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# Evolutionary Interactions in Invasive Species: the Importance of Plant-Soil Feedbacks to Local Adaptation and Rapid Evolution

Emmi Felker-Quinn
efelkerq@utk.edu

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To the Graduate Council:

I am submitting herewith a dissertation written by Emmi Felker-Quinn entitled "Evolutionary Interactions in Invasive Species: the Importance of Plant-Soil Feedbacks to Local Adaptation and Rapid Evolution." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

Jennifer A. Schweitzer and Joseph K. Bailey, Major Professor

We have read this dissertation and recommend its acceptance:

Alison Buchan, Aimee Classen, Richard Norby

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

# Evolutionary Interactions in Invasive Species: the Importance of Plant-Soil Feedbacks to Local Adaptation and Rapid Evolution

A Dissertation Presented for
the Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Emmi Felker-Quinn
August 2012

## ACKNOWLEDGEMENTS

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# ABSTRACT

Interactions between intraspecific plant variation and the environment can create evolutionary and ecosystem feedbacks, but the contribution of these feedbacks to the success of invasive plant species has rarely been explored or quantified. To test whether evolution occurs during the process of plant invasion I conducted three major experiments and a meta-analysis to test various aspects of this central question. First, I conducted a meta-analysis of studies that tested the Evolution of Increased Competitive Ability (EICA) hypothesis. The meta-analysis did not support EICA's prediction that release from herbivores leads to reduced defenses and higher performance, but it showed that evolutionary change occurs in these traits across plant invasions. To test whether soils act as selective agents for invasive plants, I grew 13 populations of the invasive tree *Ailanthus altissima* in a common garden. Phenotypic variation showed that genetic differentiation correlated with climate and soil factors has occurred among populations, indicative of rapid evolution in response to local conditions. To test how soils act as selective agents, I conducted a study in which seeds from three populations were reciprocally transplanted in soils from those populations. Genetic variation and positive feedbacks to plant performance were expressed in soils with biotic communities, but not in sterilized soils. This indicates that soil biotic communities may select for plant performance and genetic variation in future generations. To test whether intraspecific variation associated with plant nutrient availability could create ecosystem feedbacks, I conducted a decomposition experiment of leaf litter from elevated carbon dioxide and nitrogen fertilization experiments. There were feedbacks that led to faster mass loss at control sites and in the nitrogen fertilized sites, but a negative feedback led to slower mass loss in elevated carbon dioxide sites. Environmental conditions, including anthropogenic alterations to environment, can create ecosystem feedbacks between intraspecific plant variation and processes that regulate soil nutrient availability. Overall, this dissertation indicates that evolution is broadly important in invasive plant species, that it occurs in response to climatic, abiotic soil properties, and soil biotic communities and that plant-soil feedbacks to ecosystem properties vary by environment, with theoretical and applied implications for all results.

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One of the major questions of plant ecology is what determines the distribution of plant species (Clements 1916). Over the last century, as niche theory (Hutchinson 1957, Silvertown and Law 1987) and dispersal (Gleason 1927, Zobel 1997) have driven research on plant distributions, humans have complicated the concept by transporting plant species to novel environments. A fraction of these species have successfully established large and expanding populations in these new regions (Williamson and Fitter 1996). Invasion biology seeks to understand and predict such range additions. The phenomenon of plant invasion, in which a species which was recently absent from a large area becomes abundant shortly after its introduction, has been attributed to particular characteristics of the invaded community (Davis et al. 2000, Levine et al. 2004), to special traits of the invading plant species (Grotkopp et al. 2002), or to a particular kind of disturbance, usually human-mediated (Gavier-Pizarro et al. 2010). No single factor has proven powerful in explaining the majority of plant invasions, and as a result hypotheses have proliferated in the scientific literature (Catford et al. 2009). The majority of these hypotheses treat species as static entities, in which traits important to invasion success such as seedling survival, biomass production, reproduction, and resistance to enemies are fixed across the new range.

The field of ecological genetics is dedicated to connecting variation in phenotypic traits within species to processes of selection on genetic variation (Conner and Hartl

2004). The pattern and extent of genetic variation at different scales within a single species—among individuals, reproductively or geographically isolated demes, or metapopulations—changes over time in response to genetic drift, gene flow, mutation, and natural selection for advantageous phenotypic traits (Slatkin 1987, Manel et al. 2003). The processes of evolution are responsible at the largest timescale for the entire diversity of species, and at a smaller, human timescale, allow groups of organisms to maximize fitness in their environment through local adaptation. Evolution has historically been discounted as unimportant for plant invasions, which were assumed to start from small groups of individuals with overall low genetic diversity (Lee 2002). In the past decade, published population genetics studies of a variety of invasive plant species have fostered a growing awareness that there is significant genetic variation in the invaded range for traits related to plant performance and fitness (Blair and Wolfe 2004, Maron et al. 2004, van Kleunen and Fischer 2008). The search for the functional traits that make a species a successful invader must evolve to also evaluate the selective forces—interactions with the abiotic environment and with other species—that act to exaggerate or weaken these traits *in different populations* within the invaded range. I have emphasized the population-level aspect in the previous statement because one of the consequences of the interaction between environmental variation and genetic variation can be to create what John Thompson has termed the Geographic Mosaic, in which selective forces vary in strength across the landscape, leading to evolutionary hotspots and coldspots (Thompson 1997, Thompson 1999).

Intraspecific variation is not only the material upon which selection acts, it also may influence the agents of selection, creating evolutionary feedbacks. Plant functional traits (e.g., biomass production, nutrient content and secondary chemistry) not only determine the performance and fitness of individual species, but also scale to affect communities and ecosystems through their effects on aboveground (Bangert et al. 2006) and belowground (Schweitzer et al. 2008) consumers, detritivore communities and nutrient pools and fluxes (Schweitzer et al. 2004, Schweitzer et al. 2005). Intraspecific variation due to genetic variation has been demonstrated to impact plant interactions with consumers as well as to create islands of genotype-specific soil conditions and processes, most notably in the *Populus* hybrid system (reviewed in Whitham et al. 2003, Schweitzer et al. 2005). However, environmental conditions, particularly resource availability, are also an important source of intraspecific variation. Plant responses to resource availability may also lead to ecosystem feedbacks. For example, plants may respond to low resource availability by producing highly defended tissue of poor nutritional quality (Coley et al. 1985), which slows consumption rates and nutrient cycling rates (Hattenschwiler and Vitousek 2000), further constraining resource availability. Research into environmental variability is timely as anthropogenic changes to carbon and nitrogen cycling along with habitat disturbance have the potential to radically alter ecosystems (Vitousek 1994). From the standpoint of research on invasive species, attempts to classify invasible ecosystems and communities must include the consideration that the invaded environment may not be a stable entity (i.e., it evolves).

In this dissertation, I address the varied roles of intra-specific variation to address questions of whether rapid evolution contributes to the success of invasive plants, what environmental factors or biotic interactions, especially in soils, act as agents of selection, whether there is feedback between plants and soils to create evolutionary trajectories of plants and soil communities and if climatic variation can lead to differential ecosystem responses within a species (i.e., variation in intra-specific response to changes in atmospheric and soil chemistry). In Chapter 2, I address the question of whether evolution occurs broadly across invasive plant species in a meta-analytic review of the Evolution of Increased Competitive Ability (EICA) hypothesis (Blossey and Notzhold 1995). I evaluate the evidence from this literature of whether release from native herbivores causes evolution in invasive populations, particularly of increases in plant growth and competitive ability. In Chapter 3, I present results from a common-garden study of the invasive tree species *Ailanthus altissima* that address whether genetic differentiation in plant performance supports evidence of evolution in the invasive range of this species, and evaluate climate and abiotic soil factors as possible selective agents in local adaptation. Chapter 4 examines the importance of soil as a selective agent for *Ailanthus altissima* more closely, by asking whether populations create plant-soil feedbacks beneficial to their offspring, which would provide a soil-based mechanism for selection. In Chapter 5, I examine feedbacks within an ecosystem, asking whether changes in plant resource availability lead to intra-specific variation in plant litter quality within a single tree species, *Liquidambar styraciflua,* to impact leaf litter decomposition rates and changes in nutrient dynamics (i.e., nutrient immobilization and release). Chapter 6 details conclusions and future directions for research into evolutionary and ecosystem feedbacks between soils and intraspecific variation in plants.

# CHAPTER 2: **Meta-analysis reveals evolution in invasive species but little support for Evolution of Increased Competitive Ability (EICA)**

## Abstract

The Evolution of Increased Competitive Ability (EICA) hypothesis (Blossey and Notzhold 1995) proposes that evolutionary change in response to release from coevolved herbivores is responsible for the success of many invasive plant species. Studies that evaluate this hypothesis have used many different techniques to test whether plants from the introduced range allocate less to defenses and more to growth and competitive ability than do plants from the home range, with mixed results. We conducted a meta-analysis of 28 published experimental tests of EICA. Plant defenses were grouped by metrics of chemical and physical leaf traits, insect herbivore performance and development, or the damage to plants caused by herbivores. Metrics of plant performance and competitive ability were grouped by plant growth traits, reproductive traits, or direct tests of invasive plant performance under competitive conditions. We found no overall support for reduced defenses in the introduced range across all the invasive species considered. Although introduced range plants had reduced defenses against herbivores restricted to their native ranges, introduced range plants were as well defended as the home range plants against herbivores that were widely distributed or that had been introduced as biological control agents. As predicted by EICA, plants from the introduced range had higher growth and non-reproductive performance than home range plants, but there were no differences between native and introduced ranges in reproduction or competitive ability. In contrast to previous reviews, we found no broad support for the EICA hypothesis when examining a holistic suite of competitive traits (defense, growth and competitive ability). Each of the three models of plant defense and the three models of plant growth showed broad support for genetically-based changes in plant traits after introduction into new ranges but not in the manner suggested by EICA. This review suggests that evolution occurs as a result of plant introduction and population expansion in invasive plant species, and may be an important factor in contributing to the invasiveness and persistence of some introduced species.

## Introduction

In the research effort to identify and explain the success of invasive species in their new range, evolutionary explanations for invasiveness are rarely invoked. Only a small proportion of introduced species succeed in their new range, attaining greater individual size or fitness or establishing populations of greater numbers or densities, than what might be predicted from their native range (Hinz and Schwarzlaender 2004, but see Thebaud and Simberloff 2001). Although Bossdorf et al. (2005) divided research into invasiveness as seeking either an ecological or an evolutionary explanation, effort has been heavily skewed towards identifying ecological explanations. Twenty-six of the twenty-nine hypothesis of plant invasion identified in a recent review by Catford et al. (2009) explain invasions as the result of static plant traits, suitability of the invaded environment, or ecological interactions between species traits and environments. Evolutionary interactions between invasive plants and their new environment have largely been neglected as important to the invasion process, despite the importance of local adaptation in determining the distribution and success of native plants (Alvarez et al. 2009, Kawakami et al. 2011, Macel et al. 2007). The most prominent of the hypotheses of plant invasion which invoke evolution of invasives, the Enemy Release hypothesis and the Evolution of Increased Competitive Ability (EICA) hypothesis, propose that evolution of invasive populations occurs as a release from native predators, not in response to selective factors in the environment of the new range.

The EICA hypothesis has served as an important stimulus to research because it makes specific predictions about the evolutionary trajectories of multiple plant traits, and also because it is used to justify the introduction of novel herbivores as biocontrol agents. EICA was proposed by Blossey and Notzhold (1995) to explain the success of *Lythrum salicaria* in its invasive range. Blossey and Notzhold (1995) proposed that plants in their native range allocate significant carbon and energy towards synthesizing defenses against coevolved herbivores. Invasive plants, freed from the selective constraint of herbivory, allocate resources away from defenses into increased growth, shifts in allocation which become genetically fixed. EICA predicts that invasive populations will display genetically-based decreases in herbivore defenses and genetically-based increases in competitive ability (Figure 1). A number of herbivores have been introduced to novel ranges with the expectation that they will feed on these poorly-defended plants, reducing plant vigor, fitness, and population expansion, effectively controlling invasions (Muller-Scharer et al. 2004, Thomas and Reid 2007). EICA has had a large impact on the management of plant invasions, despite the fact

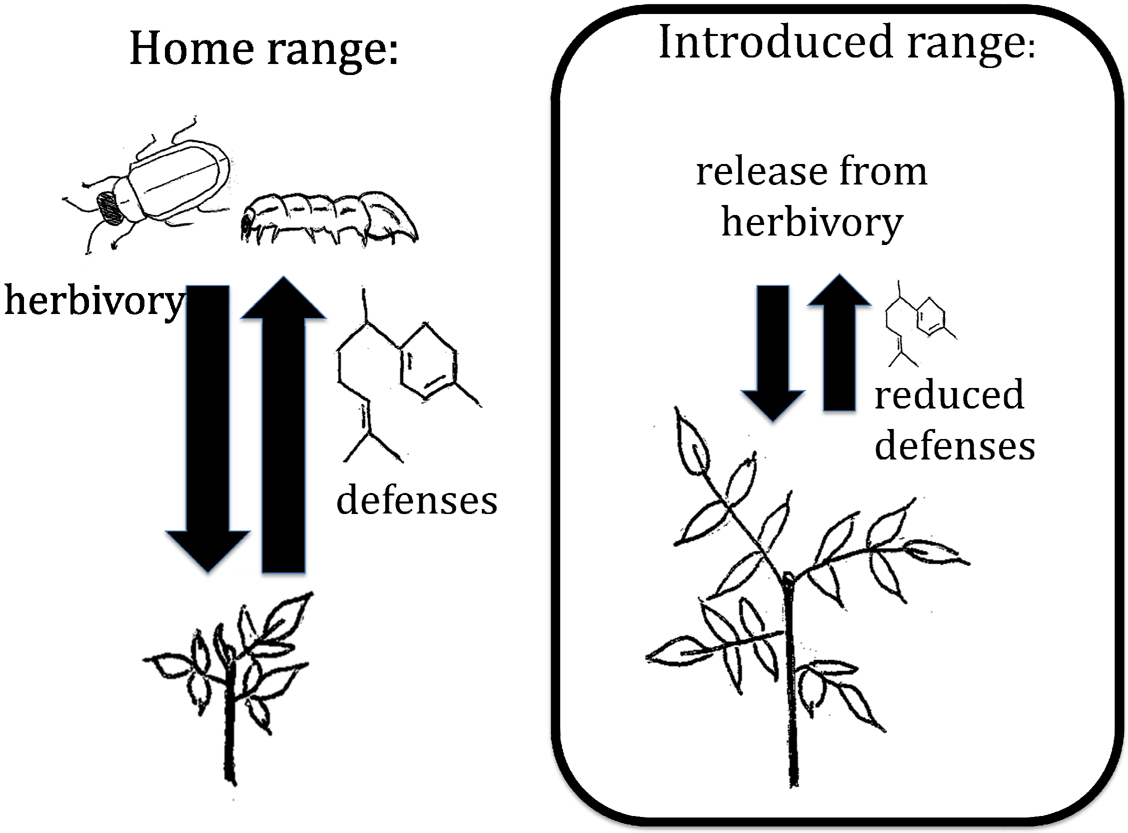

Figure 1. Schematic of plant and herbivore traits in common gardens from home and introduced ranges as predicted by the Evolution of Increased Competitive Ability (EICA) hypothesis. In the home range, selective pressure from herbivores maintains high allocation to defenses in plants, and as a result there is less allocation to plant growth, so plants are less competitive. In the introduced range, release from selection by herbivores shifts plant allocation from defenses to growth. When introduced range plants are subjected to herbivory, they will suffer greater damage than home range plants.

that its predictions have not always been satisfactorily fulfilled in experimental tests using invasive plant species.

Part of the difficulty in experimentally evaluating the EICA hypothesis lies in identifying the specific plant defenses or plant competitive traits that selection may have acted upon. Published tests of EICA rarely quantify the same specific plant traits, or use the same methods to evaluate defense or competitive ability: for example, defenses are evaluated by assessing leaf concentrations of quantitative or qualitative chemical defenses (e.g. secondary compounds), or by measuring the growth and fitness of herbivores feeding on the plants, or by quantifying the degree of damage to the herbivores themselves. This variety of results provides good justification for why published reviews of EICA have been qualitative, assessing evidence by comparing the number of significant studies for or against the hypothesis (Hinz and Schwarzlaender 2004, Bossdorf et al. 2005), or else have addressed only a single aspect of EICA, as in a recent meta-analysis that evaluated only EICA's prediction for plant chemical defenses (Doorduin and Vrieling 2011). The limitations of such reviews, however, are that qualitative studies judge evidence based on the number of significant studies, without evaluating the magnitude of trait changes in those studies. Similarly, a meta-analysis of only one measure of defense (secondary compounds and trichomes; Dooruin and Vrieling 2011) does not address the central aspect of the hypothesis, which is that lower defenses should also lead to greater plant vigor or fitness.

In order to provide a broad quantitative review, we propose to test the predictions of the EICA hypothesis using inclusive criteria for evidence of changes in plant defense and competitive ability using meta-analytic techniques. Different experimental approaches to quantifying defense or plant competitive ability can be grouped as testing separate predictions of the EICA hypothesis. For defense, EICA predicts that 1) when released from co-evolved enemies in the home range, introduced range plants will evolve lower defenses. 2) When both introduced and home range plants are subjected to feeding by a single species of herbivore, herbivores will feed with more success (fewer negative effects on development and survival) on introduced range plants, leading to 3) higher rates of herbivory damage on plants from the introduced range than the home range. In terms of competitive ability, EICA predicts that as defenses decrease, there will genetically-based shifts towards allocation to 4) higher growth, as well as 5) higher reproduction and fitness, leading to higher 6) competitive ability of introduced range plants. This paper will use meta-analytic techniques to assess the evidence from published studies for each of these predictions. However, the EICA literature also represents a rich source of data for assessing whether evolution, stochastic or selective, occurs broadly across invasive plant species. Release from herbivory may not be the singular or primary selective force on invasives; for example, many invasive plant species display latitudinal clines across their new range (Maron et al. 2004, others reviewed in Colautti et al. 2009), indicative of either adaptation to, or filtering by, climate. In addition to using meta-analysis to evaluate the EICA hypothesis, we will use data from EICA studies to evaluate whether there are genetically-based differences between home and introduced ranges in general across invasive plant species. We specifically hypothesize that while there may be evidence for evolution of reduced defense and greater plant performance traits consistent with EICA, evidence for any evolution of traits concurrent with introduction will be stronger. Such evidence of genetic change in concert with plant invasion would require greater attention in invasion research to the importance of stochastic and selective forces in the introduced range of plant species.

## Methods

In order to test the EICA hypothesis, that there are genetically-based differences between defense and growth traits of introduced versus home range populations of invasive plants, we collected published studies from peer-reviewed journals. For the purposes of this paper, we are interested in modern invasions, not in range expansions or in historical introduction events. ‘Invasion’ refers to the presence of a plant species novel to an area that was transported and introduced accidentally or intentionally by humans. We define ‘home’ range as the continent where a species has been present since at least the time of European colonial expansion (c. 1500), and ‘introduced’ range as a continent or group of continents where a species was not present preceding European colonialism.

We used the search terms ‘EICA’ or ‘evolution of increased competitive ability’ and ‘ecology’ with lemmatization to collect 45 papers from Web of Science in December 2010. In order to be included in the meta-analysis, papers had to meet each of the following criteria: 1) Papers presented data from experiments that tested at least one prediction of the EICA hypothesis using at least one invasive plant species. Thus studies that presented only the results of models, or in one case applied the EICA model to a fish system, were excluded. 2) Plants from both the introduced and home ranges of each invasive species were grown in a common environment (greenhouse or common garden) so that any variation expressed was due to underlying genetic differences, not to differences in environment or plasticity in response to environment. 3) Each of the introduced and the home ranges were represented by at least two geographically distinct populations. EICA emphasizes the difference between ranges, each of which is comprised of many populations growing under different abiotic and biotic conditions specific to geographically distinct locations. A comparison of only two populations, one from each range, confounds local, population-specific genetic structure with the genetic constraints (stochastic and selective) specific to each range. Including at least two populations from each range ensures that the question of genetic differences between groups is addressed at the scale of range and not population. Following application of these criteria, 28 studies were included in the analysis from which we collected 347 observations of the difference between home and introduced ranges of invasive species in defense, growth or competitive characteristics.

Papers reported comparisons between introduced and home ranges as F-statistics, $\chi^2$ scores, and t-scores from statistical tests, and in a few cases as mean values and standard deviations for each range. Each observation was converted to a Fischer's Z transformation of the correlation coefficient, except for observations of competitive ability, which were converted to response ratios. Positive Z-scores indicate that the value of the response variable is higher in the introduced range than the home range, and negative Z-scores indicate that the response value is higher in the home range than the introduced range. In the case of response metrics that relate to plant defense, all Z-scores were multiplied by an appropriate weighting variable (-1 or 1) so that negative scores represented higher inferred defenses in the home range plants and positive scores represented higher inferred defenses in the introduced range plants.

We characterized comparisons between introduced and home range responses as either defense or competitive traits. There were three models that addressed components of the defense hypothesis. The first defense model included quantitative and qualitatitve leaf traits, such as concentrations of secondary compounds, density of trichomes, and leaf toughness. The second defense model included the effects of herbivory in home versus introduced range plants upon herbivore performance, and included metrics from choice experiments or garden surveys such as developmental time of insects, insect mass, and number of insects. The third defense model included herbivory-induced damage upon plants using metrics such as mass of plant consumed, area of leaves consumed, and regrowth following herbivory. All traits were modeled randomly, which is appropriate for ecological studies in which variation in measured effects is comprised of biotic variation as well as error. In the case of significant summary effect sizes, fail-safe numbers ($N_R$) were calculated to indicate the number of non-significant, unpublished results that would render the summary effect size non-significant. If $N_R$ exceeded Rosenthal's identified minimum value (5n+10), the result was assumed to be robust against publication bias (Rosenthal 1979). In addition to assessing defense traits, we created models that addressed three components of the hypothesis that there is a difference between ranges in competitive ability. The first model included measures of plant performance related to growth, including height, biomass, and growth rate. These measures were taken from plants from introduced and home ranges when all are grown under non-competitive conditions, either alone in pots or in common gardens. The second model included measures of plant fitness including floral and seed mass and number, and number of vegetative offspring in plants for which asexual reproduction is important. The third model included results from direct tests of the relative competitive ability of home versus introduced range plants. Competitive ability was measured by growing target plants with intraspecific competitors, interspecific competitor plants from the introduced range, or interspecific competitor plants from the home range. Only results in which the target plant of competition was the invasive species were included, so the test for competitive ability was of the invasive species' relative ability to withstand competition from another plant. Results in which the target plant was another species from the community, which would measure the impact of the invasive species upon other species, were excluded from this analysis. Since more recently published EICA studies tended to use more appropriate nested statistical models for estimates of range differences than early studies, we also ran models using year of publication as an explanatory variable for each of the defense and growth traits. Year of publication did not significantly explain variation in any of the defense or growth effect sizes ($p>0.4$), indicating that improved statistical models did not lead to changes in estimates of effect sizes.

We also addressed the hypothesis that there were genetically-based differences or evolutionary change between ranges in defense or competitive ability, regardless of whether it was consistent with EICA. We ran random models of the three types of defense characteristics and three types of characteristics that address plant performance and competitive ability, models in which all effect sizes were positive. This allows evaluation of whether any evolutionary change has occurred concurrent with invasion and establishment of a new range across all invasive plant species. In this case, any effect size with a confidence interval that does not overlap zero indicates that there is a significant difference between home and introduced ranges in a quantitative trait, without indicating broad trends in direction of trait change.

## Results

### *Defense Characteristics in the EICA framework*

We found no broad support across invasive plant species for reduced defenses in the introduced range of invaders. There were no overall differences between home and introduced ranges within each species in defense characteristics measured as leaf chemical or physical traits (E++=-0.006, bias corrected 95% confidence interval [CI] -0.035 to 0.034, Figure 2), their effect on herbivore performance (E++= -0.044, bias corrected CI -0.140 to 0.055, Figure 3), or relative herbivore damage to plants (E++=-0.053, bias corrected CI -0.125 to 0.002, Figure 4). However, heterogeneity indices indicated that variance in each model could be explained by factors other than geographic range. Chemical and physical leaf defenses varied significantly by species, which explained 66% of the variation in effect sizes (Q=23.6765, df=8, p=0.002; Figure 2), indicating that there are a few species which support the defense predictions of EICA. We also considered whether expression of defenses would explain variation in leaf chemistry effect sizes, but found no difference in overall effect size between constitutive and induced defenses (Q=4.2512, df=3, p=0.236).

Although there were no significant differences overall by plant range for herbivore performance or plant damage inflicted by herbivores, further classification of herbivores

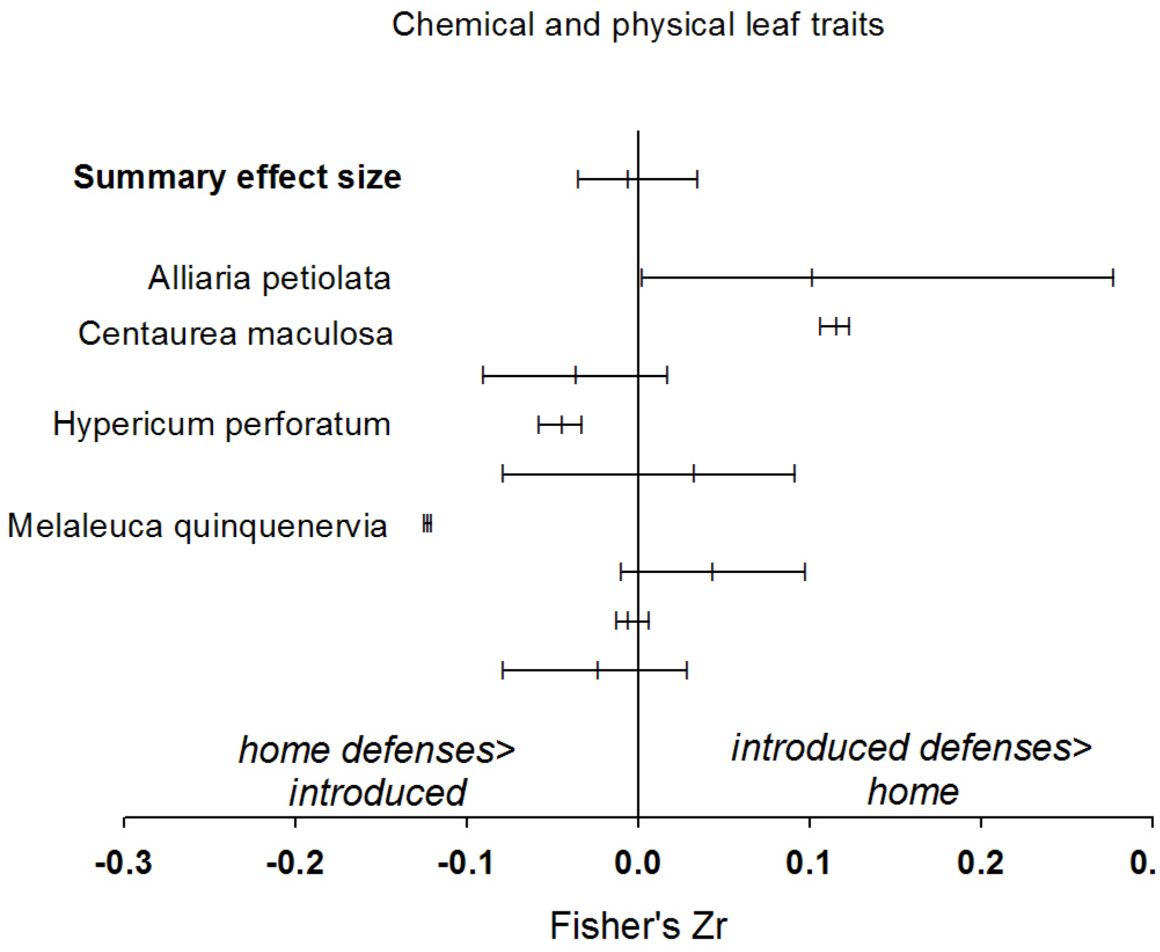

Figure 2. Meta-analysis of chemical and physical leaf traits shows no overall difference by range (summary effect size), but significant partitioning of variation by species. Points represent summary effect size with 95% bias-corrected confidence intervals. Species in which there was a significant difference by range for leaf traits are labeled by scientific name.

