THE ROLE OF PLANT-SOIL FEEDBACK IN EXOTIC PLANT INVASION: SOIL TYPE, BIOTIC OR ABIOTIC FACTORS?

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

By

KELLY D. SCHRADIN
B.S., Wilmington College, 2010

2012
Wright State University

WRIGHT STATE UNIVERSITY

SCHOOL OF GRADUATE STUDIES

March 28, 2012

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Kelly D. Schradin ENTITLED The role of plant-soil feedback in exotic plant invasion: soil type, and biotic or abiotic factors? BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science

Don Cipollini, Ph.D.
Thesis Director

David Goldstein, Ph.D., Chair
Department of Biological Sciences

Committee on
Final Examination

Don Cipollini, Ph.D.

James Amon, Ph.D.

James Runkle, Ph.D.

Andrew T. Hsu, Ph.D.
Dean, School of Graduate Studies

ABSTRACT

Schradin, Kelly D. M.S., Department of Biological Sciences, Wright State University, 2012.
The role of plant-soil feedback in exotic plant invasion: soil type, and biotic or abiotic factors?

Plants alter soil characteristics in many ways causing changes in their subsequent growth resulting in either positive or negative feedback on their own fitness. Plants in their native ranges typically experience negative feedback from natural enemies, while feedback is often positive in invaded ranges where they escape enemies, experience new beneficial mutualisms, or bring with them a novel biochemical weapon. I conducted a fully factorial greenhouse experiment to examine plant-soil feedback in the invasive shrub *Lonicera maackii* and whether or not positive feedback may contribute to its successful invasion in southern Ohio. I also investigated whether the sign and strength of the feedback changed across two distinct soil types, and whether effects were due to shifts in biotic or abiotic soil traits by analyzing soil properties, phenolic content and microbial communities. I compared *L. maackii's* response to the related native shrub, *Diervilla lonicera,* using their conditioned soils along with soil conditioned by an unrelated native tree, *Fraxinus pennsylvanica*. I hypothesized that *L. maackii* would experience positive feedback overall in both soil types. *L. maackii* showed positive feedback in Shawnee soils, but neutral to negative feedback in Wright State soils. Growth of *L. maackii* decreased and positive feedback was eliminated with sterilization in Shawnee soil which may indicate that it had benefitted from mutualisms that were destroyed by sterilization. In Wright State soil, sterilization significantly increased

growth, suggesting *L. maackii* had been released from pathogenic organisms found in live soils. Despite this, feedback became even more negative with sterilization in Wright State soil which may be a sign that its own phytochemicals hinder its growth in the absence of biotic symbioses. *Lonicera maackii* performed similarly in its own soils and in those of *F. pennsylvanica* and *D. lonicera*, regardless of soil type. Our findings also suggest native species are controlled by negative feedbacks in their own soils. *Diervilla lonicera* displayed negative feedback overall in its own unsterilized soil regardless of soil type, but sterilization eliminated or reversed feedback relationships. Growth of *Diervilla lonicera* varied little in soils conditioned by *L. maackii* and *F. pennsylvanica* in both soil types. Our results indicate that both soil type and soil microorganisms play a large role in plant-soil feedback, yet feedback in *L. maackii* is dependent on soil type. Our evidence reveals that sign and strength of feedback can vary with soil source. This is the first study to examine plant-soil feedback in *L. maackii*, one of the most important invaders in Ohio uplands.

TABLE OF CONTENTS

LIST OF FIGURES

PAGE

LIST OF TABLES

PAGE

ACKNOWLEDGEMENTS

I would like to thank my advisor and mentor, Dr. Don Cipollini, committee members, Dr. Jim Runkle, and Dr. Jim Amon, and long time mentor and friend, Dr. Kendra Cipollini for their comments and suggestions on the manuscript. I especially thank Deah Lieurance for her infinite patience, advice and support throughout every aspect of the project. Many thanks go to undergraduates Jon Ali, Alex Woodward, and Justin Sanders for their help in harvesting the plants. I also thank the Wright State University Department of Biological Sciences and Sigma Xi Grants in Aid of Research for funding. Lastly, I would like to thank my family and friends for their constant love and encouragement throughout my journey and, most of all, my mother for the strength and independence she instilled in me.

INTRODUCTION

Invasive species can incur high economic and ecological costs and continue to threaten global biodiversity (Pimentel et. al 2000). It is estimated that 50,000 nonnative species (both plants and animals) have been introduced to the United States for various reasons: as ornamental specimens, soil erosion-control, biological pest controls, and food. As a result, biodiversity suffers as our native species become threatened with extinction (Pimentel et al. 2000). Understanding the mechanisms responsible for abundance and distribution of invasive plants may lead to different management methods of control in addition to conserving rare and endangered native species and ecosystems. Nonnative invasive plant species can negatively impact native species by reducing seed germination, growth, survival and reproduction (Callaway and Aschehoug 2000; van Wilgen et al. 2004; Thorpe 2006). Invasives can have a negative impact on native plant populations through resource competition, allelopathy, and plant-soil interactions (Callaway and Aschehoug 2000; Callaway et al. 2004a; Ehrenfeld 2006; Stinson et al. 2006; Thorpe 2006; Cipollini et al. 2008; Kueffer et al. 2007; Callaway et al. 2008; Cipollini and Dorning 2008; Cipollini et al. 2008a/b). Exotic invasives gain an advantage over their native neighbors through above-ground competition (Cipollini, K. et al. 2008) and below-ground competition (Callaway et al. 2004a; Ehrenfeld 2006; Stinson et al. 2006). For example, the invasive *Centaurea maculosa* (spotted knapweed) acquires more phosphorus than surrounding native species, giving it a competitive edge (Thorpe 2006). Some invasive species possess allelopathic compounds that can alter native communities with potentially long-lasting negative impacts on neighboring plants (Kueffer et al. 2007; Callaway et al. 2008; Cipollini and Dorning 2008; Cipollini et al. 2008a/b). Allelopathic

compounds are secondary metabolites produced by a plant that negatively impact surrounding plants, soil properties and soil organisms (Beckstead and Parker 2003; Callaway et al. 2004a/b; Reinhart and Callaway, 2004; Cipollini and Dorning 2008; Cipollini et al. 2008). These allelopathic compounds from plant tissue come from sources such as volatilization and leaf and root exudation. In fact, changes in soil properties are an increasingly recognized impact of invasive species and they may leave lasting effects in the soil (Klironomos 2002;; Agrawal 2005; Hawkes et al. 2005; Ehrenfeld 2006; Stinson et al. 2006; Cipollini and Schradin 2011).

Plant-soil feedback

Research suggests that plant-soil feedback can affect plant distributions, patterns of dominance, invasion and succession (Klironomos 2002; Callaway et al. 2004a; Kueffer et al. 2007; Mangan et al. 2010; Mangan 2010). During plant growth, the soil rhizosphere (the soil surrounding a plant's root system) develops characteristics that can have effects that feed back on the plant. These changes to soil biochemical properties include altered pH and mineral and microbial composition. For instance, modified soil characteristics surrounding an invasive species can alter populations of existing soil biota. These effects can have either positive or negative feedback on the plant's own fitness (Callaway and Aschehoug 2000; Callaway et al. 2004a; Hawkes 2005; Klironomos 2002; Mangan et al. 2010; Thorpe, 2006). To determine the direction of feedback, soil is first preconditioned by the growth of a plant species. If subsequent conspecific plants perform better in the preconditioned soil than when grown in unconditioned (soil unplanted with any plants) soil, it is considered positive feedback. Negative feedback occurs when the

conspecific plants experience decreased fitness in the preconditioned soil. Often, plants experience negative feedback in their native soils due to natural enemies (pathogens) which keep the plants' growth in check. When plants are introduced to a new area, they can sometimes modify soil organisms or nutrient cycling to their advantage. Feedback is often positive in invaded ranges because they escape these enemies, experience new beneficial mutualisms, or bring with them a novel biochemical weapon. Positive feedback resulting from both direct and indirect effects of allelopathy and changes in soil characteristics is partly responsible for nonnative plants becoming invasive in introduced areas (Inderjit 2004; Ehrenfeld et al. 2006; Kulmatiski et al. 2006).

Microbial effects on plant-soil feedback

Plants are greatly regulated by soil organisms, both beneficial and pathogenic, which can control plant fitness, abundance, and distribution by influencing a plant's growth and physiological response to stress and its ability to take in nutrients and water (Klironomos 2002; Callaway et al. 2004a; Agrawal et al. 2005; Hinsinger et al. 2005; Reinhart et al. 2005; Beest et al. 2010). Beneficial examples include mycorrhizae, which are mutualistic fungi that extend a plant's root system and increase its access to water and less mobile nutrients, and which receive photosynthate from the plant roots in return (Schnepf 2008). Symbiotic nitrogen-fixing bacteria are responsible for transforming nitrogen into a usable form for many plants since plants cannot assimilate molecular nitrogen (Franch, et al. 2009). Pathogenic bacteria, nematodes and fungi are known to suppress growth of species in their native soils (Reinhart et al. 2005; Mangan et al. 2010)

Below-ground soil biota can cause shifts in plant allocation responses. Beest et al. (2010) found that allocation to stem biomass and height increased when *Chromolaena odorata* (Siam weed) was grown in soils inoculated with nonnative soil communities compared to native soil inoculations. Sterilizing soil is a way to study microbial effects as it destroys bacteria and fungi without greatly changing chemical or physical properties of the soil (Trevors 1996). Researchers grew *Centaura maculosa* in soils collected from its native range in Europe and from its invaded range in North America that were either sterilized by autoclaving or not sterilized (Callaway et al. 2004a). Growth of *C. maculosa* was 166% higher in the sterilized soils from the native range than in unsterilized soils from the native range, but only 24% higher in sterile soils from the nonnative range than in unsterilized soils of the nonnative range. Thus, plants experienced more negative feedback in their native range soils than in nonnative North American soils. Results of this sterilization treatment show how soil microbes can inhibit invasive plants in their native soils while providing an advantage in nonnative soils, possibly contributing to invasive behavior (Callaway et al. 2004a).

In their native ranges, plants are usually suppressed by soil-borne pathogens such as parasitic fungi and nematodes, but in nonnative regions, they are not exposed to these natural enemies. The process known as enemy escape is central to the "Natural Enemies Hypothesis" (or Enemy Release Hypothesis). Beckstead and Parker (2003) tested this hypothesis in invasive *Ammophila arenaria* (European beachgrass). In a greenhouse experiment, they germinated seeds in soil collected from *A. arenaria* rhizospheres from both its European native range and its invaded range in California that was either sterilized or unsterilized. Seed germination, seedling survival and biomass all decreased

in the unsterilized soils from its native range suggesting that soil-borne pathogens had a negative effect on seedling survival in its native habitat.

Feedback resulting from "enemy release" may help explain invasiveness of certain plants in introduced habitats. For example, Klironomos (2002) grew five rare plants native to North America in soils from their home range as well as in foreign soils and compared growth responses to five invasive species that were also grown in their home soils and in foreign soils. The native plants showed significant negative feedback when grown in their home soils as compared to foreign soils. In another set of experiments, he used specific microbial fractions added to both home and foreign soils. He found that the native plants suffered strong negative feedback when grown in home soil combined with pathogens from their own root systems whereas invasives did not experience similar effects.

Prunus serotina (black cherry), which is invasive in Europe, also displayed negative feedback in its native North American soils. Survival and germination rates were compared in soils collected from beneath conspecific and heterospecific trees in a greenhouse experiment. Sterilization significantly increased *P. serotina* survival in conspecific soils, showing a negative effect of soil biota. Fungicide was used to measure soil pathogen effects and its application increased survival by 27% in conspecific soils with no effect in heterospecific soils. Interestingly, seedling mortality had the least effect in the sandiest of all soils that were examined, indicating that soil texture and structure may play a role in the regulation of plant growth by soil biota (Reinhart et al. 2005). Agrawal et al. (2005) used phylogenetically related plants to examine plant-soil feedback in a range of native and nonnative species pairs. A phylogenetic comparison controls for

the variability introduced to a study of using species that are not closely related. In their study, native species experienced twice as much negative feedback as nonnatives.

Specific combinations of plants and soil microbes lead to divergent effects. *Centaurea maculosa* had an interactive effect with native grasses in a common garden experiment using soils treated with a fungicide and untreated soils. Callaway et al. (2004b) measured the biomass of *C. maculosa* grown in the presence and absence of competitors and with and without fungicide. Growth of *C. maculosa* increased in the presence of two species, but not when fungicide was applied. Its growth was inhibited when grown with a third species in the absence of fungicide but increased with the application of fungicide. This shows that specific interactive combinations between plants and soil microbes can lead to different outcomes for plant growth.

Plants in introduced habitats may profit from "enhanced mutualisms," beneficial microbial allies or mutualists they have not encountered in their native regions. In a greenhouse experiment, Reinhart and Callaway (2004) tested the effects of soil from native vs. nonnative ranges, and from conspecific vs. heterospecific competitors on seed germination, height and biomass on *Acer* species. Invasive *Acers* initially benefited from soil biota in their nonnative ranges, but feedback became increasingly negative with establishment of the species. Soil biota from the nonnative soil under heterospecifics increased biomass and height suggesting benefits from "new allies." Biomass and height were both higher in the sterilized soil from *Acer* native ranges than in nonnative ranges suggesting that microbial pathogens were important in native soils. In the nonnative range, sterilization increased growth with conspecifics but not with heterospecifics. These

results support the "Enemy Release Hypothesis," and also support "enhanced mutualists" in nonnative ranges.

Negative feedback can increase over time. Using sterilized field soil inoculated by soils from different successional stages, in addition to extracting and utilizing soil nutrients, Kardol et al. (2006) grew plant mixtures of differential successional classes in a feedback experiment in which they found that early successional species displayed negative feedback while mid-successional species had neutral feedback.

Nutrient Cycling and plant-soil feedbacks

Plants can also affect the presence of nutrient cycling microbes which in turn affect available nitrogen (N) in the soil (Ehrenfeld 2003; Hawkes et al. 2005). Hawkes et al. (2005) used native and nonnative plants grouped in monocultures and mixtures to examine effects of soil microbial activity on changes to N levels over a 4 year time span. They found that monocultures of invasive plants increased gross rates of nitrification whereas mixed groups did not.

Alliaria petiolata (garlic mustard) is native to Europe and Asia and has invaded many areas in North America. It is known to have characteristics which may increase its invasive success. For example, Rodgers et al. (2007), showed through soil analysis that the presence of *A. petiolata* in field sites significantly increased N, P, Ca and Mg availability. Interestingly, decomposing *A. petiolata* leaves increased decomposition of native leaf litter, possibly explaining why *A. petiolata* increases nutrient availability, which may facilitate its own continued invasion and could be a example of positive feedback mediated by effects on nutrient cycling (Rodgers et al. 2007).

Extracts from the invasive shrub *Lonicera maackii* (Amur honeysuckle) have been shown to limit growth and reproduction of *Arabidopsis thaliana,* a non-mycorrhizal mustard, directly and indirectly (Cipollini and Dorning 2008; Cipollini et al. 2008a). Extracts of *L. maackii* were found to directly inhibit growth of *A. thaliana* and inhibited its positive response to added nutrients (Cipollini et al. 2008a). Cipollini and Dorning (2008) also found that phenolic allelochemicals in *L. maackii* leaf and root extracts could inhibit *A. thaliana*'s response to nutrient availability. However, in this study, initial growth reductions in *A. thaliana* in *L. maackii* conditioned soils were followed by increases in that soil later suggesting that inhibitory effects degrade over time.

