experiment. Measurements of stem diameter, height, number of leaves, and number of branches were taken for all of these individuals. Then they were harvested, dried at 60°F for 72+ hours, and weighed. Multiple stepwise regressions with backward elimination were run for each species to determine which variables were significantly correlated with dry biomass of the plants. Each species analysis yielded significant predictor variables, *i.e.*, stem diameter that were then measured for each of the cuttings to be used in the experiment. Once measured, these predictor variables were then used to estimate the initial biomass of the cuttings used in the experiment.

**Soil Site Selection**

Field soils with two distinct soil histories, plowed agricultural soil and non-tilled forest soil, were used in the experiment. Soil was collected from a total of 6 sites on University of New Hampshire (UNH) property in Durham, NH. Sites were chosen by their land-use histories, soil types, and proximity to the accompanying forest or agricultural site. To ensure similarity of soil properties, all of the soil was taken from areas with glacial drift parent material: Hollis, Charlton, or Windsor soils. Site history was assessed by looking
for a plow layer in the soil horizon, examining the sites for evidence of past land use, i.e., stone or wire fences, and by coring trees for age since abandonment. The three agricultural sites had a soil profile in which there was no distinct A and B layers indicating a history of tillage. However, none of the agricultural sites had been actively farmed in approximately the last 25 years and had, therefore, been fallow for at least this long. The three “forest soil” sites were in forests older than 80 years and prior to that were probably pastured. The soil profile in these sites showed distinct O, A, and B horizons.

The top sod layer of the plowed soils was removed, as well as the forest floor litter and most of the organic layer in the forested soils. Only the top 15-20 cm of the mineral soil was used in the experiment. The soil was then sieved using a 5 mm mesh and bulked by land-use type for the purposes of this experiment. The sites were as follows: Kingman Farm (both agricultural and forest sites), West Foss Farm (both agricultural and forest sites), and College Woods (forest soil site) and Woodman Farm (agricultural soil).

*Phospholipid Fatty Acid Analysis*

The soil microbial community is made up of a variety of bacteria, fungi, and archaea, all of which may have different functions in the soil. Due to technological constraints, however, a complete picture of every individual in the soil community is not possible. There are, however, methods available that allow us to get a fingerprint of the soil community. In this study, the microbial community was assessed using PLFA, or phospholipid fatty acid analysis.
PLFA enables researchers to extract the phospholipids within a soil sample to measure fungal and bacterial biomass, as well as to identify certain groups of microbes based on their individual phospholipid chemistry. Organic solvents, trichloromethane (CHCl₃) and methanol (MeOH), were used to extract the active lipids from the freeze dried soil samples. Following this, the lipids were then methylated to make FAMEs, or fatty acid methyl esters, that were analyzed on a gas chromatograph (Varian 3800 Flame-ionization detector). The peaks on the gas chromatograph read-out identify the chemical make-up of the phospholipids. This signature is used to define groups of microbes. The area under the peak is also used to determine the biomass of the particular group. The following equation was used to convert the area under the peak to nanograms/gram (ng/g) concentration: \( \text{ng/g for the marker} = \frac{\text{[Area]} \times \text{[c19:0 area = standard's peak area]} \times \text{the concentration of that internal standard (c19:0 - 7ug)} \times \text{the total volume of sample (300 ul)}, \text{divided by the total mass of the sample (gram of dry soil)}. \]

Nanograms/gram of each lipid marker for every sample were then converted to nanomols/gram (nmol/g) by dividing the ng/g by the molecular weight of that particular lipid marker. A similar procedure can be found in Wallander et al. (2001).

The soil samples for the base soil were collected from the bulked forest or agricultural field soil using subsamples totaling 10g/sample (3 samples for each soil type). Furthermore, soil samples from each of the cultured soils of the six shrubs were collected after round 1. The soil from round 1 was bulked by species culture and mixed well. Samples were taken by subsampling from the bulked soil for a total of 10g/sample (3 samples for each species in agricultural soil/3 samples per species in forested soil). All
soil samples were taken to the Soil Microbial Ecology Laboratory at UNH, freeze dried, and stored at -80\(^{\circ}\)C.

*Soil Nutrient Analysis*

Two samples of each type of field soil, agricultural and forest, were sent to Pennsylvania State University for analysis of nutrients, texture, pH, and soil organic matter content; the nutrients analyzed were phosphorus, potassium, calcium, magnesium, copper, zinc, lead, manganese, and iron. The soil was sampled before being used in the experiment so that general differences in the two soil types were known prior to the introduction of the experimental treatments. Each sample was composed of subsamples taken from several of the bulked soil storage containers.

*Data Analysis*

All data were checked for normality before doing any statistical tests. To determine the effects of the treatments on plant growth, two-way analyses of covariance were performed for each species using SYSTAT 10 (Systat Software, Inc.; Chicago, IL) with estimated initial biomass as the covariate, final dry biomass as the dependant variable, and soil type, soil culture, and the interaction between the two as the independent variables. In addition, ANOVAs were run for each of the plant species to compare their growth in each of the other species' cultured soils. For species with significant differences in growth among the cultures of other species as determined by the ANOVAs, Tukey’s tests (α = 0.05) were used to determine which of the means were significantly different (Zar, 1999).
Soil feedback (mean home biomass-mean foreign biomass) was calculated separately for each species using the adjusted squared means from the ANCOVAs. If the difference between these mean growth measurements was positive, the plant was said to have positive feedback as this would mean that the plant grew better with its own soil community. If however, the difference between the two numbers was negative, the species was said to have a negative feedback because it had poorer growth in its own soil community. This process was completed separately for both the agricultural and forest soils. Additionally, using the adjusted means from the ANCOVAs for only those species with significant feedback effects, the magnitude of soil feedback was calculated as:

\[
\frac{(\text{mean home biomass} - \text{mean foreign biomass})}{\text{mean of the home biomass and foreign biomass}} \times 100.
\]

The average initial biomass of each species was subtracted from the mean biomass of each treatment \textit{i.e.} (mean home biomass of arrowwood - average initial biomass of arrowwood) to incorporate the effect of initial biomass on the soil feedback magnitude calculation.

Each sample was processed (42 total); the quantity of each lipid marker (14 total) contained within the sample was given in nmol/g. Gram positive bacteria corresponded to the following lipid markers: \textit{i}15:0, \textit{a}15:0, \textit{i}16:0, \textit{i}17:0, and \textit{a}17:0. Gram negative bacteria were represented by \textit{16:}1\textomega7, \textit{16:}1\textomega7c, \textit{18:}1\textomega7c. Sulfate reducing bacteria, as well as other anaerobic bacteria and actinomycetes were identified by 10Me16:0, \textit{c}y17:0, and \textit{c}y19:0. Other bacteria were represented by 15:0; 18:2\textomega6 and 18:1\textomega9c were fungal markers. Using this information, total microbial biomass (all bacteria, fungi, and actinomycetes) and fungal to bacterial ratios were derived for each of the samples.
During the PLFA process, two samples were lost: one of the base forest samples (due to procedural complications) and one of the winged euonymus agricultural samples (due to insufficient freeze drying).

The general linear model in SYSTAT 12 was used to determine whether there was a significant difference between the total soil microbial biomass and fungal to bacterial ratios of the original "base" forest and agricultural field soils (one-way ANOVA) and also to differentiate between the soil communities cultured by the different species (nested ANOVA). Using the data (nmol/g) for each of the lipid markers contained in the different soil samples, non-metric multidimensional scaling (NMDS; PC-ORD 5; MjM Software Design, Gleneden Beach, OR) was run for the two base soils (n=5), for all forest soil samples (base soil and all 6 cultured soils; n=20), and for all agricultural soil samples (base soil and all 6 cultured soils; n=20). NMDS is an ordination method that is most effective at dealing with data concerning ecological community composition (McCune and Grace, 2002). In contrast to other types of ordination like PCA, axis 1 (the x-axis in two dimensions) does not always account for the majority of the variation. The number of axes in the ordination is calculated ahead of time by the computer program based on minimizing stress. All NMDS were run with Sorensen distance measures, a random start, 20 runs with real data, 20 runs with random data, and a stability criterion of 0.0005. The NMDS run with the base soil data was processed first with 3 dimensions, then finally with one dimension that was determined using the results from the first run with the lowest stress (McCune and Grace, 2002). The NMDS for all agricultural and
forest samples were run initially with 6 dimensions. The final analysis was obtained using 2 dimensions, as recommended by the results from the initial run.

**RESULTS**

*Overall Growth*

Plant species grown in conditioned soils (round 2) differed in biomass production (Table 1.1). Two invasive species had the highest mean absolute growth (biomass production) in both soil types and soil cultures. Glossy buckthorn had the greatest mean absolute growth (biomass exceeding 8 g per plant) followed by bush honeysuckle (biomass exceeding 5g). However, the third invasive species, winged euonymus, had the smallest absolute growth of all six species in both soil types and both soil cultures (<1 g).

Absolute growth of the native plants was intermediate to that of bush honeysuckle and winged euonymus. Of the native shrubs, arrowwood had the highest absolute growth in the home soil cultures followed by common elderberry and meadowsweet. On the other hand, common elderberry had the highest absolute growth in the foreign cultured soils. Arrowwood’s absolute growth followed common elderberry’s in the foreign agricultural treatment with meadowsweet following arrowwood. In the foreign forest soil treatment, however, meadowsweet and arrowwood had similar absolute growth.
TABLE 1.1. Adjusted means of dry biomass given in grams (g). P-values resulting from ANCOVAs, where initial biomass was the co-variate. Significance was determined P ≤0.05. The culture treatment equates to biomass of species grown in their own soil (home) versus biomass of species grown in others’ soil (foreign). Soil type is either tilled agricultural land-use history or non-tilled forest. Culture X type represents the interaction term of culture and land-use history.

<table>
<thead>
<tr>
<th>Means</th>
<th>Arrowwood</th>
<th>Common Elderberry</th>
<th>Meadowsweet</th>
<th>Bush Honeysuckle</th>
<th>Glossy Buckthorn</th>
<th>Winged Euonymus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home</td>
<td>2.67</td>
<td>1.88</td>
<td>1.49</td>
<td>5.71</td>
<td>8.36</td>
<td>0.74</td>
</tr>
<tr>
<td>Foreign</td>
<td>2.16</td>
<td>3.06</td>
<td>1.55</td>
<td>6.48</td>
<td>9.13</td>
<td>0.83</td>
</tr>
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<td>Forest</td>
<td>2.87</td>
<td>2.62</td>
<td>1.78</td>
<td>7.02</td>
<td>9.98</td>
<td>0.77</td>
</tr>
<tr>
<td>Home</td>
<td>1.30</td>
<td>4.92</td>
<td>1.41</td>
<td>7.47</td>
<td>12.53</td>
<td>0.73</td>
</tr>
<tr>
<td>Foreign</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-Values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil Culture</td>
<td>≤0.001</td>
<td>≤0.001</td>
<td>0.303</td>
<td>0.099</td>
<td>0.013</td>
<td>0.689</td>
</tr>
<tr>
<td>Soil Type</td>
<td>0.236</td>
<td>≤0.001</td>
<td>0.610</td>
<td>0.003</td>
<td>≤0.001</td>
<td>0.606</td>
</tr>
<tr>
<td>Initial Biomass</td>
<td>0.009</td>
<td>0.036</td>
<td>≤0.001</td>
<td>≤0.001</td>
<td>≤0.001</td>
<td>≤0.001</td>
</tr>
<tr>
<td>CultureX type</td>
<td>0.063</td>
<td>0.108</td>
<td>0.170</td>
<td>0.677</td>
<td>0.166</td>
<td>0.375</td>
</tr>
</tbody>
</table>

**Soil Feedback**

Soil feedback is the net effect of the interactions between a plant species and its cultured soil community on the growth of that particular plant species. It is measured in relation to the growth of the species in other plants' cultured soil communities. For both the native and invasive shrubs soil feedback was species specific; no overall patterns involving native versus invasive plants were observed (Figure 1.2). Arrowwood was the only species that showed a significant positive feedback (ANCOVA; P ≤0.001).

Common elderberry and glossy buckthorn both yielded significant negative soil feedback (P ≤0.001; P = 0.013). Bush honeysuckle (invasive), winged euonymus (invasive), and meadowsweet (native) all had neutral soil feedback.

No species showed an interaction between soil type (agricultural, forest) and soil culture (home, foreign), although the P-value for arrowwood (0.063) was only marginally insignificant. Common elderberry, bush honeysuckle, and glossy buckthorn were the
only species for which soil type had a significant effect on plant growth, and in each of these cases biomass was always larger in the forest soil (Table 1.1).

![Soil feedback results graph]

**Figure 1.2.** The soil feedback results of six New England early-successional shrub species, three native and three invasive, in two soil types, tilled agricultural (black) and non-tilled forest (white). Soil feedback was calculated as the biomass of the plant species in home cultured soil minus the dry biomass of the plant species in foreign soil cultures. Significant values (p< 0.05) are represented with an asterisk, *. Species names: AW=arrowwood, CE=common elderberry, MS=meadowsweet, BH=bush honeysuckle, GB=glossy buckthorn, and WE=winged euonymus.