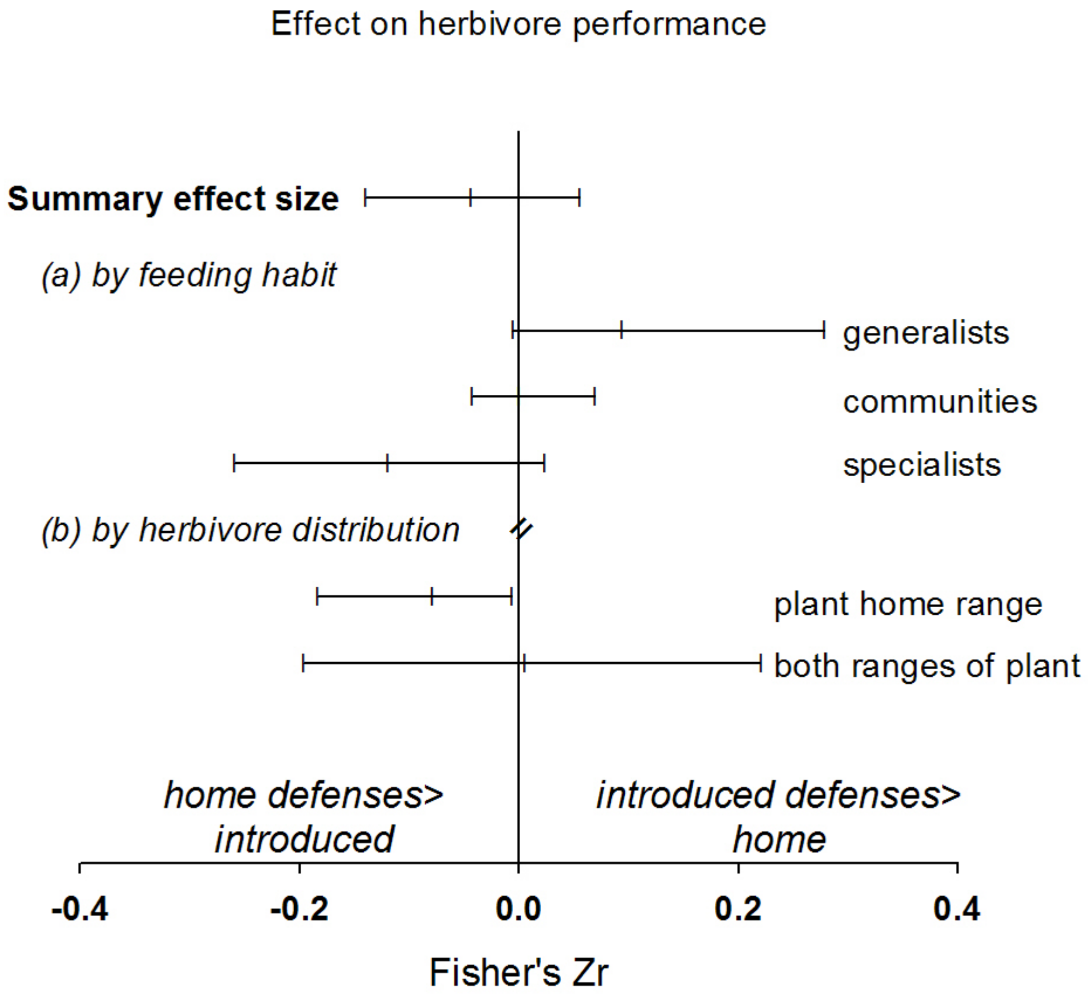

Figure 3. Meta-analysis of herbivore performance shows no overall difference by range of origin of host plant (summary effect size), but (a) herbivore feeding specialization or (b) herbivore distribution show trends in effect size variation.

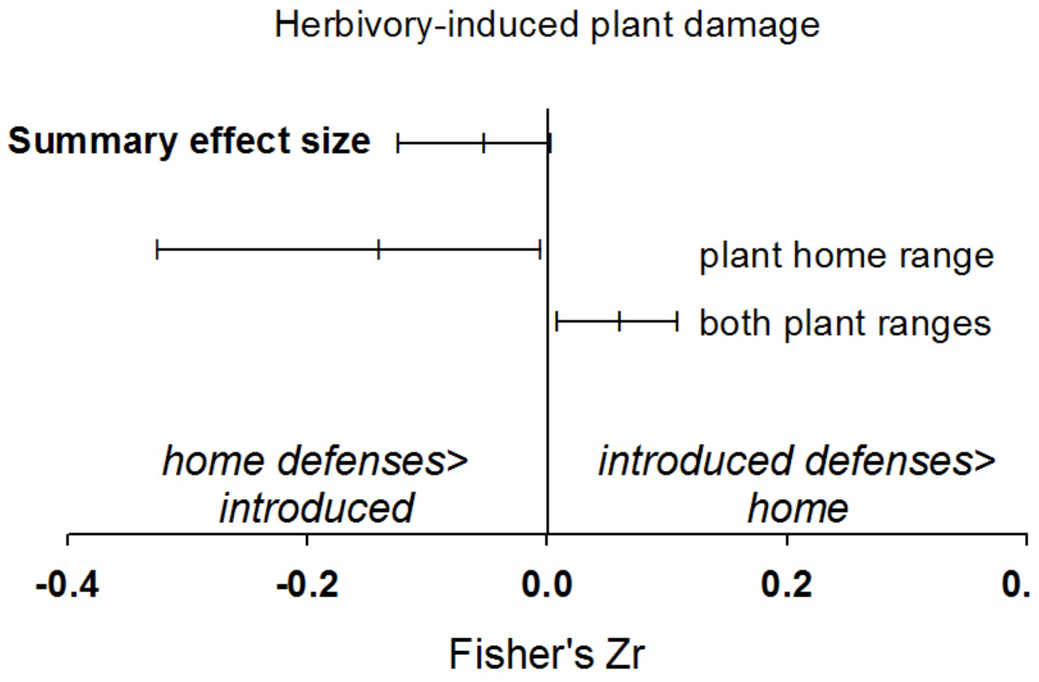

Figure 4. Meta-analysis of herbivore-induced plant damage shows no broad pattern of difference by plant range (summary effect size), but herbivore distribution significantly explains variation in effect sizes.

revealed significant variation in effect sizes for these metrics. Herbivore family did not explain variation in herbivore performance (p=0.86) or plant damage by herbivores (p=0.76), but classifying herbivores by degree of feeding specialization or geographic range did explain significant variation. Classifying herbivores by feeding habits—generalist, specialist, or unclassified communities of herbivores—predicted 67% of the variation in effect size of herbivore performance (Q=6.0923, df=2, p=0.048). There were trends towards a decline in performance of generalists and a rise in performance of specialists when both were placed on introduced plants, indicating that introduced plants tended towards higher defenses against generalists and lower defenses against specialists than home range plants (Figure 3). However, the degree of herbivore specialization did not significantly explain variation in defenses as inferred from the amount of herbivore damage sustained by plants (p=0.64). Herbivores were also categorized by geographic range; herbivores collected from the plant species' home range were more negatively impacted by feeding on home range plants (E+=-0.080, bias corrected CI -0.184 to -0.007), while herbivores present in both ranges due to universal distribution or human introduction as tools of biocontrol were equally impacted by defenses from home versus introduced range plants (E+=0.005, bias corrected CI -0.197 to 0.220). Herbivore geographic range also explained variation in plant damage by herbivores: home range plants suffered less damage from herbivores restricted from the home range, indicating higher defenses in the home range against accustomed predators, while introduced range plants suffered less herbivore damage from herbivores currently found in both ranges, indicating greater defenses in introduced range plants against universally distributed and human-introduced herbivores (Figure 4).

Plant species did not significantly predict variation in effect sizes in difference by range for herbivore performance (p=0.098) or herbivore-induced damage to plants (p=0.067).

### *Performance and competitive ability in the EICA framework*

There was mixed support for EICA's prediction that introduced range plants would have higher competitive ability than their home range relatives within each species. Grown in a common, low-competition environment, introduced range plants had significantly higher measures of non-reproductive performance and vigor than did home range plants (E++=0.066, bias corrected CI 0.019 to 0.138), with 48% of the variation in effect size explained by species ((Q=29.1494, df=15, p=0.0154, Figure 5). The fail-safe number for this result ($N_R$=1283 studies) far exceeds Rosenthal's critical minimum value (5n+10=390) for this comparison, which suggests that this effect is robust against publication biases. However, there was no corresponding difference by range in plant fitness (E++=0.0054, bias corrected CI -0.096 to 0.099) or in plant performance under competitive conditions (E++=-0.0886, CI=-0.2134 to 0.0061, Figure 6). Plant species did not significantly explain variation in fitness (p=0.811), although it did explain variation in competitive ability (p=0.02, Figure 6). The low number of studies (five studies containing 22 results) that published the results of competition experiments, along with the fact that the studies used different methods of assessing competition, means that this result should be interpreted with caution.

### *Defense and competitive ability in evolutionary framework*

All defense and competitive traits varied significantly by range when direction of response was disregarded in order to address the hypothesis that plant traits evolved in response to introduction and expansion in a new

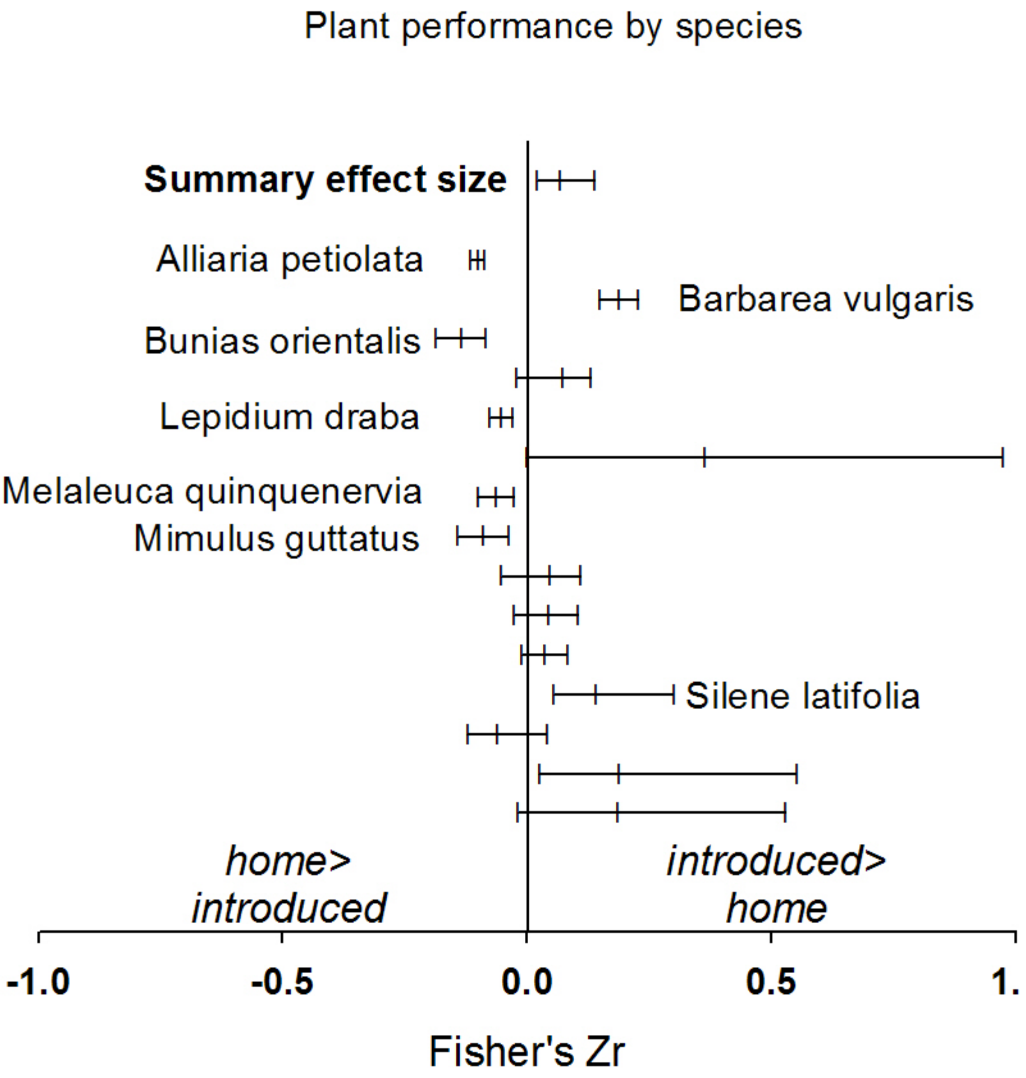

Figure 5. Meta-analysis of plant performance under non-competitive conditions shows that introduced range plants significantly outperform home range plants broadly across invasive species (summary effect size). Plant species identity significantly explains variation in effect sizes, and effect sizes of species that varied significantly by range are labeled with the species' name.

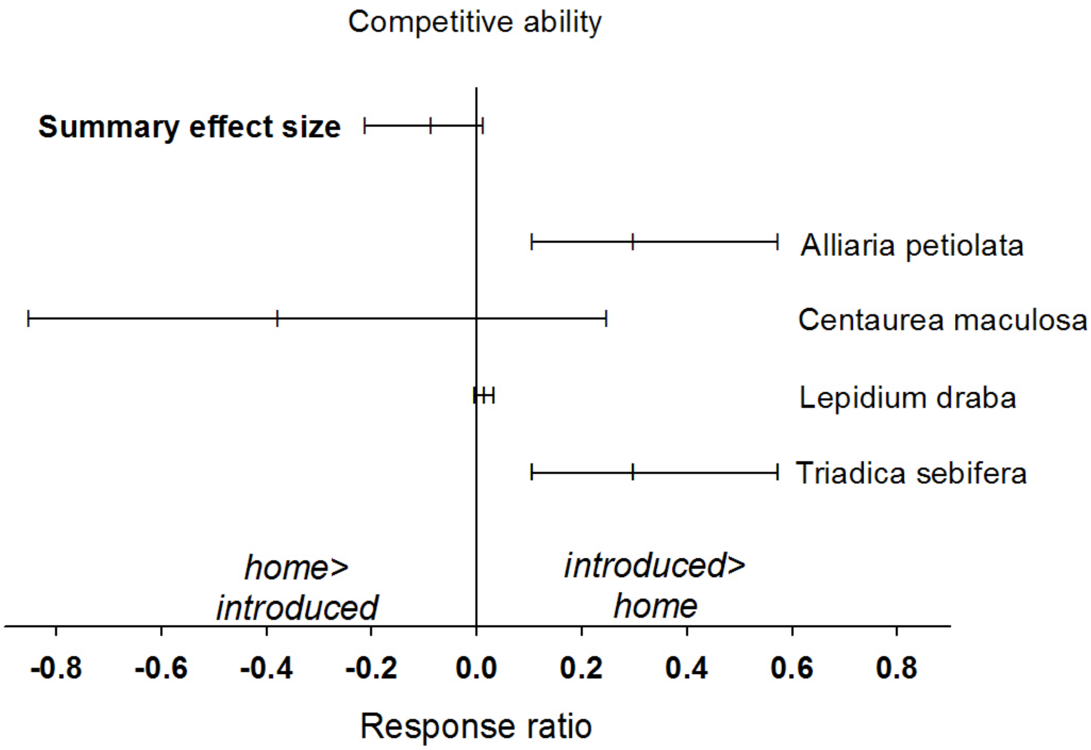

Figure 6. Meta-analysis of competitive ability shows no broad difference by range (summary effect size), although plant species explained variation in effect size.

geographic range (Figure 7). Chemical and physical leaf defense traits varied significantly by range (E++=0.0784, CI 0.0597 to 0.1000; $N_R$=1212, Rosenthal's CV=245), as did herbivore performance on plants from different ranges (E++= 0.2060, CI 0.1322 to 0.2998; $N_R$=5440, Rosenthal's CV=280) and herbivore-induced plant damage (E++=0.1292, CI 0.0829 to 0.1941; $N_R$=2913, Rosenthal's CV=385). Plant performance under non-competitive conditions varied significantly by range (E++=0.1364, CI 0.0965 to 0.2013; $N_R$=11019; Rosenthal's CV=385), as did fitness (E++=0.1672, CI 0.0919 to 0.2583; $N_R$=8099, Rosenthal's CV=200) and plant competitive ability (E++=0.1721, CI 0.0914 to 0.2935; $N_R$=623, Rosenthal's CV=120). Fail-safe numbers indicate that each of these effects is unlikely to be an artefact of publication bias.

## Discussion

This meta-analysis shows that there is little general support for the specific predictions of the EICA hypothesis across published tests of the hypothesis, but broad support for evolutionary change in general. EICA predicts that there will be reduced defenses in the introduced range, but there was no evidence for reduction in a range of defense traits in introduced ranges as categorized by leaf physical and chemical traits, effects on herbivores, and herbivore damage to plants. EICA predicts that there will be increased plant performance and competitive ability in the introduced range, and while there was higher performance in the introduced range of invasive plants, it was under non-competitive conditions. There was no support for increases in fitness or direct

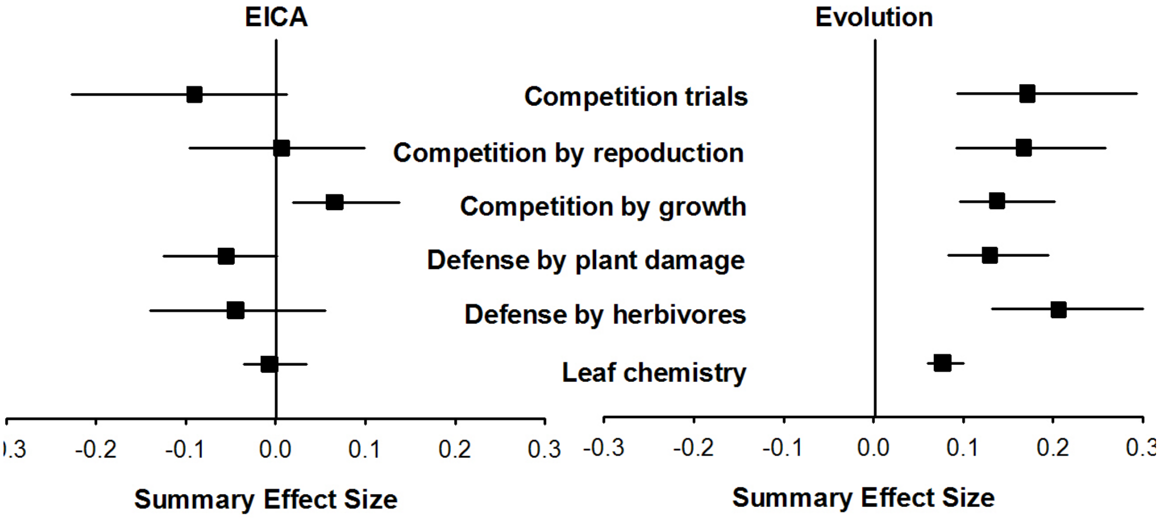

Figure 7. Meta-analysis of plant defense and competitive traits showed no broad support for EICA hypothesis, but general support for evolution of all traits in the introduced range of invasive plant species. The graph on the left shows all EICA summary effect sizes, and the graph on the right shows all summary effect sizes evaluating the hypothesis that evolution occurs with invasion. Note that all effect sizes are Fisher's Z-transformations, except for the competition trial effect sizes, which are response ratios.

measures of competitive ability in the introduced range. Although we find no broad support for EICA, each of the defense and competitive characteristics demonstrated significant divergence between home and introduced ranges across all the invasive species considered. While this meta-analysis shows that herbivores do not act as a general selective force on plant allocation between defense and growth, it does show that stochastic or selective forces are broadly important and that evolutionary divergence occurs between introduced range plants and parental range plants in the course of plant invasion.

### *Defense Traits and EICA*

Contrary to the EICA hypothesis, there was no reduction in defense in the introduced ranges of invasive species across the 19 studies in which some metric of defense was evaluated, which suggests that release from herbivory is not a powerful or the primary selective force upon plant defenses. We found no support for EICA's prediction that leaf chemical and physical traits will be lower in the introduced range, in contradiction to a recent meta-analysis which found support for higher levels of leaf toxins in introduced ranges (Doorduin and Vrieling 2011). This difference in result is due to differences in selection criteria for papers and data; for example, Doorduin and Vrieling 2011 used 13 measures of leaf toxins from 9 studies, whereas our meta-analysis of leaf chemistry and physical traits used 43 measures of secondary metabolites from 11 studies. We included all reported tests of secondary chemistry (for example, both induced and constitutive levels of defenses) in the meta-analysis, as recent work shows that selection criteria should be tested as explanatory factors in meta-analysis rather than being used to exclude data (LaJeunesse 2011), which may bias results towards supporting prominent theories (Barto and Rilling 2012). Our inclusive datasets of chemical and physical leaf traits, herbivore performance, and herbivore induced plant damage also allowed us to evaluate proposed refinements of EICA's defense predictions. For example, release from herbivory has been hypothesized to differentially affect the evolutionary trajectory of secondary chemicals based on their expression, leading to reduction in constitutive defenses and a compensatory increase in induced defenses (Koricheva et al. 2004). However, we found no differences by ranges in effect sizes based on the expression of putative defenses (constitutive versus induced). Muller-Scharer et al. (2004) suggested that since invasive plants often experience release from specialist but not generalist herbivores, introduced range plants should evolve greater defenses against generalists, and reduce defenses specific to specialists. Trends in herbivore performance support this hypothesis, but these trends did not lead to specialists or generalists causing greater plant damage based on plant range. The most powerful explanation of degree of herbivore-induced plant damage was the geographic range of the herbivore. Home range plants were more heavily defended than introduced range plants against herbivores restricted to the plant's native range, which would appear to support EICA. However, introduced range plants were more heavily defended, suffering less herbivore attack and damage, than home range plants against widely distributed herbivores, including specialist herbivores that had been introduced to the range as biocontrol agents. This suggests that introducing insect species as a means of biocontrol may result in the evolution of well-defended invasive plants rather than a reduction in invasive plant population size and success. As asserted by Thomas and Reid (2007), success of biological control agents is poorly defined, but this analysis suggests that the efficacy of biological control agents should be evaluated based on ranges where plants are well-defended as well as ranges where lower defenses have evolved. Our meta-analysis shows that the EICA hypothesis' predictions about defenses are not broadly supported across invasive species.

### *Plant growth and competitive ability in EICA*

The meta-analysis shows that plant success, but not fitness or competitive ability, is higher in the introduced range across invasive plant species as predicted by EICA. Many of the metrics of plant success reported in these studies as being greater in introduced range than home range populations have been shown to be greater in invasive species than in related native species (Grotkopp et al. 2002, McDowell 2002), or greater in invasive species than in native species from the invaded community (Pattison et al.1998, Smith and Knapp 2001). However, the importance of higher growth of introduced plants in a common environment to plant invasions may be limited by the fact that experimental conditions rarely mimic natural plant communities. Plants grown under greenhouse conditions were typically grown alone in pots and experienced no competition, while the degree and form of competition in common gardens depended on garden design as well as resource availability (see Wilson and Tilman 1993), which was rarely reported or manipulated. For the purpose of this study, we assumed that growth under common conditions where competition was not manipulated was growth under non-competitive conditions. There was no associated change in competitive ability across invasive species, which could be due to low sample size, but is consistent with a study of 14 introduced species which found differences by ranges in plant biomass under non-competitive but not under low or highly competitive conditions (Blumenthal and Hufbauer 2007). Overall, we found that introduced range plants grew more than did home range plants across invasive plant species as EICA predicts, but that this did not translate to higher competitive ability across the plant species considered.

### *Evolution of invasives and recommendation for future research*

There was broad support across these studies for evolutionary change in plant defense and performance occurring in concert with introduction and expansion in a new range, although not as predicted by EICA. Each of the six characteristics of plant defense or growth was significantly different between ranges when direction of trait change was disregarded, which suggests plant trait changes concurrent with range expansion should be considered as a component of invasion. Figure 7 shows that summary effect sizes in support of evolution are not only significant but larger in magnitude than the non-significant effect sizes generated by testing EICA. This indicates that invasive species evolve in terms of defense and performance traits without a general pattern towards greater or lower trait values across all invaders. Trait changes that confer success upon certain invasive plant species, for example, higher competitive ability, may not be crucial to the success of all invaders, for example, those plant species that establish populations in highly disturbed environments (Bossdorf et al. 2005). Evolution is an important force in invasions (Whitney and Gabler 2008), although the relative importance of selective and stochastic forces (Keller and Taylor 2008) cannot be evaluated from these studies. Stochastic events like founder’s events can limit genetic variation, which was long assumed to limit the evolutionary potential of invasive species (Lee 2002). However, successive founding events across the landscape may also result in the loss of less successful genotypes and higher mean population and range trait values (Vasemagi 2006). An important point is that EICA makes predictions about ranges, but evolution often occurs at smaller geographic scales, and is dependent upon genetic variation at the individual and population level.

Many EICA studies disregard individual- or even population-level genetic variation, lumping individuals together into population means, and populations into range means, without taking into account that large amounts of variation at a lower level of genetic organization may skew estimates of higher-level variation. Nested analyses can provide more accurate estimations, but such analyses require common gardens with replication at the individual level as well as the population level (Conner and Hartl 2004). Well-replicated common gardens will provide information about the distribution of genetic variation across individual, population, and ranges, and will provide evidence as to whether selective forces should be considered as important for plant traits.