Allelochemicals and plant-soil feedbacks

Most plants produce secondary metabolites of various classes, including phenolics, which may have a role in allelopathy and plant-soil feedbacks. Phenolic compounds, as a group, are known to play a role in plant-soil interactions through direct and indirect effects on microbial composition and nutrient cycling and are also known to cause changes in pH and mineral composition (Inderjit and Dakshini 1994; Ehrenfeld 2006; Callaway et al. 2008; Cipollini et al. 2008a; Pollock et al., 2011). Phenolics are commonly produced by plants as pathogen or herbivore deterrents, but are also used as attractants to pollinators or seed dispersers, thus providing a selective advantage. For example, coumarins (recognized as the scent of newly-mown hay) are a class of phenolics known to have antimicrobial qualities and can inhibit seed germination and inhibit plant growth. Coumarin-rich extracts of alfalfa leaves were shown to significantly reduce root growth of alfalfa and barnyard grass (Chon et al., 2002). Flavonoids, another

class of phenolics which have antifungal and antibacterial properties, are plant pigments producing yellow or red/blue pigmentation in petals used to attract pollinator animals. Tannins (found in tea and wine) are one more set of phenolic compound that have astringent properties and are known to bind proteins. These various compounds have potential allelochemical effects on plant-soil interactions.

A species in its introduced range may use biochemical weapons that inhibit neighbors directly or disturb other ecosystem properties giving the invader some advantage. These same weapons are ineffective against neighbors in its native range, which are adapted to cope with such allelochemicals. This principle is the basic concept of the "Novel Weapons Hypothesis" (Callaway and Ridenour 2004). Indeed, Callaway et al. (2008) found that these novel weapons in *Alliaria petiolata,* some of which may be phenolics, inhibit native plant growth by disrupting mycorrhizal activity. They found that *A. petiolata*'s phytochemicals were more allelopathic to arbuscular mychorrizae mutualisms in soils where it is invasive in North America than in its native European soils.

Extracts of *L. maackii* were found to inhibit seed germination of *Impatiens capensis, A. petiolata,* and *A. thaliana* in Petri dish bioassays. Interestingly, these same extracts actually increased germination of its own seeds when compared to controls, possibly explaining its invasive behavior (Dorning and Cipollini 2006). In addition, Cipollini et al. (2008b) isolated 13 of *L. maackii*'s phenolic metabolites and these extracts were found to have an inhibitory effect on *A. thaliana* seed germination. In a study designed to compare effects of *A. petiolata* and *L. maackii,* on nonmychorrizal *A. thaliana,* Cipollini et al. (2008a) showed *L. maackii* extracts significantly reduced growth

and reproduction of *A. thaliana* yet extracts of *A. petiolata* had no significant effect. Furthermore, Pollock et al. (2011) found that invasive *Centaurea stoebe* Lam. (spotted knapweed), which is known to exude (±)-catechin from its roots, inhibited microbial communities in soil from its invaded range, possibly increasing its competitive ability. The soil biota from its native region in Romania was more resistant to the inhibitory catechin.

Studies of plant-soil interactions in the context of plant invasions are increasing but many studies focus on single mechanisms (Ehrenfeld et al. 2006). Holistic examinations of plant-soil interactions give us a much better understanding of invasive species and how they alter plant communities. Invasive plants may have the ability to influence their surroundings differently from native competitors, ultimately leading to changes in community structure which may affect ecosystem processes. Knowledge of nutrient availability, microbial community structure, and soil chemistry in different soil types can provide insight to more complete understanding of how exotic invasion occurs. Knowing the influence of soil type (structure and texture) may be helpful in determining why certain areas are more vulnerable to invasion than others. Soil texture (balance of sand, silt and clay) and structure (size and shape of particles and how they aggregate) can determine the rate of water flow and nutrients through the system and can contribute to soil community dynamics which may aid in successful invasions (Hudson 1994; Ehrenfeld 2003).

Lonicera maackii and plant-soil feedbacks

Lonicera maackii (Rupr.) Maxim (Amur honeysuckle: Caprifoliaceae) is a nonnative invasive deciduous shrub found throughout most of the Midwest and eastern United States. It is one of the most important invasive species in Ohio and its abundance is increasing. It can grow in many soil types and is commonly found in old fields, forest edges and interior canopy gaps as well as in riparian zones and is often associated with disturbance (Bartuszevige et al. 2006). It can tolerate a pH of 5.5 to 8.0 (USDA 2010). *Lonicera maackii* is native to northeastern China and Korea (Luken and Thieret 1996) and was introduced to the United States by 1898, making it to Ohio in the 1960s. It was intentionally brought here for use as an ornamental, erosion control and wildlife habitat improvement. Distribution of *L. maackii* has more than doubled in the last two decades, from 21 Ohio counties in 1995 to 56 counties in 2010 (Rick Gardner, personal communication; USDA 2010). Research suggests that the American Robin (*Turdus migratorious)*, a winter frugivore, is an important seed disperser for *L. maackii* presumably due to the high availability of winter fruit (Sauer et al. 2008; Watling and Orrock 2010). This shrub negatively affects individual plants and plant communities through such mechanisms light and soil resource competition, and allelopathy and can have detrimental effects on community species abundance and richness (Hutchinson and Vankat 1997, Collier et al. 2002; Cipollini et al. 2008a/b; Cipollini and Dorning 2008; USDA 2010), but the potential role of plant-soil feedbacks in its invasive success has never been examined.

Hypotheses and predicted results

I conducted a greenhouse study to determine the extent to which the invasive shrub *L. maackii*, the native relative shrub, *Diervilla lonicera*, and the widespread native tree, *Fraxinus pennsylvanica*, had positive or negative feedback on their own fitness and how their growth affected fitness of the other species. In addition, I investigated whether the sign and strength of the feedback changed across two distinct soil types, and whether effects were due to shifts in biotic or abiotic soil traits, or some combination thereof.

I hypothesized that invasive *Lonicera maackii* would experience more positive feedback in its own soils (*Lonicera*-conditioned soil) than native species would in their own soils. I also hypothesized that a sandy-acidic soil type would show reduced positive feedback effects in *L. maackii* compared to loamy-circumneutral soil.

I predicted that each species would generally experience negative feedback in its own soil versus unconditioned soil. I predicted that each species would generally experience more negative feedback in its own soil versus soil conditioned by other species. Finally, I expected native *D. lonicera* to suffer the poorest growth in *L. maackii* soils compared to its own soil or in *Fraxinus*-conditioned soils.

I expected *L. maackii*-conditioned soils to cause bigger changes than other species in soil chemistry and microbial profiles due to its known allelochemicals. This effect is expected to be less pronounced in sandy soils than in loamy soils, possibly due to less organic material to bind allelochemicals resulting in less negative feedback for plants grown in *L. maackii*-conditioned soils. If feedback is less negative in this soil type, I would expect more growth of *L. maackii* in the preconditioned sandy soils compared to preconditioned loamy soils.

If biotic factors are responsible for feedback in either direction, sterilization should eliminate the feedback relationships. If abiotic factors are responsible for feedback, then differences in soil nutrients, pH, and allelochemistry after conditioning and in different soil types would better account for changes.

MATERIALS AND METHODS

Soil sources

The study took place at Wright State University in the laboratory and greenhouse. To determine if *L. maackii* is more successful in loamy, circumneutral soils, than in sandy-acidic soils, experiments were conducted in two types of soil, both collected in June 2010, from microsites uninhabited by any of the experimental species. Bulk soil samples were taken from multiple locations in the Wright State Woods in Dayton, Ohio and in Shawnee State Park in West Portsmouth, Ohio, and then pooled together, by location, to be used for the conditioning stage. Soil textures were determined using the Pipette method (Gee & Bauder, 1986). I added 100g of sieved soil to a 500mL beaker followed by 20mL of calgon solution and 100 ml of autoclaved deionized water and mixed thoroughly by hand. The soil slurry was transferred to a blender and mixed on lowest setting for 10 minutes. I then transferred the slurry to a 1000mL graduated cylinder and I added deionized water to the 1000mL mark. I then covered the cylinder with Parafilm® and tilted itend to end several times to mix the solution. I allowed the slurry to settle based on predetermined time periods in order for each particulate to settle out of solution. Percentages of sand, silt and clay were calculated and texture was determined using a textural triangle. The first soil type was a circumneutral loam, collected from the Wright State Biological Preserve in Dayton, OH. The Wright State Woods are located at the southern edge of Ohio's glaciated region. These soils have a circumneutral pH and contain high Ca and Mg contents due to limestone bedrock. Soils here are classified as Miamian silt-loam, characterized by low permeability (ODNR 2010; USDA: SCS 2010). The second soil type is an acidic sandy loam, collected from

Shawnee State Park, located in West Portsmouth, OH, near the Ohio River, within Shawnee State Forest in the Western Allegheny Plateau. Soils here are composed of weathered sandstone, siltstone, and shale sedimentary rock. These soils are classified as Shelocta-Brownsville, described as well drained soils formed in colluvium (transported due to gravity) and residuum (formed in place from rock) with a strongly acid subsoil (ODNR 2010; USDA: SCS 2010).

Plant Species

Diervilla lonicera

In addition to *L. maackii, D. lonicera* P. Mill., native northern bush honeysuckle, was chosen as a test species because it is also in the Caprifoliaceae and a closely related species to *L. maackii* (Jacobs 2009). Related species may have similar evolutionary traits that contribute to their behavior and distribution patterns whereas unrelated species have different characteristics. *Diervilla lonicera* is a rhizomatous shrub that is pollinated by bumble bees and hawk moths (Schoen 1977) and it has a pH tolerance of 4.8 to 7.0. Its distribution patterns are similar to those of the invasive *L. maackii* but with a greater shade tolerance. It is less abundant and is currently listed as rare in Indiana and as threatened in Tennessee (USDA 2010).

Fraxinus pennsylvanica

Fraxinus pennsylvanica Marsh. (green ash), a deciduous tree native to North America, is in the Oleaceae (olive family). It is widespread throughout the United States

and Canada east of the Rocky Mountains. This species was chosen to represent a pioneering species whose broad distribution overlaps the ranges of both *L. maackii* and *D. lonicera.* It has a very wide pH tolerance of 5.0 to 8.1 (USDA 2010). Green ash is the most common ornamental ash species presumably because it grows well in multiple landscapes having the ability to withstand drought, flooding, salt and alkaline soils. Ash are under threat of the exotic insect, emerald ash borer (EAB) (*Agrilus planipennis*) which is slowly destroying the species, and could seriously upset biodiversity in natural forests (MacFarlane and Meyer 2005).

Soil Conditioning

The soil conditioning phase of this study ran from August, 2010 through March, 2011. First season *F. pennsylvanica* seedlings were collected from a naturally growing population at Kiser Lake State Park in Conover, Ohio, in July, 2010. Both *L. maackii* and *D. lonicera* seedlings used for the conditioned phase were 12 weeks old. Plants were grown in sterilized ProMix BX potting mix without mycorrhizae (Premier Horticulture Inc. Quakertown, PA) and maintained in 1L pots and grown in a temperature-controlled greenhouse under ambient light supplemented with fluorescent lights between 0700 and 2100.

On July 27, 2010, seedlings of each species were planted in each soil type in plastic tubs for 6 months. I also maintained an unconditioned soil control that contained no plants. Field **s**oils were first mixed to ensure homogeneity and sand **(**QUIKRETE™ washed, screened and dried play sand) was mixed into soils in each tub (1:5, sand to soil) to inhibit compaction. Each tub contained 25L of soil/sand mixture into which 10-15 of

each species were planted, matched to achieve similar biomass. Plants of each species in each tub were grown in the greenhouse and all tubs (including unconditioned/unplanted control) were rotated weekly to ensure even light exposure and watered as needed with deionized water and no fertilizer was added.

Feedback Experiment

At the end of the conditioning phase, plants were removed from soils, the soils mixed within tubs and then half of each soil was sterilized by fractional sterilization (Tyndallization). The moist soil was heated in an autoclave to 100°C for 1 hour on 3 successive days. Three repetitions were needed to trigger heat-resistant spores to germinate and subsequently be destroyed in the next stages. This lower temperature sterilization technique preserves soil structure and quality better than autoclaving at 121°C (Wolf et al. 1989). I then transferred sterile soil to sterile containers and used soil immediately**.** Successful sterilization was confirmed by culturing soil extracts from unsterilized and sterilized soils from both locations. I prepared soil dilutions in sterile saline and plated the lowest dilutions (1:10000) on Tryptic Soy Agar plates (2 reps). Cultures were incubated at room temperature for 72 hours and examined for microbial growth (Trevors, 1996). Microbial growth was negative on sterilized soil plates and positive on unsterilized soil plates of both soil types (Figure 11).

In January, 2011, *L. maackii* seeds were surface sterilized by soaking in a 10% chlorine bleach solution for 10 minutes and rinsed with autoclaved water and then germinated in petri dishes on Whatman No. 2 filter paper in an incubator at 24°C, using 100mg/L concentration of gibberellic acid to hasten germination rates (Hidayati, et al.

2000). *Diervilla lonicera* seeds were purchased from Gardens North (wild collected in Canada), Annapolis Royal, NS, Canada, and germinated in petri dishes in an incubator at 24°C on autoclaved sand moistened with autoclaved deionized water in January, 2011. In February, 2011, I planted all viable germinated seeds of both species in 300mL propagation cell packs, contained in self-watering trays, purchased from BFG, using sterilized ProMix BX potting mix without mycorrhizae (Premier Horticulture Inc. Quakertown, PA). Plants were maintained in a temperature-controlled greenhouse under ambient light supplemented with fluorescent lights between 0700 and 2100. *Fraxinus pennsylvanica* plants were not used in the feedback experiment.

On March, 27, 2011, I removed seedlings of each species from cell packs and disposed of any loose potting mix then planted seedlings in 0.5L pots in each possible combination (reciprocally) of soil type, conditioning and sterilization levels. I had 8 replicates each, totaling 256 (2x2x4x2x8) pots (Figure 1). Plants were haphazardly assigned a location on tables in the greenhouse and rotated biweekly to ensure equal light exposure and to minimize microclimatic effects.

Height and basal stem diameter (BSD) were measured at the start of the experiment and biweekly thereafter. All plants were harvested after 12 weeks (June, 2011), separated from soils by rinsing under running water until roots were clean, and dried at 60°C for 48 hours before weighing roots and shoots individually. All statistical analyses were performed using SAS (Version 9.2). Final dry total biomass, root and shoot biomass, root/shoot ratios, height, and BSD of each species were compared among soil types, conditioning treatments, sterilization treatments and their interactions with three-way ANOVA. Means within conditioning, sterilization and soil types were compared

using Tukey's tests. The effects of the same factors on changes in height and BSD were analyzed with repeated measures MANOVA. Correlations between all end-of-season measures (total dry biomass, root and shoot biomass, root/shoot ratios, height, and BSD) were made using Pearson correlations.

I treated all plants periodically for spider mite infestation in mid-May through early-June 2011, using AVID® (Syngenta) miticide per manufacturer's instruction.

Soil Chemical Properties

In order to determine how each species affected the nutrient content and other soil attributes, soils were analyzed after the conditioning phase. I collected soil samples (225 g) from within each tub, sieved and packaged according to treatment level. Analyses for pH, organic matter, total N, NH_4, NO_3, available P, exchangeable K, Mg, and Ca, Cation Exchange Capacity (CEC), and percent base saturation, were performed by Spectrum Analytic, in Washington Court House, Ohio.