The relative magnitude of soil feedback was calculated to demonstrate how much each species was affected by its soil feedback in relation to its biomass growth. The following formula was used only for those species with significant soil feedback effects: 

\[ \frac{\text{mean home biomass} - \text{mean foreign biomass}}{\text{mean of the home biomass and foreign biomass}} \times 100. \]

The initial biomass of each species was taken into account by subtracting the average initial biomass the target species from each adjusted mean used in the
equation above. For example, arrowwood's relative magnitude of soil feedback was calculated as: 

$$\left\{\frac{(\text{mean home biomass of arrowwood} - \text{average initial biomass of arrowwood}) - (\text{mean foreign biomass of arrowwood} - \text{average initial biomass of arrowwood})}{\text{average of the final less initial biomass calculated in the numerator}}\right\} \times 100.$$ 

The relative magnitude was calculated separately for each soil type. The relative magnitude of arrowwood's soil feedback in agricultural soil was +31% and in forest soil was +121%. Common elderberry and glossy buckthorn both had a negative feedback of around -2.5 g in forest soil; however, the magnitude of soil feedback for these two species was very different. Common elderberry's growth was relatively more affected by soil feedback than glossy buckthorn's. The magnitude of common elderberry's soil feedback in forest soil was -91%, whereas soil feedback affected glossy buckthorn only -30% in forest soil. In agricultural soil, plant-soil feedback affected common elderberry's growth by -95% and glossy buckthorn's growth by -12%. Of the three species showing significant soil feedback, common elderberry, glossy buckthorn, and arrowwood, feedback effects were greater in forest soil than in agricultural soil for arrowwood and glossy buckthorn. Common elderberry, however, was similarly affected by soil feedback in both of the soil types. The biomass of each species growing in the foreign treatments sometimes changed in relation to which plant species had cultured the soil (Table 1.2). In the agricultural soil, the only species that had significant differences in plant growth among the soils cultured by different plant species was glossy buckthorn. It had the highest biomass in soil cultured by arrowwood (11.6 g) and winged euonymus (12.0 g) and lowest biomass in soil cultured by bush honeysuckle (7.72 g). In the forest soil, four of the six species had significant differences in biomass among the foreign treatments.
Table 1.2. Adjusted mean biomass (g) of each of six shrub species in each soil type and in all soil cultures. Asterisks indicate significant differences between home and foreign soil treatments. Letters indicate significant differences in biomass among the different foreign soil cultures; means with the same letter are not significantly different. Tukey’s tests were used to determine significant differences among species (α=0.05).

<table>
<thead>
<tr>
<th>Shrub Species that Cultured the Agricultural Soil</th>
<th>AW</th>
<th>CE</th>
<th>MS</th>
<th>BH</th>
<th>GB</th>
<th>WE</th>
<th>Average Growth in Ag Foreign Soils</th>
</tr>
</thead>
<tbody>
<tr>
<td>AW</td>
<td>2.240 (0.279)</td>
<td>1.548 (0.279)</td>
<td>2.635 (0.574)</td>
<td>2.426 (0.597)</td>
<td>1.908 (0.625)</td>
<td>2.399 (0.723)</td>
<td>2.157 (0.277)</td>
</tr>
<tr>
<td>CE</td>
<td>3.327 (0.493)</td>
<td>1.948 (0.339)</td>
<td>2.727 (0.472)</td>
<td>3.415 (0.463)</td>
<td>2.322 (0.484)</td>
<td>2.322 (0.476)</td>
<td>2.061 (0.345)</td>
</tr>
<tr>
<td>MS</td>
<td>2.039 (0.245)</td>
<td>1.655 (0.241)</td>
<td>1.891 (0.248)</td>
<td>1.906 (0.254)</td>
<td>1.428 (0.259)</td>
<td>0.928 (0.240)</td>
<td>1.545 (0.315)</td>
</tr>
<tr>
<td>BH</td>
<td>6.121 (0.560)</td>
<td>5.569 (0.560)</td>
<td>5.725 (0.613)</td>
<td>5.715 (0.601)</td>
<td>6.060 (0.592)</td>
<td>6.737 (0.560)</td>
<td>6.477 (0.368)</td>
</tr>
<tr>
<td>GB</td>
<td>11.583 (0.798)</td>
<td>9.741 (0.741)</td>
<td>10.856 (0.846)</td>
<td>7.724 (0.753)</td>
<td>8.199 (0.684)</td>
<td>11.906 (0.795)</td>
<td>9.128 (0.427)</td>
</tr>
<tr>
<td>WE</td>
<td>0.963 (0.125)</td>
<td>0.623 (0.126)</td>
<td>0.751 (0.138)</td>
<td>0.781 (0.119)</td>
<td>0.578 (0.119)</td>
<td>0.756 (0.097)</td>
<td>0.812 (0.097)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Shrub Species that Cultured the Forest Soil</th>
<th>AW</th>
<th>CE</th>
<th>MS</th>
<th>BH</th>
<th>GB</th>
<th>WE</th>
<th>Average Growth in Forest Foreign Soils</th>
</tr>
</thead>
<tbody>
<tr>
<td>AW</td>
<td>2.070 (0.290)</td>
<td>1.951 (0.289)</td>
<td>0.676 (0.209)</td>
<td>1.731 (0.201)</td>
<td>0.926 (0.204)</td>
<td>0.978 (0.200)</td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>6.340 (0.787)</td>
<td>2.617 (0.343)</td>
<td>2.491 (0.773)</td>
<td>5.895 (0.778)</td>
<td>3.081 (0.774)</td>
<td>7.847 (0.791)</td>
<td>4.916 (0.353)</td>
</tr>
<tr>
<td>MS</td>
<td>1.922 (0.400)</td>
<td>1.567 (0.391)</td>
<td>1.570 (0.323)</td>
<td>1.110 (0.410)</td>
<td>1.285 (0.370)</td>
<td>1.351 (0.370)</td>
<td>1.411 (0.151)</td>
</tr>
<tr>
<td>BH</td>
<td>10.995 (0.729)</td>
<td>6.528 (0.706)</td>
<td>6.408 (0.723)</td>
<td>7.621 (0.732)</td>
<td>8.096 (0.801)</td>
<td>9.196 (0.752)</td>
<td>9.742 (0.727)</td>
</tr>
<tr>
<td>GB</td>
<td>15.924 (1.346)</td>
<td>8.142 (1.362)</td>
<td>11.131 (1.461)</td>
<td>12.247 (1.406)</td>
<td>10.980 (1.569)</td>
<td>15.458 (1.369)</td>
<td>12.513 (0.568)</td>
</tr>
<tr>
<td>WE</td>
<td>0.494 (0.096)</td>
<td>0.459 (0.097)</td>
<td>0.633 (0.097)</td>
<td>0.625 (0.096)</td>
<td>0.837 (0.098)</td>
<td>0.766 (0.069)</td>
<td>0.753 (0.071)</td>
</tr>
</tbody>
</table>

Notes: Species abbreviations: AW=arrowwood, CE=common elderberry, MS=meadowsweet, BH=bush honeysuckle, GB=glossy buckthorn, and WE=winged euonymus. Home culture means are in light gray, foreign culture means are in white.

Arrowwood had lowest biomass in the soil culture of meadowsweet, another native plant (0.676 g), and greatest biomass in soil cultured by common elderberry (1.59 g) and bush honeysuckle (1.71 g), two species in the same family, Caprifoliaceae. Common elderberry produced the most biomass in soils cultured by arrowwood (6.34 g) and bush honeysuckle (5.95 g) and the least in meadowsweet’s (2.49 g). Bush honeysuckle’s biomass was greatest in arrowwood’s soil culture (10.60 g) but grew the least in the soil cultured by common elderberry (6.53 g) and meadowsweet (6.41 g). Lastly, buckthorn again grew best in arrowwood (15.92 g) and winged euonymus cultures (15.46 g). However, in forest soil glossy buckthorn grew the least in common elderberry’s culture (8.14 g).
Physical & Nutrient Analysis of Soil

At the start of the experiment, two soil samples were taken from the bulked tilled agricultural soil and the bulked forest soil collected from the field and all four samples were analyzed for physical characteristics and nutrient content (Table 1.3). The soils were similar in texture class (sandy loam or loam), pH (4.60 agricultural/4.95 forest), and in their micronutrient content. As expected the agricultural soil had a lower percentage of soil organic matter (4.59) than the forest (8.36). In addition the agricultural soil had higher levels of two important plant nutrients, potassium (129 ppm ag vs. 65.5 ppm forest) and phosphorus (110 ppm ag vs 41.5 ppm forest), as well as other nutrients like calcium (564 ppm ag /231 ppm forest) and magnesium (78 ppm ag/ 48 ppm forest).

<table>
<thead>
<tr>
<th></th>
<th>Forest Soil</th>
<th></th>
<th>Agricultural Soil</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard Deviation</td>
<td>Mean</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>pH</td>
<td>4.60</td>
<td>0.141</td>
<td>4.95</td>
<td>0.212</td>
</tr>
<tr>
<td>Organic Matter (%)</td>
<td>8.36</td>
<td>0.339</td>
<td>4.59</td>
<td>0.085</td>
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<tr>
<td>Clay (%)</td>
<td>8.83</td>
<td>0.283</td>
<td>10.40</td>
<td>0.424</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>50.95</td>
<td>1.909</td>
<td>49.70</td>
<td>0.424</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>40.25</td>
<td>1.626</td>
<td>39.90</td>
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</tr>
<tr>
<td>Calcium (ppm)</td>
<td>231.50</td>
<td>0.707</td>
<td>564.00</td>
<td>46.669</td>
</tr>
<tr>
<td>Magnesium (ppm)</td>
<td>48.00</td>
<td>0.000</td>
<td>78.00</td>
<td>5.657</td>
</tr>
<tr>
<td>Potassium (ppm)</td>
<td>65.50</td>
<td>2.121</td>
<td>129.00</td>
<td>7.071</td>
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<tr>
<td>Phosphorus (ppm)</td>
<td>41.50</td>
<td>0.707</td>
<td>110.00</td>
<td>1.414</td>
</tr>
<tr>
<td>Lead (ppm)</td>
<td>10.50</td>
<td>0.707</td>
<td>6.50</td>
<td>2.121</td>
</tr>
<tr>
<td>Copper (ppm)</td>
<td>0.97</td>
<td>0.212</td>
<td>1.89</td>
<td>0.396</td>
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<tr>
<td>Zinc (ppm)</td>
<td>3.04</td>
<td>0.141</td>
<td>3.33</td>
<td>0.346</td>
</tr>
<tr>
<td>Manganese (ppm)</td>
<td>15.50</td>
<td>1.414</td>
<td>12.59</td>
<td>4.398</td>
</tr>
<tr>
<td>Iron (ppm)</td>
<td>248.00</td>
<td>12.728</td>
<td>227.20</td>
<td>13.859</td>
</tr>
<tr>
<td>Texture Class</td>
<td>Sandy Loam (Sample 1)</td>
<td>Sandy Loam (Sample 1)</td>
<td>Loam (Sample 2)</td>
<td>Loam (Sample 2)</td>
</tr>
</tbody>
</table>
Soil Community Analysis

Samples from the original agricultural and forest soils taken prior to culturing and referred to here as “base agricultural” and “base forest”, differed significantly in total microbial biomass (Table 1.4; 15.3 nmol/g agricultural vs. 68.4 nmol/g forest; (one-way ANOVA P=0.012), as well as in the fungal to bacterial ratio (F:B; 0.183 agricultural vs. 0.340 forest; (one-way ANOVA; P≤0.001). After analyzing the two soils using non-metric multidimensional scaling (NMDS) based on the biomasses (nmol/g) of all the lipid markers, the two base soils had significantly different soil community compositions (ANOVA; P≤0.001). The forest and agricultural soil samples occurred at significantly different locations on Axis 1, which accounted for 93% of the variation in biomass of the various microbial markers. Forest soil samples were located at the end of Axis 1 that was associated with a greater amount of lipid markers for 18:1ω9c (fungal marker), cy17:0 and cy19:0 (anaerobic mid-branched saturated bacteria or actinomycetes), i15:0 (gram positive bacteria), and 16:1ω7t (aerobic gram negative bacteria). In fact, the agricultural base soil samples contained none of the following lipid markers: i17:0 (gram positive bacteria), 15:0 (bacteria), 18:2ω6 (fungal biomarker), or 18:1ω7c (gram negative bacteria; in all cases Kendall’s τ > 0.89, P≤0.05).
PLFA was also performed on samples taken for each of the six shrub species in the two soil types following the culturing period (round one). In agricultural soils, total microbial biomass (fungal, bacterial, actinomycetes) was significantly different for soils cultured by native (31.0 nmol/g) and invasive species (Table 1.5; 51.4 nmol/g; ANOVA with species nested in species type, i.e., native/invasive; P≤0.001). The soil cultured by native species and the base soil had similar microbial biomass (15.3 nmol/g = base). Arrowwood soil, common elderberry soil, and the base soil had the lowest microbial biomass (29.0 nmol/g max). Winged euonymus soil had the highest microbial biomass (63.8 nmol/g). The F:B also varied significantly between natives (0.139) and invasives (0.173; ANOVA with species nested in species type, i.e, native/invasive; P=0.024) in the agricultural soil.
Glossy buckthorn had the highest F:B and meadowsweet the lowest at 0.127. NMS showed that the base soil and arrowwood soil had significantly different soil community composition than the soil cultured by glossy buckthorn and winged euonymus (one-way ANOVA; Tukey's test \( \alpha = 0.05, P \leq 0.001 \)). These differences were attributed to the location of glossy buckthorn and winged euonymus' cultured soils on the left end of Axis 1 (Figure 1.3), which was associated with significantly greater quantities of the following lipid markers: \( i15:0, a15:0, i16:0, i17:0 \) (gram positive bacteria), \( 16:1\omega 7t, 16:1\omega 7c \) (gram negative bacteria), \( 10Me16:0, cy17:0, cy19:0 \) (anaerobic/actinomycete), and \( 18:1\omega 9c \) (fungal marker; Axis 1 = 86% variation; in all cases Kendall’s \( \tau > 0.56, P \leq 0.01 \)). Along Axis 2 (15% of the variation), the base soil's community composition significantly differed from bush honeysuckle, glossy buckthorn, winged euonymus, and meadowsweet (one-way ANOVA; Tukey's test \( \alpha = 0.05, P \leq 0.001 \)). These four shrub species were located at the top of Axis 2, which was associated with significantly greater quantities of the following lipid markers: \( i15:0, a15:0, i16:0, i17:0 \) (gram positive bacteria), \( 16:1\omega 7t, 16:1\omega 7c \) (gram negative bacteria), \( 10Me16:0, cy17:0, cy19:0 \) (anaerobic bacteria or actinomycete), and \( 18:1\omega 9c \) (fungal marker in all cases Kendall’s \( \tau > 0.56, P \leq 0.01 \)).
TABLE 1.5. Mean biomass (nmol/g) of 14 different lipid markers contained within the six different shrub species cultured soil (n=3 per species/per soil type). Mean total microbial biomass (nmol/g) and fungal to bacterial ratio (F:B) for all six shrub species for two soil types are also given. Standard deviations given in parentheses. All data taken from PLFA. Native shrubs are AW, CE, MS and invasives are GB, BH, and WE.