Identifying the relative importance of selective agents, environmental conditions or biotic interactions that make certain traits advantageous and result in trait change over generations, should be a central topic of further research in evolution of invasive plants. Such selective factors include climate, resource availability, and biotic interactions. Plant populations distributed across a wide geographic range may become locally adapted to climate factors correlated with latitude, both in native species (Macel et al. 2007, Kawakami et al. 2011) and in invasive species (Maron et al. 2004). Including latitude as a covariate in models of plant performance has shown that for some invasive species, evolution of increased growth in invasive populations which appeared to support EICA was more closely correlated with latitude (Colautti et al. 2009). Figure 7 shows that secondary chemistry varies by range, which in the framework of EICA suggests changes in herbivore pressure. However, models show that herbivory and resource availability may interact or act in opposition as selective forces on plant secondary metabolites (Zhang and Jiang 2006). Resource availability in the form of soil nutrients should be evaluated as a possible selective force for plant secondary chemistry, particularly as secondary metabolites can impact soil nutrient availability through effects on decomposition processes (Coley et al. 1985, Schweitzer et al. 2004). Altered nutrient cycling rates have been implicated as an ecosystem-level impact of invasive species (Ehrenfeld 2003), but should also be evaluated as an important evolutionary feedback for invasive plant species. Furthermore, while EICA only considers the biotic interactions of herbivores and plant competitors as selective forces, more recent research shows that soil biotic communities have the potential to act as selective agents, as certain tree species cultivate soil biota beneficial to their offspring (Pregitzer et al. 2011, Felker-Quinn et al. 2011). There are a number of promising avenues for research into the rapid evolution and adaptation of invasive plants.

Further tests of the EICA hypothesis should primarily address patterns of genetic differentiation between ranges, in common gardens that include both intra- and interspecific competition, in order to address current gaps in our knowledge of plant invasions. Where possible, multiple common gardens including nutrient amendments should be placed across the geographic range of the invasive species for multiple-generational studies that evaluate climate and resource availability as important selective factors. Although EICA has provided a useful framework for evaluating changes in plant species following their introduction to a new range, this meta-analysis shows that herbivores do not exert a powerful selective force on invasive plants. There were no overall shifts in allocation from plant defense to competitive ability following introduction and release from herbivory across invasive plant species.

# CHAPTER 3: **Local adaptation and rapid evolution predict performance in an invasive tree**

*This chapter was submitted to Ecosphere for publication, and is currently in review. Jennifer Schweitzer and Joseph Bailey are co-authors on the paper, as they contributed to designing the experiment and writing the paper.*

## Abstract

The roles that genetic variation and evolution play in promoting plant invasions are often invoked by using low genetic variation to explain why some clonal species become invaders, or to explain invasiveness as a result of evolutionary change between native and invaded ranges. However, high genetic variation along with rapid evolution and local adaptation may also explain the success of a species that successfully expands its range across novel environments. Previous research has shown that the tree species *Ailanthus altissima*, tree-of-heaven, has suffered no significant reduction of genetic variation in its introduction to eastern North America, and that soil biotic communities may influence the expression of this genetic variation in, as well as promote the success of, certain populations. We hypothesized that 1) there would be significant population-level genetic differentiation in the invaded range, 2) that latitude, a quantifiable proxy for climate, would explain population-level genetic patterns, indicating local adaptation, and that 3) including measures of soil and climate variables would improve modeling of population variation, providing more explanatory models of local adaptation. We collected seeds from 13 geographically distinct populations of *Ailanthus altissima*, over a range that spans 1000 km in latitude, and planted them in a common garden. We monitored a range of growth, phenology, herbivory, and disease response variables over the course of 2 years. We found that eight out of ten metrics demonstrated some level of genetic variation (at population, family, or both levels), and that five metrics (plant height, growth rate, specific leaf area, herbivory damage, and disease damage) demonstrated population level differentiation consistent with our first hypothesis. Only two metrics, plant height and growth rate, were significantly correlated with latitude, and growth rate was higher at the northern end of the range, consistent with the local adaptation noted for native species in other studies. All five metrics could be more accurately modeled by a combination of edaphic and climate data. Our results indicate that rapid evolution and selection by local soil factors and climate may explain the success of *Ailanthus altissima* across eastern North America.

## Introduction

The success with which certain introduced species invade new ranges and achieve population densities greater than those of their native ranges may be attributed to rapid evolution. Some researchers consider the process of introduction as a strong selective filter and thus as an inevitable reduction in genetic variance in the new range (Simons 2003), and there are some spectacular examples of clones that have become invasive, e.g. the alga *Caulerpa taxifolia* in the Mediterranean (Wiedenmann et al. 2001). However, an increasing number of studies have found that some invasive plant species exhibit similar amounts of genetic variation across comparable areas in their native and introduced ranges (Feret and Bryant 1974, Maron et al. 2004, van Kleunen and Fischer 2008). This genetic variation in invaders indicates that many species were introduced multiple times, and also suggests that invasive plants may undergo further adaptation to local selection pressures. One hypothesis that makes explicit predictions of how evolution may contribute to invasiveness of introduced plants is the Evolution of Increased Competitive Ability hypothesis (EICA), which posits that freed from the constraint of the herbivores and pathogens of their native ranges, invasive species will evolve weaker defenses and increased growth or plant size (Blossey and Notzhold 1995, Bossdorf 2005). This hypothesis, that changes in community-level interactions will drive adaptation in invasive species appears to be supported by the invasions of *Silene latifolia* (Blair and Wolfe 2004) and *Sapium serbiferum* (Siemann and Rogers 2001). However, other species, including the American invasion of *Lythrum salicaria* which was the basis for EICA, have not exhibited tradeoffs between defense and growth (Willis et al. 1999). Studies like the previous one have indicated that local adaptation to climate factors across the invaded geographic range may better explain variation in characteristics of plant performance.

Incremental changes in climate across wide geographic gradients can induce population-level local adaptation. This level of adaptation is observed in species that have spread and persisted over thousands of years (Linhart and Grant 1996, Olsson and Agren 2002, Kawakami et al. 2001) as well as in invasive species. Invasive species that occur in reproductively and geographically distinct populations along latitudinal gradients diverge into populations with distinct growth rates, fecundity, phenology, and general performance traits such as plant size and leaf nitrogen, changes that are genetically-based and persist when population offspring are grown under common environmental conditions (Maron et al. 2004, Maron et al. 2007, Montague et al. 2008, Coulatti and Barrett 2010). Shifts in plant phenotype associated with changes in latitude include loss of genetic variation in northern populations (Coulatti et al. 2010), earlier flowering time, smaller plant size at flowering, and lower fecundity in northern populations (Montague et al. 2008). Local adaptation in introduced North American *Hypericum perforatum* populations showed selection for a genetic by environment interaction where northern and southern populations each had higher performance when grown at a latitude close to their natal latitude (Maron et al. 2004). Including latitude as an explanatory factor in patterns of invasive evolution can reverse the conclusions of tests of the EICA hypothesis, showing that plants with native and introduced ranges do not differ due to presence or absence of herbivores, but in response to climate factors that correlate with latitude (Colautti et al. 2009).

Invasive plants, as sessile organisms depend upon local soil conditions for nutrients, water, and physical support; therefore, clines in soil conditions can result in genetic differentiation in plants even at very small geographic scales (Brady et al. 2005, Pregitzer et al. 2010). For example, research in mine tailings and serpentine soils has shown repeatedly that populations in very close proximity may have different tolerance for metals depending on the amount of metal in their soil (Linhart and Grant 1996, Brady et al. 2005). Research in serpentine soils has also demonstrated that they can change the expression of genetically-based variation among individuals, suggesting that soils play an important role in plasticity of traits and thus in exposing or shielding genetic variation to selection by the environment (Murren et al. 2006). Soil chemistry and parent material can shape and guide population expansions, as shown by a study of multiple alpine plant species which found that current geographic distributions of genetic variation correlate with locations of particular soil types in refugia during historical glaciation events, not with current climate conditions (Alvarez et al. 2009). Abiotic soil conditions can also mediate plant-soil biota interactions, altering the performance benefit plants derive from their mycorrhizal symbionts (Piculell 2008). Physical and chemical soil properties may act as further abiotic agents of selection as invasive species establish new populations across large geographic ranges.

In this study, we investigated phenotypic variation of 13 invasive populations of the tree *Ailanthus altissima* (Ait.) Swingle (tree of heaven), collected from a 1000-km gradient that spans 3 degrees of latitude in eastern North America and a significant cline in elevation, soil parameters and other factors to determine whether these factors correlate with plant performance. Seeds from each of these populations were used to start a common garden located at the southern end of the gradient, in which we monitored plant performance and community interactions for 2 years. Previous work had shown that there was both family- and population-level genetic variation for some performance traits in three of these populations (Felker-Quinn et al. 2011). We hypothesized that 1) there would be family- and population-level genetic variation for performance across the 13 populations, 2) there would be latitudinal clines in performance, with southern populations outperforming the northern populations, and 3) soil properties of the locations of parent populations would further explain variation in performance. We predict that there will be population-level and family-level genetic differentiation across the invaded range, providing support for the hypothesis that this species has undergone or may undergo rapid evolution as a result of its introduction, and that overall patterns of performance will correlate with latitude. We predict that southern populations will outperform northern populations, as the family Simaroubaceae is largely sub-tropical or tropical, and the native range of *Ailanthus altissima* is in sub-tropical through temperate Asia, and the climates associated with the southern end of our range are more similar to that of the species' native range. We predict that including other information on environmental factors, particularly climate and soils data, will improve our ability to explain variation in population performance, as soils have already been implicated as an important factor in *A. altissima* performance (Felker-Quinn et al. 2011). Such results would allow a greater understanding of what abiotic interactions shape range expansions and associated evolution of invasive species.

## Methods

### *Seed Collection*

We collected seeds from 13 field populations of *Ailanthus altissima* in total, approximately every 90 km along a latitudinal gradient from northern Pennsylvania to eastern Tennessee in the eastern United States. Seeds for this experiment were collected in January 2008 and January 2009 by clipping bunches of dried seeds produced from the most recent growing season from maternal trees located at least 50 m distant from each other (see Felker-Quinn et al. 2011 for specific seed collection details). *Ailanthus altissima* is dioecious, so although maternal identity was clear by our collection technique, the paternal contribution is undetermined; since seeds may be full or half-siblings, all seeds collected from one maternal tree are referred to as a 'family'. In January 2008, we sampled 10 families from multiple populations from Northern PA to Tennessee (see Table 1). Latitude and longitude of each population, as well as elevation of each maternal tree, were recorded using a Garmin Explorer unit (Garmin International, Olathe, KS, USA). All seeds were stored in paper sacks at 4°C after collection until they were germinated.

*Common Garden*

In order to test for genetic variation in *Ailanthus,* we established a common garden at the Knoxville campus of the University of Tennessee, TN, USA (35°57’23”N, 83°55’36”W). We germinated the plants for the common garden by planting seeds from all families in the greenhouse in February 2009. Wings were removed from all seeds, and then approximately 30 seeds from each family were planted in root trainers that had been filled with Farfard #2 potting mix (85% peat moss, perlite, Dolomitic limestone). These seedlings were grown in the greenhouse and watered daily until May of 2009. In May 2009, the seedlings were transplanted outside into a common garden. Seedlings were transplanted into pots to minimize the risk of further invasion following the experiment. From each seed family, we selected the tallest 10 plants and transplanted each into individual 20 L pots, containing a soil mixture composed largely of local sandy clay, with minor components of compost and fiber, pH 6.6-7.0 (Premium Mixture, Hines Fine Soils, Knoxville, TN, 37914). The seedlings were assigned random numbers which were used to arrange the pots into rows across the space to minimize the effects of small scale heterogeneity on performance. The first summer in which the trees were outdoors was exceptionally dry, so seedlings were

Table 1. Locations of populations of *Ailanthus altissima* sampled for the common garden experiment. The abbreviations used in the test for populations are in parentheses in the population column. The latitude and longitude reported for each population represent the exact location of one of the individuals in that population; in populations where trees were more than 200 m apart, the latitude of the northernmost grouping of trees is reported. The number of maternal trees from which seeds were collected is shown, as are the values extracted from the NRCS soil database used in the family-means database.

| **Population** | **Latitude, Longitude** | **Families** | **Elev (m)** | **Annual Precip (cm)** | **Minimum Average Annual Air T (°C)** | **Frost-free Period (days)** | **Slope (%)** | **soil pH** | **Clay (%)** |
|---|---|---|---|---|---|---|---|---|---|
| Delaware Water Gap, PA (A) | 41° 8'24.30"N, 74°55'46.50"W | 7 | 118 | 104 | 4.4 | 150 | 55 | 4.8 | 12 |
| Brooklyn, NY (B) | 40°40'29.00"N, 74° 0'31.00"W | 10 | 3 | -- | -- | -- | -- | -- | -- |
| Bethlehem, PA (C) | 40°37'18.07"N, 75°24'35.54"W | 11 | 80 | 112 | 11.7 | 175 | 12 | 6.5 | 23 |
| Belle Mead, NJ (D) | 40°29'53.39"N, 74°37'46.27"W | 4 | 21 | 117 | 7.8 | 165 | 4 | 5.0 | 15 |
| Philadelphia, PA (E) | 39°57'37.40"N, 75°10'54.93"W | 8 | 5 | 102 | 10.0 | 180 | 12 | 5.4 | 16 |
| North East, MD (F) | 39°38'7.08"N, 75°57'6.06"W | 4 | 70 | 114 | 11.1 | 200 | 8 | 5.6 | 13 |
| Manassas, VA (G) | 38°48'19.02"N, 77°34'44.16"W | 7 | 79 | 89 | 7.8 | 190 | 1 | 5.3 | 21 |
| New Market, VA (H) | 38°39'20.40"N, 78°40'18.90"W | 5 | 352 | 84 | 5.6 | 166 | 9 | 6.1 | 32 |
| Fredericksburg, VA (I) | 38°20'31.32"N, 77°29'43.62"W | 5 | 31 | 107 | 8.9 | 220 | 4 | 5.5 | 12 |
| Glen Allen, VA (J) | 37°39'15.30"N, 77°27'22.32"W | 10 | 41 | 109 | 12.2 | 186 | 1 | 5.3 | 10 |
| Warfield, VA (K) | 36°55'19.74"N, 77°46'25.27"W | 4 | 83 | 112 | 7.2 | 173 | 13 | 5.3 | 12 |
| Durham, NC (L) | 36° 1'1.67"N, 78°53'34.07"W | 6 | 102 | 125 | 15.0 | 220 | 8 | 5.8 | 12 |
| Knoxville, TN (M) | 35°56'10.55"N, 84° 0'41.64"W | 6 | 283 | 119 | 8.9 | 195 | 9 | 5.3 | 17 |

watered 2-3 times a week in equal amounts. In addition, we fertilized the seedlings with a 20-20-20 nitrogen-phosphorus-potassium fertilizer in a water solution three times, once each in June, July, and August. In the first summer, we measured plant height in July, as well as in September when we also measured percentage of leaves affected by disease and herbivory. Herbivore damage was estimated visually and scored on a scale of 0-10 (corresponding with 0% to 100%) to indicate the percentage of leaf area killed or removed due to leaf removal or evidence of chewing on leaves or rachises. Herbivory was almost exclusively by *Atteva aurea* Fitch (Lepidoptera), ailanthus webworm, a native moth species originally limited in its distribution within the United States to Texas (Becker 2009). *Atteva aurea* is now found throughout the range of this study, where it was observed in its larval stage feeding on seedlings of parent populations (field observations, Felker-Quinn). Disease damage was also estimated visually using a 0-10 scale to indicate the percentage of leaf area that was wrinkled and toughened, discolored at the leaf margins, or had shed leaflets as a result of these symptoms, which are typical of infection caused by the fungal pathogens *Verticillium albo-atrum* or *V. dahliae*, both of which have demonstrated to infect *Ailanthus altissima* (Schall 2009). In spring of 2010, we tracked the phenology of the trees by surveying individuals every 2-3 days from early in March to mid-April and recording the day of year when green buds first appeared on the trees (budding) and when the first of the leaves fully emerged (leaf out). In May 2010, when leaves had emerged on all trees and before herbivory and disease affected the leaves, we harvested the fourth leaf from the tree's apical meristem (from the tallest meristem if the tree had branching or multiple stems) and measured its length and the area of the leaflets using a CI-200 leaf area meter (CID Bio-Science Inc., Camas, WA, USA) and used area as well as the mass of the leaves to determine the specific leaf area (SLA) of the leaves. A subset of the leaves used in determining SLA were oven dried (48 h at70°C) to create an allometric equation relating wet mass of leaves to oven-dried mass; therefore SLA was presented as oven-dry SLA. In July 2010, when trees had grown considerably and before they became root-bound in their pots, we measured plant height, number of leaves, percentage herbivory and percentage disease as described above. We calculated a relative growth rate for the individual trees (a trait associated with invasive success in woody species, Pattison et al. 1998, Grotkopp et al. 2002, Zou et al. 2008) by subtracting the height in July 2009 from height in July 2010, and dividing the difference by the July 2009 height. In late September 2010, we harvested all trees, separating the shoots from the roots by cutting stems at the surface of the soil in each pot. We collected the roots by carefully shaking the dirt from the coarse roots (>2 mm). Samples were dried for 48 hours at 70°C to a constant mass and then weighed. We calculated the root to shoot mass ratio for all plants.

### *Abiotic Predictors*

In order to assess the effects of geographic variation in abiotic factors related to climate and soil on population-level divergence, we accessed soil reports collected by the National Resources Conservation Service (NRCS) available online as the Web Soil Survey (Soil Survey Staff). The latitude and longitude values reported for the populations above were entered into the data base, and ‘Soil Chemical Properties’ and ‘Soil Physical Properties’ were extracted from the Soil Reports for the predominant soil type underlying each population, with one exception. There was no soils data available for Kings County, New York, in which parent trees were observed rooted in cement or in a matrix of broken glass, refuse, and compacted soil, so the Brooklyn population was excluded from these analyses. The climate and soils data stored in these reports are reported as ranges of values. The climate data is extracted from the years 1971-2000, and a range is reported that represents the average attribute over the entire area of the soil type. The criteria used to determine the ranges reported for the chemical and physical properties are specific to each attribute and are available in Part 618 of the National Soil Survey Handbook (United States Department of Agriculture, NRCS). We used these ranges to extract a minimum and an average value for annual precipitation, annual air temperature as well as annual number of frost-free days, slope, depth to restrictive feature, cation-exchange capacity, effective cation exchange capacity, soil pH, % sand, % silt, % clay, moist bulk density, saturated hydraulic conductivity (Ksat), available water capacity, and % organic matter. If a population grew on a soil complex, the soil components had separate ranges of values for each attribute. The minimum value for the population soil for each attribute was the smallest value of the ranges for all soil components, and the average value for each attribute was the average of the averages of the ranges associated with the separate soil components. Since many of the soil characteristics extracted from the database are correlated, a correlation matrix of all these factors, as well as latitude and elevation, was created using JMP 8.0 (SAS Institute, Cary, NC, USA), and all factors that had a correlation greater than 0.7 were discarded. This left latitude (L), elevation (E), average annual precipitation (P), minimal average annual temperature (T), average number of frost-free days (F), average slope (S), clay fraction (% clay, C), and average pH (H) as possible predictor variables.

### *Statistical Analyses*

In order to test the genetic variation expressed in phenotypic differences among families and populations, we used Restricted Estimate Maximum Likelihood (REML). In this and further analyses, JMP 8.0 was used to construct mixed models for each of the common garden performance measurements with population as a fixed factor, pot number (a randomly assigned number used to order the pots in the garden) as a random variable, and family, nested within population, as a random factor in order to determine statistical significance of the population effect. To determine family effects, we used Likelihood Ratio Tests, in which the difference between the likelihood ratio of the previous model and the likelihood of the model with the family effect removed was used as a $\chi^2$ value (one-tailed $\chi^2$ distribution, df=1).

Performance metrics with significant population effects were tested for latitudinal clines. Because all abiotic response variables with the exception of elevation were available only at the population level, we used family-level averages of performance data to minimize pseudo-replication. We constructed standard least squares models with latitude, elevation, and the interaction of latitude and elevation as explanatory factors using JMP. In order to test possible effects of soil properties, we constructed generalized linear models using the statistical program 'R' and the package ‘leaps’ (Lumley 2009) to identify the most predictive model of all possible models containing one, two, three, four, five, six, or seven of the eight possible explanatory models mentioned above. The Akaike Information Criterion (AIC) score of each model was used to choose the most appropriate explanatory model for each performance metric (family-means model). We used the best-fit family-means models identified by AIC to make single-factor linear regressions or generalized linear factors of the population means (population only-models).

## Results

### *Genetic Variation of A. altissima*

There were population- or family-level genetic variation for seven of the eleven performance and community interaction metrics, and not all significant metrics display both levels of genetic variation (see Table 2). In terms of performance, genetic variation expressed varied over the course of the experiment, with significant population-level variation (p=0.0014) in plant height in the first summer (Table 2). Population D plants (Belle Mead, NJ) had the shortest average height, while the tallest population, population J (Glen Allen, VA), had a mean plant height 85% taller. By the second summer, there was no significant population-level variation (p=0.3262) even as family-level variation remained a significant predictor (p=0.0345) of plant height. When plant height was considered in terms of the growth rate, there was significant population-level (0.0167) genetic variation expressed, with increases in plant height ranging from a mean of 90% increase in population G (Manassas, VA) to a mean of 352% in population D, the fastest-growing population (Belle Mead, NJ). Specific leaf area (SLA) varied by population (p=0.0380), though variation between populations was relatively small, as the population with the smallest SLA, population J (Glen Allen, VA), had a mean SLA only 13% less than population M (Knoxville, TN), which had the highest SLA (Figure 2). Leaf length measured simultaneously on the same leaves varied significantly by population (p=0.0457) but not by family (p=0.2448). Shoot mass

Table 2. Results of statistical tests of family and population level genetic variation of *Ailanthus altissima* grown in a common garden. Population F ratios and associated probabilities (Prob>F) are reported from Restricted Likelihood tests, and log likelihood ratios and associated probabilities (p-values) are reported to assess family-level variation. Family variation was nested within populations for analyses.

| | **Population** | | **Family** | |
|---|---|---|---|---|
| **Factor** | **F Ratio** | **p-value** | **Log-ratio $\chi^2$** | **p-value** |
| Plant Height 2009 | 3.101 | 0.0014 | 215.375 | >0.0001 |
| Herbivory 2009 | 2.530 | 0.0029 | 0 | 0.5000 |
| Disease 2009 | 3.537 | 0.0004 | 0.316 | 0.2871 |
| Plant Height 2010 | (1.168)* | (0.3262)* | 3.306 | 0.0345 |
| Date of Leaf Budding 2010 | 0.543 | 0.8872 | 0 | 0.5000 |
| Date of Leaf Out 2010 | 0.815 | 0.6345 | 10.636 | 0.0006 |
| SLA 2010 | 1.845 | 0.0380 | 0 | 0.5000 |
| Leaf Length 2010 | (1.300)* | (0.2448)* | 2.849 | 0.0457 |
| Shoot mass 2010 | 1.312 | 0.2476 | 0.362 | 0.2738 |
| Root mass 2010 | 1.464 | 0.1621 | 0.644 | 0.2111 |
| Growth Rate | (2.348)* | (0.0167)* | 12.616 | 0.0002 |

*REML models do not converge, so no p-value can be estimated from these models. The F and p values reported in parentheses are calculated from ANOVAs run on family means of these metrics, which may underestimate F-ratios.

and root mass, measured directly at the end of the experiment, did not vary significantly by family or population ($p>0.1$; see Table 2 for exact p-values).

In addition to the previously presented measures of plant performance, we considered phenology and community-level interactions. The timing of spring leaf budding did not display any statistically significant variation at the population ($p=0.5000$) level. Phenology of spring leaf out did not display population-level variation ($p=0.6345$), but there was significant family-level variation for this trait ($p=0.0006$; Table 2). Herbivory damage differed significantly by population, ($p=0.0029$), ranging from approximately 10% in populations A (Delaware Water Gap, PA) and I (Fredricksburg, VA), those least consumed, to approximately 30% of leaves removed in population M (Knoxville, TN), the population most heavily affected. Disease damage to the plants also differed significantly by population ($p=0.0004$), and on average affected more of the plants' biomass and varied more across populations than did herbivory. Damage by disease affected 25-54% on average of the leaves across all populations, with populations C (Bethlehem, PA), E (Philadelphia, PA), and L (Durham, NC) experiencing the least damage, and populations H (New Market, VA) and J (Glen Allen, VA) most heavily damaged by disease.

*Population-level geographical patterns*

There were five metrics that displayed significant population variation. Of these, elevation, latitude, or their interaction were significant predictors for variation in four of these five metrics (Table 3). Plant height measured in the first summer decreased with increasing latitude (family-means model: $p=0.016$, adjusted $r^2=0.06$; population-only model: $p=0.3187$, adjusted $r^2=0.008$), while elevation holds no

Table 3. Test statistics for *Ailanthus altissima* performance metrics modelled with elevation and latitude as explanatory factors. The four response variables shown are those which displayed population-level variation as confirmed by REML (see table 2). The t ratios and associated p values are shown for each factor in the two way ANOVA: elevation (Elev), latitude (Lat), and the interaction elevation*latitude (E x L). The slope values are extracted from the previous model; a positive value indicates that the metric increases with increasing elevation or latitude. Statistically significant slopes are bolded.

| | t-ratios | | | p-values | | | Slope (beta) | |
|---|---|---|---|---|---|---|---|---|
| Factors | Elev | Lat | E x L | Elev | Lat | E x L | Elev | Lat |
| Plant Height 2009 | 0.35 | -2.46 | 1.84 | 0.729 | 0.016 | 0.070 | 0.001 | **-0.746** |
| Herbivory 2009 | -1.13 | -0.10 | -3.74 | 0.262 | 0.920 | <0.001 | -0.004 | -0.063 |
| Disease 2009 | 2.35 | -0.43 | 1.83 | 0.021 | 0.672 | 0.071 | **0.016** | -0.48 |
| SLA 2010 | 0.42 | 0.59 | -1.57 | 0.677 | 0.560 | 0.120 | 0.003 | 0.593 |
| Growth rate | -0.70 | 2.59 | -1.46 | 0.485 | 0.011 | 0.149 | >-0.001 | **0.097** |

statistically significant explanatory power ($p=0.729$), and the interaction is only marginally significant ($p=0.070$). Elevation and latitude do not significantly influence plant damage by herbivory, although the interaction of the terms is statistically significant ($p<0.001$). Population-level patterns of disease varied positively with elevation, but the slope indicates that this statistically significant effect is relatively weak (family-means model: $p=0.021$, slope=0.016; population-only model: $p=0.1611$, slope=0.037), and there is a marginally significant interaction of elevation and latitude as well ($p=0.071$). Elevation, latitude, and their interaction do not significantly predict population-level variation in specific leaf area ($p>0.1$, see table 3 for exact values). Growth rate varies significantly and positively with latitude (family-means model: $p=0.011$, slope=0.097; population means model: $p=0.0791$, slope=22.0), but not with elevation or the interaction term ($p>0.1$).

We also wanted to explicitly test the hypothesis that populations collected from sites closer to the site of the garden might have adapted to local conditions and outperform more distantly collected populations. Distance from the common garden did not correspond exactly with latitude, as populations were not collected in a strict north to south transect. We tested this alternative explanation of our results by creating linear regressions for the five measures of plant performance using the distance of each parent population from the common garden as an explanatory variable. Distance from the common garden site did not significantly explain variation in plant height, herbivory, disease, or SLA ($p>0.05$, adjusted $r^2<0.03$). Distance from Knoxville did account for a small but significant amount of variation in growth rate (family-means model: $p=0.028$, adjusted $r^2=0.044$); growth rates increased as distance from Knoxville increased.