In order to examine how putative allelochemicals varied among soils and treatments, I quantified total soluble phenolic concentrations of soil (modified from Scharfy 2010). I made soil extracts by adding 5 mL of 50% ethanol to 1 g of sieved soil and placed them on a shaker at 200 rpm for 1 h. Samples were then centrifuged at 10000 rpm for 5 minutes and the supernatant retained. I diluted a 3-mL aliquot of this extract with 2 mL of autoclaved deionized water and added 100 μL Folin-Ciocalteu-reagent followed by 300 μL of 2 M Na_2CO_3 after 8 minutes. Phenolics producing absorbance at 760 nm were detected in a microplate reader after 1 h. A standard curve for phenolics

was prepared with gallic acid. Results of the soil analyses for N, P, K, and pH and total phenolics were not analyzed statistically because of the absence of biological replication.

Community level physiological profiles (CLPP) using Biolog® EcoPlate™

The Biolog® EcoPlate™ is used by microbial ecologists to analyze microbial community footprints over time and is a good tool for analyzing changes in response to soil conditioning. The EcoPlates™ were designed for the ecological study of whole microbial communities rather than indentifying individual strains. The Biolog® microplates contain 31 carbon substrates (with 3 technical replicates) and allow measurement of substrate utilization by microbial communities. Microorganisms utilize the substrates causing changes in the color formation of the tetrazolium dye and light absorbance is measured by a spectrophotometer (Stefanowicz, 2006).

After conditioning, I collected soil samples (10 g dry weight, approximated from moist soil) from each tub of soil, first by taking core samples randomly from the tub, mixing them thoroughly and weighing the required amount. Samples were kept on ice and shaken for 60 min in 20 mL of a 10 mM Bis-Tris ($C_4H_{11}NO_3$) solution (pH 7) and allowed to settle for 30 minutes. I decanted the extracts immediately. I first made serial dilutions and then added 100 µl of the 1:1000 diluted solution to each microplate well and incubated it at 22°C. Substrate utilization was monitored by measuring light absorbance at 590nm. Measurements were made immediately following inoculation and at 12h intervals for 6 days during March, 2011. I accounted for background absorbance by subtracting the absorbance of the least utilized substrate, which varied by conditioning treatment, to prevent negative values (Hitzl et al. 1997). I used the corrected absorbance

values to calculate the average well color development (AWCD) which was 0.42. The point chosen for analysis was based on the reading that exhibited the same mean as the AWCD which best represents the optimal incubation time based on substrate utilization (Stefanowicz, 2006). Community level physiological profiles (CLPP) were analyzed by Principal Components Analysis (PCA) using R (V. 2.14.1). Community Average Metabolic Response (AMR) depicts the average respiration of carbon substrates. AMR of conditioned soils was calculated by averaging the mean difference between the absorbance value of the substrate wells and the control well (value of the least used substrate). Community Metabolic Diversity (CMD), which represents community richness, reflects the number of utilized substrates, and is calculated by adding the total number of positive responses after incubation. A positive response was established based on observed purple coloration of the wells. The threshold was set at an absorbance of 0.1. Both AMR and CMD were graphed as a function of incubation time in Sigma Plot.

RESULTS

Effects of soil type and conditioning on soil properties

Conditioned soils of both types were analyzed for pH, nutrient and phenolic levels, and other properties, but were not compared statistically due to analyzing only one sample per soil type. However, several general patterns were observed. Wright State soil had higher pH levels, more organic matter, and greater cation-exchange capacity than Shawnee soil. Soil type seemed to affect soil nutrient levels. Wright State soil had greater nutrient availability than Shawnee soil treatments after conditioning, though Shawnee soil had higher levels of Mg than Wright State soil. Interestingly, P levels, albeit low to start, did not vary in Shawnee soil treatments yet decreased by at least 20% with conditioning in Wright State soil. Calcium/magnesium ratios were twice as high in Wright State soil compared to Shawnee soil. Phenolics tended to be higher overall in Wright State soil than in Shawnee soil (Table 1).

Conditioning also appeared to have an impact on pH levels. In both soils, conditioning by all three species seemed to result in a higher pH than unconditioned soils, with a minimum increase of 0.4 in Wright State soil and a minimum increase of 0.8 in Shawnee soil treatments. Conditioning also appeared to influence nutrient levels. For instance, K and P levels decreased with conditioning when compared to unconditioned soils from Wright State soil treatments. Ca increased with conditioning, akin to the increase in soil pH. In Shawnee soils, *Fraxinus*-conditioned soil had more K and *Diervilla*-conditioned and *Lonicera*-conditioned soils had lower K than unconditioned soil. NH_4 and NO_3 levels varied widely with conditioning levels. Ca:Mg tended to increase with conditioning in both soils, but more so in Wright State soil. The trend seen

in phenolics was different among conditioning treatments. The highest phenolic level was in Wright State, *Diervilla*-conditioned soil and the lowest was in Shawnee, *Fraxinus*-conditioned soil. Phenolic levels seemed to be highest in *Diervilla*-conditioned soil in both soil types. In Wright State soil, *Fraxinus*-conditioned soil had the second highest phenolic level (Table 1).

Effects of soil type and conditioning on growth of *Lonicera maackii*

For *L. maackii*, there was a significant effect of soil type on total biomass (Table 2). Plants were larger overall in Wright State soil than in Shawnee soil (Figure 2). Conditioning alone had no significant effect on *L. maackii's* biomass; however there was a significant interactive effect between soil type and conditioning (Table 2). In general, plants responded to the conditioning treatments differently in Shawnee soil than in Wright State soil (Figure 2). For example, plants grew similarly in Wright State soil regardless of conditioning and total biomass was significantly greater in its own conditioned soil and that of *Fraxinus*-conditioned soil than unconditioned or *Diervilla*-conditioned soil in Shawnee soil treatments. Sterilization had a significant positive effect on total biomass of *L. maackii* overall (Table 2, Figure 2), but there was a highly significant interactive effect between soil type and sterilization (Table 2). Sterilizing soils significantly increased total biomass of *L. maackii* across all conditioning treatments in Wright State soils, but had an overall negative effect in Shawnee soil (Table 2, Figure 2). Finally, soil conditioning and sterilization had a significant interactive effect on total biomass (Table 2). Sterilization tended to benefit growth more in unconditioned and in *Diervilla*-conditioned soils than it did in *Lonicera*- and *Fraxinus*-conditioned soils, a

pattern seen most clearly in Wright State soil. However, the three way interaction of soil type, conditioning, and sterilization was not significant (Table 2). The patterns of significance and effects on total biomass were similarly reflected in root and shoot biomass (Table 4, Figure 2). All end-of-season measures were significantly correlated with each other, with the exception of root/shoot ratio and root biomass.

Soil type had a significant effect on root/shoot ratios, which were higher overall in Shawnee soil (Table 6, Figure 4). There was no significant effect of conditioning alone, or its interaction with soil type. Sterilization and its interaction with soil type had significant impacts on root/shoot ratios (Table 6). Sterilization increased root/shoot ratios in Shawnee soil treatments, but in Wright State soil, root/shoot ratio was not affected by sterilization. There was also a significant interactive effect between conditioning and sterilization (Table 3). Sterilizing soils increased root/shoot ratios in all conditioning levels with the exception of a decrease in *Diervilla*-conditioned soils (Figure 4). There was also a significant three way interaction, where this same pattern was seen in Shawnee soil treatments but in Wright State soil treatments, sterilization did not affect ratios across conditioning treatments (Figure 4).

Height of *L. maackii* was significantly impacted by soil type (Table 7). Plants were generally taller in Wright State soil treatments than in Shawnee soil (Figure 5). Conditioning had no independent effect, but there was a significant interactive effect between soil type and conditioning (Table 7). The tallest plants grew in unconditioned Wright State soil, while the shortest plants were in Shawnee soil conditioned by *F. pennsylvanica* and *L. maackii* (Figure 5). Sterilization alone had no significant effect on height; however, there was a significant interaction between soil type and sterilization

(Table 7). Sterilization increased height in all Wright State soil treatments, but generally decreased height in Shawnee soil (Figure 5). There was also an interactive effect between conditioning and sterilization on plant height (Table 7). Sterilization had the most positive effect on *Diervilla*-conditioned soils, and was the only conditioning treatment in which sterilization increased height in Shawnee soil (Figure 5). The three way interaction was not significant. Basal stem diameter (BSD) had similar patterns of significance and effects as height patterns (Table 7, Figure 5).

Height of *L. maackii* changed through time. Time, as a factor, significantly interacted with both soil type and sterilization, and sterilization interacted with both soil type and with conditioning (Table 8). For example, plants in unconditioned and sterilized Wright State soil started equally with other treatments, but clearly were the tallest around day 28 and were quite taller than all others. Conversely, plants in *Lonicera*-conditioned and in unsterilized Wright State soils did just the opposite. Soil type and sterilization individually significantly affected BSD through time but conditioning did not (Figure 6). There was no four way interaction on height or BSD. Patterns in BSD mirrored height patterns (Figure 6, Table 8).

Effects of soil type and conditioning on growth of *Diervilla lonicera*

Soil type had no significant effect on total biomass of *Diervilla lonicera*, but conditioning had a significant impact (Table 3). Total biomass was higher in unconditioned soil than in conditioned soil and *D. lonicera* grew significantly less in its own conditioned soil than in all other treatments (Figure 2). Soil type and conditioning had a significant interactive effect on total biomass (Table 3). Plants had the same general

patterns in both soil types, with the exception that they performed best in unconditioned Wright State soil, and in *Lonicera*-conditioned treatment in the Shawnee soil (Figure 2). Sterilization had a highly significant effect and resulted in increased biomass overall (Table 3, Figure 2). There was no significant interactive effect between soil type and sterilization but there was a significant interaction between conditioning and sterilization. Sterilization had the most positive impact in *Diervilla*-conditioned soils (Figure 2). There was no significant three-way interaction between treatment factors (Table 3).

Individually, soil type and conditioning did not significantly affect root biomass, but they had an interactive effect (Table 3). In Shawnee soil, root biomass was highest in *Lonicera*-conditioned soils, but in Wright State soils, it was highest in unconditioned soils (Figure 3). Sterilization significantly increased root biomass across all treatments (Table 3, Figure 3). Soil type and sterilization had no significant interactive effect, though conditioning did significantly interact with sterilization (Table 3). Sterilization clearly increased growth of *D. lonicera* most in *Diervilla*-conditioned and *Lonicera*-conditioned soils. The three way interaction of soil type, conditioning, and sterilization was not significant (Table 3). Shoot biomass was significantly affected by soil type (Table 3) with plants growing larger in Wright State soil than in Shawnee soil treatments. Conditioning had a significant effect on shoot biomass with *Diervilla*-conditioned soils resulting in the smallest shoots (Table 3, Figure 3). Sterilizing soils significantly increased shoot biomass across all treatments (Table 3, Figure 3). Soil type and sterilization had a significant interaction (Table 3). Sterilization was more beneficial in the Wright State soil than in Shawnee soil (Figure 3). There was a significant interactive effect between conditioning and sterilization (Table 3). Shoot biomass of plants in

Diervilla-conditioned soils responded more positively to sterilization than in other conditioning treatments (Figure 3). There was no significant three-way interactive effect between treatment factors (Table 3).

Nearly all end-of-season measures of *D. lonicera* were significantly positively correlated (Table 7). Height, BSD, root, shoot, and total biomass were all significantly positively correlated with each other (Table 7). Root/shoot ratio was not significantly correlated with height, BSD, and shoot biomass, but was significantly positively correlated to root biomass and total biomass.

The root/shoot ratio was significantly higher in Shawnee soil treatments than in Wright State soil (Table 6, Figure 4). There were no significant impacts to root/shoot ratios by conditioning or the interaction of soil type and conditioning. Sterilization significantly impacted root/shoot ratios both independently and in its interaction with soil type. Root/shoot ratios were highest in sterilized Shawnee soil treatments (Table 6, Figure 4). There was no significant three way interactive effect on *Diervilla* root/shoot ratios.

Soil type had no significant impact on height of *Diervilla*. Conditioning alone had a significant effect on height (Table 7). Plants generally grew tallest in unconditioned soils and shortest in *Diervilla*-conditioned soils (Figure 7). The interaction of soil type and conditioning significantly affected height (Table 7). For example, plants grew taller in unconditioned Wright State soil than in unconditioned Shawnee soil, but grew the least in *Diervilla*-conditioned Shawnee soil treatments (Figure 8). Sterilizing soils significantly increased overall plant height (Table 7, Figure 7). There was an interactive effect between soil type and sterilization (Table 7). Plant heights were increased by sterilization

more strongly in Wright State soil than in Shawnee soil (Table 7; Figure 7). There was a significant interactive effect between sterilization and both soil type and conditioning (Table 7). For example, plants were taller in unsterilized Shawnee soil conditioned by the three species than in unsterilized Wright State soil conditioned by the three species. However, sterilization increased height more strongly in Wright State soil than in Shawnee soil. Sterilization increased height most in Wright State soil and had the most positive effect in *Diervilla*-conditioned soil. Patterns in BSD were very similar, though there was no significant interaction between soil type and conditioning on BSD (Table 7).

Height and BSD of *Diervilla* changed through time and significantly interacted with almost every other factor, the exception being the four way interaction on both height and BSD (Table 9). For instance, plants in sterilized, Wright State, unconditioned soil were indistinguishable from other treatments at the start, but surpassed height in other treatments on day 70 and then were tallest at harvest. Plants in unsterilized, Wright State, *Diervilla*-conditioned soils grew the least throughout the experiment. BSD patterns followed suit (Figure 8).

Effects of soil type and conditioning on microbial community shifts

Biolog® data revealed that there were no major patterns in microbial communities caused by conditioning. Principal components analysis (PCA) revealed a pattern in microbial community composition based on soil type, where different communities were cultivated by the different soils (Figure 9). The first two principal components explained 59% of the variation in Biolog® data (PC1: 33.96%, PC2: 25.13%). Average metabolic response (AMR) was higher over all in Shawnee soils and highest in *Diervilla*-

conditioned soils. Unconditioned Wright State soil was the first to show a metabolic response (substrate utilization indicated by development of tetrazolium dye); however it had the lowest response at the end of incubation compared to all other treatments. Interestingly, unconditioned Shawnee soil and *Lonicera*-conditioned Wright State soil had nearly the same final metabolic response, though their patterns over time differed considerably (Figure 10). Community functional richness was generally higher in Shawnee soils. *Fraxinus*-conditioned soils cultivated the highest community functional richness in both soil types. *Diervilla*-conditioned Shawnee soils, *Lonicera*-conditioned Shawnee soils and *Fraxinus*-conditioned Wright State soils had similar richness levels at the end of incubation, but differed in their development over time. *Fraxinus*-conditioned Wright State soils maintained the highest richness from 48 hours through120 hours, only to be surpassed at the last observation by *Fraxinus*-conditioned Shawnee soils. Unconditioned Wright State soils cultivated the lowest community richness (Figure 10).