<table>
<thead>
<tr>
<th>Lipid Marker</th>
<th>AW (B)</th>
<th>CE (F)</th>
<th>MS (A)</th>
<th>GB (B)</th>
<th>BH (B)</th>
<th>WE (B)</th>
</tr>
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<tr>
<td>15:0</td>
<td>3.316</td>
<td>3.961</td>
<td>7.099</td>
<td>7.206</td>
<td>7.103</td>
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<tr>
<td>16:0</td>
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<td>2.507</td>
<td>3.754</td>
<td>3.824</td>
<td>3.533</td>
<td>5.249</td>
</tr>
<tr>
<td>17:0</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
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<td>2.638</td>
<td>3.893</td>
</tr>
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<td>1.855</td>
<td>2.119</td>
<td>2.081</td>
<td>2.495</td>
<td>2.656</td>
</tr>
<tr>
<td>16:0t7c</td>
<td>5.955</td>
<td>7.331</td>
<td>12.337</td>
<td>13.736</td>
<td>11.636</td>
<td>15.171</td>
</tr>
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<td>2.721</td>
<td>2.466</td>
<td>2.687</td>
<td>3.755</td>
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<td>17:0</td>
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<td>0.971</td>
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<td>1.703</td>
<td>1.709</td>
<td>2.516</td>
</tr>
<tr>
<td>18:0</td>
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<td>0.606</td>
<td>0.595</td>
<td>1.161</td>
<td>0.588</td>
<td>2.297</td>
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<td>19:0</td>
<td>0.610</td>
<td>1.147</td>
<td>1.283</td>
<td>1.959</td>
<td>1.948</td>
<td>2.524</td>
</tr>
<tr>
<td>18:1</td>
<td>0.000</td>
<td>0.292</td>
<td>0.000</td>
<td>2.648</td>
<td>0.425</td>
<td>3.002</td>
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<tr>
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<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
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<tr>
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<td>29.028</td>
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<td>21:0</td>
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</tr>
<tr>
<td>22:0</td>
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<td>0.606</td>
<td>0.595</td>
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<td>2.297</td>
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<tr>
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<td>1.283</td>
<td>1.959</td>
<td>1.948</td>
<td>2.524</td>
</tr>
<tr>
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<td>0.000</td>
<td>2.648</td>
<td>0.425</td>
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<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>26:0</td>
<td>2.188</td>
<td>3.436</td>
<td>4.566</td>
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<td>4.388</td>
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<tr>
<td>Total F, B, A</td>
<td>20.079</td>
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<td>43.939</td>
<td>37.886</td>
<td>44.081</td>
<td>44.169</td>
</tr>
<tr>
<td>F:B</td>
<td>0.133</td>
<td>0.159</td>
<td>0.125</td>
<td>0.200</td>
<td>0.135</td>
<td>0.180</td>
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Cultured Forest Soil

<table>
<thead>
<tr>
<th>Lipid Marker</th>
<th>AW (B)</th>
<th>CE (A)</th>
<th>MS (B)</th>
<th>GB (B)</th>
<th>BH (B)</th>
<th>WE (B)</th>
</tr>
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<tbody>
<tr>
<td>15:0</td>
<td>5.815</td>
<td>7.661</td>
<td>15.127</td>
<td>11.512</td>
<td>15.018</td>
<td>11.229</td>
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<tr>
<td>17:0</td>
<td>0.312</td>
<td>1.091</td>
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<td>0.000</td>
<td>0.657</td>
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<tr>
<td>18:0</td>
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<tr>
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<td>2.654</td>
<td>2.538</td>
<td>2.515</td>
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<tr>
<td>21:0</td>
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<td>5.574</td>
<td>5.750</td>
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<tr>
<td>22:0</td>
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<td>2.632</td>
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<td>2.657</td>
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<tr>
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<td>Total F, B, A</td>
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<tr>
<td>F:B</td>
<td>0.232</td>
<td>0.226</td>
<td>0.210</td>
<td>0.214</td>
<td>0.213</td>
<td>0.187</td>
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</table>

Notes. The type of lipid marker is given in parentheses next to the name of the lipid marker. Abbreviations for the table are as follows: B=bacterial marker, F=fungal marker, A=actinomycete marker, AW=arrowwood, CE=common elderberry, MS=meadowsweet, GB=glossy buckthorn, BH=bush honeysuckle, and WE=winged euonymus.
Soil microbial biomass in forest soil did not differ among the base soil, invasive cultures, and native cultures. However, arrowwood (53.5 nmol/g) and common elderberry (63.6 nmol/g) soils had significantly lower microbial biomass than meadowsweet soil (Table 1.5; 117 nmol/g; ANOVA; P=0.008). Invasive and native species had similar F:B ratios (0.206 & 0.222, respectively); both types of plant species’ (natives/invasives) F:B were also significantly different from the base soil (0.340; ANOVA; P $\leq$ 0.001). In fact, all of the individual shrub species had similar F:B ratios (0.187-0.230), which were all significantly different from the base forest soil (0.340; ANOVA; P $\leq$ 0.001). The soil
community composition of the unconditioned base soil, arrowwood, and common elderberry were similar as seen with NMS (Figure 1.4). Yet, these soil cultures differed significantly from meadowsweet (ANOVA; $P=0.012$). Meadowsweet was located on the right end of Axis 1, which was associated with a significantly greater amount of the lipid markers $i15:0$, $a15:0$, $i16:0$, $i17:0$, $a17:0$ (gram positive bacteria), $16:1ω7t$ (gram negative bacteria), $10Me16:0$, $cy17:0$, $cy19:0$ (anaerobic/actinomycete), and $18:1ω9c$ (fungal marker; Axis 1 = 82% variation; in all cases Kendall’s $τ > 0.56$, $P<0.01$ to determine significance of differences in lipid biomarkers among species’ soil communities). On Axis 2 (17% of the variation), arrowwood’s soil community was significantly different from meadowsweet’s, as the latter was located at the top of Axis 2, which was associated with the lipid markers $i15:0$, $a15:0$, $i16:0$, $i17:0$, $a17:0$ (gram positive bacteria), $16:1ω7t$ and $16:1ω7c$ (gram negative bacteria), $10Me16:0$, $cy17:0$, $cy19:0$ (anaerobic bacteria/actinomycete), and $18:1ω9c$ (fungal marker; Axis 1 = 82% variation; in all cases Kendall’s $τ > 0.56$, $P<0.01$).

Soil community biomass may be related to root mass. As root systems get bigger, the potential root surface increases as well as pocket habitat space for soil microbes; therefore, the microbial biomass may also increase. To test this hypothesis, an ANCOVA was run in SYSTAT 10 to determine if there was a positive relationship between root biomass and microbial biomass (averages of the root biomass and microbial biomass for each plant species in two soil types were used in the ANCOVA; microbial biomass was the dependent variable and root biomass was the independent variable; soil
type was run as the co-variate). The test revealed that there was no significant relationship between the two variables ($P > 0.05$).

**FIGURE 1.4.** The means of all forest soil samples, base and six cultured soils, as ordinated by non-metric multidimensional scaling (NMDS) and taken from PLFA. Significant differences (ANOVA; $P < 0.05$) were found between the soil cultures of the group of samples at the right end of Axis 1 (AW, CE, BASE) and MS. The lipid markers associated with the right end of Axis 1 were i15:0, a15:0, i16:0, i17:0, a17:0 (gram positive bacteria), 16:1ω7t (gram negative bacteria), 10Me16:0, cy17:0, cy19:0 (anaerobic/actinomycete), and 18:1ω9c (fungal marker; Kendall’s $r > 0.56$, $P < 0.01$). AW differed significantly from MS along Axis 2. The biomass of the following biomarkers increased significantly from the bottom to the top of Axis 2: i15:0, a15:0, i16:0, i17:0, a17:0 (gram positive bacteria), 16:1ω7t and 16:1ω7c (gram negative bacteria), 10Me16:0, cy17:0, cy19:0 (anaerobic bacteria /actinomycete), and 18:1ω9c (fungal marker; Axis 1 = 82% variation; in all cases Kendall’s $r > 0.56$, $P < 0.01$). Species names have been shortened: AW=arrowwood, CE=common elderberry, MS=meadowsweet, GB=glossy buckthorn, BH=bush honeysuckle, WE=winged euonymus, and BASE=uncultured soil.

**DISCUSSION**

**Invasive Versus Native**

The effect of soil feedback on the six shrub species was species specific. There were no overall patterns of response for invasive versus native shrubs. This result contrasts with results from studies of grassland species and from a soil-feedback meta-analysis.
Soil Feedback

Instead of clear relationship between invasive/native status and soil feedback, the interactions between a plant and its cultured soil community seemed to affect these six

(Kulmatiski et al., 2008), where native species were negatively or neutrally affected by their soil communities and the invasive species were positively or neutrally affected (Klironomos, 2002; Van der Putten et al., 2007). Given this, I expected that the native shrubs in my experiment would all have negative feedback effects, as native species have been shown to accumulate more pathogens than invasive species in host ranges (Klironomos, 2002; Mitchell and Power, 2003; Van der Putten et al., 2007). Contrary to the expectation, the direction of soil feedback varied in the three native species, arrowwood (positive), common elderberry (negative), and meadowsweet (neutral). Additionally, although arrowwood and common elderberry had opposite soil feedback effects (positive and negative, respectively), the growth of both species was affected to a great extent by soil feedback. The magnitude of arrowwood's soil feedback in agricultural soil was +31% and in forest soil was +121%, and common elderberry's was -95% in agricultural soil and -91% in forest soil. Hence, the native species not only had different directions of soil feedback but, when feedback occurred, the effects on growth were substantial. Consistent with previous studies, two of the invasive species did, indeed, have neutral soil feedback effects. However the third, glossy buckthorn, showed a significant negative soil feedback response, contradicting the results from grassland studies. Overall the soil feedback patterns of native and invasive plants in this study did not correspond with results from previous research.
shrub species differently. Arrowwood had a positive feedback and therefore may have cultured a beneficial soil community (Bever et al., 1997). This was unexpected since previous research showed that native species accumulated more soil pathogens than non-native species; a result which would yield a negative feedback (Klironomos, 2002; Mitchell and Power, 2003; Van der Putten et al., 2007). However, it is conceivable that this native species may have developed a relationship with beneficial soil microbes over evolutionary time. The PLFA data, however, did not suggest that certain types of microbes were uniquely present or abundant in arrowwood's soil culture. Because fungi are more likely than bacteria to form facilitative symbiotic relationships with plant species (Paul, 2007), I anticipated that the PLFA analysis would show that arrowwood had higher fungal marker biomass than all the other species. Yet, this was not the case (Table 1.5). In addition, NMS did not show any unique differences attributed to fungal markers for arrowwood's soil culture. Therefore, I can only speculate based on the soil feedback results that arrowwood may have cultured a beneficial soil community.