However, latitude explained almost twice as much of the variation in growth rate (adjusted $r^2$=0.078), indicating that the performance of plants at the common garden was better correlated with the locations of their parent populations than by whether the conditions of their parent populations more closely resembled that of the common garden. Overall, changes in elevation, latitude, or the interaction of elevation and latitude explain some of the population-level patterns of plant growth alone in *Ailanthus altissima.*

Including other abiotic factors, including soil characteristics, as possible explanatory models for the five metrics that displayed significant population variation produced more accurate models, according to Akaike's Information Criterion (Table 4). Population variation in plant height was best predicted by a seven factor model which includes latitude, elevation, average precipitation, number of frost free days, average slope, average pH, and clay fraction (family-means model: AIC=418, adjusted $r^2$=0.30). The best model for herbivory, for which latitude and elevation had no statistically significant predictive power as main effects, contained three explanatory factors: air temperature, soil pH, and clay fraction (AIC=557, adjusted $r^2$=0.17). While elevation alone significantly predicted population-level variation in disease, a three-factor model that also included precipitation and clay fraction improved model fit (AIC=645, adjusted $r^2$=0.15), and the best single-factor model for predicting disease based on AIC scores actually contained soil pH, not elevation, as the explanatory factor (AIC=652, adjusted $r^2$=0.07). The most appropriate model for population-level variation in specific leaf area, for which latitude and elevation had no explanatory power, was the single-factor model of air temperature (AIC=628, adjusted $r^2$=0.09), although the two-factor model which

Table 4. Best one- through eight-factor models as selected by minimum AIC scores, for the five metrics of plant performance that displayed population-level variation. For each plant performance metric, models are arranged from best fit (lowest AIC score) to worst fit (highest AIC).

| **Response** | **Model** | **Family means AIC** | **Family means $r^2$** | **Pop only $r^2$** |
|---|---|---|---|---|
| Plant Height 2009 | Latitude*Elevation*Precip*FrostFree*Slope*pH*Clay | 418.86 | 0.303 | 0.567 |
| | Latitude*Elevation*Precip*MinAirT*FrostFree*Slope*pH*Clay | 420.25 | 0.293 | |
| | Latitude*Precip*Clay | 424.79 | 0.273 | |
| | Elevation*Precip*Slope*Clay | 426.52 | 0.306 | |
| | Latitude*Precip | 428.28 | 0.188 | |
| | Elevation*Precip*Slope*pH*Clay | 428.54 | 0.313 | |
| | Elevation*Precip*FrostFree*Slope*pH*Clay | 428.54 | 0.312 | |
| | Latitude | 441.19 | 0.063 | |
| Herbivory 2009 | MinAirT*pH*Clay | 557.12 | 0.171 | 0.467 |
| | Precip*MinAirT*pH*Clay | 560.84 | 0.176 | |
| | Precip*MinAirT*FrostFree*pH*Clay | 560.84 | 0.171 | |
| | Elevation*Slope | 561.92 | 0.066 | |
| | Slope | 565.31 | 0.042 | |
| | Latitude*Precip*MinAirT*FrostFree*pH*Clay | 567.68 | 0.159 | |
| | Latitude*Precip*MinAirT*FrostFree*Slope*pH*Clay | 567.70 | 0.148 | |
| | Latitude*Elevation*Precip*MinAirT*FrostFree*Slope*pH*Clay | 581.22 | 0.136 | |
| Disease 2009 | Elevation*Precip*Clay | 645.06 | 0.150 | 0.214 |
| | pH*Precip | 647.54 | 0.115 | |
| | Elevation*Precip*FrostFree*Clay | 651.76 | 0.160 | |
| | pH | 652.58 | 0.070 | |
| | Elevation*Precip*FrostFree*Slope*Clay | 659.48 | 0.219 | |
| | Latitude*Elevation*Precip*MinAirT*FrostFree*Slope*pH*Clay | 667.07 | 0.218 | |
| | Latitude*Elevation*Precip*MinAirT*FrostFree*Slope*Clay | 668.19 | 0.228 | |
| | Latitude*Elevation*Precip*FrostFree*Slope*Clay | 669.07 | 0.227 | |
| SLA 2010 | MinAirT | 628.40 | 0.085 | 0.327 |
| | MinAirT*FrostFree | 629.32 | 0.102 | |
| | Precip*MinAirT*FrostFree | 630.66 | 0.100 | |
| | Latitude*Elevation*Precip*MinAirT*FrostFree*Slope*Clay | 630.90 | 0.145 | |
| | Latitude*Elevation*Precip*MinAirT*FrostFree*Slope*pH*Clay | 634.25 | 0.139 | |
| | Precip*MinAirT*FrostFree*Clay | 635.59 | 0.107 | |
| | Latitude*Elevation*Precip*MinAirT*Clay | 637.77 | 0.144 | |
| | Latitude*Elevation*Precip*MinAirT*Slope*Clay | 639.19 | 0.149 | |
| Growth Rate | Latitude*Precip | 271.72 | 0.175 | 0.600 |
| | Latitude*Elevation*Precip*MinAirT*Slope*pH*Clay | 273.19 | 0.186 | |
| | Latitude*Precip*Clay | 274.63 | 0.208 | |
| | Latitude*Elevation*Precip*MinAirT*FrostFree*Slope*pH*Clay | 274.65 | 0.174 | |
| | Latitude*Elevation*Precip*MinAirT*pH*Clay | 274.93 | 0.198 | |
| | Latitude | 277.36 | 0.078 | |
| | Latitude*Precip*pH*Clay | 284.88 | 0.202 | |
| | Latitude*Elevation*Precip*pH*Clay | 288.92 | 0.208 | |

included number of frost free days as well as air temperature was fairly similar in terms of goodness of fit (AIC=629, adjusted $r^2$=0.10). Variation in growth rate, which was significantly correlated with latitude (AIC=277, adjusted $r^2$=0.08), was better correlated with a two-factor model containing precipitation as well as latitude (AIC=271, adjusted $r^2$=0.18).

## Discussion

This study overall illustrates a case of population differentiation in a recently introduced species whose range is still expanding, and shows that climate and soil factors correlate more strongly with important performance metrics than does latitude. We had hypothesized that there would be population-level genetic differentiation for the metrics considered, and we found statistical evidence of such differentiation in five out of the ten metrics considered. Our hypothesis that latitude would correlate with performance received only partial support; only plant height and growth rate correlated with latitude, although the interaction of latitude with elevation proved at least marginally significant for three of the five metrics. Also, the two metrics for which latitude was a significant explanatory factor were better modeled by including other factors as well. This supports our third hypothesis, that including climate and soil factors would improve the fit of our explanatory models. We were even able to construct explanatory models using climate and soils data for the three metrics where latitude did not significantly correlate with population performance. This suggests that climate and soils data may be useful in predicting the success of a species even when latitude, generally perceived to be autocorrelated with these factors, cannot predict population performance.

### *Evidence for rapid evolution of* Ailanthus altissima

Rapid evolution of *Ailanthus altissima* could be demonstrated by reduced family-level variation for characters under selection. Many previously published studies of variation in invasive plants across their ranges do not consider family-level genetic variation; the population is the smallest unit of interest (but see Blair and Wolfe 2004, Van Kleunen and Fischer 2008). There are sound practical reasons for this, as a study with multiple populations and replicates of multiple families within populations can quickly become unmanageably large. However, partitioning variation allows for more powerful modeling of data, reducing the chance of concluding that there are population level processes at work when individual level responses vary so widely as to create the appearance of population-level variation, and the method is standard practice in population genetics studies (Conner and Hartl 2004). We found evidence of population but not family variation for herbivory, disease, and SLA. This pattern may result from local adaptation, from genetic drift due to small population size, or from founding events, in which the different populations are descended from unrelated individuals. We do not have genetic data drawn from neutral markers for our populations that would allow for the most rigorous test of whether this pattern represents selective or stochastic processes, but there is relevant information from the common garden and from other studies. An isoenzyme analysis of five North American populations and five populations from the native range in China found that the sum of genetic variation at the family and population level was indistinguishable for the native and introduced ranges, which suggests that the species has not suffered genetic bottlenecks as a result of introduction (Feret and Bryant 1974). Feret and Bryant (1974) also note that a representative sample of the original British collection of *Ailanthus altissima* seeds was sent to America for cultivation, so information on how the seeds were disseminated via cultivation could provide information about population structure. Simons (2003) and Vasemagi (2006) note that founder effects can produce genetically distinct populations if a series of successive introductions consist of only a subset of the parent population's genetic variation. Floral records indicate that by the late nineteenth century, *Ailanthus altissima* had already escaped cultivation in Tennessee, the southern limit of our study, and was "perfectly naturalized, spreading widely over the state" (Gattinger 1887). This indicates that *Ailanthus altissima* has been present in southern locations for a substantial period of time, and that any founder effects are more likely to be the result of human cultivation than successive genetic bottlenecks. Some of the metrics we studied showed variation at both the family and population level, which suggests that the traits may have been subject to historical selection, and may still be subject to selection, although again drift cannot be dismissed as an important factor. Plant height and growth rate display population- and family-level variation. The *Ailanthus altissima* populations that we tested have significant genetic variation at the individual level for a number of traits, which makes founder effects less probable as a possible explanation of population-level patterns. The EICA hypothesis suggests that invasive plants undergo relaxation of selection by herbivory, which allows stronger selection for competitive ability, in their invaded range. This study has not directly tested the EICA hypothesis, as we did not include any populations from the native range of *Ailanthus altissima*. However, we did observe significant levels of herbivory across invaded populations, which did not correlate at all with growth rate (adjusted $r^2$=0.01, p=0.60), a metric used in EICA studies as a measure of competitive ability (Handley et al. 2008). Within the limits of this geographic range of *Ailanthus altissima*, there is no evidence of a trade-off between herbivory and growth rate, one of the assumptions of the EICA hypothesis. Factors other than selection by herbivory are responsible for the pattern of family or population-level variation, or both, for several quantitative traits *of Ailanthus altissima* grown in a common garden. The genetic differentiation which demonstrates that rapid evolution has occurred in the North American range of *Ailanthus altissima* is due to genetic drift or to local adaptation to invaded environments.

### *Population-level adaptation explained by latitude, climate, and edaphic factors*

Latitudinal clines of performance, phenology, and fitness characteristics are often cited as evidence of local adaptation (Endler 1977). We hypothesized that metrics, which exhibited population-level genetic variation, would vary with climate, a strong selective force. Latitude is assumed to correlate with climate, which is composed of multiple interacting factors and is more difficult to quantify. Of the five metrics with significant population-level genetic variation, only plant height in the first year and growth rate showed a significant correlation between latitude and population mean. To the best of our knowledge, this is the first record of an invasive tree species which demonstrates a latitudinal cline for metrics related to growth. In a review of studies testing the EICA hypothesis, Colautti et al. (2009) found that 14 of 34 tested invasive species showed evidence of latitudinal clines for at least one trait; the single tree species of the significant 14 species, *Melaleuca quinquenervia*, showed a latitudinal cline only for defense traits. It is difficult to identify latitude as the sole selective factor for defense traits, as herbivory can be affected by and interact with latitude to select defense traits (Garibaldi et al. 2011). The patterns of population means in *Ailanthus altissima* height and growth rate are similar to that of *Helianthus maximiliani* in its native range, with latitudinal clines where the largest plants tend to be in the south and the fastest growth rates tend to be found in northern populations (Kawakami et al. 2011). This indicates local adaptation in northern populations to a shorter growing season. Another herbaceous plant, *Hypericum perforatum* has established latitudinal clines in leaf size in its invasive range (Maron et al 2004). It is worth noting that there is significant family-level variation for both plant height and growth rate, indicating that these traits are amenable to further selection. In light of these latitudinal clines, it was surprising that there were no genetically-based differences among populations in the phenology of leaf out. However, a previous study of *Ailanthus altissima* demonstrated that there was high plasticity in spring bud break in response to temperature (Kowarik and Saumel 2007), so plasticity in response to timing of spring warming may overwhelm selective pressure from climate. The latitudinal clines of plant performance metrics in *Ailanthus altissima* are unique in that they represent an invasive plant with a relatively longer generation time that has established these clines within approximately 200 years since introduction.

We hypothesized that including soil characteristics and more explicit metrics related to climate (rainfall, annual temperature) would increase our ability to predict variation in *Ailanthus altissima* and allow us to identify possible selective factors important in local adaptation. Elevation can powerfully affect climate at the local scale, and many plant species, including both tree and herbaceous species, display genetic and genetically-based performance differences in populations across altitudes, even on a relatively small spatial scale (Linhart and Grant 1996, Ohsawa and Ide 2008). Elevation, or the interaction of elevation by latitude, was at least marginally significant for three variables (see table 2). We had predicted that including further variables in our models of population patterns would improve the predictive power of the models. This was supported to the extent that a seven-factor model including latitude and elevation held the most predictive power for plant height, and the best model for growth rate by AIC selection was a two-factor model that included latitude and average annual precipitation. However, we were also able to create predictive models of the characteristics that did not show latitudinal clines. The best model for herbivory was one which included minimum air temperature as well as a measure of soil chemistry (pH). Soil pH controls a broad range of other factors relating to nutrient availability and soil biological communities, and also often correlates with soil texture in the form of clay fraction, which will affect water retention and nutrient availability (Cote et al. 2000, Fierer and Jackson 2006). One mechanism through which soils may select for resistance to herbivory is by altering leaf palatability. Plant populations growing on soils of differing fertility may adapt to maintain optimal concentrations of nutrients in leaf tissue, and nutrient availability may also affect concentrations of secondary compounds in leaf tissue (Cunningham et al. 1999, Wright et al. 2001). *Ailanthus* produces a number of secondary compounds, which include tannins, alkaloids, and quassinoids, a class of proteins peculiar to the Simaroubaceae with demonstrated phytotoxic and putative herbivore-inhibiting effects (Kowarik and Saumel 2007). The plant populations in this study were grown in a common garden with a single soil type, so if this correlation reflects biological patterns, soil characteristics of the parent populations have created population-level patterns of herbivory via selection for traits that alter herbivore behavior. Disease severity on *Ailanthus altissima* was predicted by a combination of geography, climate, and soil characteristics (Table 3). The disease symptoms that we observed were consistent with a fungal pathogen, so a mechanism by which elevation, average annual precipitation, and clay might select for genetic differences between populations is by altering traits that affect the vascular system. Overall, we find that including climate and soil information in models of traits that follow a latitudinal cline improves the predictive power of the models, and even traits that do not conform to a latitudinal cline can be modeled using climate and soil data.

### *Soil conditions as a selective force in plant evolution*

The hypothesis that soils may select certain traits to create local adaptation in plants is most strongly supported by research done in serpentine soils, where high concentrations of metals select for any number of unique adaptations by populations of many plant species (Bergland et al 2001, Bergland et al. 2004, Brady et al. 2005). However, serpentine soils represent a fairly harsh environment, and the role that more fertile soils play in local adaptation is less well understood. Our correlation coefficients for the soil and climate models of population-level variation are significant, but not very high, indicating that there is still considerable unexplained variation within *Ailanthus altissima.* This could be due to the paternal contribution to the plants, which we did not know and thus could not quantify (although the plants are obligate out-crossers). This could also be due to the fact that we used soil and climate data that were summarized from a database that reports ranges of data for soil formations, rather than exact measurements of soils and microclimate at the locations of the trees from which we happened to collect seed. Our approximation of soil and climate may not capture the full variation of these factors, and thus may underestimate their importance as selective factors. Pregitzer et al. (2010) found that *Populus angustifolia* seedlings had the highest levels of survival in the soils in which their parent populations grew. A study of plant-soil feedbacks in three populations each of two European plant species found that the number of fruits produced by the legume species showed selection by soil but not climate, but that growth metrics of the legume did not show selection by soil (Macel et al. 2007). These studies show that more fertile soils than the high metal serpentine soils may foster local adaptation, but that they may not provide as strong of a selective force as do serpentine soils. This study of *Ailanthus altissima* demonstrates that soil factors may play an important role in the evolution of a plant invading a novel range, even when soils are not comprised so as to act as very strong selective forces.

### *Conclusions*

This study represents preliminary research into the role that soil and climate factors play in fostering local adaptation as a invasive plant expands in metapopulation size and range. Further work in this system could include observations of the parent populations and other *Ailanthus altissima* populations for the metrics in which we have identified genetic variation, in order to determine the role that plasticity may play in aiding or preventing selection. The soils and climate data used were collected over a long period of time and at a larger scale than may be biologically relevant to these populations, so studies in which microclimate and local soils are directly measured could considerably improve our understanding of the selective forces at work. This study shows that testing for range, population, and family levels of genetic variation in invasive plants, not just genetic differences between native and invaded ranges, may deepen our understanding of why invasive species become problematic across large novel ranges.  Rapid evolution and interactions with local environment may result in many different locally successful genotypes of an invader, suggesting that to view plant invasions as monolithic events, each with a unified explanation for the species' success, will not provide useful theories or management solutions.

# CHAPTER 4: **Soil biota drive expression of genetic variation and development of population-specific feedbacks in invasive plant.**

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## Abstract

Invasive plant species alter soils in ways that may affect the success of subsequent generations, creating plant-soil feedbacks. *Ailanthus altissima* is an invasive tree introduced two centuries ago to North America. We hypothesized that geographically distinct populations of *A. altissima* have established feedbacks specific to their local environment, due to soil communities cultivated by *A. altissima.* We collected seeds and soils from three populations in the Eastern United States, and in the greenhouse reciprocally planted all families in all collected soils as well as in a control mixed soil, and in soils that had been irradiated for sterilization. There were positive plant-soil feedbacks for two populations in the live field-collected soils, but strong negative feedbacks for the third population. There were no population-level performance differences or feedbacks in the sterilized population locale soils, supporting a soil biotic basis for feedbacks and for the expression of genetic differentiation in *A. altissima*. If populations of *Ailanthus altissima* vary in the extent to which they benefit from and promote these plant-soil biota feedbacks, the interaction

between invader and invaded community may be more important in determining the course of invasion than are the characteristics of either alone.

## Introduction

Invasive plant species defy the ecological assumption that organisms are most successful when they have adapted to local abiotic and biotic factors. A proportion of the plant species introduced to a novel environment do not struggle to survive, reproduce, and maintain a small population, but instead become invasive. Invasives often attain high densities, produce large numbers of offspring, thus increasing their population size to the detriment of native communities. The mechanisms by which invasive plant species successfully recruit sufficient numbers to not only maintain but also aggressively increase their population have been studied in terms of demographics and competitive interactions between plants, such as allelopathy (Jongejans et al. 2008, Inderjit et al. 2008). For example, the ability to expand populations of invasive plant species that depend on wind for seed dispersal or pollination depends in part on density of existing populations (Marchetto et al. 2010, Davis et al. 2004). Interactions between invasive seedlings and the soil communities in which they grow may be an important aspect of invasion, particularly as many invasive plants cause dramatic alterations in soils (Ehrenfield et al. 2001, Ehrenfield 2003).

Invasive plants cultivate soil environments distinct from soils associated with native plant communities, changing soil pH (Gomez-Aparicio and Canham 2008a), rates of soil organic matter accumulation, and rates of nutrient cycling (Stock el al. 1995). Invasives often exude chemicals that disrupt symbioses between native plant species and beneficial microbes (Wardle et al. 1994, Wolfe et al. 2008) or otherwise alter the composition of soil microbial communities (Batten et al. 2008). These distinctive invaded soils may affect the success of subsequent generations of plants growing at that site, creating a feedback loop in which soil conditions promoted by an invasive species promote further invasion (a positive plant-soil feedback; Callaway et al. 2004, Klironomos 2002, Rout and Callaway 2009). Plant-soil feedbacks are abiotic or biotic soil-based mechanisms that account for shifts in plant population size and community composition (van der Putten et al. 1993, Bever 1994). Negative plant-soil feedbacks prevent species from persisting at fixed locations or at high abundances (Klironomos 2002). Positive plant-soil feedbacks are more often suggested as mechanisms of invasion or local adaptation (Johnson et al. 2010). Certain introduced plants benefit from immunity to naïve pathogens in the new range (van Grunsven et al. 2007), reaping the benefits of mutualisms while escaping the costs of attack by pathogens and saprobes. However, soil communities and the population of the introduced plant may vary by geographic location and thus invasive species may experience variation in feedbacks in different parts of their introduced range.

To explore the impact that invasive-amended soil may have on the establishment and persistence of the invasive species *Ailanthus altissima*, we designed a greenhouse experiment that would allow us to test the effects of plant genetic family (i.e., within-population genetic variation), plant population, soil origin, and soil biota on seedling performance. *Ailanthus altissima* raises soil pH and nutrient availability (Gomez-Aparicio and Canham 2008a), and grows in persistent monocultural stands with no apparent reduction in performance, suggesting the presence of a positive plant-soil feedback (pers. obs.). Such a feedback would provide a mechanism that explains how current populations of *A. altissima* are able to expand their ranges, but it does not explain the success of geographically and genetically distinct populations of *A. altissima*, which have managed to establish themselves under different climactic and soil conditions. We collected seeds and soils from three geographically distinct and established populations in the eastern United States (across a 1000 km latitudinal gradient), and reciprocally planted seeds from all populations in sterile and non-sterile soils collected from all locations (using mixed soil from all populations as controls). We hypothesized that: 1) *A. altissima* populations have genetically based (as opposed to environmentally based) differences in plant performance, when grown in the control soil; 2) each population produces seeds which will perform best when grown in soil from their parental population (positive plant-soil feedbacks, specific to each population); and 3) the feedbacks are due to the biotic components of the soils rather than the physical characteristics of each soil. The confirmation of these hypotheses would indicate population-level, locally adapted positive feedbacks between *A. altissima* and soil microbial communities.

## Methods

### *Study species*

*Ailanthus altissima* (P. Mill.) Swingle, commonly known as tree of heaven, is native to southeast Asia and was planted in North American cities following its introduction to Philadelphia two centuries ago, where its tolerance for pollution made it a popular shade tree (Kowarik and Saumel 2007). Abundant seed production, fast growth rates, and resistance to drought have made *A. altissima* a persistent naturalized presence in American cities, and it has spread along transportation corridors into forested ecosystems. It is a dioecious, wind-pollinated tree species, which also reproduces via clonal growth, as the surface root system can develop into adventitious stems when damaged. *A. altissima* has also been shown to raise the soil pH and increase soil calcium and net nitrogen (N) mineralization rates within the tree's litter shadow (Gomez-Aparicio and Canham 2008a). While *A. altissima* has been shown to possess allelopathic, herbicidal properties, the active compound, ailanthone, is not thought to be auto-toxic or to persist in the soil (Heisey and Heisey 2003).

*Seed population and soil collections*

To address the hypothesis that local feedbacks promote persistence across the landscape (i.e., each population has a soil-based 'homefield advantage'), we collected seeds and soils from three populations of *A. altissima* across a latitudinal gradient that stretches along the Blue Ridge of the Appalachian Mountains. We collected seeds (half to full-sibling) from 10 females (hereafter referred to as seed families) from a population in Philadelphia, Philadelphia County, PA; from 5 females from a population in New Market, Shenandoah County, VA; and from 10 females from a population in Knoxville, Knox County, TN. Female trees were randomly selected at a minimum of 10 m distance from each other, and separated by a tree of another species where possible, to minimize the possibility that two stems were clones. Each population consisted of several stands of *A. altissima* monoculture as well as several *A. altissima* stems growing in isolation (i.e. at least 100 m from any other *A. altissima* stem). We collected seeds by clipping them from each tree in the winter of 2007, and stored them at 4°C until planting.

In April 2008, we collected soils from each of the populations. We collected a shovelful of mineral soil (top 15 cm) in each of the cardinal directions less than 0.5 m from the trunk beneath each selected mother tree. We collected soils exclusively from beneath female trees because we were interested in the specific influence of *A. altissima* on soils and thus on the next generation of *A. altissima* to grow in these soils. Soils from each site were pooled to form a PA, a VA, and a TN field soil. Approximately 8 L of soil was collected from each location; half of each soil was stored as collected, and will be referred to as 'field soil.' We also mixed soils from the three populations to create a control 'mixed field soil' in order to test for population genetic variance under common conditions These soils were stored at the 4°C within 24 h of being collected, and a subsample taken within 48 h of collection was used in microbial biomass and soil enzyme analyses. To separate the effects of microbial community composition and activity from the effect of soil physical and chemical properties on growth, the remaining 4 L of soil collected at each location was sterilized. Soils were transported to Steris Isomedix Services (Spartanburg, SC) and sterilized using gamma irradiation for 48 h at 30 kGy; these soils are hereafter referred to as 'sterile soils,' and include sterile population soils as well as a sterile mixed soil.

We quantified soil pH, texture (i.e., particle size), microbial biomass carbon (C) and N pools, and extracellular enzyme activity for each of the field soils following protocols recently utilized in Stritar et al. (2010; Table 5). Air-dried soil from each site

Table 5. Properties of Field Soils. The chemical, physical, and biotic properties of the pooled field soils by population (Pennsylvania [PA], Virginia [VA], Tennessee [TN]); where analyses allow, standard errors are shown as 1 standard error of the mean, and letters in parentheses indicate grouping of means as shown by Tukey's HSD.

| | Population Locale | | |
|---|---|---|---|
| Response Variables | PA | VA | TN |
| pH | 7.2 | 6.7 | 7.4 |
| Microbial biomass C (mg C/kg soil) | 155 ± 23 **(B)** | 185 ± 32 **(B)** | 298 ± 15 **(A)** |
| Microbial biomass N (mg C/kg soil) | 50.1 ± 4.3 **(B)** | 72.1 ± 9.6 **(AB)** | 97.2 ± 4.6 **(A)** |
| Microbial biomass C:N | 3.10 **(A)** | 2.56 **(B)** | 3.06 **(A)** |
| Potential NAGase activity (nmol/h/g soil) | 55.9 ± 26.3 **(A)** | 23.4 ± 2.2 **(A)** | 82.9 ± 11.2 **(A)** |
| potential phosphatase activity (nmol/h/g soil) | 66.1 ± 45.0 | 14.2 ± 14.2 | 7 ± 7 |
| Texture (% Sand-% Silt- %Clay) | 6-69-25 | 57-32-11 | 25-39-36 |

was used to determine soil pH using the 0.1 M $CaCl_2$ method (Hendershot et al. 1993); additionally, we measured particle sizes of the field soils by determining soil texture using the hydrometer method (Gee and Bauder 1986). We quantified soil microbial C and N pools using the chloroform fumigation extraction method. These samples were digested by a micro-Kjedahl process, and the digests were run using a Schimadzu TOC-V csh TNM-1 multi-carbon, nitrogen analyzer (Shimadzu Scientific Instruments, 7102 Riverwood Drive, Columbia, MD, USA) in order to determine pool sizes of microbial biomass C and N associated with each population. To determine the activity of microorganisms in soils from each population and their relative limitation by C, N, and phosphorus (P), we measured the potential activity of C, N, and P degrading extracellular enzymes using methylumbelliferone (MUB)-linked substrates in a fluorometric assay (Molecular Devices, Gemini XPS, Sunnyvale, CA). We ran assays using three different substrates: 4-MUB-ß-D-cellobioside (EC 3.2.1.91), to determine the activity of cellobiohydrolase, which breaks cellulose into cellobiose dimers; 4-MUB-N-acetyl- ß-D-glucosaminide (NAGase, EC 3.2.1.14), to determine the activity of NAGase, which acts upon chitin and is involved in nitrogen cycling; and 4-MUB-phosphate (EC 3.1.3.1), which releases phosphate from phosphomonoesters, to quantify acid phosphatases in the soil (Sinsabaugh et al. 2008). Values obtained were recorded in units of µmol enzyme $g^{-1}$ $h^{-1}$.