Feedback effects of soil type, conditioning and sterilization on plant growth

In Shawnee soil treatments, total biomass of *L. maackii* was higher in both conspecific and heterospecific soils that were unsterilized than in unconditioned soils, showing evidence of positive feedbacks, but showed the opposite pattern in Wright State soils conditioned by *L. maackii* or *F. pennsylvanica*. However, sterilization generally led to decreased biomass of *L. maackii* in Shawnee soil, thereby changing feedback direction in that soil. In Wright State soil, sterilization made the negative feedback even stronger (Figure 2). *Diervilla lonicera* experienced strong negative feedback in unsterilized in Wright State soil conditioned by both conspecifics and heterospecifics, but experienced

negative feedback in its own soil in unsterilized Shawnee soils. Total biomass was lower in *Diervilla*-conditioned soils than unconditioned soil in both soil treatments, but was ~80% lower in Wright State soil compared to ~50% lower in Shawnee soil. Sterilization increased biomass of *Diervilla* overall and eliminated evidence of feedback in Wright State soil and resulted in positive feedback in Shawnee soil treatments (Figure 3).

DISCUSSION

Feedback effects of soil type and conditioning on growth of *Lonicera maackii*

Our results indicate that both soil type and soil biota play a large role in plant-soil feedback. I hypothesized that *L. maackii* would experience positive feedback overall in both soils because it is not native to North America. Usually plants display negative feedback in their native soils and positive feedback in nonnative soils (Klironomos 2002; Beckstead and Parker 2003; Callaway et al. 2004a; Reinhart and Callaway 2004; Van Grunsven et al. 2007). *Lonicera maackii* showed positive feedback in unsterilized Shawnee soil, growing almost twice as much in its own soil versus unconditioned soil in accordance with predictions. I expected plants in Shawnee soils to show reduced positive feedback compared to Wright State soil, but contrary to prediction, feedback was relatively neutral to slightly negative in Wright State soils. I found *L. maackii* to be less affected by conditioning alone than by soil type and its interactions with conditioning and sterilization. The interaction between sterilization and soil type was the most significant factor affecting the nature of feedback in *L. maackii*. In Wright State soil, *L. maackii* grew similarly in unsterilized soil whether it was conditioned or not but responded very positively to soil sterilization. This may indicate that this soil type contained pathogens that suppressed its growth, indicating that biotic controls were more important than abiotic controls. Others have found that invasives initially benefit from soil biota in nonnative regions, but over time the soil microbial community becomes inhibitory (Reinhart and Callaway, 2004). Indeed, Kardol et al., (2006) found that mid-successional plant species displayed neutral feedback which might mean that this exotic has achieved

peaked invasion in this region and may be on a decline. *Lonicera maackii* may have experienced positive feedback in unsterilized Shawnee soils by taking advantage of mutualists not previously encountered in this soil type. Reinhart and Callaway (2004) found increased benefit from mutualisms when invasives have escaped natural enemies. Others have found increased mycorrhizal formation in soils beneath *L. maackii* compared to soils beneath natives which were uninhabited by *L. maackii* (Alverson, unpublished). Because sterilization reversed the sign of feedback between soil types, it suggests that different biotic factors were important in each soil type. Patterns of growth of *L. maackii* in Shawnee soil supported that it had benefitted from mutualisms that were destroyed by sterilization. Patterns of growth in Wright State soil developed during conditioning suggesting that it was negatively affected by pathogens that accumulated during conditioning that were killed by sterilization, even in unconditioned soils. Despite this, feedback became even more negative with sterilization in Wright State soil partly because plants in unconditioned soils responded so positively to sterilization. This also suggests that *L. maackii's* phytochemicals may somehow suppress its growth in the absence of biotic symbioses. In Wright State soils, *L. maackii* performed similarly in its own soils and in *F. pennsylvanica* and *D. lonicera* soils. In Shawnee soils, *L. maackii* did better in its own soil than in other soils supporting our argument that feedback is dependent on soil type. Our results show that *L. maackii* is more negatively affected by organisms in soils where it has invaded than in soils where it is not prevalent. This might be because microbial richness was lower in Wright State soils than in Shawnee soils, but also because different microbial communities exist in the two soils as indicated by the

Biolog data. This could also mean that microbes in the Shawnee area are naïve to *L. maackii* and have not yet evolved to interact and limit growth of *L. maackii*.

Feedback effects of soil type and conditioning on growth of *Diervilla lonicera*

Native species may be more susceptible to biotic factors than exotics. Our findings suggest that a native species was controlled by negative feedbacks in its native soil. *Diervilla lonicera* displayed negative feedback overall in its own unsterilized soil and in heterospecific soils in the Wright State soil type. This finding is similar to other studies and is most likely due to accumulated soil pathogens, or "natural enemies" (Klironomos, 2002; Beckstead and Parker, 2003). Sterilization enabled us to observe changes in plant growth caused by biotic conditions. Sterilization affected growth more so than any other factor for *D. lonicera*. Sterilizing soils generally eliminated evidence of negative feedback in either soil type. This finding is consistent with other research of native species in native soils (Mangan, et al., 2010) and suggests that local soil biota may be a key factor in the decline of a less abundant native plant species. It is important to mention that sterilization can release nutrients into the soil and it is often controlled for by fertilization (Troelstra et al., 2001). However, because I had different responses to sterilization between species, I feel that the results of sterilization are not due to nutrient release caused by sterilization. By using lower sterilization temperature, I was able to minimize nutrient and phenolic conversion effects and I confirmed that sterilization effectively eliminated microbes by culturing soil extracts. Biolog® data revealed that there were no obvious patterns in microbial community changes caused by conditioning, meaning that species generally cultivated the same communities and species-specific soil

microbial communities did not appear to be a major factor in feedback effects. However, there seemed to be a pattern in microbial community composition based on soil type. Different communities were cultivated by the different soil types, most likely having a stronger influence on growth of *L. maackii* and *D. lonicera.* This pattern is supported by community level physiological profiles (CLPP) which showed that Shawnee soils cultivated microbial communities with higher average metabolic response (AMR) and community metabolic diversity (CMD) than Wright State soils. Biolog results should be interpreted with caution because they may not fully represent species diversity or richness. An experiment with added nutrients may allow for clearer interpretation of these results.

Effects of soil type and conditioning on root/shoot ratios of *Lonicera maackii*

Low root/shoot ratios is a trait associated with many invasive plants (Ehrenfeld, 2003). Conditioning alone had little impact on root/shoot ratios but sterilization increased root/shoot ratios of *L. maackii* in Shawnee soils, with the exception of *Diervilla*-conditioned soils. In Wright State soil, where sterilization benefitted biomass, root/shoot allocation did not change with sterilzation. *Lonicera maackii* is known to display plasticity in resource allocation (Luken 1988, Luken 1997). Indeed, exotic invasives can be more plastic than native species when not limited by resources (Davidson, et al., 2011). *Lonicera maackii* put more resources into root biomass in sterilized Shawnee soils where sterilization presumably destroyed mutualists and caused negative feedback. This may have been particularly important because of the poorer soil nutrient profile in

this soil type. Controlling resource allocation clearly provides a competitive advantage. Plasticity could strengthen its invasive capability through evolutionary change.

Effects of conditioning on growth of both species

I expected more growth of both species in soil conditioned by other species than in their own conditioned soil. My findings support this for native *D. lonicera*, which grew significantly less in its own conditioned soil across both soil types. However *L. maackii* promoted its own growth in one soil type but not in the other, but effects on itself were largely similar to effects caused by heterospecifics. While *L. maackii* is not prevalent in the Shawnee area at present time, this positive feedback implies that it can successfully invade the area if given a chance. Allelopathic compounds are known to cause changes in microbial communities and vice versa (Inderjit, 2005: Callaway et al. 2004a; Callaway et al. 2008) and both allelochemicals and microbes have effects on nutrient cycling (Ehrenfeld 2003; Hawkes et al. 2005) and these interactions can affect ecosystem feedbacks and thereby composition (Klironomos 2002; Beckstead and Parker 2003; Callaway et al. 2004a; Hawkes, et. al, 2005; Reinhart et al. 2005; Beest et al. 2010). Both species performed similarly in unsterilized *Lonicera*-conditioned soil and in *Fraxinus*-conditioned soil, which suggests that they modify soils similarly In fact, *F. pennsylvanica* is invasive in Hungary where research showed evidence of reduced germination rates and shoot and root length of white mustard (*Sinapis alba* L) caused by green ash extracts compared to a control (Csiszár, 2009). Using multiple plant species to first condition soil allows us to make predictions about patterns of invasion based on current ecosystem

composition. It appears that *L. maackii* has no different effects than a widespread tree, but its responses might vary.

Effects of conditioning on soil chemical properties

I expected *L. maackii* to cause large changes in soil chemistry and microbial profiles due to its documented allelopathy (Cipollini and Dorning 2008; Cipollini et al. 2008a/b). In Shawnee soil, phenolic levels were lower in *Lonicera*-conditioned soils than in unconditioned soils, but they increased overall in Wright State soils. Different soil microorganisms degrade or magnify allelochemicals differently, possibly explaining differences seen between soil types (Inderjit, 2005). Though it has not been well studied, some phenolic compounds are known to be oxidized by high heat (Daskalaki et al., 2009) so the net effect of allelopathy may not be observable with sterilization. Phenolics should be compared before and after sterilization to better account for their putative effects. Interestingly, unsterilized *Diervilla*-conditioned soil generally had the highest total phenolics in both soil types.

Implications, conclusions and future research

Often, studies are missing key components when examining successful invasions. For example, they may only test "Enemy Release Hypothesis" (Beckstead and Parker, 2003; Reinhart and Callaway, 2004) rather than exploring both abiotic factors, such as soil type and biotic factors such as microbial changes using Biolog® Ecoplates. Because there were so many significant interactions in this study with soil type, it is important that studies consider accounting for soil attributes. Using two distinct soil types allowed us to

compare potential feedback effects if plants are introduced to new areas which can help make predictions of their success or failure to invade that particular habitat. Conducting studies for adequate growth periods and taking measurements throughout the study rather than just end of season measures is important, as I noted significant differences through time. For instance, if I had stopped midway through our experiment, I would not have detected how dynamic plant-soil interactions can be over time. If I had stopped at day 28, where the height of *L. maackii* in sterilized, unconditioned Wright State soils, was similar to other Wright State soil treatments, I would not have detected the significant effect of sterilization that was evident at the end of the experiment.

Study into the field of plant-soil feedback is still lacking, but is growing in popularity as new evidence is uncovered. In light of our results, I cannot say that plant-soil feedback plays any more significant role in *L. maackii's* successful invasion, though there was a tendency for feedbacks in unsterilized soils to be neutral to positive for *L. maackii*. In unsterilized soils where this species dominates, feedback was neutral, but strongly negative in sterilized soils. Both biotic and abiotic factors can influence plant-soil feedback in general. By using sterilization, I was able to demonstrate that native species success is heavily reliant upon microbes.

Though our results indicate that both soil type and soil microorganisms play a large role in plant-soil feedback, feedback in the invasive success of *L. maackii* is dependent on soil type. Most importantly, our evidence reveals that sign and strength of feedback can vary with soil source in native versus non-native species. More in depth analysis of soil properties, including proper biological repetition, would provide a better understanding of the impact of conditioning. It would also be beneficial to compare

growth of *L. maacki* in soils from its native and invaded regions. I suggest that these elements be considered in future research.

TABLES

Table 1: Effects of soil conditioning by three different plant species in two soil types on soil properties. Several plants of each species were first grown in containers of each soil type for six months to condition soil, while an unconditioned soil for each soil type was maintained in the same manner.

	Wright State soil				Shawnee Soil			
Factors	DL	FP	LM	UN	DL	FP	LM	UN
pH	7.9	7.8	8.0	7.4	7.0	6.9	7.1	6.1
Organic matter (%)	1.3	1.8	1.5	1.9	1.3	1.6	1.2	1.4
Total N (%)	0.2	0.55	0.14	0.21	0.37	0.29	0.24	0.40
NH_4 (ppm)	20	8	7	1	7	9	4	8
NO_3 (ppm)	7	9	8	43	5	48	6	65
Available P (ppm)	37	34	30	46	4	3	3	3
Exchangeable K (ppm)	104	103	84	163	44	64	48	62
Exchangeable Mg (ppm)	285	286	258	368	205	257	252	192
Exchangeable Ca (ppm)	3451	3549	3779	3010	1163	1559	1535	1010
CEC	15.3	15.6	16.2	14.3	6.9	9.2	8.5	5.3
K (% BS)	1.5	1.4	1.1	2.4	1.4	1.5	1.2	2.5
Mg (% BS)	13.7	13.4	11.6	18.8	21.9	20.5	21.8	26.4
Ca (% BS)	84.8	85.2	87.2	78.7	63.6	63.5	67.9	71.1
Phenolics ($\mu g\ g^{-1}$ soil)	0.206	0.180	0.158	0.173	0.181	0.125	0.165	0.126

Table 2: Results of three-way ANOVA of soil type, soil conditioning, and soil sterilization on root, shoot and total biomass on *Lonicera maackii*.

		Root biomass		Shoot biomass		Total biomass	
Factors	df	F	P	F	P	F	P
Soil Type (T)	1	31.52	**<0.0001**	52.95	**<0.0001**	47.14	**<0.0001**
Condition (C)	3	1.94	0.1277	2.06	0.1101	1.81	0.148
T x *C*	3	6.50	**0.0004**	9.27	**<0.0001**	8.52	**<0.0001**
Sterilization (S)	1	11.4	**0.0010**	6.87	**0.0100**	6.68	**0.0101**
T x *S*	1	59.78	**<0.0001**	95.57	**<0.0001**	87.05	**<0.0001**
C x *S*	3	3.64	**0.0150**	3.81	**0.0121**	3.60	**0.015**
T x *C* x *S*	3	0.68	0.5661	2.16	0.0967	1.57	0.200
Error	112						

Table 3: Results of three-way ANOVA of soil type, soil conditioning, and soil sterilization on root, shoot and total biomass on *Diervilla lonicera*.

		Root biomass		Shoot biomass		Total biomass	
Factors	df	F	P	F	P	F	P
Soil Type (T)	1	2.03	0.1574	15.94	**<0.0001**	2.76	0.0998
Condition (C)	3	0.47	0.7035	26.05	**<0.0001**	15.73	**<0.0001**
T x *C*	3	4.40	**0.0058**	18.31	**<0.0001**	15.58	**<0.0001**
Sterilization (S)	1	166.58	**<0.0001**	412.03	**<0.0001**	426.27	**<0.0001**
T x *S*	1	2.67	0.1054	14.9	**0.0002**	3.02	0.0854
C x *S*	3	2.67	**<0.0001**	15.68	**<0.0001**	20.87	**<0.0001**
T x *C* x *S*	3	0.81	0.4912	2.56	0.0589	2.53	0.0614
Error	106						

Table 4: Correlation matrix of *Lonicera maackii* end-of-season measures. Numbers represent: Pearson Coefficients, P-value and sample size.

End of season measures	Height	Basal stem diameter	Root biomass	Shoot biomass	Total biomass	R:S
Height		0.88268	0.79493	0.89415	0.89965	-0.41990
		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
		128	128	128	128	128
Basal stem diameter			0.85516	0.89904	0.91692	-0.22397
			<0.0001	<0.0001	<0.0001	0.0110
			128	128	128	128
Root biomass				0.92006	0.95115	-0.04522
				<0.0001	<0.0001	0.6123
				128	128	128
Shoot biomass					0.99384	-0.34184
					<0.0001	<0.0001
					128	128
Total biomass						-0.27039
						0.0020
						128
R:S						

Table 5: Correlation matrix of *Diervilla lonicera* end-of-season measures. Numbers represent: Pearson Coefficients, P-value and sample size.