Common elderberry and glossy buckthorn had negative feedback responses in both soil types; additionally, common elderberry was more negatively affected than glossy buckthorn by the feedback effect. This may have been due to an accumulation of soil pathogens, which might also explain the differences in the magnitude of the response for both species (Bever et al., 1997; Klironomos, 2002). Common elderberry is a native species and has had more time in this ecosystem to develop relationships, both harmful and beneficial, with the native soil biota (Gleason and Cronquist, 1991; Haines and Vining, 1999). Glossy buckthorn, on the other hand, was recently introduced to this area.
(Frappier et al., 2003) and may not have had time to accumulate as many species specific pathogens as common elderberry, which could explain the differences in magnitude of response (Mitchell and Power, 2003). In early-successional sites in New England, glossy buckthorn is often one of the most abundant shrubs. Therefore, this species must have the ability to overcome its negative feedback response and could possibly accomplish this by being a strong competitor (Chapter 2). Powdery mildew was present in the greenhouse on common elderberry and meadowsweet; this pathogen may have affected the growth of both species but could not have been the only cause of the negative soil feedback; powdery mildew spreads by wind and was present on all plants, not just the individuals planted in home soil (Agrios, 2005; Sinclair and Lyon, 2005). The soil community composition of the two species was significantly different based on the NMS results. However, one can only compare differences in large groups of microbes, i.e., gram negative bacteria using PLFA. Thus, the specific differences (i.e., at the level of microbial species) in community composition and pathogen load of the two plant species cannot be assessed.

Resource Competition

The feedback patterns found in this study may have been influenced by nutrient depletion through resource competition (Keddy, 1989). If species are limited by the same suite of resources, they tend to form a competitive hierarchy, with the best competitor accumulating the most biomass and the poorest competitor accumulating the least biomass (Tilman, 1988). In a competitive hierarchy, all species compete for the same resources. The good competitors use more resources than the weaker competitors,
leaving fewer resources for these other species (Keddy, 1989; Silvertown and Charlesworth, 2001). If a competitive hierarchy were responsible for this study’s results, the hierarchy would have been ordered based on biomass accumulation, with glossy buckthorn as the best competitor, as this species was able to accumulate the most biomass out of all species, and winged euonymus with the smallest biomass accumulation as the weakest competitor (initial biomass; Chapter 2). Under this assumption, glossy buckthorn should have grown the least in its own soil because it would have depleted most of the resources in this soil during the first round of the experiment. Additionally, winged euonymus should have done best in its own soil because there would have been ample resources left in its home soil and poorly in soils conditioned by other species more effective at depleting nutrients. However, neither of these results was true. When compared with the biomass in its own (home) soil glossy buckthorn had a lower biomass in bush honeysuckle’s soil culture in the agricultural soil and in common elderberry’s soil culture in the forest soil. Furthermore, in agricultural soil winged euonymus accumulated more biomass in arrowwood and bush honeysuckle’s soil cultures and in forest soil it had its greatest biomass in soil cultured by its best competitor, glossy buckthorn. These results did not support the idea that resource allocation under a competitive hierarchy was responsible for the outcomes of the experiment.

Alternatively, it is possible that the six species had somewhat different niches, requiring resources in different quantities or ratios (Tilman, 1988). If the species competed for different resources, then the community would be organized by niche differentiation (Silvertown and Charlesworth, 2001). In this case, all of the species would have less
biomass accumulation in their own soil cultures, as intraspecific competition for resources would be greater than interspecific competition (Silvertown and Charlesworth, 2001; Vila and Weiner, 2004). There were only two species, however, common elderberry and glossy buckthorn, that had negative soil feedback effects, growing better in soils conditioned by other species than in their home soils. As stated previously, glossy buckthorn had lower biomass accumulation in bush honeysuckle’s soil culture and common elderberry’s soil culture in agricultural and forest soils, respectively. On the other hand, in agricultural soil intraspecific competition for resources may have been responsible for common elderberry’s negative feedback, as it had the lowest biomass in its own soil culture (Chapter 2). In forest soil, common elderberry accumulated less biomass in meadowsweet’s soil culture. Competition for resources under niche differentiation may be responsible for common elderberry’s agricultural soil feedback response but would not explain the results for the rest of the species or for common elderberry in forest soil.

**Allelopathy**

Allelopathy, or the release of chemicals that inhibit another species growth, is a method of interference competition (Weir, 2007). If allelopathy were at work in this experiment, the plant species other than the one that produced chemicals, and possibly even the chemical producing species, would have grown poorly in the allelopathic soil. There were no soil cultures in which all plant species, or just the other species that had not cultured the soil, demonstrated suppressed growth. Assuming no species specific allelochemicals, none of the results support the suggestion that allelopathy is responsible
for the feedback responses seen here. However, allelochemicals may influence soil microbes, as well as directly interfering with other plants growth (Weir 2007; Callaway et al., 2008; Cipollini et al., 2008; Wolfe et al., 2008). In consequence, it may be difficult to disentangle allelopathy and soil feedback in this study.

**Combined Effect**

As stated previously, it would be difficult to tease apart the effect of the interactions of the shrub species with the cultured soil communities, resource competition, and allelopathy. Soil biota are a major factor contributing to the availability of resources for plants, and allelopathy can alter microbial communities (Callaway et al., 2008; Cipollini et al., 2008; Weir, 2007; Wolfe et al., 2008). When creating the theoretical framework for soil feedback responses, Bever et al. (1997) assumed that plants share identical resources and are competitively equivalent. If his assumptions were correct and if the effects from allelochemicals could be ignored, the soil feedback responses seen here would be due to differences in soil biota alone. However, additional experiments have shown that the shrub species used in this experiment were not competitively equivalent (Chapter 2), and therefore, the responses were probably due to a combination of competitive ability, including the use of allelochemicals, and interactions with the soil microbial community.

**Growth in Soils Cultured by Other Species**

Each of the six shrub species was not only grown in its own soil culture but also in the soil cultures of the other plant species (Table 1.2). For the species that showed
significantly different accumulations of biomass among the cultures of the other species (glossy buckthorn in agricultural soil and arrowwood, common elderberry, bush honeysuckle, and glossy buckthorn in forest soil), glossy buckthorn, bush honeysuckle, and common elderberry showed the greatest biomass in the soil cultures of arrowwood and winged euonymus. Arrowwood also had the greatest biomass in its own soil culture but did not grow well in winged euonymus' soil culture. If most species grow well in soils cultured by winged euonymus, areas invaded by this species may be more easily restored. However, the importance of this trend seems to diminish in agricultural tilled soils where most species accumulate the same amount of biomass regardless of the "other" species by which it was cultured.

Three of the six shrub species are members of the same plant family and are, therefore, more closely related to each other than to the other shrub species. One would expect these confamilials to exhibit some of the same patterns of growth in other species' soil cultures because of this relationship. Arrowwood, common elderberry, and bush honeysuckle are all Caprifoliaceae (Haines and Vining 1999). Arrowwood and common elderberry had significantly greater biomass in the cultures of the species within their same family than in cultures of the other species in the forest soil. Additionally, these two native shrubs also had the lowest amount of biomass in meadowsweet's soil culture. Bush honeysuckle, the invasive Caprifoliaceae, did not show the same pattern of accumulating more biomass in cultures of confamilial species; it had the greatest biomass in arrowwood's soil culture, as did most species, but had the lowest biomass in both meadowsweet and common elderberry's soil culture. Meadowsweet's soil culture may
include organism(s) harmful to this family because all three Caprifoliaceae plants had the least amount of biomass in this species' culture.

Land-use History

The land-use history of the two different soil types altered the magnitude, but not the direction, of soil feedback for arrowwood and glossy buckthorn. The magnitude of soil feedback was greater in the non-tilled forest soils than in the plowed agricultural soils for both of these species. The PLFA data from the base agricultural and base forest soils demonstrated that the base agricultural soil had a lower total microbial biomass and a lower fungal to bacterial ratio than the forest soil. In addition, the physical characteristics and nutrient analysis tests of the base field soils showed that the agricultural soil had a lower content of soil organic matter (SOM) than the forest soil. SOM supplies a majority of the soil biota with needed nutrients and energy for growth (Paul, 2007). By decreasing the amount of SOM in a soil through agricultural practices such as tillage, the nutrient and energy base for these soil species is also reduced which may affect the microbial biomass and community composition (Santiago et al., 2008; Paul, 2007; Kulmatiski et al., 2006; Frey et al., 1999). The decrease in total microbial biomass could cause a decrease in the magnitude of the effect of the soil community on plant growth. Due to lower availability of nutrients and energy, as well as the altered structure and composition of the soil, a plant may be able to culture only a fraction of the microbes in the tilled agricultural soil as in the non-tilled forest soils. In this case, the effect of the soil community on plant growth might also decrease in the tilled soils.
Like glossy buckthorn, common elderberry also had a significant negative feedback with its soil community. However, neither the magnitude nor the direction of common elderberry's negative soil feedback was altered by the land-use history of the soil. This species' growth showed a considerable reduction when grown in its own soil culture (-95% in tilled soil and -91% in non-tilled soil) versus its growth in the cultures of the other species. If a soil pathogen is responsible for this growth reduction, perhaps it is just as efficient at harming the plant in small quantities in the tilled soil as it would be in increased quantities in the non-tilled soil. This reasoning would account for the fact that there is no change in the magnitude of the negative soil feedback for common elderberry despite the obvious differences in land-use history, soil characteristics, and soil microbial biomass between the two soil types.

The base field soils, agricultural and forest, had significantly different microbial biomass, community composition, and fungal to bacterial ratios (F:B). The base agricultural soil had a much lower microbial biomass and F:B ratio than the base forest soil. These patterns were most likely due to the differences in land-use history for the two soil types (Frey, 1999). Tilling soil tends to decrease the amount of fungi by continuously breaking up the fungal hyphae and compacting the soil. Compaction and a lower fungal biomass may also cause reductions in the amount of total microbial biomass and diversity by eliminating different types of habitat available for soil biota (Frey et al., 1999; Paul, 2007). Fungi are responsible for forming soil aggregates or pockets of habitat for different soil biota (Elliot, 1986; Simpson et al., 2004; Paul, 2007; Alvaro-Fuentes et al., 2008). With a reduction in fungi, there may also be a reduction in soil aggregates. In
addition, compaction reduces soil pore space, can inhibit gas exchange between the air and soil, and may alter the movement of water into and through the soil (Paul, 2007). All of these factors can cause a decrease in microbial community biomass by homogenizing the soil habitat, reducing niches for different soil biota to live, and altering biotic processes necessary to obtain the required nutrients.

Soil Community Analysis

PLFA did not yield any conclusive results to explain the differences in the direction of feedback among species. Arrowwood and common elderberry's soil microbial profiles were similar; yet, arrowwood had a positive soil feedback and common elderberry had a negative soil feedback. Furthermore, glossy buckthorn, which also showed negative soil feedback, had a comparable soil microbial community and biomass to winged euonymus, bush honeysuckle, and meadowsweet. All of these other species (winged euonymus, bush honeysuckle, and meadowsweet) had neutral soil feedback effects, meaning that different soil communities (home vs. foreign) had similar effects on the species' growth. On the other hand, this may lend support to the nutrient depletion hypothesis as an explanation of glossy buckthorn's negative feedback versus soil community composition.

Literature suggests that invasive and native species culture significantly different soil microbial communities (Kourtev et al., 2003; Callaway et al., 2004; Batten et al., 2006; Li et al. 2006). However, NMDS did not show any significant differences in the composition between native and invasive soil communities of the shrubs used here. As natives have been established in this area for a much longer time period than the non-
native invasive species, one would expect that they have very specific pathogens and symbionts in their soil cultures. Therefore, native species' soil communities should be very different from a non-native's. In addition, some invasive species have the ability to alter soil properties like pH which may in turn, change the soil community composition from native to a non-native culture (Kourtev et al., 2003). Meadowsweet, a native species, had a similar soil community composition to the invasive species and a significantly different soil community composition than other two native species. Additionally, meadowsweet was unaffected by soil feedback, as was bush honeysuckle and winged euonymus. This may be due in part to meadowsweet's overall poor growth during the experiment, which might also explain similarities to winged euonymus' soil community (Table 1.2). Winged euonymus also had poor growth during the study. Yet, this would not account for the similarity to glossy buckthorn and bush honeysuckle, two species that grew the most overall. In the future, a more in depth soil assay should be conducted to better differentiate among the cultured soil communities.