*Greenhouse study*

To determine the effects of plant population and soil origin on seed germination and seedling performance, we planted all seeds reciprocally into all soils. Each soil treatment was established in book planter type root trainers (Tinus Roottrainers, Spencer-Lemaire, Edmonton, Alberta, Canada) by filling the root trainers approximately 15 cm of potting mix (equal ratios of peat, vermiculite and perlite), and filling the top 3 cm of the cells with the treatment soil. There were eight soil treatments in total: field PA, field VA, field TN, mixed field control, sterile PA, sterile VA, sterile TN, and mixed sterile control soil. Twenty random seeds from all seed families from each population (25 families total) were planted into each soil treatment. A month after planting, seedlings were thinned to 5 seedlings from each family. We watered every 3 days, and randomly shifted the planters monthly to minimize the effects of variations in light or moisture. We measured seedling performance to assess phenotypic differences in growth based on seed population origin. From June to August, approximately every three weeks we measured total germination, total number of leaves, stem height (cm, measured from soil to apical meristem), stem width (mm, measured at cotyledon scars), leaf length and width (cm, both measured on the third leaf from the apical meristem). When plants were 16 weeks old the trees were sacrificed to determine the biomass of leaves, stems, and roots. The root mass and leaves were clipped from the stem, and all were oven-dried for 48 h at 70°C and before weighing.

### *Statistical analyses*

Performance metrics showed that the trees were at the peak of their growth on 1 July, and that thereafter they began to decline, so we used the metrics from this date as well as the final biomass data for all analyses. We analyzed the data collected using mixed effect models and Restricted Maximum Likelihood (REML) using the statistical program JMP 7. All analyses were run separately for field-collected and sterile soils.

To determine quantitative genetic differences among our seed populations we constructed mixed models which included seed population as a fixed effect, family nested within seed population as a random effect, and replicate as a random effect based on performance measures in mixed field soil. The test statistic for family-level effects was determined by Likelihood Ratio Tests, in which the difference between the likelihood ratio of the model described above and the likelihood of the same model with the family effect removed was used as a $\chi^2$ value (one-tailed $\chi^2$ distribution, df=1). To test for seed population x soil interactions (statistical evidence of feedbacks), we ran mixed models for the seedlings grown in soils collected from each population (the control mixed soil was excluded) that included seed population, soil origin, seed population x soil origin as fixed effect, and family nested in seed population as a random effect. For the mixed models, we used Tukey's HSD post-hoc test to determine how performance means differed by seed population or soil type where appropriate. We did not have sufficient statistical power to use post-hoc tests on family-level means nested within population.

We compared the performance of each population when grown in soil collected from underneath its established invasive population (i.e., 'home' soils hereafter, even though this does not refer to soils where *A. altissima* is native) to when grown in soils collected from other populations (i.e., 'away' soils, hereafter) in order to evaluate plant-soil feedbacks. To assess the magnitude of plant-soil feedbacks, we calculated Hedge's d for following the methods described in Kulmatiski et al. (2008), which compares the mean performance of each population grown in "home" soil to the mean performance of the seed populations grown in "away" soils while adjusting for variance and sample size. A value of 0 for Hedge's d indicates no difference between "home" and "away" and neutral feedback, values between 0.2 and 0.5 indicate weak effects, 0.5 and 0.8 indicate moderate effects, 0.8 and 1.0 indicate strong effects, and a value of d over 1.0 indicates a very large effect (Kulmatiski et al. 2008). Positive values indicate positive plant-soil feedbacks, and negative values indicate negative plant-soil feedbacks to plant performance.

## Results

### *Seed population and soil origin effects on A. altissima performance*

In a greenhouse environment, one of the three geographically distinct populations of *A. altissima* displayed genetic differences in performance traits, but not in germination rates. Seeds planted in the common environment of the mixed field control soils differed significantly in performance as measured by stem height, leaf mass, stem mass, and total aboveground biomass (Figure 8, Table 6). PA and VA populations were similar, but TN plants were 20 or 30% shorter than PA and VA plants, respectively, and produced approximately 40% less biomass than the other two populations. Likelihood Ratio Tests show that there was no significant genetic variation among families in mixed field soil. In mixed sterile soil, there were no significant population difference in performance for stem height or stem mass, but there were significant differences by population in leaf mass and aboveground biomass (Table 7).

### *Plant-soil feedbacks*

Feedbacks to performance are specific to each population. The four performance traits that showed genetic divergence among populations when seeds were grown in the mixed control soil all had significant seed population x soil origin effects in field soils (Table 8). In other words, in a common environment plant traits that showed evidence

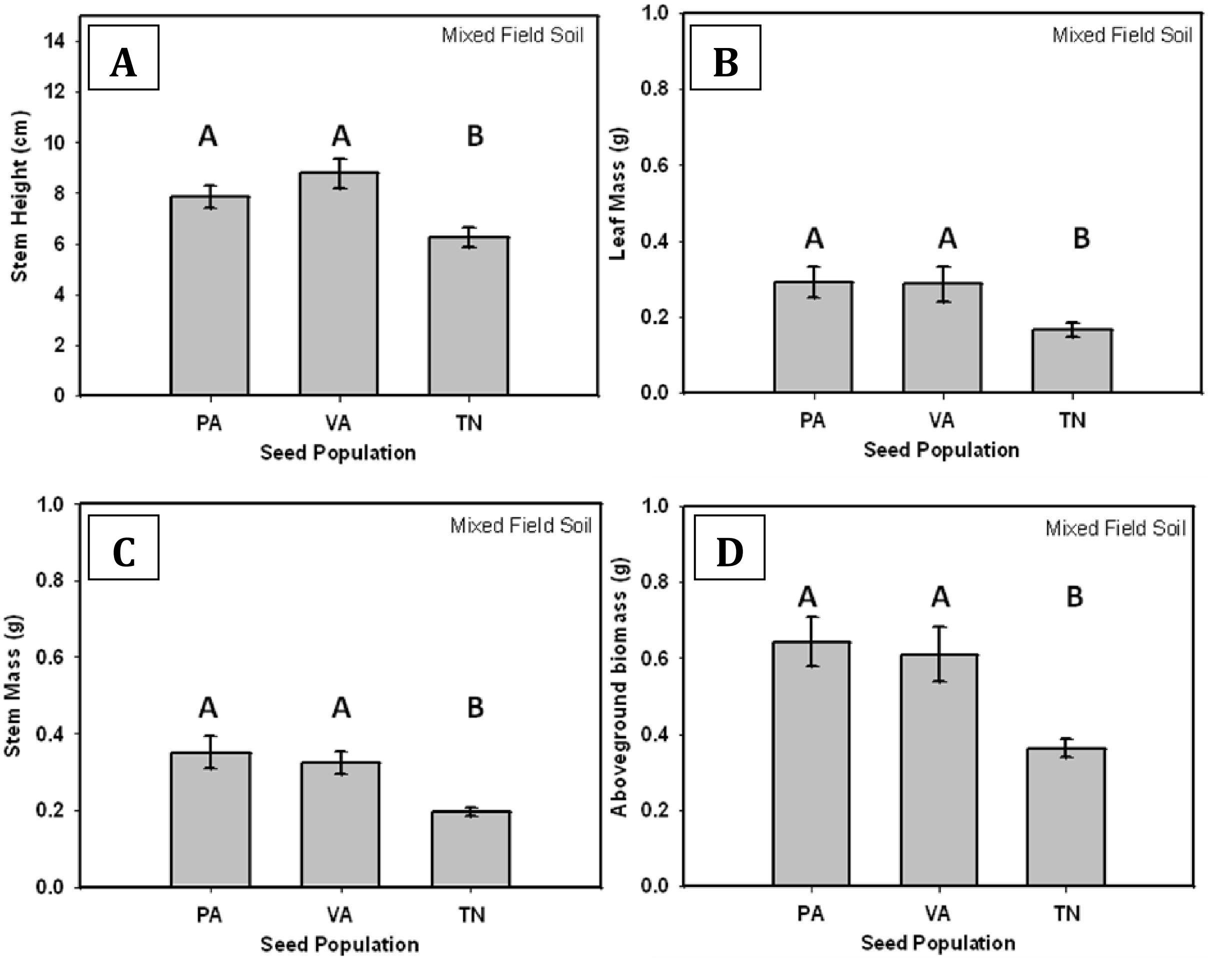

Figure 8. Seed populations are significantly different when grown in control (mixed) soil. For the performance metrics of stem height (A), leaf mass (B), stem mass (C), and aboveground mass (D), VA and PA populations significantly differ from the TN population (letters above bars indicate significant groupings indicated by Tukey’s HSD).

Table 6. *Ailanthus altissima* grown in field collected soils from geographically distinct populations. Performance metrics of *Ailanthus altissima* seeds grown in soils collected from three parent *A. altissima* populations. Performance means (± standard error) for each population are reported, along with seed population statistics as shown by mixed models and family-level statistics as shown by likelihood ratio tests (LRT). Asterisks(*) indicate significant differences between populations, while crosses(†) indicate significant family-level variation.

| | | | | **Seed Population** | | **Family** | |
|---|---|---|---|---|---|---|---|
| **Performance Indices** | **PA Population** | **VA Population** | **TN Population** | **F ratio** | **p-value** | $\chi^2$ | **p-value** |
| **Mixed Field Soil** | | | | | | | |
| Germination (%) | 0.33 ± 0.05 | 0.39 ± 0.03 | 0.41 ± 0.07 | 0.825 | 0.4524 | - | - |
| Number Leaves | 7.25 ± 0.41 | 6.76 ± 0.47 | 6.36 ± 0.27 | 1.083 | 0.3646 | 0.62 | 0.2152 |
| Stem Height (cm)* | 7.84 ± 0.56 | 9.00 ± 0.64 | 6.26 ± 0.29 | 6.898 | 0.0070 | 0.80 | 0.1863 |
| Petiole Length (cm) | 2.09 ± 0.13 | 2.55 ± 0.27 | 2.37 ± 0.12 | 1.765 | 0.1994 | 0.00 | 0.4874 |
| Leaf Width (cm) | 5.43 ± 0.40 | 6.34 ± 0.47 | 5.52 ± 0.31 | 0.895 | 0.4243 | 1.12 | 0.1455 |
| Leaf Mass (g)* | 0.29 ± 0.04 | 0.29 ± 0.05 | 0.17 ± 0.02 | 4.546 | 0.0150 | 0.00 | 0.5000 |
| Stem Mass (g)* | 0.35 ± 0.04 | 0.32 ± 0.03 | 0.20 ± 0.01 | 6.292 | 0.0149 | 0.47 | 0.2463 |
| Aboveground biomass (g)* | 0.64 ± 0.06 | 0.61 ± 0.07 | 0.36 ± 0.02 | 8.894 | 0.0005 | 0.00 | 0.5000 |
| Root biomass (g) | 1.23 ± 0.15 | 1.08 ± 0.16 | 0.80 ± 0.15 | 2.168 | 0.1421 | 0.11 | 0.3712 |
| **PA Field Soil** | | | | | | | |
| Germination | 0.48 ± 0.07 | 0.65 ± 0.04 | 0.57 ± 0.07 | 1.347 | 0.2807 | - | - |
| Number Leaves† | 6.69 ± 0.21 | 6.25 ± 0.29 | 6.27 ± 0.17 | 0.594 | 0.5614 | 11.19 | 0.0004 |
| Stem Height* | 6.50 ± 0.26 | 8.58 ± 0.49 | 6.19 ± 0.23 | 8.068 | 0.0024 | 7.01 | 0.0040 |
| Petiole Length*† | 2.07 ± 0.09 | 2.40 ± 0.11 | 1.92 ± 0.09 | 5.022 | 0.0017 | 0.17 | 0.3396 |
| Leaf Width* | 4.78 ± 0.26 | 5.92 ± 0.42 | 4.37 ± 0.21 | 5.952 | 0.0095 | 0.37 | 0.2704 |
| Leaf Mass* | 0.09 ± 0.01 | 0.07 ± 0.02 | 0.05 ± 0.01 | 3.536 | 0.0479 | 0.74 | 0.1953 |
| Stem Mass* | 0.12 ± 0.01 | 0.16 ± 0.02 | 0.11 ± 0.01 | 5.346 | 0.0135 | 0.00 | 0.5000 |
| Aboveground biomass* | 0.22 ± 0.01 | 0.23 ± 0.03 | 0.16 ± 0.02 | 3.779 | 0.0400 | 0.28 | 0.2987 |
| Root biomass | 0.67 ± 0.05 | 0.91 ± 0.11 | 0.78 ± 0.08 | 1.968 | 0.1459 | 0.00 | 0.5000 |
| **VA Field Soil** | | | | | | | |
| Germination | 0.33 ± 0.06 | 0.56 ± 0.08 | 0.46 ± 0.07 | 1.587 | 0.4302 | - | - |
| Number Leaves† | 7.17 ± 0.33 | 6.71 ± 0.34 | 6.92 ± 0.21 | 0.334 | 0.6930 | 8.58 | 0.0017 |
| Stem Height† | 9.92 ± 0.56 | 10.67 ± 0.73 | 10.53 ± 0.37 | 0.321 | 0.7178 | 7.89 | 0.0025 |
| Petiole Length* | 2.62 ± 0.11 | 2.73 ± 0.19 | 3.10 ± 0.10 | 4.271 | 0.0297 | 0.43 | 0.2562 |
| Leaf Width | 6.09 ± 0.38 | 6.48 ± 0.53 | 6.98 ± 0.33 | 1.421 | 0.2691 | 0.02 | 0.4438 |

***Table 6 cont'd***

| | | | | Seed Population | | Family | |
|---|---|---|---|---|---|---|---|
| **Performance Indices** | **PA Population** | **VA Population** | **TN Population** | **F ratio** | **p-value** | **$\chi^2$** | **p-value** |
| ***VA Field Soil cont'd*** | | | | | | | |
| Leaf Mass*† | 0.24 ± 0.03 | 0.34 ± 0.04 | 0.11 ± 0.02 | 11.712 | 0.0004 | 3.11 | 0.0390 |
| Stem Mass† | 0.29 ± 0.02 | 0.38 ± 0.03 | 0.29 ± 0.02 | 1.834 | 0.3028 | 11.79 | 0.0003 |
| Aboveground biomass*† | 0.53 ± 0.05 | 0.72 ± 0.07 | 0.40 ± 0.03 | 5.767 | 0.0224 | 7.08 | 0.0039 |
| Root biomass† | 1.24 ± 0.13 | 1.43 ± 0.14 | 0.93 ± 0.09 | 2.398 | 0.1328 | 5.78 | 0.0081 |
| **TN Field Soil** | | | | | | | |
| Germination | 0.40 ± 0.09 | 0.50 ± 0.06 | 0.34 ± 0.05 | 0.877 | 0.4302 | - | - |
| Number Leaves* | 7.27 ± 0.32 | 5.50 ± 0.45 | 6.40 ± 0.36 | 3.945 | 0.0259 | 0.00 | 0.5000 |
| Stem Height | 7.32 ± 0.42 | 6.06 ± 0.65 | 5.94 ± 0.41 | 1.769 | 0.2039 | 0.33 | 0.2837 |
| Petiole Length | 2.39 ± 0.11 | 2.07 ± 0.21 | 2.29 ± 0.13 | 0.674 | 0.5224 | 0.56 | 0.2263 |
| Leaf Width | 6.41 ± 0.20 | 5.54 ± 0.76 | 5.83 ± 0.45 | 0.560 | 0.5835 | 0.03 | 0.4347 |
| Leaf Mass | 0.25 ± 0.04 | 0.15 ± 0.04 | 0.22 ± 0.02 | 1.238 | 0.3235 | 0.38 | 0.2677 |
| Stem Mass | 0.22 ± 0.02 | 0.18 ± 0.03 | 0.23 ± 0.03 | 0.849 | 0.4352 | 0.00 | 0.5000 |
| Aboveground biomass | 0.47 ± 0.06 | 0.34 ± 0.06 | 0.45 ± 0.04 | 1.215 | 0.3316 | 0.07 | 0.3968 |
| Root biomass† | 1.02 ± 0.07 | 1.10 ± 0.25 | 0.92 ± 0.17 | 0.670 | 0.5279 | 6.25 | 0.0062 |

Table 7. *Ailanthus altissima* grown in sterilized soils collected from geographically distinct populations. Performance metrics of *Ailanthus altissima* seeds collected from three populations, Pennsylvania (PA), Virginia (VA), and Tennessee (TN), and grown in soils collected from the three parent populations and sterilized by gamma irradiation. Performance means (± standard error) for each population are reported, along with seed population statistics as shown by mixed models and family-level statistics as shown by likelihood ratio tests (LRT). Asterisks(*) indicate significant differences between populations, while crosses(†) indicate significant family-level variation. Bolded p-values indicate performance metrics that were significantly different for the populations when seeds were grown in field-collected soil (see Table 6).

| | | | | **Seed Population** | | **Family** | |
|---|---|---|---|---|---|---|---|
| **Performance Indices** | **PA Population** | **VA Population** | **TN Population** | **F ratio** | **p-value** | **$\chi^2$** | **p-value** |
| **Mixed Sterile Soil** | | | | | | | |
| Germination (%) | 0.11 ± 0.04 | 0.12 ± 0.05 | 0.17 ± 0.06 | 0.827 | 0.4552 | - | - |
| Number Leaves | 5.89 ± 0.32 | 5.20 ± 0.43 | 5.64 ± 0.26 | 0.433 | 0.6574 | 1.83 | 0.0882 |
| Stem Height (cm) | 6.52 ± 0.42 | 6.07 ± 0.56 | 6.25 ± 0.33 | 0.176 | **0.8414** | 0.15 | 0.3493 |
| Petiole Length (cm) | 2.15 ± 0.15 | 2.26 ± 0.21 | 2.20 ± 0.12 | 0.094 | 0.9108 | 0.61 | 0.2181 |
| Leaf Width (cm) | 5.64 ± 0.45 | 5.77 ± 0.61 | 5.66 ± 0.36 | 0.016 | 0.9843 | 0.24 | 0.3117 |
| Leaf Mass (g)* | 0.48 ± 0.04 | 0.14 ± 0.06 | 0.27 ± 0.04 | 11.608 | **0.0001** | 0.00 | 0.5000 |
| Stem Mass (g)† | 0.48 ± 0.04 | 0.38 ± 0.05 | 0.31 ± 0.03 | 3.142 | **0.0884** | 2.88 | 0.0449 |
| Aboveground biomass (g)* | 0.96 ± 0.07 | 0.53 ± 0.09 | 0.58 ± 0.06 | 10.931 | **0.0001** | 0.00 | 0.5000 |
| Root biomass (g) | 1.27 ± 0.16 | 1.59 ± 0.22 | 1.27 ± 0.13 | 0.907 | 0.4106 | 0.00 | 0.5000 |
| **PA Sterile Soil** | | | | | | | |
| Germination | 0.17 ± 0.05 | 0.11 ± 0.03 | 0.13 ± 0.03 | 0.635 | 0.5408 | - | - |
| Number Leaves | 5.78 ± 0.46 | 4.67 ± 0.46 | 5.80 ± 0.25 | 1.327 | 0.2959 | 1.21 | **0.1361** |
| Stem Height | 5.97 ± 0.57 | 5.17 ± 0.57 | 6.69 ± 0.31 | 2.892 | **0.0658** | 0.00 | **0.5000** |
| Petiole Length* | 1.90 ± 0.23 | 1.87 ± 0.23 | 2.47 ± 0.12 | 3.992 | **0.0255** | 0.00 | 0.5000 |
| Leaf Width | 5.39 ± 0.62 | 4.44 ± 0.62 | 5.97 ± 0.34 | 2.098 | **0.1499** | 0.91 | 0.1698 |
| Leaf Mass | 0.42 ± 0.08 | 0.38 ± 0.07 | 0.44 ± 0.04 | 0.316 | **0.7355** | 2.18 | 0.0699 |
| Stem Mass | 0.30 ± 0.05 | 0.28 ± 0.05 | 0.34 ± 0.03 | 0.705 | **0.5184** | 0.33 | 0.2837 |
| Aboveground biomass | 0.72 ± 0.12 | 0.66 ± 0.11 | 0.78 ± 0.07 | 0.460 | **0.6429** | 1.39 | 0.1190 |
| Root biomass | 1.26 ± 0.24 | 1.11 ± 0.19 | 1.41 ± 0.11 | 0.971 | 0.3891 | 0.00 | 0.5000 |
| **VA Sterile Soil** | | | | | | | |
| Germination | 0.09 ± 0.03 | 0.09 ± 0.02 | 0.12 ± 0.02 | 0.034 | 0.9670 | - | - |
| Number Leaves | 5.60 ± 0.44 | 5.33 ± 0.81 | 6.55 ± 0.30 | 1.189 | **0.4245** | 0.00 | **0.4822** |
| Stem Height | 8.56 ± 0.62 | 8.33 ± 1.12 | 8.31 ± 0.42 | 0.944 | **0.9437** | 0.00 | **0.5000** |
| Petiole Length† | 2.21 ± 0.26 | 2.40 ± 0.47 | 2.15 ± 0.17 | 0.240 | **0.7901** | 7.95 | 0.0024 |
| Leaf Width | 6.79 ± 0.43 | 7.67 ± 0.79 | 6.72 ± 0.29 | 0.211 | 0.8143 | 0.55 | 0.2300 |
| Leaf Mass† | 0.37 ± 0.07 | 0.60 ± 0.13 | 0.51 ± 0.05 | 1.649 | **0.2240** | 3.48 | **0.0311** |
| Stem Mass | 0.51 ± 0.05 | 0.54 ± 0.10 | 0.44 ± 0.04 | 0.569 | **0.5786** | 1.37 | **0.1208** |

***Table 7 cont'd***

|  |  |  |  | Seed Population |  | Family |  |
|---|---|---|---|---|---|---|---|
| **Performance Indices** | **PA Population** | **VA Population** | **TN Population** | **F ratio** | **p-value** | **$\chi^2$** | **p-value** |
| ***VA Sterile Soil cont'd*** |  |  |  |  |  |  |  |
| Aboveground biomass† | 0.88 ± 0.11 | 1.14 ± 0.20 | 0.96 ± 0.08 | 0.490 | **0.6248** | 3.06 | **0.0402** |
| Root biomass | 1.46 ± 0.18 | 1.41 ± 0.32 | 1.60 ± 0.14 | 0.203 | **0.8180** | 1.16 | **0.1405** |
| **TN Sterile Soil** |  |  |  |  |  |  |  |
| Germination | 0.11 ± 0.04 | 0.10 ± 0.04 | 0.06 ± 0.02 | 0.414 | 0.6683 | - | - |
| Number Leaves | 5.08 ± 0.37 | 4.75 ± 0.65 | 5.19 ± 0.28 | 0.079 | **0.9249** | 0.44 | 0.2543 |
| Stem Height† | 5.74 ± 0.44 | 4.25 ± 0.77 | 5.10 ± 0.34 | 0.947 | 0.4080 | 4.29 | 0.0192 |
| Petiole Length | 2.54 ± 0.25 | 2.58 ± 0.43 | 2.62 ± 0.19 | 0.019 | 0.9817 | 0.81 | 0.1845 |
| Leaf Width | 6.23 ± 0.54 | 5.43 ± 0.93 | 5.62 ± 0.41 | 0.267 | 0.7705 | 0.88 | 0.1737 |
| Leaf Mass | 0.39 ± 0.06 | 0.53 ± 0.13 | 0.41 ± 0.05 | 0.635 | 0.6349 | 0.00 | 0.4917 |
| Stem Mass | 0.40 ± 0.06 | 0.33 ± 0.11 | 0.36 ± 0.04 | 0.183 | 0.8346 | 0.02 | 0.4466 |
| Aboveground biomass | 0.79 ± 0.11 | 0.86 ± 0.22 | 0.77 ± 0.09 | 0.068 | 0.9348 | 0.00 | 0.5000 |
| Root biomass† | 1.23 ± 0.14 | 1.20 ± 0.28 | 1.15 ± 0.11 | 0.092 | 0.9122 | 0.00 | **0.5000** |

Table 8. Statistical evidence of plant-soil feedbacks in field, not sterile, soils. Mixed Model interaction of seed population x soil origin shows evidence of plant-soil feedbacks in field soils, but not in sterile soils. For each of the performance indices recorded for this experiment, the F-ratio and p-values of the interaction term (seed population x soil origin) from the mixed models that included both seed population and soil origin. Significant p-values indicating a plant-soil feedback for that performance metric in field soil are bolded (α=0.05); there are no statistically significant interactions in sterile soil.

| | Field Soils | | Sterile Soils | |
|---|---|---|---|---|
| Performance Indices | F ratio | p-value | F ratio | p-value |
| Germination | 0.47 | 0.7604 | 0.54 | 0.7068 |
| Number Leaves | 1.41 | 0.1228 | 0.70 | 0.6113 |
| Stem Height | 3.82 | **0.0042** | 0.75 | 0.4260 |
| Petiole Length | 3.36 | **0.0004** | 0.95 | 0.3744 |
| Leaf Width | 2.17 | **0.0155** | 0.90 | 0.2724 |
| Leaf Mass | 5.45 | **<0.0001** | 3.84 | 0.5456 |
| Stem Mass | 4.35 | **0.0239** | 1.50 | 0.6299 |
| Aboveground Biomass | 5.05 | **<0.0001** | 2.60 | 0.8265 |
| Root Biomass | 3.44 | 0.1514 | 0.76 | 0.8091 |

of population-level genetic differentiation changed in their expression when grown in soils collected from different populations, evidence of plant-soil feedback. The Hedge's d effect sizes indicate the presence of population-specific feedbacks (Figure 9). The PA seed population experiences strong to very strong negative feedbacks (Hedge's d of -.90 to -1.16) in their home soil ranging from a 25% decrease in stem height to a 62% decrease in leaf mass. The VA population experiences strong to very strong positive feedbacks (Hedges d of 0.94 to 1.40), growing 45% taller and producing up to 200% more biomass (leaf biomass) in VA soils compared to PA and TN soils. TN populations experience mixed positive and negative feedbacks (stem height d=-0.79, leaf mass d=1.27, stem mass d=0.26, aboveground mass d=0.83), with TN seedlings 29% shorter but producing 60% more aboveground biomass in their home soils.