End of season measures	Height	Basal stem diameter	Root biomass	Shoot biomass	Total biomass	R:S
Height		0.67569	0.55717	0.87792	0.82128	-0.02157
		<0.0001	<0.0001	<0.0001	<0.0001	0.8136
		122	122	122	122	122
Basal stem diameter			0.49240	0.73588	0.69880	0.01299
			<0.0001	<0.0001	<0.0001	0.8871
			122	122	122	122
Root biomass				0.68571	0.87770	0.72080
				<0.0001	<0.0001	<0.0001
				122	122	122
Shoot biomass					0.94878	0.02323
					<0.0001	0.7995
					122	122
Total biomass						0.33142
						0.0002
						122
R:S						

Table 6: Results of three-way ANOVA of soil type, soil conditioning, and soil sterilization on root/shoot ratio of *Lonicera maackii* and *Diervilla lonicera*.

		Lonicera maackii		*Diervilla lonicera*	
Factor	df	F	P	F	P
Soil Type (T)	1	8.65	**0.004**	10.55	**0.0016**
Condition (C)	3	0.58	0.6278	2.05	0.1112
T x *C*	3	1.45	0.2316	1.52	0.2127
Sterilization (S)	1	7.01	**0.0093**	6.59	**0.0117**
T x *S*	1	11.00	**0.0012**	9.01	**0.0033**
C x *S*	3	5.92	**0.0009**	1.18	0.3197
T x *C* x *S*	3	2.76	**0.0457**	0.04	0.9881
		Error: 112		Error: 106	

Table 7: Results of three-way ANOVA of soil type, soil conditioning, and soil sterilization on height and BSD of both species.

		Lonicera maackii				*Diervilla lonicera*			
		Height		BSD		Height		BSD	
Factors	df	F	P	F	P	F	P	F	P
Soil Type (T)	1	23.89	**<0.0001**	30.20	**<0.0001**	2.99	0.0867	5.58	**0.0200**
Condition (C)	3	0.65	0.5874	1.08	0.3586	32.69	**<0.0001**	11.65	**<0.0001**
T x *C*	3	6.19	**0.0006**	5.76	**0.0011**	13.74	**<0.0001**	0.24	0.8690
Sterilization (S)	1	0.08	0.7730	1.31	0.2549	222.11	**<0.0001**	84.66	**<0.0001**
T x *S*	1	57.86	**<0.0001**	45.45	**<0.0001**	26.75	**<0.0001**	0.50	**<0.0001**
C x *S*	3	5.69	**<0.0001**	1.74	0.1621	16.52	**<0.0001**	7.52	**0.0001**
T x *C* x *S*	3	0.82	0.4873	0.53	0.6614	2.59	0.0564	1.07	0.3644
			Error:112					Error:106	

Table 8: Results of Repeated measures MANOVA with Wilks' lambda test (W) for the effect of time and its interactions with soil type, soil conditioning, and soil sterilization on height and BSD on *Lonicera maackii.*

Subject		Height			BSD		
	df	W	F	P	W	F	P
Time	6	0.047	354.94	**<0.0001**	0.056	298.35	**<0.0001**
Time x Soil Type (T)	6	0.692	7.92	**<0.0001**	0.761	5.6	**<0.0001**
Time x Condition (C)	6	0.778	1.56	0.0701	0.800	1.38	0.1389
Time x *T* x *C*	18	0.816	1.25	0.2201	0.715	2.12	**0.0055**
Time x Sterilization (S)	6	0.717	7.01	**<0.0001**	0.877	2.49	**0.0268**
Time x *T* x *S*	6	0.666	8.92	**<0.0001**	0.709	7.29	**<0.0001**
Time x *C* x *S*	18	0.729	1.99	**0.0101**	0.775	1.58	0.0626
Time x *T* x *C* x *S*	18	0.807	1.32	0.1739	0.828	1.16	0.2945
Error	112						

Table 9: Results of Repeated measures MANOVA with Wilks' lambda test (W) for the effect of time and its interactions with soil type, soil conditioning, and soil sterilization on height and BSD on *Diervilla lonicera.*

Factors	df	Height			Basal stem diameter		
		W	F	P	W	F	P
Time	6	0.013	1252.34	**<0.0001**	0.031	515.76	**<0.0001**
Time x Soil Type (T)	6	0.701	2.13	**0.0054**	0.670	2.41	**0.0013**
Time x Condition (C)	6	0.701	2.13	**0.0054**	0.670	2.41	**0.0013**
Time x *T* x *C*	18	0.621	2.91	**<0.0001**	0.741	1.77	**0.0284**
Time x Sterilization (S)	6	0.451	20.47	**<0.0001**	0.675	8.08	**<0.0001**
Time x *T* x *S*	6	0.810	3.94	**0.0014**	0.888	2.11	**0.0581**
Time x *C* x *S*	18	0.548	3.76	**<0.0001**	0.661	2.50	**0.0008**
Time x *T* x *C* x *S*	18	0.831	1.07	0.3819	0.895	0.63	0.8743
Error	106						

FIGURES

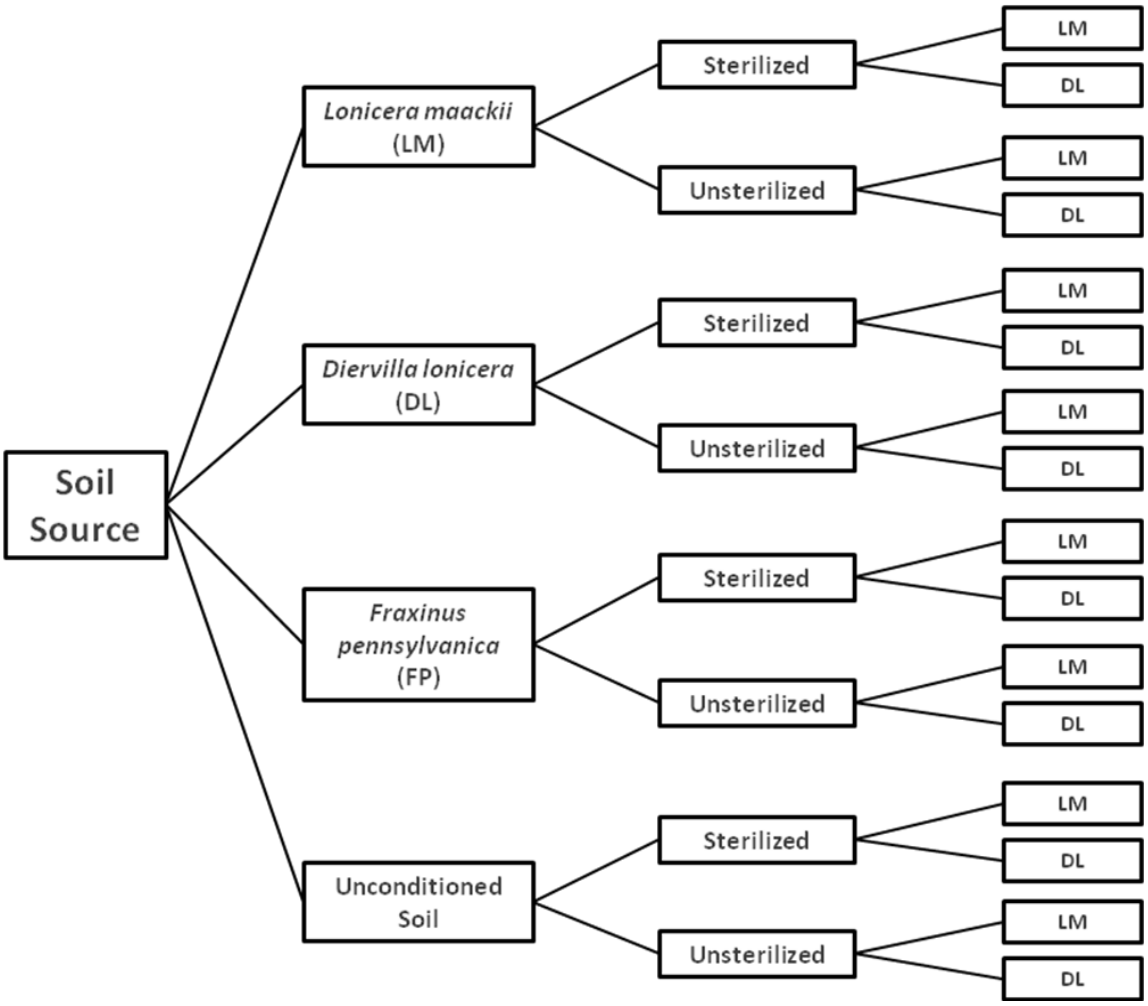

Figure 1: Full factorial design for effects of soil conditioning by three different species, and soil sterilization, in two soil types on *Lonicera maackii* and *Diervilla lonicera.*

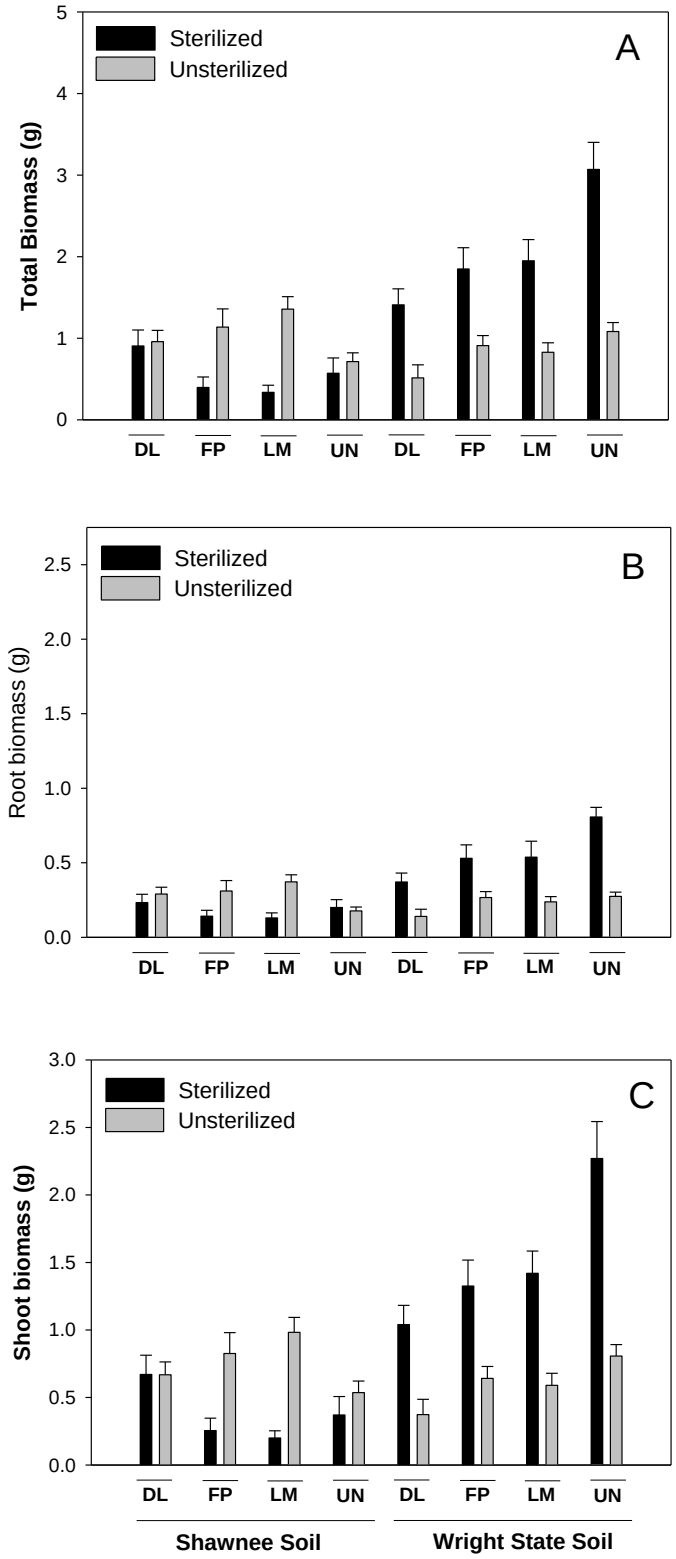

Figure 2: Mean (+ 1SE) dry (A) total biomass, (B) root biomass and (C) shoot biomass of *Lonicera maackii* in response to soil sterilization, and soil conditioning by three different species, and two soil types.

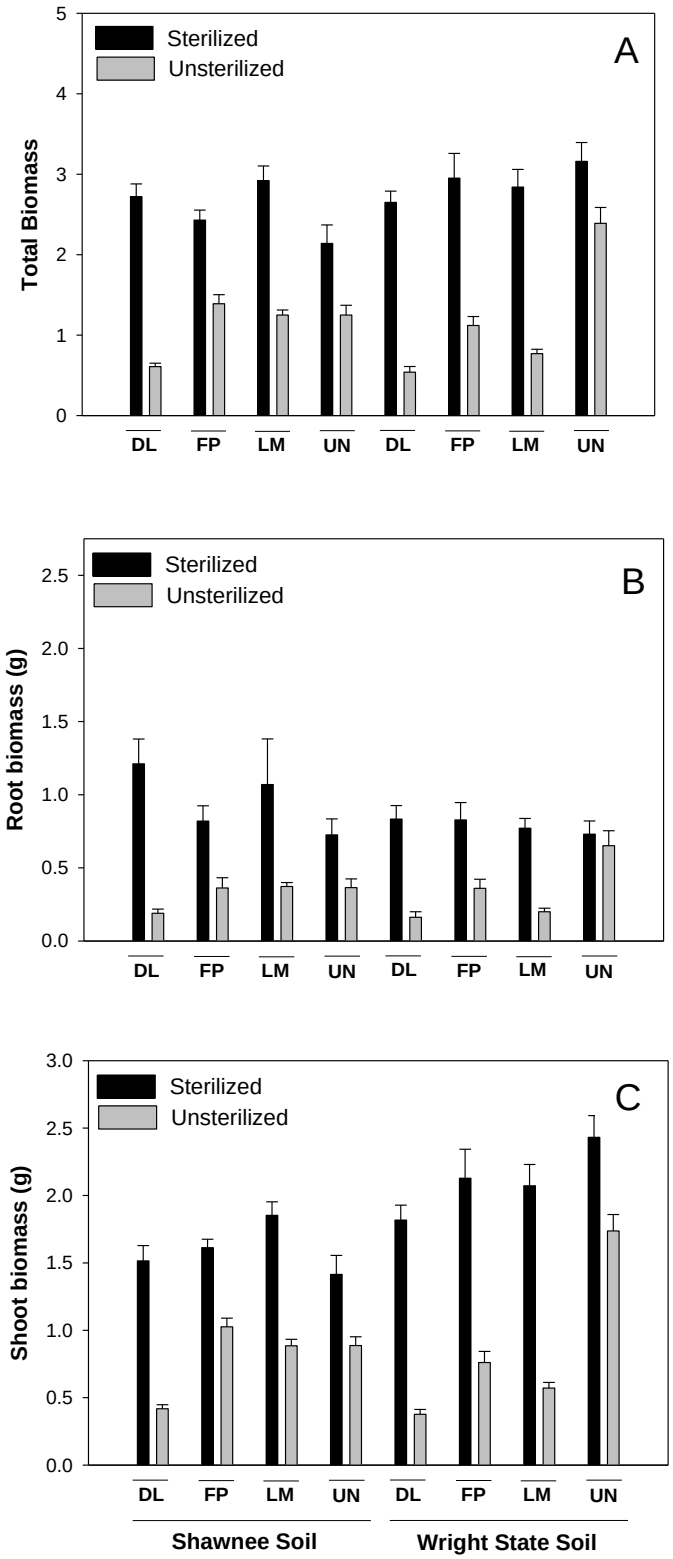

Figure 3: Mean (+ 1SE) dry (A) Total biomass, (B) Root biomass and (C) Shoot biomass of *Diervilla lonicera* in response to soil sterilization, and soil conditioning by three different species, and two soil types.