**CONCLUSION**

As our native shrublands age into forests or become new housing developments, conservation and land management agencies in the northeast are actively creating and restoring early-successional habitat for those species that depend on shrublands for shelter, nourishment, and breeding. During this restoration process, the establishment of invasive species should be prevented or at least controlled to ensure that the quality of the native habitat remains intact. Therefore, it is crucial that we have an understanding of the factors that influence shrubland invasions.
In this greenhouse experiment, I examined whether the effect of soil feedback, or the
interactions between plants and soil biota on plant growth, may facilitate invasion in
early-successional areas. Although I showed that a cultured soil community affected the
growth of three of six native and non-native invasive early-successional shrub species,
the response to soil feedback was species specific and no overall trends were seen for
native and invasive species. Patterns of biomass production did not indicate a major role
of soil nutrient depletion (resource competition) or allelopathy, although a combination of
resource depletion and microbial feedback may have been involved. The effects of soil
feedback were greater in non-tilled forest soil versus tilled agricultural soil for glossy
buckthorn and arrowwood (two of the three species with significant soil feedback). Soil
type, tilled and non-tilled, did not affect the magnitude of common elderberry's feedback
response. Biomass growth of the remaining three species, bush honeysuckle, winged
euonymus, and meadowsweet, was not significantly affected by the cultured soil
communities. PLFA revealed differences between the soil communities of both base
field soils. These differences suggest that agricultural practices such as tillage can alter
soil community composition. Microbial communities also differed among soils cultured
by different species, which supports the idea that plants culture unique soil communities.
While soil feedback was not found to influence invasion in this ecosystem, the cultured
soil community of some species did greatly affect plant growth, both negatively and
positively, and therefore should be still be considered as an important factor when
managing early-successional habitat.
CHAPTER 2

A COMPETITIVE EDGE: ASSESSING THE INFLUENCE OF COMPETITION IN SHRUBLAND INVASION
INTRODUCTION

Early-successional areas composed of native shrub species are becoming increasingly rare in New England (Litvaitis, 2003). Forest maturation, fire suppression, and human development are responsible for the total area of shrubland lost (Litvaitis, 1993; Litvaitis, 2003). However, the invasion of non-native shrub species into these areas may cause additional losses, in this case, to the quality of the habitat. Because invasive species are suspected of altering community interactions and suitability, it is important, for the purpose of conservation management, to identify factors that enable invasion (Vitousek et al., 1987; Vitousek and Walker, 1989; Woods, 1993; Brown and Mitchell, 2000; Fagan and Peart, 2003; Frappier et al., 2003; Frappier et al., 2004; Johnson et al., 2006).

Competition, the harmful effects that one organism has on another by utilizing or restricting access to a limited resource (Keddy, 1989), is a fundamental factor shaping ecosystem dynamics (Tilman, 1988). Plants that use limited resources such as light, water, and nutrients more efficiently than their neighbors may have additional resources to use for growth, reproduction, and herbivore defense. Furthermore, individuals that block other species from acquiring these resources will leave more for themselves and less for their neighbors, inhibiting growth (Silvertown and Charlesworth, 2001). As invasive shrubs immigrate into early-successional areas, these non-native species and our native shrubs are likely to compete for the same resources. Superior competitive ability—a competitive edge—could give non-native invasive species the ability to overcome and suppress their native counterparts (Crawley, 1990).
In a meta-analysis of thirty-six studies testing whether invasive plant species exhibited superior competitive ability over natives, Vila and Weiner (2004) found that a majority of studies showed invasive plants to be better competitors. These results, however, may not be readily applicable to early-successional habitats in New England. Only a few of the studies in this meta-analysis focused on shrublands (Witkowski, 1991; Aplet and Laven, 1993) and of these, none were located in habitats similar to northeastern early-successional areas or used species found in these areas. Yet, there is evidence that some of the invasive shrubs in New England may have a competitive edge over native species. Bush honeysuckle [*Lonicera maackii* (Rupr.) Herder] (Gorchov and Trisel, 2004; Hartman and McCarthy, 2004) and glossy buckthorn (*Frangula alnus* Mill.) (Fagan and Peart, 2004; Frappier et al., 2004) have been shown to suppress the growth of native tree seedlings and herbs. While this supports the conclusion that competition may facilitate invasion, these studies were located in forest habitats and did not test shrub-shrub competition. In fact, there has been no research to date on whether non-native shrub invaders can outcompete their native shrub counterparts in New England’s early-successional habitats.

With the decline in early-successional habitat, it has become necessary to create and maintain native shrubland to provide habitat for species that are thicket-dependent, such as the New England cottontail (*Sylvilagus transitionalis*), chestnut-sided warbler (*Dendroica pensylvanica*), and least flycatcher (*Empidonax minimus*; Hill and Hagan, 1991; Litvaitis, 1993; DeGraaf and Yamasaki, 2003). As competition plays a major role in ecosystem dynamics and may be a key factor in species distribution, it is essential that
we recognize the potential that competition has to influence invasion in shrubland habitats (Tilman, 1988). Information about the competitive interactions between native and invasive shrubs would facilitate the process of restoring, maintaining, and monitoring early-successional areas. This study served as an investigation into these competitive interactions.

The purpose of this greenhouse experiment was to examine the effect of simple pair-wise competition on three native and three invasive early-successional shrub species commonly found in southeastern New Hampshire. My goal was to determine whether these invasive shrubs are competitively superior to their native counterparts. Individuals of all six species were grown in pots either alone or with individuals of the same species to measure the effect of no competition and intraspecific competition, respectively. In addition, individuals of each native species were paired up with individuals of each invasive species to determine interspecific interactions.

Interspecific interactions were assessed in two ways, by measuring the "competitive effect" and "competitive response" for all species (Goldberg and Fleetwood, 1987; Gurevitch et al., 1990; Silvertown and Charlesworth, 2001; Schmidt et al., 2008). The competitive effect for a target species was measured as the effect of competition from that particular species on its neighbor's growth, i.e., an invasive species effect on native neighbor's growth or a native species effect on invasive neighbor's growth. To determine competitive effect, the neighbor's change in growth in the presence of the competitor was considered.
In contrast to competitive effect, the competitive response was evaluated for each target species as the growth response of that particular species when in competition with a neighbor, i.e., a invasive species growth response to competition from native neighbors or a native species growth response to competition from their invasive neighbors. For competitive response, the change in growth of the target species when in competition was the variable examined. As these non-native shrubs are often seen dominating early-successional areas and have the ability to invade, my hypothesis was that the invasive species would, in fact, be better competitors than the native species and would also respond less to competition from native species than native species would respond to their invasive neighbors.

Root to shoot ratios were evaluated to determine whether the invasive and native shrubs allocated resources differently to their above-ground and below-ground biomass, as well as to identify any changes to this ratio under the different competition treatments for all species. Previous studies demonstrated that a plant's root to shoot ratio was affected by nutrient levels and by the presence of certain competitors (Chapin, 1980; Gurevitch et al., 1990; Aplet and Laven, 1993; Nilsson, 1994; Genney et al., 2002). Lower nutrient levels caused plants to apportion additional resources to their roots, instead of to their shoots (Chapin, 1980). In addition, a study of four Hawaiian shrubs showed that in the absence of root competition plants increased the proportion of above-ground biomass in relation to below-ground biomass (Aplet and Laven, 1993). The shrub species in this study were year old plants grown from cuttings. Given the greenhouse environment, planting regime
(see Methods, below), and the consequent probability that light was not a limited resource for any of the species, below-ground competition was most likely more important to a plant's growth. Therefore, I hypothesized that root to shoot ratios would be higher in competition than without competition for all species.

Although a simple pair-wise design conducted in a greenhouse does not take into account the different densities at which plant species occur in the field, nor the diversities of competing neighbors encountered there, the relatively uncomplicated design carried out in a controlled environment was chosen to provide a foundation for future competition experiments (Gibson et al., 1999; Cousens, 2000; Freckleton and Watkinson, 2000). The invasive shrub species chosen for this experiment are abundant in early-successional areas in New England, but the factors aiding their establishment and invasion ability remain relatively unknown (Johnson et al., 2006; Searcy et al., 2006; Von Holle and Motzkin, 2007).

**METHODS**

*Experimental Design*

This study ran from mid-May 2008 until September 15, 2008 at the University of New Hampshire’s research greenhouse, Durham, NH. The experimental design was a randomized block with three competition treatments for each of the six species, three native and three invasive. Treatments were: 1) one individual of the species was planted alone in a pot (no competition), 2) two individuals of the same species were planted together in one pot (intraspecific competition), and 3) two individuals, each of a different
species, were planted together in one pot (interspecific competition). In the interspecific competition treatments, however, natives were only planted with invasive and invasives were only planted with natives; native/native and invasive/invasive were not planted in interspecific pairs due to the lack of individual plants available for the experiment. Thus, each block contained each species grown alone (6 pots), each species grown in intraspecific competition (6 pots), and each of the three native species grown with each of the three invasive species (9 pots). In all, there were 7 blocks and 21 pots in each block for a total of 147 pots and 252 individual plants.

Greenhouse Procedure

Each pot was filled to about 13 cm in depth with agricultural field soil taken from Woodman Farm. After shaking the potting soil from their roots, the plants were then transplanted into the pots (1200 squat custom pots; Nursery Supplies, Inc., Chambersburg, PA). Each plant was tagged with an identification number (7" Yellow Flexible Vinyl Labels, AMTSystems, Cheshire, CT), and allowed to grow in ambient temperature and natural light. The plants were not fertilized, were watered as needed, and were also treated for spider mites and powdery mildew when necessary (Appendix A). Directly after transplanting, measurements of each individual's stem diameter, height, number of leaves, and number of branches were taken and recorded for later use in estimating initial biomass (see below). At the beginning of September, 2008, the plants were harvested in two parts, roots and shoots, dried at 60°C for three days, and weighed to measure final above and below-ground dry biomass (in grams) so that competition indices could be calculated and compared.
Plant Species

All plant species selected for this study were early-successional shrubs commonly found in thickets and abandoned fields in southeastern New Hampshire (Gleason and Cronquist, 1991; Haines and Vining, 1999; Howard and Lee, 2002; Johnson et al., 2006).

Arrowwood (*Viburnum dentatum* L.), common elderberry (*Sambucus canadensis* L.), and meadowsweet (*Spiraea alba* Du Roi) were the native species used, and glossy buckthorn (*Frangula alnus* Mill.), bush honeysuckle (referred to here as *Lonicera* sp.), and winged euonymus (*Euonymus alatus* (Thunb.) Siebold) were the non-native invasives. My *Lonicera* material was of the *Lonicera morrowii* – *L. tartarica* complex, i.e., *L. morrowii* A.Gray., *L. tatarica* L., or their hybrid *L. x bella* Zabel. In SE New Hampshire, the two species are commonly intermixed and appear to hybridize freely.

The individual plants used in this experiment had been used to culture soil in another experiment; they were all grown from cuttings in the summer of 2007 and planted in field soil for approximately three months. After being removed from the field soil after round one of the soil-feedback experiment in November 2007 (see Chapter 1), they were placed into pots containing a peat:perlite mixture (Fafard Growing Mix F-15; Conrad Fafard, Inc., Agawam, MA) and spent the winter in an apple cooler (at approximately 1-2 °C) located at the UNH Woodman Farm, Durham, NH. At the end of April 2008, all of the plants were removed from the cooler and returned to the greenhouse. At the onset of bud-break, plants were transplanted into the pots for the experiment as described above.
Initial Biomass Measurements

The dry biomass measurements taken at the end of the experiment represented the final biomass of the individual plants. However, it was also necessary to estimate the initial biomass, as some of the variation in the final biomass may have been due to variation in initial plant biomass (Gibson et al., 1999). Dimension analysis was used to estimate the initial biomass of the individual plants. Twenty individuals (10 in the case of glossy buckthorn and winged euonymus due to insufficient supply of plants) representing a wide range of sizes were chosen to be used for the initial biomass estimates. Measurements of stem diameter, height, number of leaves, and number of branches were taken for all of these individuals. Then they were harvested, dried at 60 °F for 72+ hours, and weighed (grams). Multiple stepwise regressions with backward elimination were run for each species to determine which variables were significantly correlated with dry biomass of the plants. Each species analysis yielded significant predictor variables, i.e., stem diameter that were then measured for each of the individuals used in the experiment. Once measured, these predictor variables were then used to estimate the initial biomass of the plants used in the experiment.

Soil Selection

Field agricultural soil was used for planting, as abandoned agricultural lands in NH are more frequently invaded than forested areas (Johnson et al., 2006). The field soil was taken from the UNH Woodman Farm, was derived from glacial drift, and mapped as Windsor loamy, fine, sand. After being removed from the field, it was sieved using a 5 mm mesh and put into bins for storage until used in the experiment.
Data Analysis

The effects of competition between species were determined using competition indices (CI). Three CIs were chosen for comparison and use in this experiment: the relative competition index (RCI), the relative neighbor effect (RNE) index, and the relative interaction intensity (RII) index (Wilson and Keddy, 1986; Markham and Chanway, 1996; Armas et al., 2004; respectively). Wilson and Keddy developed the RCI in 1986, and since then it has been used extensively in the literature although not without criticism (Armas et al., 2004); I chose it because its widespread use would allow the results of this study to be compared with others with ease. RNE and RII were chosen because both indices are bound between 1 and -1. In addition, both indices allow for an unbiased comparison between the values expressed as competition and facilitation because they are symmetric around zero (Armas et al., 2004).

The RCI of a species was calculated as the change in growth due to competition in relation to the growth of the species without competition or 
\[
\frac{g_{no\,competition} - g_{w/competition}}{g_{no\,competition}}
\]
where "g" equals growth or dry biomass (calculated two ways: final and final less initial biomass were used). Using this index, all positive values indicate a competitive effect; the closer this number is to 1 the stronger the competitive effect. Facilitation results in a negative value.

RNE is similar to RCI and uses the same numerator as RCI, yet the denominator changes depending on whether the plant's growth is greater with competition or without competition. The greater of these two growth values (\(g_{no\,competition}\) or \(g_{w/competition}\)) is
placed in the denominator. Calculating the proportion of loss in relation to the greatest
growth allows for a more balanced distribution of means around zero and also gives the
index an upper limit and a lower limit (Markham and Chanway, 1996; Armas et al.,
2004). In similarity to RCI, positive values represent competition with an upper limit of
1, and negative values equate to facilitation. However with RNE, there is a lower limit of
-1. The equation for RNE is \((g_{\text{no competition}} - g_{\text{w/competition}})/g_x\), where "g" equals dry
biomass (calculated two ways: final and final less initial biomass were used) and "x" is
the greater of the two growth values in the numerator.