### *Putative mechanisms*

We grew seedlings in gamma-irradiated sterile soils from each population to test the hypothesis that plant-soil feedbacks are related to biotic factors in the soil. When we examined the seed population effects on stem height, leaf mass, stem mass, or aboveground biomass, in the PA, VA, and TN sterilized soils, we found no significant population effects on performance (Table 7). There were also no significant interactions of seed population x soil origin, in other words no statistical evidence for plant-soil feedbacks, in the sterilized soils (Table 8). This suggests that any plant-soil feedbacks in the sterilized soils were neutral (no overwhelming negative or positive effects on plants), in contrast to the results in live field soils. There were significant effects of soil origin on seedling performance in sterilized PA, VA, and TN soils, indicating differences in soil nutrient quality. Post-hoc tests (Tukey's HSD) revealed that the traits of stem

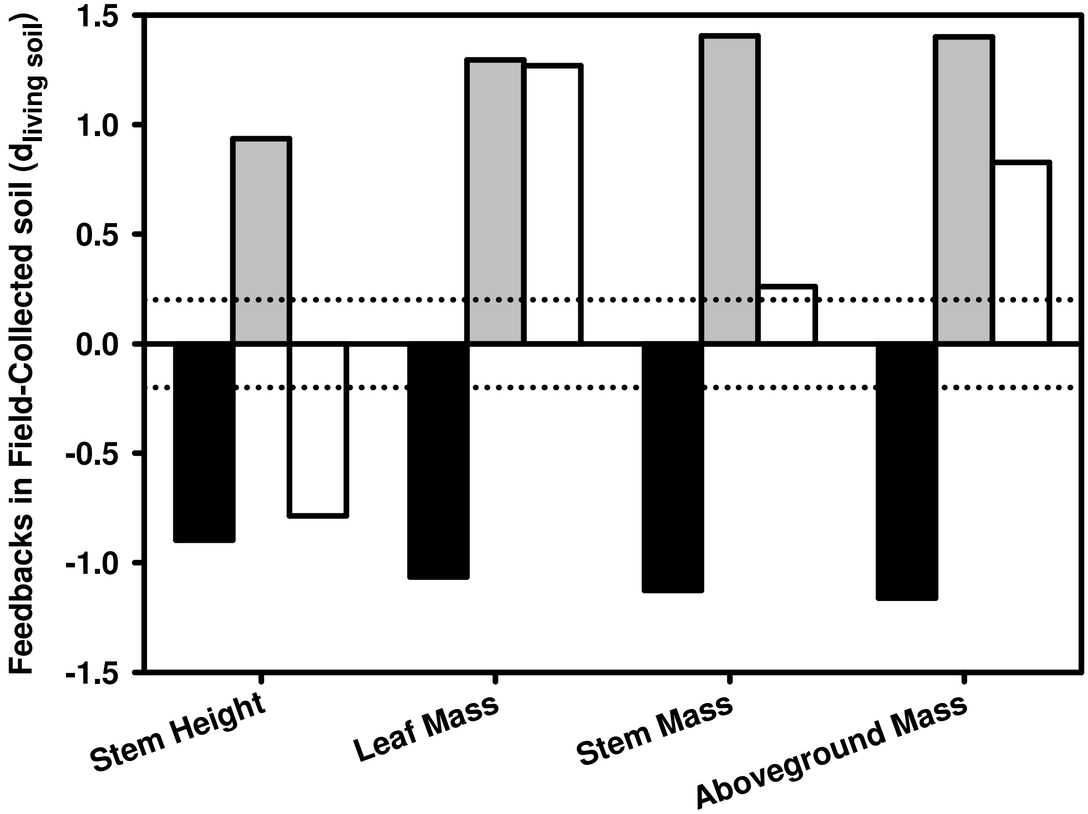

Figure 9**.** Population-specific feedbacks to *Ailanthus altissima* performance in field-collected soils. The Hedge's d calculated for each seed population (performance in "home" soils-performance in other population soils) is shown for the performance metrics of stem height, leaf mass, stem mass, and aboveground mass. The dotted line (±0.20) indicates minimum value of significant weak plant-soil feedback; values above 0 indicate positive feedback while values below 0 indicate negative feedbacks. PA populations (black bars) experience negative feedbacks grown in their own soils. VA populations (grey bars) experience positive feedbacks. TN populations (white bars) experience negative and positive feedbacks grown in their own soils.

height, leaf width, and stem mass of all (PA, TN, and VA) seedlings were significantly higher in sterile VA soils and aboveground mass was significantly higher in sterile VA than sterile PA soils. Analysis of the field soils showed differences in microbial biomass pools and the activity of extra-cellular enzymes, indicating that there may be differences in biologically available nutrients. Virginia soils had the lowest microbial C:N ratio and the lowest potential NAGase activity, indicating that the Virginia soils are likely to have the most biologically available N of the three populations. If the physical characteristics of each soil were responsible for the feedbacks observed in the field-collected soil, we would have expected to see similar patterns of growth in the sterile soil. Instead, the feedbacks in the sterile soil were neutral, and no expression of genetic variation in performance traits in sterile soil.

## Discussion

The results of this experiment demonstrate that 1) the *Ailanthus altissima* populations express genetically-based differences in performance traits in mixed and population soils (Figure 8) and; 2) there is a geographic mosaic of plant-soil feedbacks of varying direction and magnitude specific to each population in its home soil. The VA population experiences positive feedbacks and the PA population experiences negative feedbacks in their home soils; and 3) the feedbacks are not present in sterile soil, supporting the soil biota as the mechanism for the feedback. These results provide support for the first and third of our original hypotheses, that there is population differentiation in *A. altissima* and that the feedbacks are biotically based, and suggest that plant-soil feedbacks for this invasive species may be more idiosyncratic and soil biota mediate invasion success.

*Population-level genetic variance*

In mixed field soil, seed populations were genetically distinct for the performance traits of stem height, leaf mass, stem mass, and aboveground biomass, indicating population level genetic differentiation (Seifert et al. 2009), although phenotypic differences do not allow us to separate selection from genetic drift as possible causal forces in this evolution. There was no family-level variation in the mixed field soil. In contrast, seeds grown in field soils collected from PA, VA, or TN populations displayed different combinations of population and even family-level variation (Table 7). The pattern of genetic variation expressed in performance was unique to each field soil type, providing evidence that the soils collected from different parental populations have a strong influence over the expression of varying levels of genetic variation in seedlings.

We were also interested in differentiating between the physical structure of the soil and the soil biota as the causal factor in the observed patterns. Soil type can affect interactions between plants and soil biota such as the strength of the mutualism between coevolving mycorrhiza and plants (Johnson et al. 2010). However, our results suggest that the soil biota play a key role in *A. altissma* expression of population genetic differentiation. The seedlings grown in the sterilized mixed soils showed no population-level differentiation in stem height and stem mass, and the differences in leaf mass and aboveground biomass, do not mirror the results of the mixed field soils. In the mixed field soils, VA populations had the highest leaf and aboveground biomass, but in the sterile mixed soils, it was the PA populations that had the highest biomass (Table 7).

This suggests that the combined soil biotic communities represented in the mixed field soil enhance the performance of the VA but not the PA population. This evidence of varying interactions across populations between soil biota and *A. altissima* fits into the framework of the Geographic Mosaic Theory of Coevolution (Thompson 1997), in which the magnitude and direction of biotic interactions vary across the landscape depending upon the interacting species. Our results show that soil type and communities affect the expression of genetic variation in *A. altissima* grown under greenhouse conditions, and suggest that the expression of genetic variation, and by extension the potential for selection in this species, may be dependent upon the soil and soil biota in which it grows.

### *Plant-soil feedbacks*

The magnitude and direction of the plant-soil feedbacks are unique to each *Ailanthus altissima* population, in contrast to our initial hypothesis. We hypothesized before conducting the experiment that there would be positive plant-soil feedbacks for each of the populations. The VA population was the only population with strong positive feedbacks in its own soil for each performance characteristic considered. There are strong negative feedbacks for the PA population in its own soil, and a mix of feedbacks of varying magnitude and effect for the TN population. These contrasting feedbacks highlight the importance of studying invasive plants within the context of existing plant-soil interactions. When the performance of populations grown in their natal field-collected soils are compared to their performance in soil collected from other populations, it is clear that VA seedlings grew more aggressively in their home soils than do either PA or TN seedlings in their home soils. Based on this result, VA populations might increase in number of individuals and in landscape area invaded faster than other populations. Positive feedbacks may allow VA populations to grow in more dense monocultures, which is of concern for associated native species as allelopathy of *A. altissima* increases as stems grow denser (Gomez-Aparicio and Canham 2008b). Moreover, since this experiment was conducted under the artificial climatic conditions of the greenhouse, it tested only the soil conditions associated with each site, not the different climatic conditions. Much of the previous research on invasive species' plant-soil feedbacks focuses on differences between native and introduced ranges or compares invasive to native species. Comparisons of invasives with conspecifics from their native range (Callaway et al. 2004) or heterospecifics in the invaded range (Klironomos 2002) show that in general, invasive species experience positive plant-soil feedbacks or are simply less dependent on mutualists in the soil (Seifert et al. 2009). In focusing our study on populations within the invaded range rather than on sampling the invasive range as a homogeneous entity, we have found that in contrast to earlier research and theoretical predictions (Rout and Calloway 2009), invasive populations may experience a range of feedbacks across their invaded range. Different feedbacks may result in varying rates of expansion for different populations.

### *Biotic mechanisms*

The use of sterilized soils allowed us to test our hypothesis that the feedbacks are due to the soil biota present in the field soils, rather than to the physical properties and nutrient levels common to both field and sterilized soils. The results support a biotic feedback, as there were no statistically significant seed population by soil origin (genetic X environment) effects in sterilized soil, and virtually no difference between populations grown in the sterilized PA, VA, or TN soil (the one exception was for petiole length in sterile PA soil). The positive feedbacks associated with VA (for the traits of stem height, leaf mass, stem mass, and aboveground biomass) and TN (leaf mass, stem mass, and aboveground biomass) populations, and the negative feedbacks associated with PA (stem height, leaf mass, stem mass, and aboveground biomass) and TN (stem height) populations, were associated with a component of the unsterilized soil, an effect consistent with differences in the soil biotic communities or their activities. The population-specific feedbacks may be due to pre-invasion soil structure and microbial communities, as suggested by a study of invasive plants across a broad geographic range which shows that the extent to which invasive species alter invaded soils depends in part on pre-invaded soil conditions (Dassonville et al. 2008). Plant-soil feedbacks have been associated with the activity of nematodes, bacteria, mutualistic and pathogenic fungi found in the soil (Bever 2003). The pattern of plant-soil feedbacks that we observed could be due to differences in the soil communities associated with each population as well as to the variation in local adaptation that *A. altissima* has made to these conditions. Alternatively, the populations may have been established at different times and are in different stages of accumulating mutualistic or antagonistic biotic interactions (Strayer et al. 2006). However, we were not able to locate introduction records of these populations, therefore conclusions about how these feedbacks change over time is unknown.

### *Conclusions/Implications*

The different directions of plant-soil interactions shown by the populations emphasize the importance of understanding how ecological interactions shape population dynamics. Soil-specific feedbacks have been demonstrated with invasive species, in studies in which plants experienced positive feedbacks in their introduced range (e.g., Klironomos 2002). However, this is the first study, to our knowledge, that provides evidence of population-specific plant-soil feedbacks for an invasive species in its new range. If populations of *Ailanthus altissima* vary in the extent to which they benefit from feedbacks with soil biota as well as in their ability to promote these feedbacks, this suggests that the interaction between invader and invaded community may be far more important in determining the success of invasion than are the characteristics of either component alone. Monitoring these populations over time may allow us to determine how important these feedbacks are in facilitating the further spread of this species, and could focus management plans on populations in locations where the invasive species experiences a significant benefit from its interactions with the soil biota.

# CHAPTER 5: **Environmental influences on litter quality lead to within-species specialization in decomposition, or home-field advantage**

*This chapter will be submitted to Journal of Ecology, with Drs. Colleen M. Iversen, Richard J. Norby, and Jennifer A. Schweitzer as co-authors. The research project was conducted at sites set up and maintained by CMI and RJN, who also contributed to writing the paper. JAS assisted in experimental design and contributed to writing the paper.*

## Abstract

Decomposition of leaf litter occurs due to interactions of abiotic factors (leaching, fragmentation) as well as through the activities of soil biota feeding on litter. Recent research on microbial specialization shows that litter produced by different plant species can promote microbial communities most efficient at promoting mass loss, an effect called home-field advantage (HFA). We used two ecosystem experiments in closely situated *Liquidambar styraciflua* plantations in Oak Ridge, Tennessee, USA to compare the effects of elevated carbon dioxide ($CO_2$) and nitrogen (N) addition on leaf litter decomposition, in order to test whether intra-specific changes in litter alone can foster HFA. We collected litter from ambient $CO_2$ plots, elevated $CO_2$ plots, unfertilized ambient N plots, fertilized elevated N plots, and reciprocally placed bags containing the different litter origins into all sites. We found that elevated $CO_2$ decreased litter quality (%N, lignin:N), and N fertilization increased litter quality (% lignin, lignin:N). Elevated $CO_2$ litter had a slower mass loss than ambient $CO_2$ litter, but ambient N and elevated N litters lost mass at the same rate. The location where the litter bags of differing litter origin were decomposed primarily affected N and P dynamics (i.e., immobilization and release) of decomposing litter, but had no effect on mass loss. There was a significant HFA for ambient $CO_2$ litter decomposed in its treatment of origin, as well as for ambient N and elevated N litters. In contrast, there was a significant home-field disadvantage (20%) for elevated $CO_2$ litter decomposed in its treatment of origin. Our results demonstrate the importance of interactions between plant nutrient content and soil biota and conditions in determining litter decomposition, a process which returns plant-available nutrients to the soil. We have demonstrated that HFA, (i.e. decomposer specialization in local litter resulting in faster mass loss) can occur even when the litter varies only in lignin and N content, not by differences among species.

## Introduction

The process of leaf litter decomposition is often described as a chemical reaction, the sum of a variety of chemical and physical process, whose rate is determined primarily by the features of its substrate (Chapin et al. 2002). However, with increasing use of enzyme assays, researchers are able to look more closely at the role that soil communities, the ‘microbial gatekeepers’ of decomposition, play in determining rates of mass loss and nutrient release (DeForest et al. 2004; Hofmockel et al. 2007; Sinsabaugh 2010). Recent studies on ‘home-field advantage’ (hereafter referred to as HFA) in litter decomposition show that many types of litter decompose fastest on the soils in which they were grown (“home” sites relative to “away” sites), suggesting that substrate preference and specialization can occur in detritivore communities (Hunt et al. 1988; Gholz et al. 2000; Vivanco and Austin 2006; Ayres et al. 2009a, b). Laboratory work has shown that it is the microbial community rather than physical conditions that is responsible for this phenomenon (Strickland et al. 2009a, b). However, direct tests of the HFA have compared litter from different species, litter that varied in quality as well as in leaf structure and secondary metabolites (e.g. Strickland et al. 2009a, b). Such studies confound aspects of litter quality such as nitrogen and lignin content, which can vary in response to environment, with the leaf structures and secondary metabolites that are specific to particular species. A more stringent test of the hypothesis would use litter from a single or from closely related species to explore whether variation in litter quality alone can change microbial specialization and HFA.

Decomposition of leaf litter is a crucial process in ecosystems as it allows relatively fast turnover of limiting nutrients such as nitrogen. As we attempt to understand and predict the response of temperate forested ecosystems to anthropogenic climate change, it becomes increasingly important to focus on processes such as decomposition, which cycle relatively small amounts of nutrients but play important roles in determining plant productivity and future ecosystem pools. Litter decomposition has been commonly studied under elevated $CO_2$ and in response to N fertilization manipulations. Plants growing under elevated $CO_2$ increase fixation of C, and if this additional C remains in leaf tissue or is used to produce more leaves in an N-limited environment it will raise the C:N ratio of the litter and lower overall litter quality (Knops et al. 2007). Changes in litter produced under elevated $CO_2$ have been demonstrated to lead to unchanged (Norby et al. 2001a; Hall et al. 2006; Finzi and

Schelsinger 2002) or slower (Cotrufo et al. 2005; Hoorens et al. 2003; Zheng et al. 2010) decomposition rates in different systems. Inconsistent results have been attributed to variation of responses in different species and ecosystems, or to the variety of experimental designs utilized in different studies (Norby et al. 2001a). Plants grown in soils fertilized with N may have a lower C:N ratio in their leaves and higher litter quality due to increased plant uptake of N and incorporation into tissues (Knorr et al. 2005). A meta-analysis found that litter produced in response to N fertilization decomposes at unchanged or faster rates in different systems, depending largely upon the species' litter quality under ambient conditions: N fertilization increases litter quality and decomposition rates of species that produce low-quality litter under ambient conditions (low-quality litter defined by the author as >20% lignin), but does not generally affect species with high-quality litter (Knorr et al. 2005). Many studies have addressed the question of whether increased atmospheric $CO_2$ or increased soil N availability affect leaf litter decomposition, but results vary by species and ecosystem, making it difficult to assess broad patterns in order to determine whether microbial specialization in decomposition may even be relevant to discussions of anthropogenic change to ecosystems.

Many multi-year experimental manipulations of forested ecosystems by practical design consider only one factor of climate change, or consider several putatively interacting variables in a factorial design. The first type of experiment has been instrumental in addressing uncertainties about the specific effects of changes such as warming (Harvard Forest, Frey et al. 2008) or elevated atmospheric $CO_2$ (ORNL Free Air $CO_2$ Enrichment, or FACE, Norby et al. 2001b) upon ecosystem pools. The second type of experiment has been useful for addressing the relative importance of interacting ecosystem alterations: $CO_2$ with factors such as soil nutrient availability (Duke FACE, McCarthy et al. 2010) or atmospheric concentrations of the biologically reactive gas ozone (Aspen FACE, Dickson et al. 2001). However, a rigorous study of leaf decomposer specialization under different nutrient availabilities, such as might occur with anthropogenic global change, requires a forest in which several single-factor manipulations have occurred on otherwise similar tree species and soil conditions. The Oak Ridge National Environmental Research Park contains two multi-year experiments in plantations of closed-canopy *Liquidambar styraciflua* L., one experiment in which atmospheric concentrations of carbon dioxide was elevated, and the other experiment in which soil N availability was increased by the application of urea-N fertilizer. We used both of these experiments as a model system to address the role of intra-specific variation in response to climate change factors. Specifically we hypothesized that 1) increases in $CO_2$ concentrations and in soil N availability in each experiment have altered litter quality to the extent that 2) litters from different origins decompose at different rates. We further hypothesized that as a cumulative result of changes in litter quality over the course of the experiment, 3) sites under elevated $CO_2$ or N fertilization treatments will have altered decomposition processes resulting in site-specific decomposition rates. We further hypothesized that altered litter quality and site-specific decomposition conditions will interact so that 4) there will be quantifiable HFAs for each litter origin. We predict that elevated $CO_2$ will decrease litter quality (C:N ratio) compared to litter from ambient $CO_2$ plots, resulting in slower litter decomposition rates for the elevated $CO_2$ litter and for all litters at the elevated $CO_2$ site. In the N fertilization experiment, we predicted that N fertilization will increase litter quality compared to litter grown without fertilization, and that this enriched litter will decompose faster. We further predicted that N-fertilized sites will have slower rates of decomposition than unfertilized sites, consistent with studies that show that higher available soil N decreases microbial biomass and respiration (Liu and Greaver 2010). We predicted that each litter origin will decompose fastest in the site type from which it was collected. Such results would show that the biotic communities responsible for decomposition are sensitive to plant intraspecific variation in the form of quality of litter inputs, and would suggest a much broader applicability to natural systems of HFA and plant-soil feedbacks than at the biome or species levels in which much of the research to date has explored.

## Methods

To address hypotheses on the role of plant resource availability (C and N) in decay dynamics and home-field advantage, we utilized $CO_2$ and N addition studies whereby atmospheric $CO_2$ and soil N were manipulated for 11 and 3 years, respectively. Both research experiments were established in *Liquidambar styraciflua* monocultures that were planted as one year-old saplings in 1988 in the Oak Ridge National Environmental Research Park. The soils are a silty clay loam texture, classified as an Aquic Hapludult, and are moderately well-drained (Norby et al. 2001b). Soil pH is slighty acidic (5.5-6.0). Mean annual air temperature is 13.9°C, and the site receives on average 1371 mm of precipitation annually. There are slight differences between the two experimental sites: in the Free Air $CO_2$ Enrichment (FACE) experiment, the trees were planted 2.4 × 1.3 m apart, while in the Nitrogen Fertilization Experiment (NFE), the rows were 2.2 × 1.7 m. The total soil N content at the time the experiments were initiated was lower at the FACE site (11 Mg $ha^{-1}$) than at the NFE site (16 Mg $ha^{-1}$; Norby et al. 2001b).

The FACE experiment was established in 1996 and fumigation with elevated $CO_2$ occurred from 1998 to 2009 (April to November of each year). The rings were 25-m in diameter, and air was blown into the rings at canopy level by blowers on each ring's perimeter. Two rings received the elevated $CO_2$ treatment at a target level of approximately 565 ppm $CO_2$ during daytime hours during the growing season. Three control rings were established, two of which were utilized in this experiment: one in which ambient $CO_2$ of approximately 390 ppm was blown into the canopy and another in which the tower apparatus was installed but the blowers were not used (Norby et al. 2001b). In the early years of the experiment *L. styraciflua* in the elevated $CO_2$ plots demonstrated increased net primary productivity compared to ambient plots, with much of the additional fixed C being allocated belowground to fine roots (Norby et al. 2004). However, this response declined in latter years of the study (Norby et al. 2010b). Leaf litter N concentration was 10% lower in elevated $CO_2$ than ambient $CO_2$ plots (Norby and Iversen 2006), but more leaf litter was produced in the elevated $CO_2$ plots (Norby et al. 2003). The elevated $CO_2$ rings had a greater understory biomass and shifted faster from an herbaceous to a woody understory than the ambient $CO_2$ rings (Souza et al. 2010). There have been few studies examining effects of $CO_2$ enrichment on soil microbial community or function at this site. Austin et al. (2009) found no significant differences in soil extra-cellular enzyme activity or soil bacterial community composition, but a more recent study by Iversen et al. (2012) found that there was reduced microbial biomass N across the soil profile (0-90 cm) in the elevated $CO_2$ plots.

The NFE began in 2004. Twelve 12 × 16 m plots were established in a generalized randomized block design of three blocks. For this study, we utilized only four of the plots, one fertilized and one ambient plot each from two of the blocks to match the number of FACE plots; the plots utilized were chosen because they contained an invasive shrub, *Elaeagnus umbellata*, used in a concurrent experiment. Two plots in each block were untreated and served as unfertilized controls, while two were fertilized annually with 200 kg $ha^{-1}$ of N in the form of urea from 2004 to 2009. Fertilization was done by hand every March before leaf flush, and total soil inorganic N availability in the soil peaked early in the growing season with a 26-fold increase over ambient levels before declining to near ambient levels at the end of the growing season in 2005, the only growing season with monthly soil N data (Iversen and Norby 2008). Nitrogen fertilization increased *L. styraciflua* woody growth by 38%, and leaf litterfall by 11% over control plots (Iversen and Norby 2008). Nitrogen fertilization also increased the N concentration of these tissues, with wood containing 81-113% higher N and litterfall 14-30% higher N than ambient plots (Iversen and Norby 2008). Fertilization reduced soil respiration by 30% during the growing season in 2006 (Felker-Quinn, *unpublished data*).

Naturally senesced leaf litter from *L. stryaciflua* from both experiments was collected on two occasions in late October and early November 2008 to be used in the litterbag experiments. The topmost and most recently fallen litter was collected from litter traps and directly from the forest floor following leaf drop (which occurred in the

FACE plots from 27 September to 19 November). Collected litter was pooled by site of collection into four types, hereafter referred to as litter origins: elevated $CO_2$, ambient $CO_2$ (control FACE sites only), elevated N (NFE fertilized plots), and ambient N (NFE unfertilized sites only). Litter was air-dried and stored in the laboratory until placed in litter bags. Litter bags were constructed of nylon mesh, using 2 mm mesh on the top of the bag to allow litter-dwelling invertebrates access to the inside of the bags, and 0.25 mm mesh on the bottom to prevent the loss of litter to the soil surface via fragmentation. We filled litterbags with 5 g litter from the four sites, an amount that is consistent with average total litterfall by area (Norby et al. 2010a). The bags were installed at the sites in mid-December, 2008. Bags of all litter origins were reciprocally placed at each of the sites (4 litter origins × 8 sites × 3 collection dates × 3 or 4 replicates = 306 bags). Intact litterbags were collected after 2 (*n* = 88 bags), 6 (*n* = 97 bags) and 11 (*n* = 93 bags) months in the field. Elevated $CO_2$ was blown over the elevated C plots from April-October 2009, and N fertilization occurred in spring 2009. Initial litter quality of each litter origin was assessed by measuring lignin using the acid-fibre detergent method and by measuring total N and phosphorus (P) using a modified micro-Kjeldahl digestion (Parkinson and Allen 1975). Litter dynamics of N and P were assessed for all three collection dates using the same technique. At each collection date, the leaf litter was carefully removed from the litterbag, oven-dried (72 °C for 48 h), and a subsample of each bag's contents was ash-corrected (combusted at 500 °C for 5 h). The micro-Kjeldahl digestions were analyzed for total N and total P using a Lachat AE Flow Injection Analyzer using the salicylate and molybdate-ascorbic acid methods, respectively (Lachat Industries, Loveland CO, USA); apple leaves (SRM 1515, 2.25%

N by mass, National Institute of Standards and Technology, 100 Bureau Drive, Stop 1070, Gaithersburg, MD, USA) and digested "blanks" that contained no litter were used as internal standards.

We conducted separate statistical analyses for the FACE and NFE experiments because the initial site conditions were different (see above), and because $CO_2$ elevation and N fertilization took different forms, the first as an increase in atmospheric partial pressure, and the second as a solid fertilizer applied to the soil. We reduced heteroscedascity in the datasets by arcsine square root-transforming all mass remaining data, and log-transforming N and P remaining data, and used transformed data in all statistical analyses. We used a statistical comparison of mass loss data, rather than a comparison of decomposition rate constants (k-constants) as suggested by Wieder and Lang (1982), because the low number of sites (two per site type) allowed us to calculate only two k-constants per litter per site type. We calculated k-constants for each decomposition location of each *L. stryaciflua* litter origin as the linear slope of the natural-log-transformed mass loss data, and also by the exponential slope of the untransformed data. Analyses showed that these two methods produced similar k constants, and produced the same statistical results; therefore, for simplicity, only the k-constants calculated by the first method for each litter type at each site type are reported. To test for home-field advantage of different litter origins decomposed in the location where the leaf litter were produced, we modified the formula outlined in Ayres et al. (2009a) for a fully reciprocal transplant of four litter origins i, j,k, and l, placed at site types I, J, K, and L:

$HFA_i = HDD_i - ADD_i – H$ eqn. 1

$HDD_i = (D_{iI} - D_{iJ}) + (D_{iI} - D_{iK}) + (D_{iL} - D_{iL})$ eqn. 2

$ADDi = (D_{iJ} - D_{jJ}) + (D_{iK} - D_{kK}) + (D_{iL} - D_{lL})$ eqn. 3

$H = (HDD_i + HDD_j + HDD_k + HDD_l)/ (N-1)$ eqn. 4

where $HFA_i$ is the home-field advantange of litter i decomposing at site I, $HDD_i$ is a measure of litter i's performance in its home site I (litter i at 'home' versus all the 'away' litters at site I), $ADD_i$ is a measure of litter i's performance at other sites (litter i when it is 'away' versus j,k,l litters at 'home'), H is an adjusted average of the home performance (HDD) of each of the litters, and N is the number of litter origins (four). We calculated HFA using percent mass remaining data from the last removal date as D. A positive $HFA_i$ indicates that litter i decomposes faster at its home site i than would be predicted from the average litter mass loss rate across all sites and from the average rate specific to the site for all litter origins. A negative $HFA_i$ indicates that litter i decomposes more slowly at home than would be predicted from the litter's average rate across all sites or from the average rate of all litters at the home site.