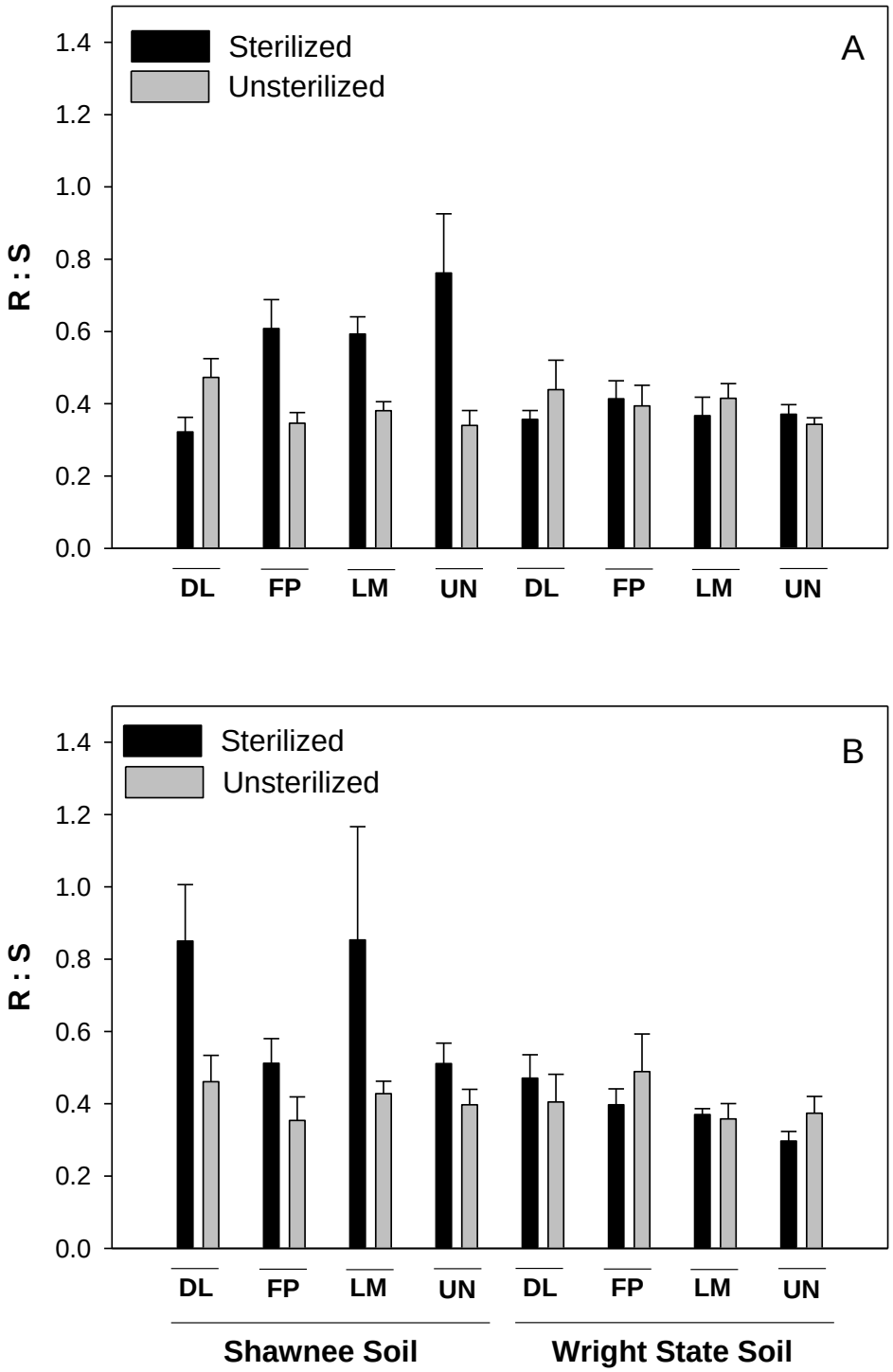

Figure 4: The effect of soil sterilization, and soil conditioning by three different species, in two soil types on root/shoot ratio (R:S) (mean ± 1SE) of (A) *Lonicera maackii* and (B) *Diervilla lonicera*

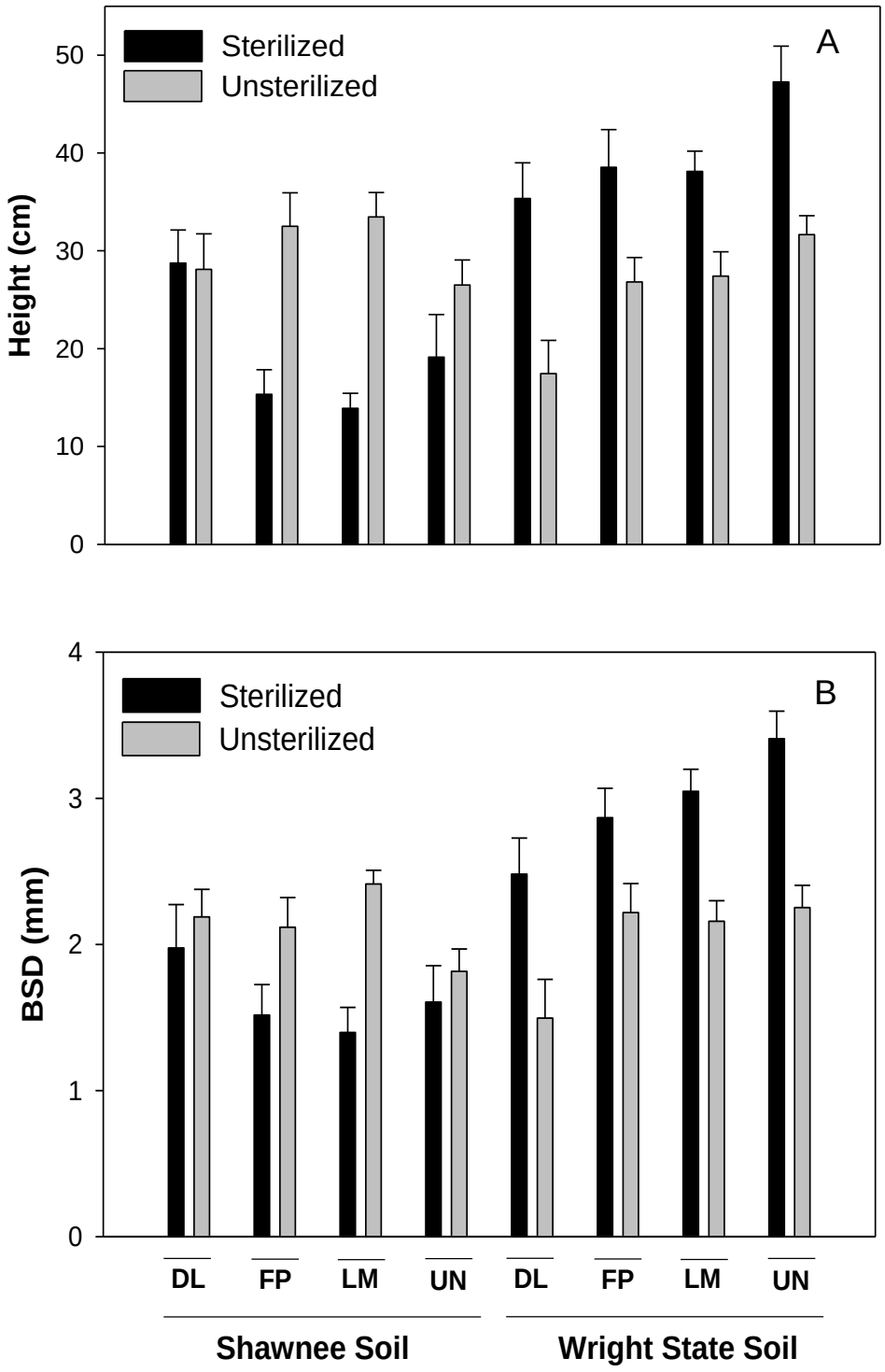

Figure 5: Mean (+ 1SE) final (A) height and (B) BSD of *Lonicera maackii* in response to soil sterilization, and soil conditioning by three different species and two soil types

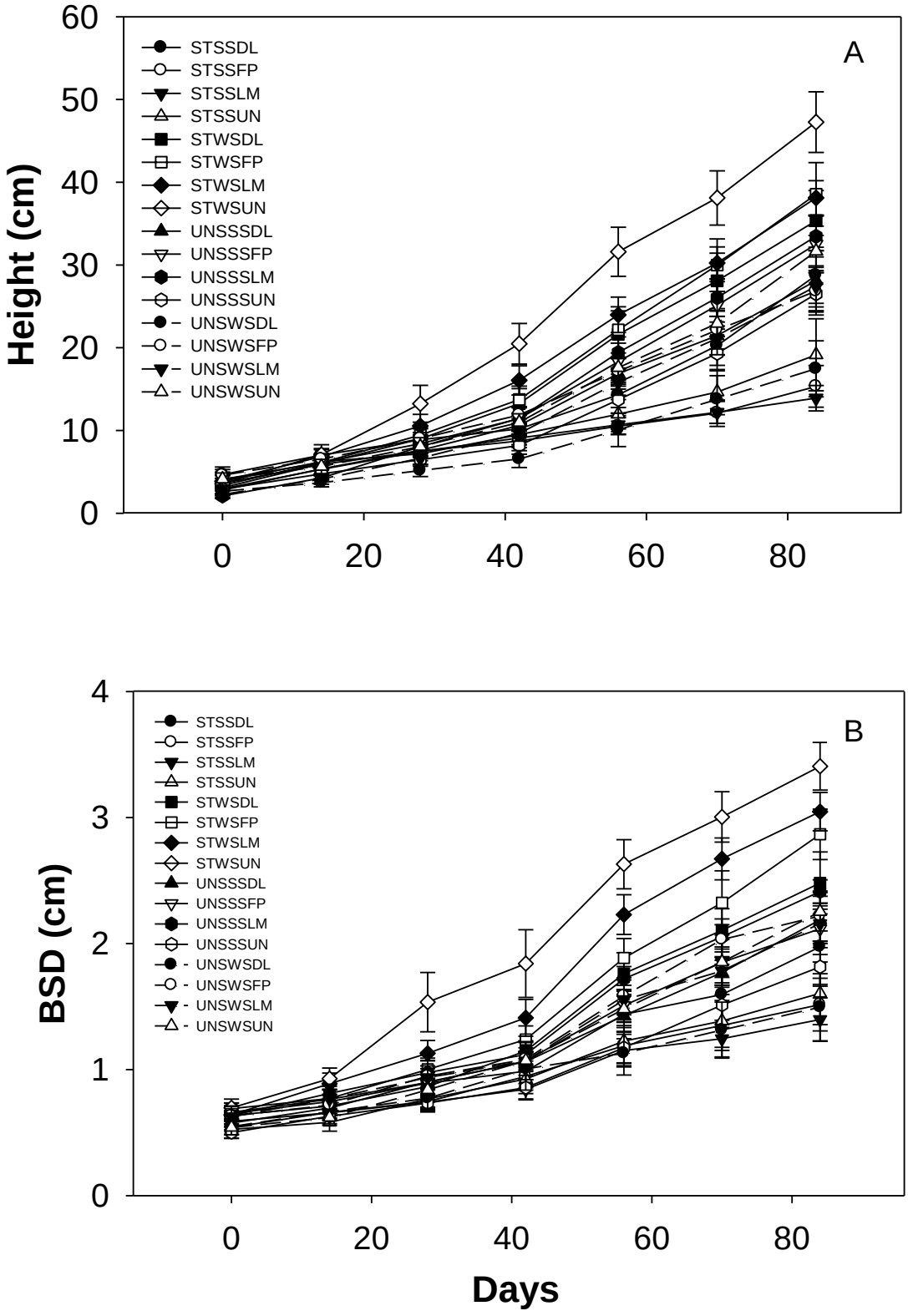

Figure 6: Change in final (A) height and (B) BSD, (mean ± 1SE) in *Lonicera maackii* in response to sterilization, two different soil types, and soil conditioning by three different species

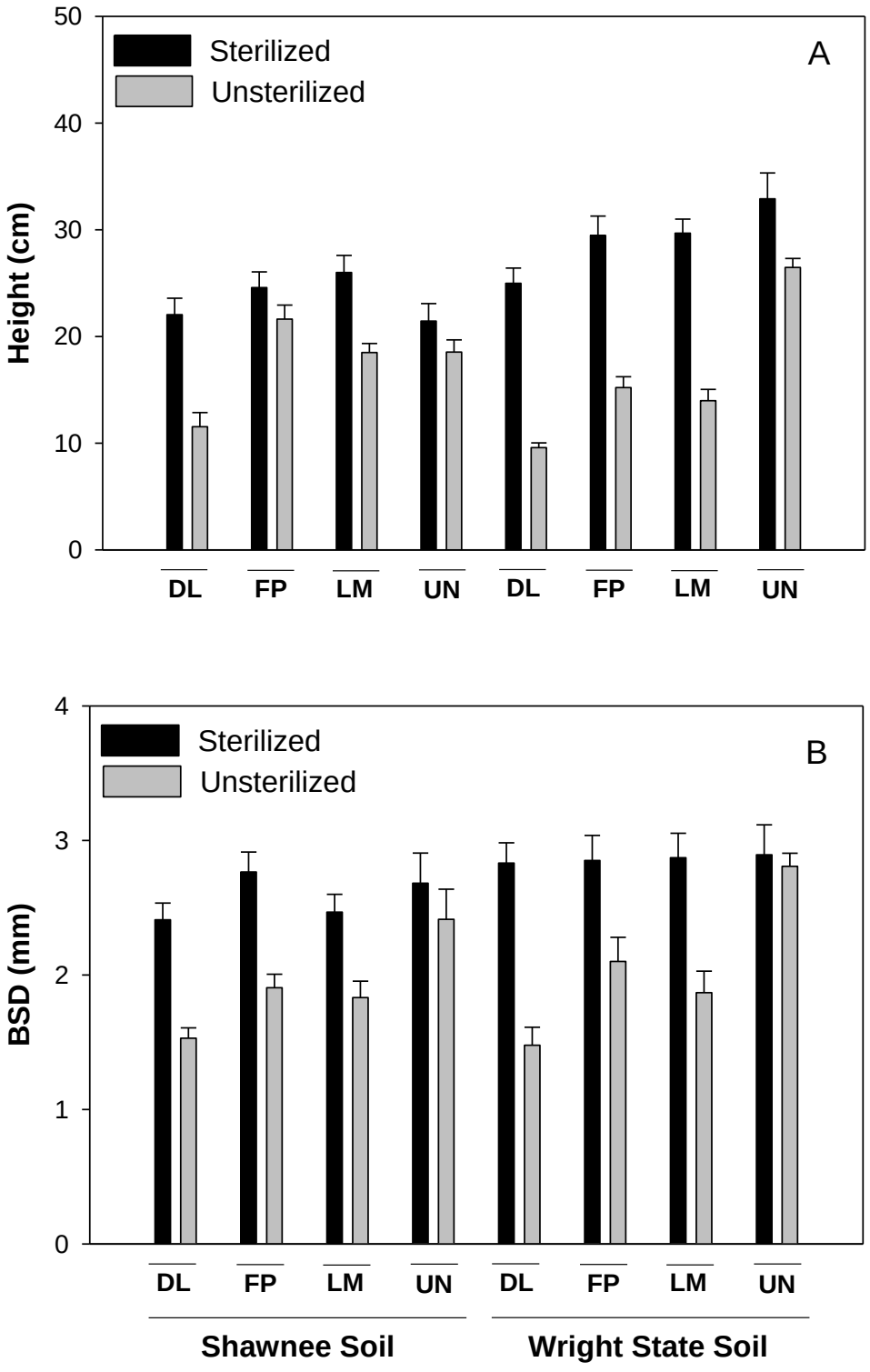

Figure 7: Mean (+ 1SE) final (A) height, and (B) BSD of *Diervilla lonicera* in response to soil sterilization, and soil conditioning by three different species and two soil types