RII is a competition index that measures the net loss of growth relative to the net gain and
is calculated as such: \((g_{\text{w/competition}} - g_{\text{no competition}})/(g_{\text{w/competition}} + g_{\text{no competition}})\), where "g"
equals growth or dry biomass (calculated two ways: final and final less initial biomass
were used). Competition is negative using this index with -1 as the lower limit, whereas
facilitation is positive with 1 as the upper limit. For more information and a comparison
of the indices, see Armas et al. (2004).

CIs were calculated for every individual competing interspecifically within a block. For
example, glossy buckthorn was in competition with each of the three native species in
every one of the seven blocks. The biomass of glossy buckthorn in competition with
each of the natives in a block would be compared separately to the biomass of the glossy
buckthorn individual growing alone in that block. Each of the individual CIs (RCI, RNE,
RII) was calculated for each species. In addition, the indices were calculated one of two
ways: 1) with final biomass only or 2) with final minus the estimated initial biomass.
The latter measure accounted for the variation in mass at the beginning of the experiment. Every individual in interspecific competition competed with just one individual of a different species per pot, *i.e.*, one glossy buckthorn matched with one arrowwood, which yielded two competition measurements, one measuring glossy buckthorn’s effect on arrowwood and the other measuring arrowwood’s effect on glossy buckthorn. Yet, each of these was calculated using three different indices equating to 3 CIs/interspecific individual. In addition, the CIs were calculated using two different types of biomass for 6 competition measurements/interspecific individual per block. There were 7 blocks; therefore, each individual interspecific competition treatment, *i.e.*, glossy buckthorn X arrowwood or arrowwood X glossy buckthorn had 42 CIs associated with it. When calculating competition for a species (glossy buckthorn matched with each one of the natives), there would be 3 glossy buckthorn individuals competing interspecifically in each block for a total of 3 competition measurements (*n*=3 for each of the CIs) per block. Most of the analyses were processed with *n*=21, which is the 3/block multiplied by the number of blocks.

After calculating all the CIs for each species, the data were checked for normality. Then *competitive effect* and *competitive response* were determined for each species. To measure the differences in competitive effect, the CIs for each species in competition with the target species were used. For example, if arrowwood were the target species, the CIs of glossy buckthorn, bush honeysuckle, and winged euonymus in competition with arrowwood were used in the subsequent statistical analyses (3 competition measurements/block X 7 blocks; *n*=21 for each species. These CIs measure the effect of
competition from arrowwood on the growth of each of the other species. Statistical analyses were run for all three CIs (RCI, RNE, RII) and both biomass measurements (see below). In contrast, to measure competitive response, the CIs of the target species in competition with each of its neighbors were used. Again if arrowwood was the target species, the CIs of arrowwood in competition with glossy buckthorn, bush honeysuckle, and winged euonymus would be used. These CIs measure the effect of competitors on arrowwood's growth.

ANOVAs with a nested design (SYSTAT 12; Systat Software, Inc., Chicago, IL) were run to test whether competitive effects and competitive responses varied between invasive and native species. The measurement of the competition indices was the dependent variable, and the independent variables were species type (invasive/native) and species (i.e., arrowwood) nested in species type. These models were run with each CI as the dependent variable, which included final and final-less-initial biomass calculations of the CIs. If there were a significant difference among the different species in these groups, a one-way ANOVA (species as independent variable) was run and Tukey's tests ($\alpha = 0.05$) were used to distinguish where those differences were (Zar, 1999).

Root to shoot ratios (R:S) were calculated for all species in each competition treatment at the end of the experiment using the masses of the separate fractions of dry biomass, above and below ground. The R:S were compared in order to determine whether competition had any affect on the root growth in relation to the shoot growth of the individual plant species and also to see if there was a general difference between invasive
and native species or among species. A one-way ANOVA (n=7/species) using just the R:S of the plant species with no competition was used to test the difference in root to shoot ratios between native and invasive species, as well as among the individual species. R:S was the dependent variable and species type/species was the independent variable. Individual one-way ANOVAs were also performed for each species to compare R:S among competition treatments i.e. no competition, intraspecific, or with each of the competitors (competition treatment was the independent variable and R:S the dependent; n=7 per competition treatment per species). In addition, Tukey's tests (α = 0.05) were used to compare R:S among species and among competition types if significant differences (P≤ 0.05) were obtained with the ANOVAs (Zar, 1999).

Overall biomass production among species and between natives and invasives was compared using a one-way ANOVA, but only using the biomass of the species when it was growing alone without competition (n=7/species). This was not done using a nested design due to the low sample size. Means were calculated using the initial, final, and final less initial biomass for each of the plant species under no competition. To compare differences among species, a one-way ANOVA was run with biomass as the dependent variable and species as the independent variable. Tukey's tests (α = 0.05) were used to distinguish differences between means (Zar 1999). All analyses were carried out on SYSTAT, versions 10 and 12 (Systat Software, Inc., Chicago, IL).
RESULTS

Overall Biomass Production

Grown without competition, native species had significantly higher final biomass than invasives (38.14 g natives vs. 27.33 g invasives; P=0.03). However when initial estimated biomass and final-less-initial biomass were assessed, there was no significant difference in overall biomass between invasive and native shrubs (Table 2.1). At the start of the experiment, common elderberry had the highest biomass (13.36 g) and winged euonymus had the lowest (1.24 g). At the end of the experiment, after growing for three months without competition, winged euonymus still had the lowest biomass (3.77 g), but arrowwood, glossy buckthorn, meadowsweet, and bush honeysuckle all had similarly large biomasses (36.61 g - 47.05 g). When initial biomass was accounted for in the final biomass, the same four species had the largest biomasses (25.60 g - 37.97 g), arrowwood always with the most. Winged euonymus and common elderberry both had the smallest biomasses (2.53 g and 10.34 g, respectively).

TABLE 2.1. Mean total dry biomass (g) of six shrub species grown in pots in a greenhouse with no competition. Initial, final, and final-less-initial biomass are presented. Significance of biomass difference among all species was determined by P≤0.05 (one-way ANOVA with biomass as the dependent variable and species as the independent). Means sharing the same letter are not significantly different from one another based on Tukey's test (α=0.05). n=7 per species grown alone.
**Competitive Effect**

Invasive shrub species had a greater competitive effect on native shrubs than natives had on invasives. Specifically, invasive shrubs were significantly better at reducing the growth of their native competitors than native shrub species were at reducing the biomass of their invasive neighbors (nested ANOVA; P < 0.001). This was true over all three competition indices and with both growth measures – final biomass and final-less-initial biomass (Table 2.2). Using the means for RII with final biomass, where competition is indicated by negative values and is maximal at -1, the mean value of the effect of native species on invasives was -0.035; the mean effect of invasives on natives was -0.158. This pattern can be seen for all CIs, varying slightly in the magnitude of the difference. Despite strong overall differences in competitive ability between natives and invasives, there were no significant differences in competitive ability among species within the invasive group or within the native group (Table 2.2; ANOVA; P = 0.40).

**Competitive Response**

Competitive response is a measurement of the growth response (increasing, decreasing, stable) of a species to competition from its neighbor. Native species growth was affected more negatively by competition than invasive species growth, meaning that native species had a greater negative response to competition than the invasive shrubs for all indices and both types of biomass (Table 2.3). In addition, when the native and invasive labels were ignored, the competitive response varied significantly among the six species for all CIs (Table 2.3). The significance of these differences did not change when using final biomass or final less initial biomass to calculate the CIs. Arrowwood, common
elderberry, meadowsweet, glossy buckthorn, and bush honeysuckle were all affected similarly by competition. In contrast, winged euonymus was facilitated by its neighbor in all cases. This indicates that winged euonymus accumulated more biomass when growing with other species than when growing alone.

### TABLE 2.2

<table>
<thead>
<tr>
<th></th>
<th>Final Biomass</th>
<th>Final Less Initial Biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RCI</td>
<td>RNE</td>
</tr>
<tr>
<td><strong>P-values</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All native vs. all invasive</td>
<td>≤0.001</td>
<td>≤0.001</td>
</tr>
<tr>
<td>Within invasive &amp; native groups</td>
<td>0.916</td>
<td>0.927</td>
</tr>
<tr>
<td><strong>Means</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All invasives</td>
<td>0.253</td>
<td>0.257</td>
</tr>
<tr>
<td>All natives</td>
<td>-0.030</td>
<td>0.060</td>
</tr>
<tr>
<td><strong>Invasives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bush Honeysuckle</td>
<td>0.285</td>
<td>0.288</td>
</tr>
<tr>
<td>Glossy Buckthorn</td>
<td>0.256</td>
<td>0.256</td>
</tr>
<tr>
<td>Winged Euonymus</td>
<td>0.219</td>
<td>0.229</td>
</tr>
<tr>
<td><strong>Natives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arrowwood</td>
<td>-0.019</td>
<td>0.068</td>
</tr>
<tr>
<td>Common Elderberry</td>
<td>-0.007</td>
<td>0.076</td>
</tr>
<tr>
<td>Meadowsweet</td>
<td>-0.076</td>
<td>0.035</td>
</tr>
</tbody>
</table>

**Notes:** The names of the competition indices have been shortened: RCI=relative competition index, RNE=relative neighbor effect, RII=relative intensity index.
TABLE 2.3. Competition indices of the competitive response of native and invasive competitors grown pairwise in pots in a greenhouse. Data analyzed using an ANOVA with a nested design (species nested in species type; native/invasive). The competitive response for each invasive species was calculated by taking the mean of the CIs for that invasive species as it was affected by the three native species (3n/block; 7 blocks; n=21/species). The competitive response for each native species was calculated by taking the mean of the CIs for that native species as it was affected by the three invasive species (3n/block; 7 blocks; n=21/species). Significance was given to all P-values ≤ 0.05. Letters were used to show differences among species determined in the Tukey’s tests (α =0.05); means with the same letters are not significantly different.

<table>
<thead>
<tr>
<th></th>
<th>Final Biomass</th>
<th>Final Less Initial Biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RCI</td>
<td>RNE</td>
</tr>
<tr>
<td>P-values</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All native vs. all</td>
<td>≤0.001 ≤0.001 ≤0.001</td>
<td></td>
</tr>
<tr>
<td>invasive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All invasives</td>
<td>-0.030 0.060 -0.035</td>
<td>-0.138 0.079 -0.057</td>
</tr>
<tr>
<td>All natives</td>
<td>0.253 0.257 -0.158</td>
<td>0.263 0.330 -0.246</td>
</tr>
<tr>
<td>Invasives</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bush Honeysuckle</td>
<td>0.166a 0.213a -0.129a</td>
<td>0.017a 0.246a -0.172a</td>
</tr>
<tr>
<td>Glossy Buckthorn</td>
<td>0.180a 0.188a -0.118a</td>
<td>0.238a 0.252a -0.180a</td>
</tr>
<tr>
<td>Winged Euonymus</td>
<td>-0.413b -0.214b 0.142b</td>
<td>-0.669b -0.262b 0.179b</td>
</tr>
<tr>
<td>Natives</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arrowwood</td>
<td>0.370a 0.370a -0.231a</td>
<td>0.411a 0.410a -0.260a</td>
</tr>
<tr>
<td>Common Elderberry</td>
<td>0.176a 0.189a -0.177a</td>
<td>0.123a 0.325a -0.319a</td>
</tr>
<tr>
<td>Meadowsweet</td>
<td>0.213a 0.213a -0.125a</td>
<td>0.256a 0.262a -0.158a</td>
</tr>
</tbody>
</table>

Notes: The names of the competition indices have been shortened: RCI=relative competition index, RNE=relative neighbor effect, RII=relative intensity index.

**Root to Shoot Ratios**

Root to shoot ratios (R:S) of individuals grown without competition did not differ between native and invasive species (GLM; P=0.29). When invasive status was ignored, however, there was a difference in R:S among species grown without competition (GLM; P<0.001; Table 2.4). All of the species had R:S greater than 1, meaning that all species allocated more resources to their roots. Certain species, however, had root to shoot ratios very close to 1, i.e., winged euonymus (1.009); these species did not apportion as much biomass to their roots as glossy buckthorn, which had the highest root to shoot ratio (2.43).
When comparing the R:S for the individual species under the different competition treatments (no competition, intraspecific competition, and interspecific competition), only arrowwood showed a significant effect on R:S for the different treatments (Table 2.5). Arrowwood's R:S was smallest with no competition, as well as in competition with winged euonymus and largest in competition with bush honeysuckle. Root to shoot ratios are known to increase in the presence of root competition. I hypothesized that the differences in arrowwood R:S seen were due to arrowwood the competitive effect of the species arrowwood was grown with. Specifically, I expected that bush honeysuckle, the competitor causing arrowwood to allocate most heavily to root, to have the greatest competitive effect on arrowwood, and winged euonymus to have the least. I calculated the effect of each invasive on arrowwood to see if there was a difference (Table 2.6). All of the invasive species, however, had a similar effect on arrowwood's growth while in competition.