We used Markov-chain Monte Carlo analysis to resample mass remaining data from each litter at each site (1000 iterations) to calculate HFA, using the values of the individual decomposition bags rather than site mean mass remaining values as D. Since the variance within and among sites are included in the calculations, error terms can also be calculated by this resampling method. All statistical analyses were completed using JMP 7.0.1 (SAS Institute, Cary, NC, USA) except for the Monte Carlo analysis, which was done in Excel using supplemental package PopTools, version 3.2.3 (Hood 2010).

## Results

### *Litter Quality*

T-tests of chemical concentrations of collected litter showed that its quality declined significantly when it was produced under elevated $CO_2$. Elevated $CO_2$ altered litter quality in two of the four metrics quantified (Table 9). Litter lignin:N was 15% greater in the elevated $CO_2$ treatment when compared with the ambient $CO_2$ treatment ($P = 0.004$). Nitrogen concentration of leaf litter was 14% lower in the elevated $CO_2$ treatment ($P = 0.003$). Phosphorus concentration of litter was 12% lower in the elevated $CO_2$ treatment when compared with the ambient $CO_2$ treatment, but this difference was only marginally significant ($P = 0.07$).

Leaf litter produced in the plots fertilized with N was of higher quality than litter produced in the unfertilized control. T-tests on litter quality showed a 15% decline in litter lignin concentration in the fertilized plots compared with the unfertilized control ($P = 0.03$). While litter N concentration was only marginally higher (e.g., 10%) in the fertilized plots ($P = 0.07$), N fertilization reduced litter lignin:N by 24% ($P = 0.001$) when compared with the unfertilized control. Nitrogen fertilization did not significantly affect litter P concentration ($p=0.18$).

### *Litter decomposition and nutrient loss*

Elevated $CO_2$ litter decomposed differently than ambient $CO_2$ litter in terms of mass loss and P dynamics, but not N dynamics. Elevated $CO_2$ litter had more mass remaining at each collection date during decomposition than did ambient $CO_2$ litter ($P = 0.03$; Figure 10, Figure 11). Litter origin (ambient or elevated $CO_2$) had no significant

Table 9**.** *Liquidambar stryaciflua* grown under different conditions produces leaf litter of different quality. Initial concentrations of lignin, nitrogen (N), and phosphorus (P) are reported as percentage of dry mass of litter; the lignin:N ratio is calculated using the concentrations of lignin and N. Error terms indicate standard error, and n=5 for each litter origin. An asterisk (*) next to an elevated litter origin value indicates that there is a significant ($p>0.05$) difference between litter grown under ambient versus elevated conditions for that experiment.

| Litter Origin | % Lignin | % N | % P | Lignin: N |
|---|---|---|---|---|
| Ambient $CO_2$ | 37.8 ± 1.4 | 1.21 ± 0.04 | 0.19 ± 0.01 | 31.2 ± 0.7 |
| Elevated $CO_2$ | 37.3 ± 0.6 | 1.04 ± 0.03* | 0.16 ± 0.01 | 35.9 ± 0.7* |
| | | | | |
| Ambient N (unfertilized) | 35.3 ± 1.8 | 1.50 ± 0.07 | 0.16 ± 0.02 | 23.7 ± 0.7 |
| Elevated N (fertilized) | 30.0 ± 1.2* | 1.68 ± 0.07 | 0.12 ± 0.02 | 17.9 ± 0.7* |

main effects or interactive effects on N dynamics during decomposition (*P* > 0.10, Table 10, Figure 12), indicating that although litter produced in the elevated $CO_2$ treatment decomposed more slowly, similar microbial processes of N immobilization occurred in both $CO_2$ treatments.

Litter origin also had no significant main effect on litter P dynamics, but there was a significant interaction of litter origin and decomposition location (*P* = 0.03, Figure 13). Compared with litter produced under ambient $CO_2$, elevated $CO_2$ litter immobilized more P when placed in the elevated $CO_2$ sites for decomposition, and litter produced under ambient $CO_2$ immobilized more P than elevated CO2 litter when both were placed in the ambient $CO_2$ treatment.

Changes in litter quality in response to N fertilization did not lead to changes in litter decomposition in terms of mass loss, but did affect N and P dynamics of decomposing litter. There was no significant difference in mass loss between elevated N litter and ambient N litter (P>0.10; Table 11). There was no main effect of litter origin on litter N dynamics, but there was a significant interaction of litter and time (*P* < 0.0001), with N immobilization in elevated N litter peaking at the second removal date, while the ambient N litter had the largest amount of N immobilized at the third removal date (Figure 12). The P dynamics of decomposing litter also were affected by litter origin (*P* < 0.001), with elevated N litter containing approximately 10-30% more P at each stage of decomposition than ambient N litter, regardless of where the litter was placed to decompose (Figure 13).

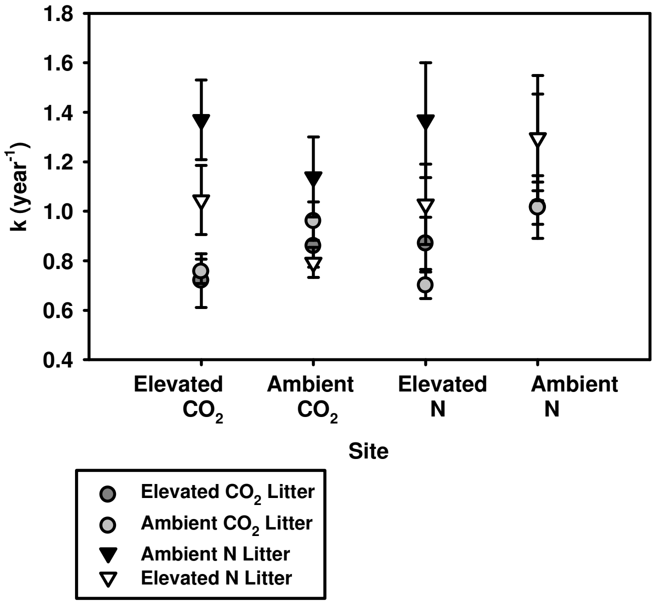

Figure 10. Litter grown under ambient or elevated carbon dioxide ($CO_2$) treatments, or in unfertilized ambient N or fertilized elevated N treatments, decomposed at different rates. Decomposition rate constants (k, year$^{-1}$) were calculated separately for each litter origin placed for decomposition in each treatment. Error bars represent standard errors, and *n* = 264 litter bags.

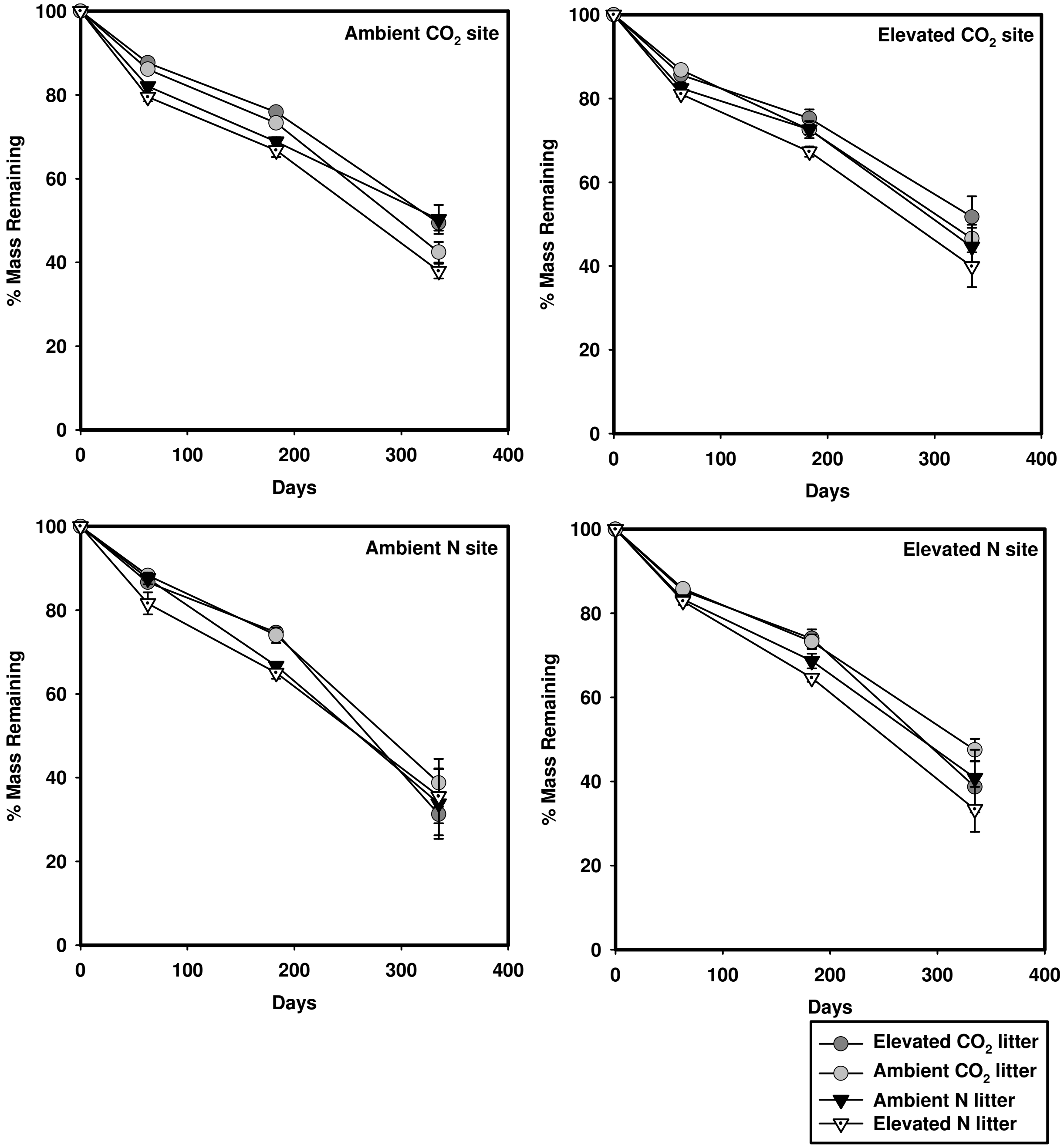

Figure 11. Mass loss of all *Liquidambar styraciflua* litter origins decomposed at all site types. Each point represents the mean of 3-7 litterbags, and error bars represent standard errors.

Table 10. Explanatory factors in ANOVAs for mass loss, N dynamics (% initial N), and P dynamics (% initial P) at the Free-air $CO_2$ enrichment (FACE, elevated $CO_2$ and ambient $CO_2$) experiment. An asterisk (*) next to a p-value indicates a statistically significant effect ($\alpha$=0.05).

| | | Mass loss (% initial mass) | | N dynamics (% initial N) | | P dynamics (% initial P) | |
|---|---|---|---|---|---|---|---|
| Factor | df | F-ratio | p-value | F-ratio | p-value | F-ratio | p-value |
| Time | 2 | 313.6 | <0.0001* | 153.5 | <0.0001* | 219.1 | <0.0001* |
| Litter origin | 1 | 4.4 | 0.03* | 0.1 | 0.78 | <0.1 | 0.83 |
| Location of Decomposition | 1 | 0.1 | 0.71 | 0.1 | 0.77 | 0.4 | 0.55 |
| Litter × Location | 1 | 0.4 | 0.52 | 2.4 | 0.12 | 4.7 | 0.03* |
| Litter × Time | 2 | 1.3 | 0.27 | 1.8 | 0.18 | 0.1 | 0.13 |
| Location × Time | 2 | 0.9 | 0.41 | 4.0 | 0.02* | 0.1 | 0.11 |
| Litter × Location × Time | 2 | 0.2 | 0.86 | 1.9 | 0.15 | 0.3 | 0.30 |

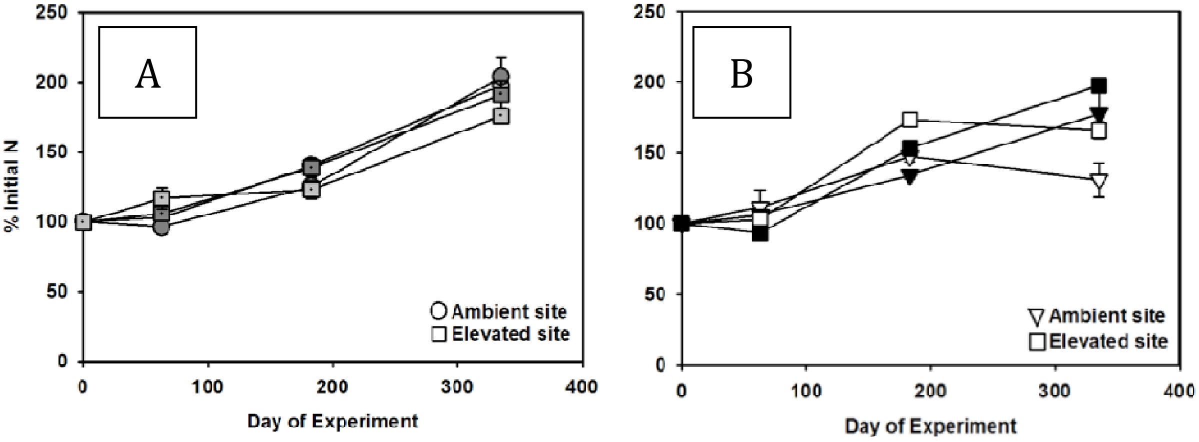

Figure 12. Nitrogen (N) dynamics in decomposing *Liquidambar styraciflua* litter. The N remaining in the litter at each removal is expressed as a percentage of the N originally contained in litter collected from elevated $CO_2$ and ambient $CO_2$ treatments (A), or litter collected from unfertilized ambient N and fertilized elevated N treatments (B). Different colored symbols represent different litter origins: dark grey represents litter produced under elevated $CO_2$, light grey represents ambient $CO_2$ litter, white represents elevated N litter, and black represents ambient N litter. Different shapes of symbols represent where litter was placed for decomposition: square symbols in (A) indicate litter was placed in elevated $CO_2$ plots, circular symbols indicate that litter was placed in ambient $CO_2$ plots; square symbols in (B) indicate litter placed in fertilized elevated N plots, and triangular symbols indicate litter placed in unfertilized ambient N plots. Error bars represent one standard error of the mean.

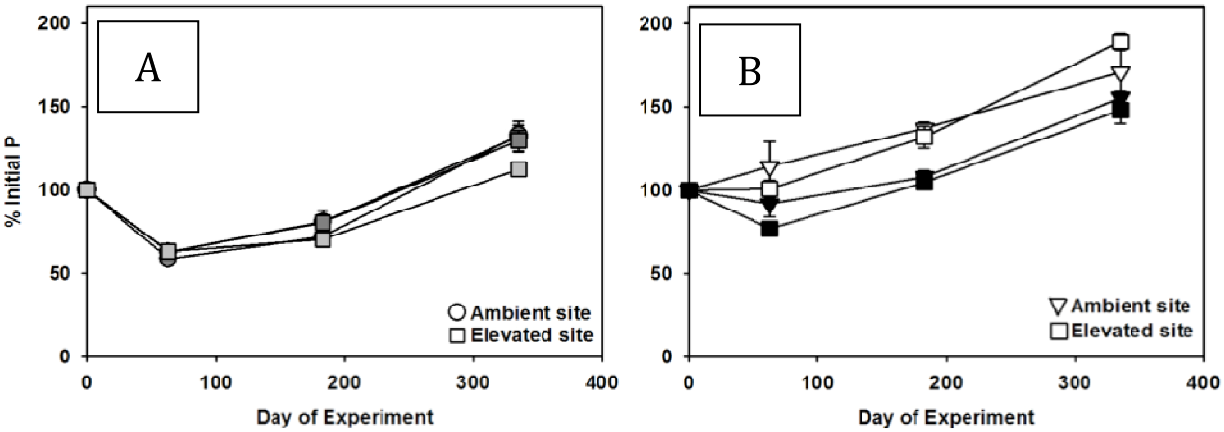

Figure 13. Phosphorus (P) dynamics in decomposing *Liquidambar styraciflua* litter. The P remaining in the litter at each removal is expressed as a percentage of the P originally contained in the litter collected from elevated $CO_2$ and ambient $CO_2$ treatments (A), or litter collected from unfertilized ambient N and fertilized elevated N treatments (B). Different colored symbols represent different litter origins: dark grey represents litter produced under elevated $CO_2$, light grey represents ambient $CO_2$ litter, white represents elevated N litter, and black represents ambient N litter. Different shapes of symbols represent where litter was placed for decomposition: square symbols in (A) indicate litter was placed in elevated $CO_2$ plots, circular symbols indicate that litter was placed in ambient $CO_2$ plots; square symbols in (B) indicate litter placed in fertilized elevated N plots, and triangular symbols indicate litter placed in unfertilized ambient N plots. Error bars represent one standard error of the mean.

Table 11. Explanatory factors in ANOVAs for mass loss, N dynamics (% initial N), and P dynamics (% initial P) at the N fertilization experiment (NFE, unfertilized ambient N and fertilized elevated N). An asterisk(*) next to a P-value indicates a statistically significant effect ($\alpha = 0.05$).

| | | Mass loss (% initial mass) | | N dynamics (% initial N) | | P dynamics (% initial P) | |
|---|---|---|---|---|---|---|---|
| Factor | df | F-ratio | p-value | F-ratio | p-value | F-ratio | p-value |
| Time | 2 | 119.7 | <0.0001* | 75.8 | <0.0001* | 80.6 | <0.0001* |
| Litter origin | 1 | 1.6 | 0.21 | 0.5 | 0.46 | 36.8 | <0.0001* |
| Location of Decomposition | 1 | <0.1 | 0.86 | 6.0 | 0.02* | 1.9 | 0.17 |
| Litter × Location | 1 | 0.1 | 0.79 | 1.4 | 0.24 | 1.2 | 0.29 |
| Litter × Time | 2 | <0.1 | 0.98 | 13.7 | <0.0001* | 0.6 | 0.54 |
| Location × Time | 2 | 0.3 | 0.76 | 7.4 | <0.01* | 2.1 | 0.13 |
| Litter × Location × Time | 2 | 0.8 | 0.46 | 0.3 | 0.72 | 0.7 | 0.49 |

### *Effects of site on decomposition rates and nutrient dynamics*

In addition to testing whether litter produced by plants under altered $CO_2$ or N availability would decompose at different rates (see above), we tested the hypothesis that $CO_2$ and N treatments would alter site-specific decomposition rates. The location at which litters were allowed to decompose had no main effects on mass loss, N dynamics, or P dynamics in the FACE experiment ($P >$ 0.10). Mass loss rates were statistically indistinguishable between the elevated $CO_2$ and ambient $CO_2$ plots (Table 10). There was a significant decomposition location × time interaction for N dynamics (P=0.02), with higher immobilization in litters placed in elevated $CO_2$ sites at the first removal date, no difference between sites in N immobilization at the second removal dates, and highest N immobilization in litters placed at the ambient $CO_2$ sites at the third removal date. As mentioned in the previous section, there was a significant litter origin × location for the elevated $CO_2$ and ambient $CO_2$ sites ($P = 0.03$) for P dynamics. Although there was no main effect of elevated CO2 treatment in creating mass, N, or P decomposition rates specific to each location, interactions indicate that there were site-specific effects contingent upon decomposition stage for litter N dynamics and initial litter quality for litter P dynamics.

In the N-fertilization experiment, only litter N dynamics were affected by where litter was placed for decomposition. The location where decomposition took place (N-fertilized versus unfertilized ambient N plots) had no significant main or interaction effects on mass loss or P dynamics ($P > 0.10$; Table 10). However, there was a significant effect of decomposition location on N dynamics

($P = 0.02$), with decomposing litter immobilizing more N in fertilized compared with unfertilized control plots (Figure 12). There was also a significant interaction between decomposition location and time ($P < 0.01$), as all litters (both elevated N and ambient N litters) placed in the ambient N plots immobilized more N at the first removal date than litters placed in the N-fertilized plots, while at the second and third removal dates, litter placed in the N-fertilized plots immobilized more N.

### *Home-field Advantage*

We found home-field advantages for litter produced and decomposed in the ambient $CO_2$, the unfertilized ambient N, and the N-fertilized treatments; and a home-field disadvantage for litter produced and decomposed in the elevated $CO_2$ treatment. However, an ANOVA model for mass loss that included all litters in all sites showed no significant litter origin × decomposition location effect ($P = 0.70$), and neither did a model that contained only mass loss data from the FACE experiment ($P = 0.52$; Table 10), or the model of mass loss data from the NFE ($P = 0.79$; Table 11). When we compared the decomposition rates of the different litter origins at all locations with re-sampling approaches, there was evidence of site specialization in three litter origins at their home sites: ambient $CO_2$ litter lost mass 17.5 ± 1.9% faster than would be predicted from the average decomposition rate of that litter placed at other decomposition locations or the average decomposition rate of litter from other origins placed for decomposition in the ambient $CO_2$ treatment. Litter produced and decomposed under the unfertilized ambient N treatment experienced 8.4 ± 3.8% faster mass loss, and litter produced and decomposed in the fertilized N treatment lost mass 5.3 ±

3.1% faster (Figure 14). However, litter produced and decomposed in the elevated $CO_2$ treatment experienced slower mass loss than would be predicted from its decomposition elsewhere or overall decomposition rates in the elevated $CO_2$ treatment, resulting in a home-field "disadvantage" of 20.9 ± 2.6% (Figure 14).

## Discussion

This study compared leaf litter produced in plots fumigated with elevated $CO_2$ or in plots fertilized with N with litter produced in associated ambient $CO_2$ or unfertilized ambient N plots as a model system to test the importance of intra-specific variation for HFA. We hypothesized that elevated plant-available C or N would lead to changes in litter quality, changes in decomposition process rates specific to each litter origin, and changes in process rates specific to each location, and that as a result there would be positive home-field advantages (HFA) for litter decomposing in its site of origin. As hypothesized, litter produced in the elevated $CO_2$ treatment was of reduced quality (lower %N, higher lignin:N) compared with the ambient $CO_2$ treatment, and litter produced in the N-fertilized treatment was of higher quality (lower % lignin, lower lignin:N) than litter produced in the unfertilized ambient N treatment. We predicted that elevated $CO_2$ or elevated N litter would decompose at different rates from litter produced in associated ambient treatments, which was supported by slower mass loss and by a litter by location interaction in P dynamics at the FACE experiment, and by a litter by time interaction in N dynamics at the NFE. Our hypothesis that different

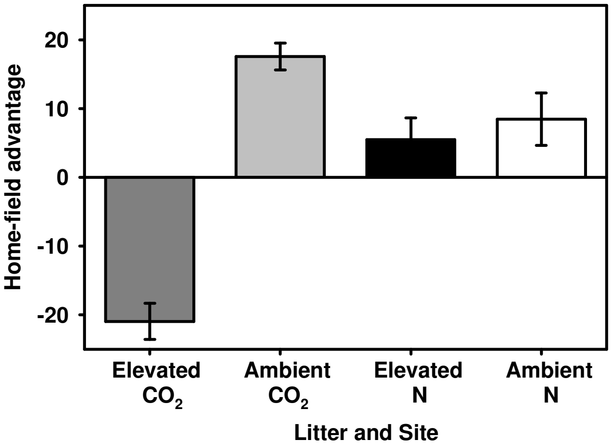

Figure 14**.** Home-field advantage (HFA) calculated for each litter origin. A positive HFA indicates that the litter decomposed more quickly in its location of origin than did other litters placed there or than does the litter placed in other locations. A negative HFA indicates that the litter decomposes more slowly in its location of origin than in foreign locations and also more slowly than do foreign litters placed in its location of origin. Error bars represent one standard deviation calculated by re-sampling (unable to calculate standard errors) of the distributions of HFAs.

locations (i.e., ambient and elevated [$CO_2$], or N-fertilized and unfertilized ambient N plots) would promote different decomposition rates was not supported by mass loss in either experiment. It was supported by the interactions of location with other factors for P dynamics and N dynamics in the FACE experiment, and by both main and interactive effects of location for N dynamics in the NFE. Alterations to litter and locations are expected to interact such that there would be positive HFA for each litter origin, and the results support the existence of a home-field effect for each of the litter origins. However, one of the litter types experienced a negative HFA, not positive as hypothesized: litter produced and decomposed in the elevated $CO_2$ treatment decomposed more slowly than would be predicted from average site or litter rates. This is despite the fact that while the elevated $CO_2$ treatment reduced the overall quality of the litter, resulting in slowed mass loss (but no change in N or P dynamics), there was no statistically significant effect of elevated $CO_2$ upon site mass loss rates. These results suggest that biological interactions (e.g. the HFA) may exist when standard statistical procedures fail to detect them. They also suggest that plant-soil feedbacks between litter quality, decomposition rates, and plant-available nutrients are sensitive to plant intraspecific variation, not just to differences between species as has been previously demonstrated.

### *The effects of elevated $CO_2$ on leaf litter decomposition*

We hypothesized that litter produced under elevated $CO_2$ would decompose more slowly than ambient $CO_2$ litter, and that elevated $CO_2$ sites would have slower decomposition rates than ambient $CO_2$ sites. Elevated atmospheric $CO_2$ caused shifts in the quality of leaf litter, which altered the overall rate of mass loss and, through interactions with location, the P dynamics of litter, but no changes in N during leaf litter decomposition. The initial quality of the litter (specifically, %N) that we collected in 2008 was higher than would be predicted from the declining trends in litter N concentration and the previous years' data (Norby et al. 2008). Leaching of soluble sugars in the time between leaf fall and litter collection might explain the high initial % N, although the sites received only 7 cm precipitation in two events between 27 September, when leaf fall began, and 11 November, the last date on which we collected litter (Riggs et al. 2010). Higher levels of % N may be due to the fact that our litter samples are ash-corrected and previously reported data were not, or that we used different analytical techniques. Neither explanation would completely account for the difference, as the change in litter [N] without ash-correction only explains 20% of the difference between litter from 2008 and 2009, and we used the same internal standards as the previous studies.