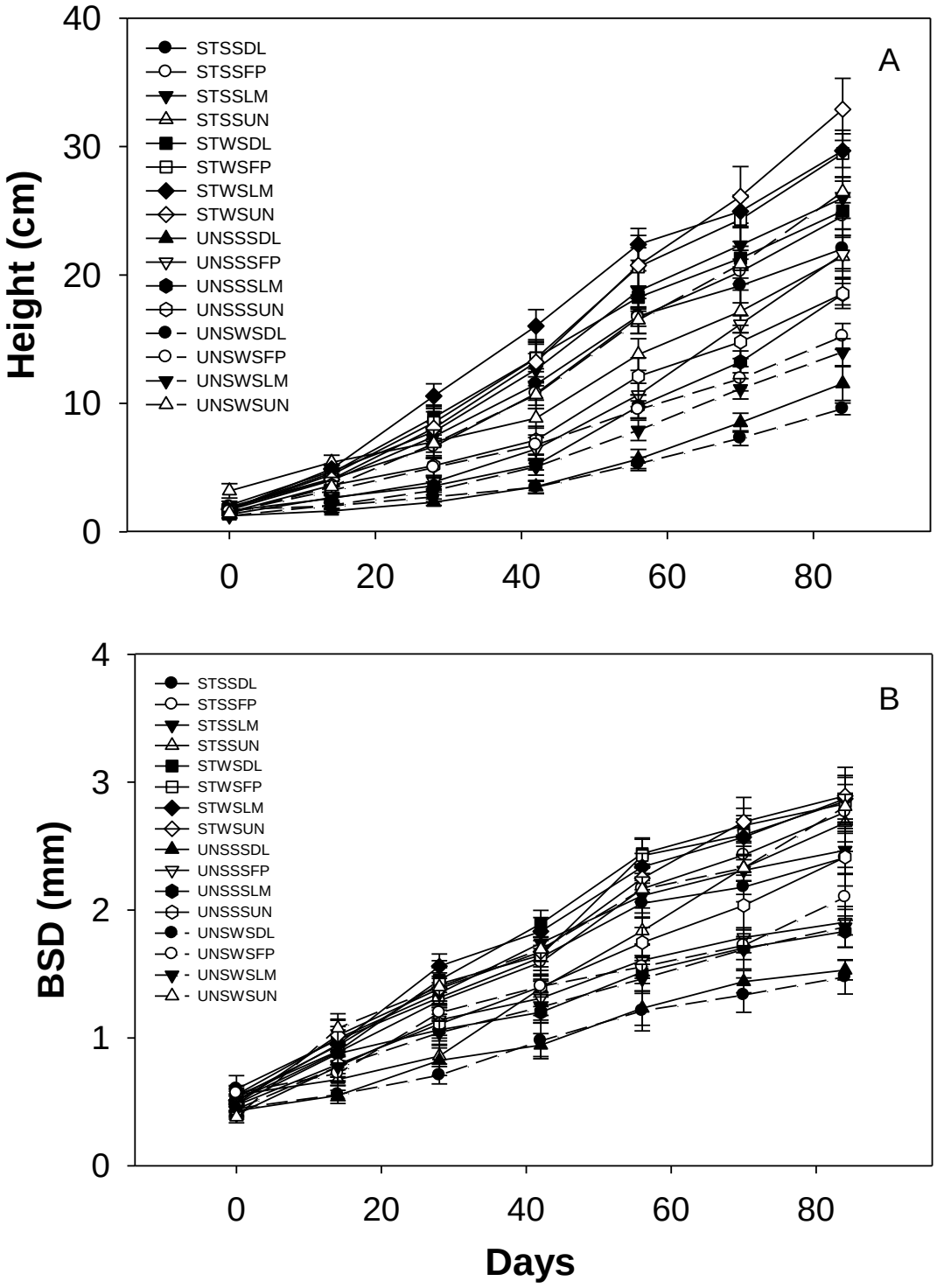

Figure 8: Change in final (A) height and (B) BSD, (mean ± 1SE) in *Diervilla lonicera* in response to sterilization, two soil types, and soil conditioning by three different species

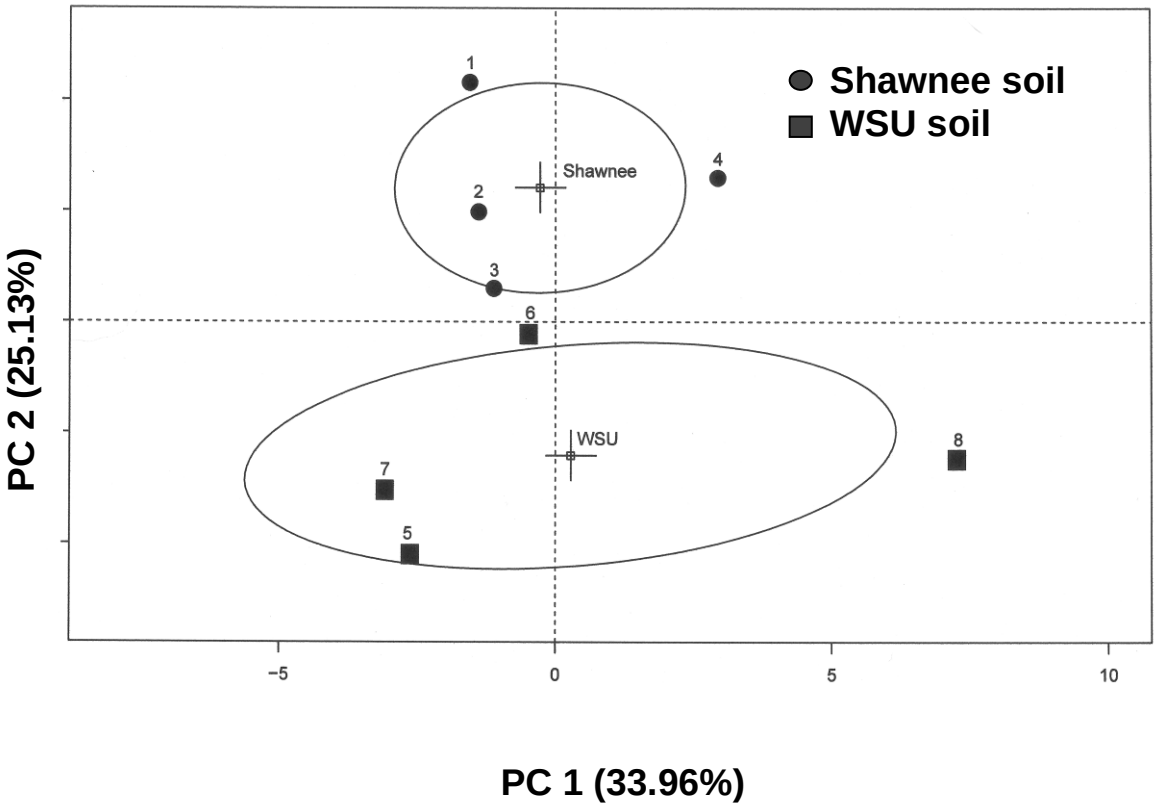

Figure 9: Mean values of principal components (PC 1 and PC 2) in response to two soil types with 95% CI. Percent of explained variance are in parentheses.

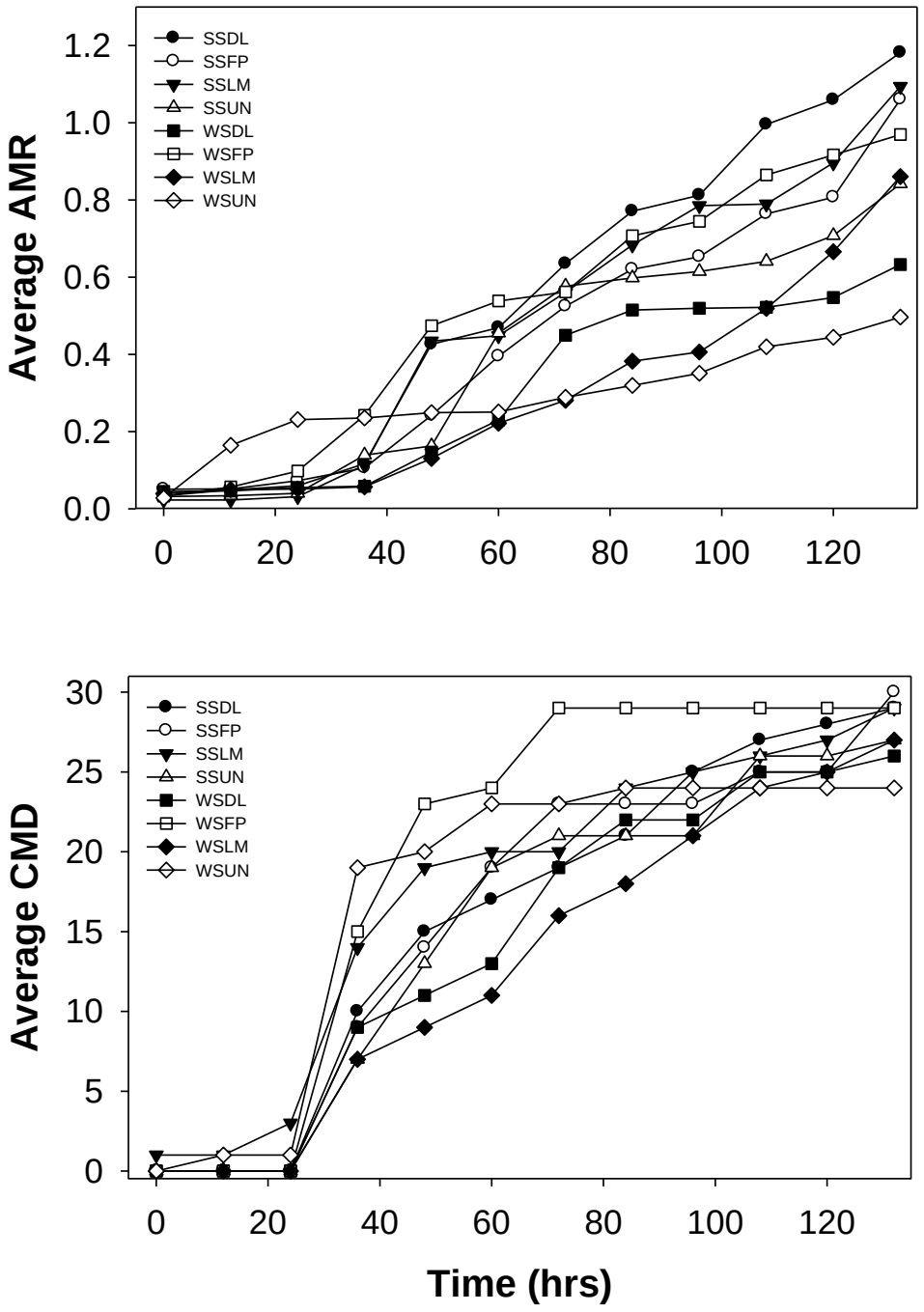

Figure 10: CLPP comparing the AMR and CMD in response to soil conditioning by three different species and unconditioned soil control in two soil types.

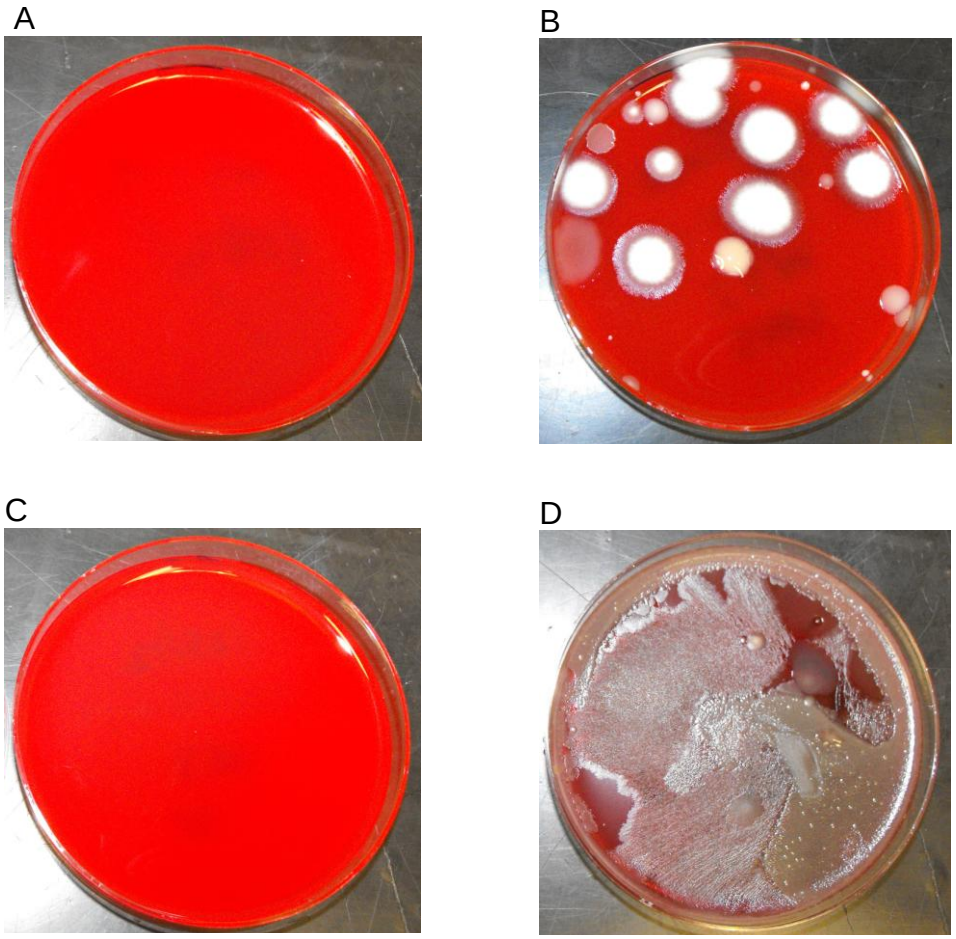

Figure 11: Soil sterilization confirmation: soil extracts incubated on TSA plates for 72h (A) Wright State sterilized soil extract (B) Wright State unsterilized soil extract (C) Shawnee sterilized soil extract (D) Shawnee unsterilized soil extract.

LITERATURE CITED

Agrawal, A. (2005). Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. *Ecology*, *86*(11), 2979-2989.