<table>
<thead>
<tr>
<th>Competition Treatments</th>
<th>No Competition</th>
<th>Intra Bush Honeysuckle</th>
<th>Glossy Buckthorn</th>
<th>Winged Honeysuckle</th>
<th>Means with the same letters are not significantly different (Tukey's tests, α = 0.05).</th>
</tr>
</thead>
<tbody>
<tr>
<td>R:S Mean</td>
<td>1.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.57&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.48&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

### TABLE 2.4. Root to shoot ratios (R:S) for six different species. Interspecific differences in R:S analyzed using a one-way ANOVA with R:S as the dependent variable and species as the independent variable. For comparison of all species, means were calculated from the biomass of individuals grown without competition (n=7 per species). Significance was given to P-values < 0.05. Means with the same letters are not significantly different (Tukey's tests, α = 0.05).

<table>
<thead>
<tr>
<th>Species</th>
<th>R:S Mean among species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arrowwood</td>
<td>1.496&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Common Elderberry</td>
<td>1.786&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Meadowsweet</td>
<td>1.145&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glossy Buckthorn</td>
<td>2.534&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bush Honeysuckle</td>
<td>1.523&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Winged Euonymus</td>
<td>1.082&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

### TABLE 2.5. Root to shoot ratios (R:S) for arrowwood, the only species with significant differences in R:S among competition treatments, i.e., no competition, intraspecific (one-way ANOVA with R:S as dependent variable and the competition treatments as the independent variable; P<0.05). Means with the same letters are not significantly different (Tukey's tests, α = 0.05).

<table>
<thead>
<tr>
<th>Competition Treatments</th>
<th>No Competition</th>
<th>Intra Bush Honeysuckle</th>
<th>Glossy Buckthorn</th>
<th>Winged Euonymus</th>
</tr>
</thead>
<tbody>
<tr>
<td>R:S Mean</td>
<td>1.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.57&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.48&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

68
TABLE 2.6. A comparison of the effect that competitors had on the arrowwood. Significance was given to all P-values ≤ 0.05 (one-way ANOVA with CIs as the dependent variable and each invasive as the independent variable). Means were obtained by using the CIs for arrowwood when in competition with the invasive species. Final and final-less-initial biomass were used to calculate the indices (n=21 total).

<table>
<thead>
<tr>
<th>Competitors</th>
<th>Final Biomass</th>
<th></th>
<th></th>
<th>Final Less Initial Biomass</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RCI</td>
<td>RNE</td>
<td>RII</td>
<td>RCI</td>
<td>RNE</td>
<td>RII</td>
</tr>
<tr>
<td>Bush Honeysuckle</td>
<td>0.327</td>
<td>0.327</td>
<td>-0.199</td>
<td>0.403</td>
<td>0.403</td>
<td>-0.260</td>
</tr>
<tr>
<td>Glossy Buckthorn</td>
<td>0.369</td>
<td>0.369</td>
<td>-0.226</td>
<td>0.434</td>
<td>0.434</td>
<td>-0.267</td>
</tr>
<tr>
<td>Winged Euonymus</td>
<td>0.414</td>
<td>0.414</td>
<td>-0.270</td>
<td>0.396</td>
<td>0.396</td>
<td>-0.259</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.293</td>
<td>0.293</td>
<td>0.299</td>
<td>0.768</td>
<td>0.768</td>
<td>0.971</td>
</tr>
</tbody>
</table>

Notes: The names of the competition indices and plant species have been shortened: RCI=relative competition index, RNE=relative neighbor effect, RII=relative intensity index.

DISCUSSION

Competitive Effects

In support of my hypothesis and the conclusions of Vila and Weiner (2004), the invasive shrub species in this study were better competitors than their native counterparts.

Although the mechanisms underlying the competitive abilities of these native and invasive shrubs were not identified, I offer four possible explanations for the differences seen here.

First, invasive shrubs may be more efficient at using limited resources than native species. This would enable the invasives to use fewer resources to accomplish the same biological objectives as the natives, and may increase their growth rates, survival, and fecundity in relation to the natives. Different plant species are known to vary in their nutrient use efficiencies (Chapin, 1980). Furthermore, recent studies have revealed that some invasive shrubs have higher net CO₂ assimilation rates and can photosynthesize more efficiently than natives (Pattison et al., 1998; Stewart and Graves, 2006).
The second possible explanation for an invasive species' better competitive ability is related to resource allocation. Tilman (1988) determined that the competitive ability of a plant depends on the proportional allocation of biomass to leaf, root, and shoot. If this were the mechanism enabling invasives to be competitively superior, the invasive species should have shown different morphologies from that of the native species. However all species, native and invasives, had greater root biomass than shoot biomass, and arrowwood (native) and bush honeysuckle (invasive) had similar R:S ratios. Therefore, resource allocation is probably not responsible for these invasives being better competitors than the native species.

Allelopathy may also play a role in the invasive species' superior competitive ability. Allelopathy is a method of interference competition by means of chemicals released into the soil (Weir, 2007). These chemicals can directly interfere with neighboring plants' growth or inhibit beneficial soil fungi (Callaway et al., 2008; Cipollini et al., 2008; Weir, 2007; Wolfe et al., 2008). Cappuccino and Arnason (2006) determined that invasive species were associated with a greater amount of unknown secondary chemicals that could potentially harm plant neighbors. Yet it is highly suspect that all of the invasives competed using allelochemicals, as species like winged euonymus actually grew more in the presence of a competitor (same species or different species) but grew poorly alone.

Lastly, Tilman (1988) states that simply having less herbivory and disease would enable a species to become a better competitor. When plant species are transported from their native range to a new area, the species may leave their natural predators behind; this
includes soil pathogens (Elton, 1958; Mitchell and Power, 2003; Callaway et al., 2004). The idea of a reduction in natural enemies for non-native species and a subsequent increase in abundance and spread of those species is the basis of the enemy release hypothesis (ERH; Elton, 1958; Mack, 2000; Keane and Crawley, 2002; Mitchell and Power, 2003; DeWalt et al., 2004). Disease and herbivory reduces the resource use efficiency of the target plant, raising its equilibrium resource level, or $R^*$, a measure of its competitive ability (Tilman, 1988). Although my competition study took place in a greenhouse, I was not able to exclude all pathogens and herbivores, especially those in the soil. The foliar pests powdery mildew and spider mites were present for portions of the experiment before being sprayed. Therefore, a dearth of enemies is a plausible mechanism for invasive superior competitive ability.

Implications for Shrubland Habitat

If the results of this study are also true for the field and invasive shrubs are competitively superior to native species under natural conditions, what would be the implications of this dynamic for our early-successional habitats? Tilman (1988) demonstrated that good competitors will eventually exclude poorer competitors from the community; this may happen in successional systems over a long period of time. The disappearance of native plant species from thicket habitats could indirectly affect many different types of species, including the New England cottontail (Litvaitis, 1993). However, perhaps over time native plants will adapt to competition with invasive species and acquire new defenses against allelopathy and parasitism. At the same time, invasive species may develop a
greater amount of harmful associations with herbivores and diseases. A combination of these two scenarios might enable species coexistence (Tilman, 1988).

Is Parasitism at work?

Contrary to what much of the literature claims (Goldberg and Fleetwood, 1987; Crawley, 1990; Gurevitch et al., 1990), the degree to which a species can competitively affect its neighbor is not necessarily positively correlated with its size. Studying competition in an old-field habitat, Gurevitch et al. (1990) determined that species with greater biomass had a stronger competitive effect on their neighbors and were also least affected by competition from their neighbors. Winged euonymus, however, had the smallest biomass (final and final-less-initial) of all the species used in this study (Table 2.1); yet, it was able to suppress native species growth to the same degree as bush honeysuckle and glossy buckthorn, two species with substantial biomasses (Table 2.2). In addition, winged euonymus achieved greater biomass growth in competition with a neighbor of the same or different species than it did in when grown without competition. The presence of a neighbor actually facilitated winged euonymus’ growth (Table 2.3).

When in competition, winged euonymus harmed its neighbors and, at the same time, its growth was facilitated. Silvertown and Charlesworth (2001) aptly described similar relationships as parasitism. Although this study did not investigate the mechanisms behind winged euonymus’ competitive ability, other studies have explored the potential for plant species to indirectly parasitize their neighbors through mychorrhizal associations in the soil (Marler et al., 1999). Watkins et al. (1996) and Chiariello et al.
(1982) showed that mycorrhizal fungi have the capability of moving resources such as phosphorus and carbon through the soil and from plant to plant (Chiariello et al., 1982; Watkins et al., 1996). Furthermore, Marler et al. (1999) demonstrated that spotted knapweed (*Centaurea maculosa* Lam.), an invasive forb commonly found in the northwestern United States, may parasitize its native neighbors indirectly via mycorrhizal interactions. Perhaps mycorrhizal fungi also enable winged euonymus to indirectly garner nutrients from its neighbor's roots.

**Comparing Indices**

In general, all competition indices yielded similar results with minor variation among groups. Competitive effect and competitive response measurements were similar in order and magnitude for all indices (Tables 2.2 & 2.3). However, the literature states that the relative competition index (RCI) may produce exaggerated negative values due to lack of a lower limit (Markham and Chanway, 1996; Armas et al., 2004). I found this to be true in comparing RCI to the relative neighbor effect index (RNE), which has a lower limit. For example, the competitor effect for the native species was slightly negative, indicating facilitation when assessed by RCI (with final biomass) (Table 2.2). Yet, when assessed with RNE, the same data generated positive values, corresponding to a small competitive effect. RII was the easiest index to read because negative values expressed by the index represent competition and positive values equate to facilitation. Therefore, a negative effect on growth corresponds to a negative number and vice versa, whereas for the other two indices, RCI and RNE, the opposite is true.
Experimental Limitations and Future Research

Greenhouse experiments allow for a greater degree of control by reducing external variability and enabling researchers to assess the effects of specific treatments on target variables (Freckleton and Watkinson, 2000). Due to the nature of their artificial design, conclusions drawn from greenhouse experiments should not be used to explain dynamics in the field (Gibson et al., 1999). However, greenhouse studies can be used to generate results that can then be assessed under natural conditions (Freckleton and Watkinson, 2000). As shrubs are perennial plants that have long lifespans, a study of the competitive interactions of these species over a longer period of time would be beneficial to our understanding of these species and their competitive potential. This greenhouse study lasted for only three months. In addition, studies examining the competitive effect of invasives and natives in differing densities would provide information about possible changes in the magnitude of competitive response and effect as species grow in abundance in the community. Research addressing the effects of competition on invasive and native shrub fecundity, seedling survival, and germination rates would also help to connect these competitive interactions to the overall success of a species.

CONCLUSION

When managing shrubland habitat, it is essential to understand the ecosystem processes that contribute to the structure and function of the system, as well as to recognize the mechanisms responsible for its degradation. In this greenhouse study, I found that invasive, non-native shrub species were better competitors than the native shrubs. Additionally, I established that the size of a species does not determine its competitive
ability. The shrub species with the smallest initial and final biomass had the same effect on competitors as the species with the largest biomass. For the most part, root to shoot ratios were not altered by the different competition treatments but did differ among species. All species put more resources into their roots, as root competition was most likely predominant in this study. In order to properly restore, create, and manage native early-successional habitat in New England, we must understand what drives a non-native species' ability to invade. This foundational study determined that one of these mechanisms may be an invasive species' competitive edge.
CHAPTER 3

DO INVASIVE AND NATIVE SHRUBS GROW DIFFERENTIALLY IN THICKET HABITATS?
INTRODUCTION

Previous research has shown that invasive species are better competitors than native species (Vila and Weiner, 2004). In addition, invasive species may also avoid herbivory (Elias et al., 2006) and a majority of pathogens in their new range (Mitchell and Power, 2003; Klironomos, 2002). All of these factors could lead to greater growth, as well as superior survivorship and fecundity for invasive shrubs in relation to their native counterparts. Furthermore, a combination of these factors may facilitate invasion in shrubland habitats. The purpose of this field study was to assess whether there was a difference in the annual growth of native and invasive shrub species in an early-successional field in southeastern New Hampshire.

Specific objectives of the study were to 1) compare shoot length (cm) and dry biomass (g) of annual extension growth of two invasive and four native shrub species and 2) to measure the density of the different species in the study area. I hypothesized that the invasive shrubs would have greater annual biomass and new growth in relation to the native species. In addition, the density of the two invasives should be greater than the native shrubs.

METHODS

Site Description

This study was conducted during the month of August 2008 in an early-successional field (~1 ha; 43.1338° north latitude, 70.9681°, east longitude) in Lee, New Hampshire.
Average annual rainfall in Lee is approximately 107 cm. The average annual high temperature is 28.4 °C, and the low is -10.6 °C. The field is situated behind a residence and is bordered by grassland on two sides and forest on the others. The area is managed by the current owner and has been mowed using a flail mower every year in October, most recently in 2007. A small mowed trail transects the field in the northwestern corner. The site was chosen for its homogeneity of plant age/size class and soil type (Elmwood fine sandy loam 0-3% slope), detailed account of prior management actions, and plant community composition.

Prior to the study, the field was surveyed to determine whether native and invasive shrubs were both present (Haines and Vining, 1999; Gleason and Cronquist, 1991). The shrub community consisted of three invasive species: glossy buckthorn (*Frangula alnus* Mill.), autumn olive (*Elaeagnus umbellata* Thunb.), and multiflora rose (*Rosa multiflora* Thunb.) and five native shrub species, arrowwood (*Viburnum dentatum* L.), gray dogwood (*Cornus racemosa* Lam.), silky dogwood (*Cornus amomum* Mill.), choke cherry (*Prunus virginiana* L.), and low bush blueberry (*Vaccinium angustifolium* Aiton). All of these species commonly occur in early-successional areas in southeastern New Hampshire (Howard and Lee, 2002; Haines and Vining, 1999; Gleason and Cronquist, 1991).

*Experimental Procedure*

Sample points were selected by establishing a grid on the field with the north-south edge as the X-axis and the east-west edge as the Y-axis. A buffer of one pace (~1.5 m) was
fixed around the field so that no sample point would be located near the edge.

Coordinates for the sample points were selected from a random number chart. Pacing began at the south-west corner of the field. Eleven random points were used in the study. If a point landed in the trail, it was moved one pace in the westerly direction.

Given the likelihood of high variation in extension grown among plants, I decided that a minimum of ten individuals of each of the following species would be needed to measure invasive and native annual growth: arrowwood, gray dogwood, silky dogwood, choke cherry, glossy buckthorn, and autumn olive. Multiflora rose was not used in the study because only four individuals were identified in the field. In addition, low bush blueberry was also not included due to its prostrate growth, a different growth form than the other species. When the eleventh random point was established, ten individuals of each of the species except for arrowwood and autumn olive had been sampled. More random points were selected to assess whether this method would yield individuals of these two species. However, after fifteen more points were visited and none contained arrowwood or autumn olive individuals, a new method for sampling these species was utilized. The field was surveyed again; this time just for arrowwood and autumn olive. All individuals were flagged and marked with a number beginning with 1. If the individual was already accounted for by the random point method, it was not flagged. Five individuals of arrowwood and autumn olive were selected at random by choosing their number from a random number chart. These individuals were then used in the study.
At every random point, individuals of each of the shrub species were identified. Some species were not present at every point (see above). New growth of one stem (cm; which included only this year’s growth), total growth of the same stem (cm; which was this year’s growth plus any additional growth from previous years), and distance to the random point were measured for one individual of each species closest to the random point. For the individuals chosen randomly by the tagging method (see above), only growth measurements were taken because there was no point from which to measure distance (n=10, 5 arrowwood, 5 autumn olive). After recording all measurements, the new growth from each individual was clipped, bagged, tagged, and brought to a forest ecology laboratory at the University of New Hampshire. All plant biomass was dried (60°C) for 72 hours then weighed (g).

Data Analysis

All data were checked for normality. The dry biomass of the annual growth clipped in the field was compared for invasive versus native species using a nested ANOVA. Dry biomass (g) was the dependent variable (n=10 for each species), as well as species type (invasive or native) and species (i.e., arrowwood, autumn olive; total of 6 species, 2 invasive and 4 native) nested in species type were the independent variables. If the P-value of the species nested in species type was less than 0.05, then a Tukey’s test (α=0.05) was performed to identify significant differences among individual species' means. The same nested general linear model was used for new growth (cm; n=10 for each species). New growth was the dependant variable and the independent variables
were the same as above. Tukey’s tests were used in the same manner as in the dry biomass analysis (Zar, 1999).

Density of each shrub species in the field was obtained by first calculating the mean distance (cm) of each of the species from the random points using the distance measurements taken in the field (n=10 for all species except for arrowwood and autumn olive n=5; see Experimental Procedure). This resulted in one mean for each species, which will be referred to here as “D”. Using “D” for each species, the land area of each species was calculated with the following formula: \( A = (2D)^2 \). This yielded shrub density per square foot. In order to get shrub density per hectare, the number of square feet in a hectare (107639 \( f^2 \)) was divided by “A”.

**RESULTS**

Invasive shrub species had significantly greater dry biomass and new growth length than the native shrubs (Table 3.1). Autumn olive had the greatest of both dry biomass and length (18.65 g and 97.40 cm). Gray dogwood had the smallest biomass and new growth (1.65 g and 16.50 cm). However, gray dogwood was the most abundant species in the study site with a density of 3.307 individuals per hectare. Arrowwood and autumn olive had the lowest densities at 0.206/ha and 0.314/ha, respectively. This reflects the difficulty of finding these species in the study area.
TABLE 3.1. Dry Biomass (g) and height (cm) of new annual growth, as well as density per hectare for six shrub species in an early-successional field. Means and P-values for dry biomass and new growth were obtained using separate nested ANOVA (species nested in species type; n=10/species). Density means were calculated using the formula: A=(2D)^2 and dividing the number of feet² in a ha by A. Significance was determined as P<0.05. For individual species, means with different letters are significantly different (Tukey's test; α=0.05).

<table>
<thead>
<tr>
<th>Invasive</th>
<th>Dry Biomass (g)</th>
<th>New Growth (cm)</th>
<th>Density (per ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn Olive</td>
<td>18.65^a</td>
<td>97.40^a</td>
<td>0.314</td>
</tr>
<tr>
<td>Glossy Buckthorn</td>
<td>5.96^b</td>
<td>62.50^b</td>
<td>0.885</td>
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<tr>
<td>Native</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arrowwood</td>
<td>3.30^b</td>
<td>30.50^c</td>
<td>0.206</td>
</tr>
<tr>
<td>Choke Cherry</td>
<td>2.49^b</td>
<td>17.09^c</td>
<td>1.003</td>
</tr>
<tr>
<td>Silky Dogwood</td>
<td>4.90^b</td>
<td>28.36^c</td>
<td>1.465</td>
</tr>
<tr>
<td>Gray Dogwood</td>
<td>1.65^b</td>
<td>16.50^c</td>
<td>3.307</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.012</td>
<td>0.007</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

In accordance with the results of previous literature (Stewart and Graves, 2006; Pattison et al., 1998), the invasive shrub species in this study had greater annual biomass and growth extension than the native species. Stewart and Graves (2006), as well as Pattison et al. (1998) attributed higher growth rates of invasive species to higher net assimilation rates, meaning that the invasive shrubs were able to take in more CO₂ from the environment to use for photosynthesis than the natives. In addition, Pattison et al. (1998) states that invasive shrubs in Hawaii were able to obtain and use the resources from light with less energy than the native species, i.e. at the same dark respiration rate, invasive species photosynthesized more than the native species. Unfortunately, photosynthetic rates were not measured in this field study.
Other factors that may have led to increased growth of invasive versus native shrubs are a lack of disease and herbivory, as well as certain site characteristics. Previous studies have shown that invasive species have a reduced pathogen load as compared with native species (Mitchell and Power, 2003; Klironomos, 2002). In fact, some of the choke cherry individuals in this study demonstrated what may have been signs of disease with small, brightly colored, misshapen red leaves. Pathogens can cause decreases in growth rates, fecundity, and survivorship (Agrios, 2005; Sinclair and Lyon, 2005), as the plant must allocate more resources toward defense against the disease. Invasive species may also have increased growth due to the avoidance of herbivory predators such as white-tailed deer (Odocoileus virginianus; Elias et al., 2006). Nonetheless, I noticed deer browse on all species in the study including both of the invasive species. Finally because the study took place at only one site, there was no replication. Given the initial criteria for finding a site i.e. homogeneity of age and size class, it was difficult to find early-successional areas that met all the specifications. However, the criteria had to be met in order to reduce the variation that would have been associated with individuals of different ages and sizes, as well as with varying soil properties which could affect plant growth in different ways. The limitations of a non-replicated study are that the results cannot be extended to other similar early-successional areas because it is possible that they could be attributed to certain site characteristics that are unique to the study area. For example, Sanford et al. (2003) examined the growth rates of arrowwood, glossy buckthorn, silky dogwood, and autumn olive at two sites in Sunderland, Massachusetts. Although, glossy buckthorn had the highest relative growth rate, autumn olive had the lowest growth rate
out of all the species (Sanford et al., 2003). These findings contradict the results seen at the study site in Lee, NH.

CONCLUSION

In a managed early-successional field in southeastern New Hampshire, invasive shrub species showed greater biomass and new growth over one growing season than did native shrub species. These results may support the idea that invasive species are better competitors than native shrubs (Chapter 2). If invasive species can acquire more above-ground biomass annually, then over time they will be larger than the native species. This disparity in size could enable invasive shrubs to obtain more light and thus photosynthates, to be used for additional growth, herbivore defense, or for reproductive material such as flowers, fruits, and seeds (Pratt et al., 2005). Eventually, the larger and more fecund invasive species could competitively exclude their native counterparts (Tilman, 1988). The ability to utilize light resources efficiently (Stewart and Graves, 2006; Pattison et al., 1998) and to escape predators/pathogens (Elías et al., 2006; Mitchell and Power, 2003; Klironomos 2002) may contribute to the greater annual growth of invasive species. However, the results seen here may also be attributed to certain site specific factors, as the study was not replicated.
Invasive shrubs have the potential to drastically alter native early-successional ecosystems, and in doing so, they negatively affect the flora and fauna living in these habitats. Management of these species is of utmost concern to a majority of natural resource leaders, scientists, and practitioners. However, in order to control the current invasive species and prevent future invasions, we must identify and understand the factors that contribute to a non-native species ability to invade. In two greenhouse studies, soil feedback and competition were assessed as possible mechanisms facilitating the invasion of non-native shrubs into thicket habitats.

Although soil feedback differed among species, the trend seen in previous research, that natives were more negatively affected by soil pathogens, was not seen here. In this study, soil feedback was species specific, and therefore, is likely not a factor which influences invasion in early-successional habitats. Furthermore, land-use history altered the magnitude of the effect of soil feedback for two of the three species with significant soil feedback results; the effects were greater in non-tilled forest soil versus tilled agricultural soil. Soil community biomass and composition was evaluated using phospholipid fatty acid analysis (PLFA). PLFA revealed differences between the non-tilled and tilled soil communities’ biomass and composition, as well as among species in both soil types. While soil feedback is likely not a mechanism allowing non-native shrubs to invade, competition may be.

Invasive shrubs were better competitors and were less affected by competition than their native neighbors. A small field study revealed that invasive shrubs accumulate more
annual biomass than native shrubs, which could be an indication of their superior competitive ability. As this study was conducted at only one field site and the competition experiment in a controlled greenhouse environment, the conclusions drawn here should be tested under natural conditions at several field sites.

Although each experiment focused on a different factor potentially influencing shrubland invasion, general conclusions can be drawn which incorporate the results of both studies. For example in the competition experiment, winged euonymus was relatively unaffected by competition and actually grew better when grown with a neighbor. As stated previously, this ability to grow well in the presence of a competitor may be a result of winged euonymus utilizing soil microbes to parasitize neighboring plants. The results from the PLFA in the soil feedback experiment show that winged euonymus had the most total microbial biomass in the agricultural soil (the type of soil used in the competition experiment). Perhaps the greater amount of microbial biomass is somehow related to this species' ability to grow well in competition, or parasitize its neighbors. In addition, the soil feedback experiment showed no trend linking plant-soil feedback to invasive species. Yet, invasive species were shown to be better competitors than the native shrubs in Chapter 2. Given the importance of the soil community to plant growth, one would have expected soil feedback to play a more important role in invasive shrubs competitive ability. However, the conflicting results of the two studies show otherwise.
LIST OF REFERENCES


## Appendix A

### Table of Greenhouse Products

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Manufacturer</th>
<th>Active Ingredient</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latron</td>
<td>Loveland Industries; Greeley, CO</td>
<td>modified phthalic glycerol alkyd resin</td>
<td>surfactant</td>
</tr>
<tr>
<td>Ovation</td>
<td>Scotts-Sierra Crop Protection Company; Marysville, OH</td>
<td>Clofentezine</td>
<td>spider mite control</td>
</tr>
<tr>
<td>Endeavor</td>
<td>Syngenta Crop Protection Canada, Inc.; Guelph, ON</td>
<td>Pymetrozine</td>
<td>aphids</td>
</tr>
<tr>
<td>Capsil</td>
<td>Aquatrols; Paulsboro, NJ</td>
<td>Blend of Polyether-polymer and nonionic surfactant</td>
<td>surfactant</td>
</tr>
<tr>
<td>Floramite</td>
<td>Uniroyal Chemical Company, Inc.; Middlebury, CT</td>
<td>Bifenazate</td>
<td>spider mite control</td>
</tr>
<tr>
<td>Shuttle</td>
<td>Arysta LifeScience North America Corporation; Cary, NC</td>
<td>Acequinocyl</td>
<td>spider mite control</td>
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<tr>
<td>MilStop</td>
<td>BioWorks; Victor, NY</td>
<td>Potassium bicarbonate</td>
<td>powdery mildew</td>
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<tr>
<td>Phyton27</td>
<td>Source Technology Biologicals, Inc.; Edina, MN</td>
<td>Copper Sulphate Pentahydrate</td>
<td>powdery mildew</td>
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<tr>
<td>Judo</td>
<td>OHP; Mainland, PA</td>
<td>Spiromesifen</td>
<td>spider mite control</td>
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</tbody>
</table>