Ahonsi, M. (2002). Effects of soil pasteurisation and soil N status on severity of *Striga hermonthica* (Del.) Benth. in maize. *Soil Biology & Biochemistry*, *34*(11), 1675-1681.

Alverson, S., Friese, C. (2010) Assessing the implications of arbuscular mycorrhizal colonization in the invasive shrub Amur Honeysuckle (*Lonicera maackii)*, unpublished.

Bartuszevige, A.M., D.L. Gorchov, and L. Raab. (2006). The relative importance of landscape and community features in the invasion of an exotic shrub in a fragmented landscape. Ecography 29: 213-222.

Beckstead, J. and Parker, I (2003). Invasiveness of *Ammophila arenaria:* release from soil-borne pathogens? *Ecology:* 84(11), 2824-2831.

Beest, M., Stevens, N., Olff, H., and van der Putten, W.H. (2009). Plant-soil feedback induces shifts in biomass allocation in the invasive plant *Chromolaena odorata*. *Journal of Ecology*, 97:1281-1290.

Boivin, M.E.Y., (2005). Diversity of Microbial Communities in Metal-polluted Heterogeneous Environments. PhD thesis, Vrije University, Amsterdam.

Brinkman, E., Van der Putten, W., Bakker, E., Verhoeven, K. (2010). Plant-soil feedback: experimental approaches, statistical analyses and ecological interpretations. *Journal of Ecology*, *98*:1063-1073.

Burke, G. S. (1923). Studies on the thermal death time of spores of *Clostridium botulinum*. *The Journal of Infectious Diseases*, 32(6), 433-438

Callaway, R. and Aschehoug, E (2000). Invasive plant versus their new and old neighbors: a mechanism for exotic invasion. *Science* 290:521–523.

Callaway R., Thelen G., Rodriguez A., Holben W. (2004a). Soil biota and exotic plant invasion. *Nature* 427: 731–733.

Callaway, R., Thelen, G., Barth, S., Ramsey, P., Gannon, J. (2004b). Soil fungi alter interactions between the invader *Centaurea maculosa* and North American natives. *Ecology*: 85 (4), 1062-1071.

Callaway, R. M. and W. M. Ridenour. (2004c). Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Eco and the Environment* 2:436-443.

Callaway, R. M., Cipollini, D., Barto, K., Thelen, G., Hallett, S., Prati, D., Stinson, K., and Klironomos, J. (2008). Novel Weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. *Ecology*, *89*(4), 1043-1055.

Cipollini, D., and Dorning, M. (2008) Direct and indirect effects of conditioned soils and tissue extracts of the invasive shrub, *Lonicera maackii,* on plant performance. *Castanea* 73 (3): 000-000.

Cipollini, D., Stevenson, R. and Cipollini. K. (2008a). Contrasting direct and indirect effects of allelochemicals from two invasive plants on the performance of a non-mycorrhizal plant. *International Journal of Plant Science.* 169:371–375.

Cipollini, D., Stevenson, R. Enright, S. Eyles, A. and Bonello. P. (2008b). Phenolic metabolites in leaves of the invasive shrub, *Lonicera maackii*, and their potential phytotoxic and anti-herbivore effects. *Journal of Chemical Ecology* 34:144–152.

Cipollini K, McClain G, Cipollini D. (2008) Separating above- and belowground effects of *Alliaria petiolata* and *Lonicera maackii* on the performance of *Impatiens capensis*. *American Midland Naturlist* 160:117-128.

Cipollini, K. and Schradin, K. (2011). Guilty in the court of public opinion: Testing presumptive impacts and allelopathic potential of *Ranunculus ficaria. American Midland Naturalist, in press.*

Chodak, M. and Niklinska, M. (2010). Effect of texture and tree species on microbial properties of mine soils. *Applied Soil Ecology*, *46*(2), 268-275.

Chon, S., Choi, S., Jung, S., Jang, H., Pyo, B. , & Kim, S. (2002). Effects of alfalfa leaf extracts and phenolic allelochemicals on early seedling growth and root morphology of alfalfa and barnyard grass. *Crop Protection*, *21*(10), 1077-1082

Collier, M.H., J.L. Vankat, and M.R. Hughes. (2002). Diminished plant richness and abundance below *Lonicera maackii*, an invasive shrub. *American Midland Naturalist.* 147:60–71.

Csiszár, Á. *(2009)* Allelopathic Effects of Invasive Woody Plant Species in Hungary. *Acta Silvatica et Lignaria Hungarica, 5:9-17*

Daskalaki, D., Kefi, G., Kotsiou, K., & Tasioula-Margari, M. (2009). Evaluation of phenolic compounds degradation in virgin olive oil during storage and heating. *Journal Of Food & Nutrition Research*, *48*(1), 31-41

Davidson, A. M., Jennions, M., Nicotra, A.B. (2011). Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters* (2011) 14: 419–431

Davidson, E., and Swank, W. (1986). Environmental parameters regulating gaseous nitrogen losses from two forested ecosystems via nitrification and denitrification. *Applied Environmental Microbiology* 52:1287-1292.

De Leij, F. A. A. M, Whipps, J. M. Lynch, J. M. (1994). The Use of Colony Development for the Characterization of Bacterial Communities in Soil and on Roots. *Microbial Ecology*, 27(1), 81-97

Dorning, M. and Cipollini, D. 2006. Leaf extracts of the invasive shrub, *Lonicera maackii* inhibit seed germination of three herbs with no autotoxic effects. *Plant Ecology*, 184:287–296

Ehrenfeld J.G. (2003). Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems* 6: 503–523

Ehrenfeld, J.G., Ravit, B, and Elgersma1, K. (2005) Feedback in the plant-soil system. *Annual Review of Environment and Resources* 30:75–115

Ehrenfeld J.G. (2006). A potential source of information for screening and monitoring the impact of exotic plants on ecosystems. *Biological Invasions* 8:1511–1521.

Franche, C., Lindström, K., and Elmerich, C. (2009). Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. *Plant and Soil*, *321*(1-2), 35 - 59

Gardner, R., *Lonicera maackii* Ohio Distribution Map as of 2010. Ohio Department of Natural Resources, Columbus, Ohio. Personal Communication, January 2011

Garland, J. and Mills, L. (1991) Classification and Characterization of Heterotrophic Microbial Communities on the Basis of Patterns of Community-Level Sole-Carbon-Source Utilization *Applied And Environmental Microbiology*, Aug. 1991, p. 2351-2359

Gee, G., and Bauder, J. 1986. Particle-size analysis. p. 383–411. *In* A. Klute (ed.) Methods of soil analysis. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA,Madison, WI.

Harrison, K. A. and R. D. Bardgett (2010). Influence of plant species and soil conditions on plant–soil feedback in mixed grassland communities. *Journal of Ecology*, *98*(2), 384-395

Hawkes, C., Wren, I., Herman D., Fireston, M. (2005). Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. *Ecology Letters*, *8*(9), 976.

Hongqing Hu. (2005). Role of Phenolics and Organic Acids in Phosphorus Mobilization in Calcareous and Acidic Soils. *Journal of Plant Nutrition, 28*(8), 1427-1439.

Hidayati, S., Baskin, J. and Baskin, C. (2000). Dormancy-breaking and germination requirements for seeds of four *Lonicera* species (Caprifoliaceae) with underdeveloped spatulate embryos. *Seed Science Research* 10:459-469.

Hinsinger P, Gobran GR, Gregory PJ, Wenzel WW (2005). Rhizosphere geometry and heterogeneity arising from root mediated physical and chemical processes. *New Phytologist* 168:293–303

Hitzl W., Ranggera A., Sharma S., Insam H. (1997) Separation power of 95 substrates of the Biolog system determined in various soils. FEMS *Microbiol. Ecology* 22**:** 167-174

Hudson, B. D. (1994) Soil organic matter and available water capacity. *Journal of Soil and Water Conservation* 49:189-194

Hutchinson T.F., and J.L. Vankat. (1997). Invasibility and effect of Amur honeysuckle in southwestern Ohio. *Conservation Biology* 11(5): 1117-1124.

Inderjit and Dakshini, K.M.M. (1994). Allelopathic effect of *Pluchea lanceolata* (Asteraceae) on characteristics of four soils and tomato and mustard growth. *American Journal of Botany* 81(7): 799-804.

Jacobs, B. (2009). Evolution of fruit and seed characters in the *Diervilla* and *Lonicera* clades (Caprifoliaceae, Dipsacales). *Annals of Botany*,*104*(2), 253-276.

Kardol, P., Bezemer T., van der Putten, W. (2006) Temporal variation in plant–soil feedback controls Succession. *Ecology Letters*, 9: 1080–1088

Kiers, T., Palmer, T., Ives, A., Bruno, J., Bronstein, J. (2010). Mutualisms in a changing world: an evolutionary perspective. *Ecology Letters*, *13*(12), 1459-1474

Klironomos, J. (2002). Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, *417*(6884), 67.

Kueffer, C., Schumacher, E., Fleishmann, K., Edwards, P., & Dietz, H. (2007). Strong below-ground competition shapes tree regeneration in invasive *Cinnamomum verum* forests. *Journal of Ecology, 95:* 273-282.

Levine, J. M., Pachepsky, E., Kendall, B. E., Yelenik, S. G., & Lambers, J. (2006). Plant–soil feedbacks and invasive spread. *Ecology Letters, 9*(9), 1005-1014

Luken, L.O., and W. Thieret. 1996. Amur honeysuckle, its fall from grace. *BioScience* 46(1): 18-24.

Luken, J.O., L.M. Kuddes, T.C. Tholemeier and D.M. Haller. 1997. Comparative responses of *Lonicera maackii* (Amur Honeysuckle) and *Lindera benzoin* (Spicebush) to increased light. American Midland Naturalist 138(2): 331-343

Luken, J.O. 1988. Population structure and biomass allocation of naturalized shrub *Lonicera maacki*i (Rupr.) Maxim. in forest and open habitats. American midland naturalist 119(2): 258-267.

Mangan, S., Schnitzer, A., Herre, E., Keenan, M., Valencia, M., Sanchez, E., Bever, J. (2010) Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature* 466: 752–755.

Mangan, S. A. (2010). Specificity between Neotropical tree seedlings and their fungal mutualists leads to plant-soil feedback. *Ecology*, *91*(9), 2594-2603

Markham, J. H. (2009). Reciprocal interactions between plants and soil in an upland grassland. *Ecological Research*, *24*(1), 93-98.

McCarthy-Neumann, S. (2010). Conspecific and heterospecific plant–soil feedbacks influence survivorship and growth of temperate tree seedlings. *Journal of Ecology*, *98*(2), 408-418

MacFarlane, D.W. and Meyer, S.P. (2005) Characteristics and distribution of potential ash tree hosts for emerald ash borer. *Forest Ecology and Management,* 213(1-3), 15-24.

Ohio Department of Natural Resources (ODNR), (2010) Division of Soil and Water Resources - Soil Regions http://www.dnr.state.oh.us/ Accessed 21 December 2010

Pimentel, D., Lach, L., Zuniga, R., Morrison, D. (2000). Environmental and economic costs of nonindigenous species in the United States. *Bioscienc*e, 50(1): 53-65.

Pollock, J., Kogan, L., Thorpe, A., Holben, W. (2011) (±)-Catechin, a root exudate of the invasive *Centaurea Stoebe* Lam. (spotted knapweed) exhibits bacteriostatic activity against multiple soil bacterial populations. *Journal of Chemical Ecology* 37:1044–1053

Reinhart, K. and Callaway, R. (2004) Soil biota facilitate exotic *Acer* invasions in Europe and North America. *Ecological Applications*: 14(6), 1737-1745.

Reinhart, K., Royo, A., Van Der Puttent, W., and Clay, K. (2005). Soil feedback and pathogen activity in *Prunus serotina* throughout its native range. *Journal of Ecology*, *93*(5), 890-898.

Rodgers, V., Wolfe, B., Werden, L., Finzi, A. (2008) The invasive species *Alliaria petiolata* (garlic mustard) increases soil nutrient availability in northern hardwood-conifer forests. *Oecologia*, 157:459–471.

Ros, M. (2008). 16S rDNA analysis reveals low microbial diversity in community level physiological profile assays. *Journal of Microbiological Methods*, [No Volume/Issue], 221-226. doi:10.1016/j.mimet.2008.01.003

Sauer J., Hines J., Fallon J (2008) The North American Breeding Bird Survey, results and analysis 1966–2007. Version 5.15.2008. USGS Patuxent Wildlife Research Center, Lauren, MD

Scharfy, D. (2010). Invasion of *Solidago gigantea* in contrasting experimental plant communities: effects on soil microbes, nutrients and plant-soil feedbacks. *Journal of Ecology*, *98*(6), 1379-1388.

Schoen, D. J. (1997). Floral biology of *Diervilla lonicera* (Caprifoliaceae). *Bulletin of the Torrey Botanical Club* 104(3) 234-240.

Schnepf, A., Roose, T., and Schweiger, P. (2008). Impact of growth and uptake patterns of arbuscular mycorrhizal fungi on plant phosphorus uptake—a modelling study. *Plant and Soil*, *312*(1-2), 85 - 99

Stefanowicz, A. (2006). The Biolog Plates Technique as a Tool in Ecological Studies of Microbial Communities. *Polish Journal of Environmental Studies*, *15*(5), 669-676.

Stinson, K., Campbell, S., Powell, J., Wolfe, B., Callaway, R., Thelen, G., Hallett, S., Prata, D., and Klironimos, J., (2006). Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *PLoS Biology,* 4(5):e140.

Thorpe, A. (2006). The invasive forb, Centaurea maculosa, increases phosphorus availability in Montana grasslands. *Applied Soil Ecology 32*: 118-122.

Trevors, J. (1996) Sterilization and inhibition of microbial activity in soil. *Journal of Microbiological Methods*, 26: 53-59

Troelstra, S., Wagenaar, R., Smant, W. Peters, B. (2001) Interpretation of bioassays in the study of interactions between soil organisms and plants: involvement of nutrient factors. *New Phytologist*, **150**, 697–706.

US Department of Agriculture (USDA). (2010). The PLANTS Database. http://plants.usda.gov. Accessed 21 December 2010.

US Department of Agriculture Soil Conservation Service (USDA:SCS). (2010). Soil Survey of Scioto County, Ohio. http://soildatamart.nrcs.usda.gov/ Accessed 21 December 2010.

Van Grunsven, R., Van Der Putten, W., Bezemer, T., Tamis, W., Berendse, F., and Veenendaal, E. (2007). Reduced plant–soil feedback of plant species expanding their range as compared to natives. *Journal of Ecology*, *95*(5), 1050-1057.

van Wilgen, B, de Wit, M, Anderson, H, Le Maitre, D, Kotze I, Ndala S, et al. (2004) Costs and benefits of biological control of invasive alien plants: case studies from South Africa. *South African Journal of Science 100*: 113-122.

Watling, J., and Orrock, J. (2010) Measuring edge contrast using biotic criteria helps define edge effects on the density of an invasive plant. Landscape *Ecology* 25:69–78

Wolf, D.C.; Dao, T.H.; Scott, H.D.; Lavy, T.L. (1989). Influence of sterilization methods on selected soil microbiological, physical and chemical properties. *Journal of Environmental Quality* 18:39-44.

Zhang, Z. (2010). Isolation, identification and characterization of soil microbes which degrade phenolic allelochemicals. *Journal of Applied Microbiology*, *108*(5), 1839-1849