Leaf litter produced under elevated $CO_2$ decomposed more slowly than litter produced under ambient $CO_2$, as might be predicted from its lower initial N content and higher initial ratio of lignin:N. This result is in contrast to most litter decomposition studies conducted under elevated $CO_2$, which find that there is no difference in decomposition rates between litters grown under elevated or ambient $CO_2$ (Norby et al. 2001a; Finzi and Schlesinger 2002; but see Cotrufo et al. 2005). One possible explanation for this discrepancy is that we conducted our study after nearly a decade of $CO_2$ fumigation, during which time N limitation decreased the concentration of foliar N in the maturing trees under both ambient and elevated $CO_2$ conditions (Norby et al. 2010b). Responses to elevated plant resource availability such as elevated $CO_2$ may depend on duration of experimental conditions (Liu et al. 2009) or stand development in the forest studied (Finzi and Schlesinger 2003; Zak et al. 2000). Finzi and Schlesinger (2002) included *L. styraciflua* litter from trees in the understory in a decomposition study at Duke FACE, where the litter initially contained 19.3 and 21.0% lignin from ambient and elevated $CO_2$, respectively, whereas the litter we collected from the closed-canopy *L. styraciflua* contained about 38% lignin, about twice as much as the litter from Duke FACE. Leaf chemistry and soil N availability may both change as a result of progressive nitrogen limitation (the hypothesis that rising $CO_2$ exacerbates N limitation in plants, so that as $[CO_2]$ increases, plant response is more constrained by N demand), and it appears that at the ORNL FACE experiment, plant-available N (measured as $^{15}N$ fraction in leaf litter) has declined more quickly over time in the elevated $CO_2$ plots than in the ambient plots (Garten et al. 2011). It should be noted that Garten et al. (2011) detected the difference in leaf litter $^{15}N$ between ambient and elevated $CO_2$ treatments only as a trend over several years, and predicted that at current rates of change, it would take another ten years to detect a statistically significant difference at a single collection date between elevated and control litters. This is particularly interesting since along with the change in decomposition rate and the diminishing net primary productivity responses to the $CO_2$ treatment (Norby et al.

2010b), as soil N availability constrains growth of the trees, the resultant litter will be more recalcitrant, further limiting soil N pools.

While litter quality decreased by elevated $CO_2$ affected mass loss rates specific to litter origin, the effects of lower litter quality had not resulted in any detectable alterations to site-specific mass loss. Phosphorus and N dynamics generally indicate microbial activity and exploitation of litter mineral nutrients. There were significant effects of elevated $CO_2$ on nutrient dynamics, with a significant location by time effect on N dynamics and a significant location by litter type effect on P dynamics. Litter placed at the elevated $CO_2$ sites had immobilized less N at the third removal date than litter at the ambient sites, and only ambient $CO_2$ litter had immobilized less P at the third removal date than the litters at the the ambient sites. This is in contrast to the results of Cotrufo et al. (2005), who found that litter placed in elevated $CO_2$ sites decomposed faster and also immobilized more N than did litter placed in control sites at the POP FACE experiment in Italy. A study conducted at ORNL FACE found no differences between soil bacterial community sequences or soil functional enzymes between elevated $CO_2$ and ambient $CO_2$ sites (Austin et al. 2009), and a recent study on soil N mineralization throughout the soil profile also found no difference between elevated $CO_2$ and ambient plots (Iversen et al. 2011). At the AspenFACE experiment, elevated $CO_2$ produced similar results, with no differences between elevated $CO_2$ and ambient plots in mass loss during decomposition, or changes in fungal abundance or community composition (King et al. 2005; Chung et al. 2006; Liu et al. 2007). Given that it would take approximately 20 years of elevated $CO_2$ treatment before the difference between elevated $CO_2$ and ambient $CO_2$ plots in plant-available N in the soil would become large enough to produce significant differences in $^{15}N$ concentrations of litter within a single season (Garten et al. 2011), it appears that the interaction between litter and decomposition conditions at the elevated $CO_2$ site as revealed by the home-field disadvantage of the elevated $CO_2$ litter are more strongly driven by the quality of plant inputs. However, a recent study by Iversen et al. (2012) shows that microbial biomass N at the elevated $CO_2$ plots is significantly lower than in the ambient $CO_2$ plots, which indicates that the microbial community is nitrogen-limited. After more than a decade of elevated $CO_2$, the treatment has significantly altered litter quality compared to ambient conditions, and has only begun to affect the ecosystem by altering the function of dependent decomposer communities, as measured by litter N and P dynamics and associated properties of nutrient cycling.

### *Effects of N fertilization on leaf litter decomposition*

We hypothesized that elevation of N through soil fertilization would result in alterations to litter quality and to litter decomposition rates, as well as to site-specific decomposition rates. The patterns we found in the NFE were distinct from those in the FACE experiment. Despite significant differences in litter quality between the unfertilized control and the fertilized plots, leaf litter mass loss rates were not explained by differences in litter origin, decomposition location, or any interactive effects. However, main and interactive effects of litter origin and site type on N and P dynamics indicate that microbial decomposers are reacting to N fertilization. In contrast to our results, the effects of N fertilization on litter decomposition in other studies, as summarized in a meta-analysis by Knorr et al. (2005), were that N fertilization in amounts similar to that applied here inhibited mass loss by about 9%. Our mass loss results also contradict earlier data from the NFE which show that N fertilization decreased soil respiration by about 25% (Felker-Quinn unpublished data), although this may be because root respiration is responsible for a large fraction of soil respiration (and root biomass declined somewhat in the fertilized plots; Iversen and Norby 2008). This apparent discrepancy may be explained by studies that show that effect of N fertilization on soil communities may differ from its effect (or lack thereof) upon the communities that colonize the litter layer. While microbial communities in soil shift in response to N fertilization, with decreases in fungal:bacterial ratios (Feng et al. 2010), other studies have shown that basidiomycetes can differ significantly in community composition between the litter layer and the surface soil (Hofmockel et al. 2007), and this class of fungus is responsible for a large portion of leaf litter decomposition (Boberg et al. 2011). Given the significant interactions between litter origin, decomposition location, and time for N dynamics, a reasonable explanation of our results would be one which explains how microbial community metabolism of litter C remains unchanged while N allocation or enzyme production are affected by N fertilization. A decrease in substrate use efficiency by basidiomycetes in response to higher litter quality (Voriskova et al. 2011), along with suppression by elevated soil N availability of basidiomycete oxidative enzymes (DeForest et al. 2004) in the fertilized elevated

N treatment sites, may explain why overall mass loss does not vary by litter origin or decomposition location despite the altered N dynamics in the decomposing litter. Cloning and sequencing of rRNA has demonstrated that N fertilization can alter the composition of actinobacterial and fungal communities in litter, both important groups of decomposers (Zak et al. 2011). Litters produced and decomposed in the unfertilized ambient N or the N-fertilized treatments each experienced minor home-field advantages, which provides support for a scenario in which decomposer communities have altered their function, composition, or both, in order to specialize in decomposing home litter.

### *Home-field Advantage of litter decay*

We hypothesized that there would be positive HFA for each of the litters in its home treatment. Home-field advantages indicative of decomposer community specialization in home litter or optimized conditions at home locations were present in the ambient $CO_2$ treatment, the unfertilized ambient N treatment, and the fertilized elevated N treatment, but there was a significant home-field disadvantage for litter produced under elevated $CO_2$. The magnitude of home-field effects were comparable to the range of HFA (-9% to 29%) calculated in other studies (Ayres et al. 2009b, Jacob et al. 2010), which compared changes in the decomposition rates of different species in reciprocal designs. This indicates that variation in litter quality within a species may have effects of comparable magnitude to species effects on detritivore community specialization (see Madritch and Lindroth 2011), as lab work by Strickland indicates that HFA effects are due to microbial communities (Strickland et al. 2009a, b). The HFA we calculated were not supported by a significant interaction of site and litter origin in our statistical models of mass loss, as Ayres et al. (2009a) suggests is necessary to confirm significant HFA. This may be due simply to high variation in mass loss within specific litter origins at different sites (Fig. 1), which would affect statistical analyses more strongly than the re-sampling techniques that we used to calculate HFA. Gholz et al. (2000) reported a strong HFA for the hardwood species *Drypetes glauca* decomposed in broadleaf versus conifer forests, despite an insignificant statistical interaction of site and litter on mass loss. The authors point out that magnitude of differences in mass loss between litter types may vary by site while the overall pattern of which litter type has lost the most mass is the same across sites, which would result in statistical insignificance (Gholz et al. 2000), even though the relative differences between litter types in mass loss cannot be predicted for one site from another site in such a scenario.

The home-field disadvantage of litter produced and decomposed in the elevated $CO_2$ treatment contradicts the predictions of HFA studies, but responses to elevated $CO_2$ at the ecosystem level may provide some explanation for this result. HFA studies generally find strongest positive home field advantages for the most recalcitrant litter under study (Ayres et al. 2009a, Strickland et al. 2009a,b). However, the most recalcitrant litter in this study (litter produced under elevated $CO_2$), decomposed more slowly in its home treatment than other litters. One possible explanation is that litter quality in the elevated $CO_2$ treatment has continually declined over the course of the experiment due to increasing N limitation (Norby et al. 2010), which could mean that detritivore communities have shifted to those that specialize in breaking down litter of lower quality but do so at slower rates. It is also possible that detritivore communities remain unchanged in composition and function as litter inputs increase, the result being a slower decomposition rate per unit of litter mass even as the decomposition per unit area of litter layer remains the same (Finzi et al. 2006). Increased leaf litter as a result of increased productivity under elevated $CO_2$ may lead to dilution of detritivore activity, so that total ecosystem decomposition rates slow even as detritivore community structure and function remain unchanged.

### *Conclusions*

Our results support theories, which hold that ecosystem responses to shifts in plant resources such as C and N will be non-linear and will depend heavily upon current nutrient limitation of plant and microbial biomass. Moreover, our results support the hypothesis that within species variation, demonstrated in this study in response to climate change, can result in HFA, which extends current research suggesting that large species-level differences are required for HFA to occur. Changes in litter quality did not scale to changes in mass loss, as litter produced under elevated $CO_2$ (15% greater lignin:N ratio) lost mass more slowly than litter produced under ambient $CO_2$, but litter produced under N-fertilization (25% lower lignin:N ratio) lost mass at the same rate as litter produced under unfertilized ambient N. We also found that HFA exists in the ambient $CO_2$ and unfertilized ambient N treatments, and to a lesser extent in the fertilized elevated N treatment. However, litter produced and decomposed in the elevated $CO_2$ treatment experienced a reduction in mass loss to such an extent that it had a home-field disadvantage. These data demonstrate that intra-specific variation, due to climate change factors, can alter phenotypes that give rise to home-field advantage (and disadvantage), suggesting that species-level variation is not required for local adaptation but that variation at finer levels can also have this same result.

# CHAPTER 6: **Conclusions and future directions**

This dissertation has shown the occurrence and importance of evolutionary processes in invasive plants species, including *Ailanthus altissima*. Moreover, I found that evolutionary and ecosystem feedbacks can occur between plant intraspecific variation and soils. However, there are several avenues of future research that can extend the questions addressed and raised in the dissertation.

Chapter 2 suggests that evolution occurs with plant invasion, but that herbivores do not play a primary role in the evolution of invasive plants. Multi-factor experiments that include molecular evaluation of range and population genetic structure would address the relative importance of stochastic and selective forces in invasive evolution, and would be useful in identifying selective factors. Greenhouse studies, over multiple generations of an annual invasive, manipulating plant resource availability through soil amendments, herbivore presence/absence, and temperature, could help to identify the relative importance, as well as interactions between, selective agents. Sequencing of neutral molecular markers could identify genetic isolation or founder's events that constrain local evolution of different populations, and comparison of genotypic variation ($F_{ST}$) and quantitative phenotype variation ($Q_{ST}$) would allow evaluation of how stochastic and selective forces shape the evolution of invasive plants.

In the case of *Ailanthus altissima,* there are a number of fundamental questions that were not directly addressed in this research. Chapter 3 shows that certain traits important to invasiveness vary among populations when plants are grown in a common garden. However, it would be important to evaluate whether these genetic differences are expressed similarly when the populations are embedded in their source environments, or whether there are genetic by environment interactions that result in similar relative performance of *Ailanthus altissima* across the landscape. Results from the first scenario would indicate that certain populations are more aggressive invaders, and should be targeted in management schemes. The second scenario would suggest that invasive *A. altissima* is capable of locally adapting to restrictive environmental conditions, as do some native plants. This research could be conducted by planting common gardens of reciprocal transplants across the range of *A. altissima*, although the persistence of *A. altissima* individuals, despite eradication efforts, necessitates care in garden design and maintenance.

The specialist herbivore *Atteva aurea* caused significant damage, both within the common garden and on seedlings in the source populations of *A. altissima*. Although Chapter 1 indicates that this interaction is unlikely to be important in shaping the evolution of invasive *A. altissima*, it does represent the unusual case of a native insect taking advantage of a plant invasion to widely expand its range. In the course of writing Chapter 3, I encountered a paucity of information on *Atteva aurea*. The natural history of this species, which as an adult may act as a pollinator of native plant species, bears further investigation. *Atteva* may serve as a model species to researchers interested in insect range expansion under climate change scenarios, as the introduction of *A. altissima* has allowed the moth to expand its range from Texas, the northernmost limit of its previous host plant, into the northern United States.

Chapter 4 shows that plant-soil feedbacks, mediated by soil biota promote the performance and expression of genetic variation in the offspring of certain *A. altissima* populations. However, I did not experimentally show that this feedback would act as a selective force over successive generations of *A. altissima*. The minimum generation time of *A. altissima* is twelve years, so further research on the importance of positive plant-soil feedbacks in promoting invasion should be conducted using a more appropriate invasive species, preferably an annual. Positive plant-soil feedbacks have been identified at the species-level for a number of invasive species (see Klironomos et al. 2002), but population-specific feedbacks represent an exciting application of plant-soil feedbacks as a mechanism for evolutionary selection, not community composition.

Chapter 5 shows that intraspecific plant variation, due to climate change factors, can create feedbacks between litter quality and location that promote or slow decomposition of specific litters. While other studies have identified the soil microbial community as responsible for this effect, I did not identify the mechanism responsible for home-field advantage (HFA) at the FACE and NFE. The mechanism for HFA could be tested by pairing field decomposition studies with lab incubations in which litters are sterilized or inoculated with microbial samples. Although the FACE experiment has been terminated, it would be interesting to repeat the experiment and consider both the effects of plant genotype and plant resource availability in promoting HFA. An experiment using litter from taxonomically paired invasive and native species would also allow testing of the mechanisms for the ecosystem-level impacts of plant invasions.

This dissertation has suggested exciting new directions for research into the causes and evolutionary impacts of invasive plant species. I have shown that evolution occurs in defense and performance traits across invasive plants. I found evidence of genetic differentiation in invasive populations of *Ailanthus altissima* consistent with local adaptation, and evidence that populations can promote the establishment of their offspring. My experiment with *Liquidambar stryaciflua* showed that intraspecific plant variation caused by resource availability can promote ecosystem feedbacks. Further work in the field of the evolution of invasive plants should focus on identifying selective forces that promote traits associated with plant invasion, and exploring interactions between soils and plant genetics in promoting intraspecific variation and selection in invasive and native plants.

# REFERENCES

Abhilasha, D. and J. Joshi. 2009. Enhanced fitness due to higher fecundity, increased defence against a specialist and tolerance towards a generalist herbivore in an invasive annual plant. Journal of Plant Ecology-Uk 2:77-86.

Agrawal, A. A. 2011. Current trends in the evolutionary ecology of plant defence. Functional Ecology 25:420-432.

Agrawal, A. A., P. M. Kotanen, C. E. Mitchell, A. G. Power, W. Godsoe, and J. Klironomos. 2005. Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. Ecology 86:2979-2989.

Alvarez, N., C. Thiel-Egenter, A. Tribsch, R. Holderegger, S. Manel, P. Schoenswetter, P. Taberlet, S. Brodbeck, M. Gaudeul, L. Gielly, P. Kuepfer, G. Mansion, R. Negrini, O. Paun, M. Pellecchia, D. Rioux, F. Schuepfer, M. Van Loo, M. Winkler, F. Gugerli, and C. IntraBioDiv. 2009. History or ecology? Substrate type as a major driver of partial genetic structure in Alpine plants. Ecology Letters 12:632-640.

Austin, E. E., H. F. Castro, K. E. Sides, C. W. Schadt, and A. T. Classen. 2009. Assessment of 10 years of $CO_2$ fumigation on soil microbial communities and function in a sweetgum plantation. Soil Biology & Biochemistry 41:514-520.

Ayres, E., H. Steltzer, S. Berg, and D. H. Wall. 2009. Soil biota accelerate decomposition in high-elevation forests by specializing in the breakdown of litter produced by the plant species above them. Journal of ecology 97:901-912.

Ayres, E., H. Steltzer, B. L. Simmons, R. T. Simpson, J. M. Steinweg, M. D. Wallenstein, N. Mellor, W. J. Parton, J. C. Moore, and D. H. Wall. 2009. Home-field advantage accelerates leaf litter decomposition in forests. Soil Biology & Biochemistry 41:606-610.

Bangert, R. K., R. J. Turek, B. Rehill, G. M. Wimp, J. A. Schweitzer, G. J. Allan, J. K. Bailey, G. D. Martinsen, P. Keim, R. L. Lindroth, and T. G. Whitham. 2006. A genetic similarity rule determines arthropod community structure. Molecular Ecology 15:1379-1391.

Barto, E. K. and M. C. Rillig. 2012. Dissemination biases in ecology: effect sizes matter more than quality. Oikos 121:228-235.

Batten, K. M., K. M. Scow, and E. K. Espeland. 2008. Soil microbial community associated with an invasive grass differentially impacts native plant performance. Microbial Ecology 55:220-228.

Becker, V. O. 2009. A review of the New World *Atteva* Walker moths (Yponomeutidae,Attevinae). Revista Brasileira De Entomologia 53:349-355.

Berglund, A. B. N., S. Dahlgren, and A. Westerbergh. 2004. Evidence for parallel evolution and site-specific selection of serpentine tolerance in *Cerastium alpinum* during the colonization of Scandinavia. New Phytologist 161:199-209.

Berglund, A. B. N., A. Saura, and A. Westerbergh. 2001. Genetic differentiation of a polyploid plant on ultramafic soils in Fennoscandia. South African Journal of Science 97:533-535.

Bever, J. D. 1994. Feedback between plants and their soil communities in an old-field community. Ecology 75:1965-1977.

Bever, J. D. 2003. Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. New Phytologist 157:465-473.

Blair, A. C. and L. M. Wolfe. 2004. The evolution of an invasive plant: An experimental study with *Silene latifolia*. Ecology 85:3035-3042.

Blossey, B. and R. Notzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. Journal of Ecology 83:3.

Blumenthal, D. M. and R. A. Hufbauer. 2007. Increased plant size in exotic populations: A common-garden test with 14 invasive species. Ecology 88:2758-2765.

Boberg, J. B., T. Näsholm, R. D. Finlay, J. Stenlid, and B. D. Lindahl. 2011. Nitrogen availability affects saprotrophic basidiomycetes decomposing pine needles in a long term laboratory study. Fungal Ecology 4:408-416.

Bossdorf, O., H. Auge, L. Lafuma, W. E. Rogers, E. Siemann, and D. Prati. 2005. Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144:1-11.

Bossdorf, O., H. Auge, L. Lafuma, W. E. Rogers, E. Siemann, and D. Prati. 2005. Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144:1-11.

Bossdorf, O., A. Lipowsky, and D. Prati. 2008. Selection of preadapted populations allowed *Senecio inaequidens* to invade Central Europe. Diversity and Distributions 14:676-685.

Bossdorf, O., D. Prati, H. Auge, and B. Schmid. 2004. Reduced competitive ability in an invasive plant. Ecology Letters 7:346-353.

Brady, K. U., A. R. Kruckeberg, and H. D. Bradshaw. 2005. Evolutionary ecology of plant adaptation to serpentine soils. Pages 243-266 Annual Review of Ecology Evolution and Systematics.

Buschmann, H., P. J. Edwards, and H. Dietz. 2005. Variation in growth pattern and response to slug damage among native and invasive provenances of four perennial Brassicaceae species. Journal of Ecology 93:322-334.

Callaway, R. M., G. C. Thelen, A. Rodriguez, and W. E. Holben. 2004. Soil biota and exotic plant invasion. Nature 427:731-733.

Carmona, D., M. J. Lajeunesse, and M. T. J. Johnson. 2011. Plant traits that predict resistance to herbivores. Functional Ecology 25:358-367.

Carreiro, M. M., R. L. Sinsabaugh, D. A. Repert, and D. F. Parkhurst. 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81:2359-2365.

Catford, J. A., R. Jansson, and C. Nilsson. 2009. Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Diversity and Distributions 15:22-40.

Chapin, F. S., P.A. Matson, and H.A. Mooney. 2002. Principles of Terrestrial Ecosystem Ecology. Springer.

Chung, H. G., D. R. Zak, and E. A. Lilleskov. 2006. Fungal community composition and metabolism under elevated $CO_2$ and $O^-_3$. Oecologia 147:143-154.

Cipollini, D., J. Mbagwu, K. Barto, C. Hillstrom, and S. Enright. 2005. Expression of constitutive and inducible chemical defenses in native and invasive populations of *Alliaria petiolata*. Journal of Chemical Ecology 31:1255-1267.

Clements, F. E. 1916. An Analysis of the Development of Vegetation. Carnegie Institution, Washington.

Colautti, R. I. and S. C. H. Barrett. 2010. Natural selection and genetic constraints on flowering phenology in an invasive plant. International Journal of Plant Sciences 171:960-971.

Colautti, R. I., C. G. Eckert, and S. C. H. Barrett. 2010. Evolutionary constraints on adaptive evolution during range expansion in an invasive plant. Proceedings of the Royal Society B-Biological Sciences 277:1799-1806.

Colautti, R. I., J. L. Maron, and S. C. H. Barrett. 2009. Common garden comparisons of native and introduced plant populations: latitudinal clines can obscure evolutionary inferences. Evolutionary Applications 2:187-199.

Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. Science 230:895-899.

Conner, J. K. and D. L. Hartl. 2004. A primer of ecological genetics. Sinauer Associates, Sunderland, Mass.

Cornwell, W. K., J. H. C. Cornelissen, K. Amatangelo, E. Dorrepaal, V. T. Eviner, O. Godoy, S. E. Hobbie, B. Hoorens, H. Kurokawa, N. Perez-Harguindeguy, H. M. Quested, L. S. Santiago, D. A. Wardle, I. J. Wright, R. Aerts, S. D. Allison, P. van Bodegom, V. Brovkin, A. Chatain, T. V. Callaghan, S. Diaz, E. Garnier, D. E. Gurvich, E. Kazakou, J. A. Klein, J. Read, P. B. Reich, N. A. Soudzilovskaia, M. V. Vaieretti, and M. Westoby. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters 11:1065-1071.

Cote, L., S. Brown, D. Pare, J. Fyles, and J. Bauhus. 2000. Dynamics of carbon acid nitrogen mineralization in relation to stand type, stand age and soil texture in the boreal mixedwood. Soil Biology & Biochemistry 32:1079-1090.

Cotrufo, M. F., P. De Angelis, and A. Polle. 2005. Leaf litter production and decomposition in a poplar short-rotation coppice exposed to free air CO2 enrichment (POPFACE). Global Change Biology 11:971-982.

Cotrufo, M. F., P. Ineson, and A. P. Rowland. 1994. Decomposition of tree leaf litters grown under elevated $CO_2$—Effect of litter quality. Plant and Soil 163:121-130.

Cripps, M. G., H. L. Hinz, J. L. McKenney, W. J. Price, and M. Schwarzlander. 2009. No evidence for an 'evolution of increased competitive ability' for the invasive *Lepidium draba*. Basic and Applied Ecology 10:103-112.

Cunningham, S. A., B. Summerhayes, and M. Westoby. 1999. Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. Ecological Monographs 69:569-588.

Dassonville, N., S. Vanderhoeven, V. Vanparys, M. Hayez, W. Gruber, and P. Meerts. 2008. Impacts of alien invasive plants on soil nutrients are correlated with initial site conditions in NW Europe. Oecologia 157:131-140.

Davis, H. G., C. M. Taylor, J. G. Lambrinos, and D. R. Strong. 2004. Pollen limitation causes an Allee effect in a wind-pollinated invasive grass (*Spartina* *alterniflora*). Proceedings of the National Academy of Sciences of the United States of America 101:13804-13807.

Davis, M. A., J. P. Grime, and K. Thompson. 2000. Fluctuating resources in plant communities: a general theory of invasibility. Journal of ecology 88:528-534.

DeForest, J. L., D. R. Zak, K. S. Pregitzer, and A. J. Burton. 2004. Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests. Soil Science Society of America Journal 68:132-138.

Dickson, R. E., M. D. Coleman, P. Pechter, and D. Karnosky. 2001. Growth and crown architecture of two aspen genotypes exposed to interacting ozone and carbon dioxide. Environmental Pollution 115:319-334.

Ehrenfeld, J. G. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6:503-523.

Ehrenfeld, J. G., P. Kourtev, and W. Z. Huang. 2001. Changes in soil functions following invasions of exotic understory plants in deciduous forests. Ecological Applications 11:1287-1300.

Eigenbrode, S. D., J. E. Andreas, M. G. Cripps, H. Ding, R. C. Biggam, and M. Schwarzlander. 2008. Induced chemical defenses in invasive plants: a case study with *Cynoglossum officinale* L. Biological Invasions 10:1373-1379.

Endler, J. A. 1977. Geographic variation, speciation, and clines. Princeton University Press, Princeton, N.J.

Felker-Quinn, E., J. K. Bailey, and J. A. Schweitzer. 2011. Soil biota drive expression of genetic variation and development of population-specific feedbacks in an invasive plant. Ecology 92:1208-1214.

Feng, X. J., A. J. Simpson, W. H. Schlesinger, and M. J. Simpson. 2010. Altered microbial community structure and organic matter composition under elevated $CO_2$ and N fertilization in the duke forest. Global Change Biology 16:2104-2116.

Feret, P. P. and R. L. Bryant. 1974. Genetic differences between American and Chinese *Ailanthus* seedlings. Silvae genetica 23:5.

Fierer, N. and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America 103:626-631.

Finzi, A. C., D. J. P. Moore, E. H. DeLucia, J. Lichter, K. S. Hofmockel, R. B. Jackson, H. S. Kim, R. Matamala, H. R. McCarthy, R. Oren, J. S. Pippen, and W.

H. Schlesinger. 2006. Progressive nitrogen limitation of ecosystem processes under elevated $CO_2$ in a warm-temperate forest. Ecology 87:15-25.

Finzi, A. C. and A. H. Schlesinger. 2002. Species control variation in litter decomposition in a pine forest exposed to elevated $CO_2$. Global Change Biology 8:1217-1229.

Finzi, A. C. and W. H. Schlesinger. 2003. Soil-nitrogen cycling in a pine forest exposed to 5 years of elevated carbon dioxide. Ecosystems 6:444-456.

Franks, S. J., P. D. Pratt, F. A. Dray, and E. L. Simms. 2008. No evolution of increased competitive ability or decreased allocation to defense in *Melaleuca quinquenervia* since release from natural enemies. Biological Invasions 10:455-466.

Frey, S. D., R. Drijber, H. Smith, and J. Melillo. 2008. Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biology & Biochemistry 40:2904-2907.

Garibaldi, L. A., T. Kitzberger, and A. Ruggiero. 2011. Latitudinal decrease in folivory within *Nothofagus pumilio* forests: dual effect of climate on insect density and leaf traits? Global Ecology and Biogeography 20:609-619.

Garten, C. T., C. M. Iversen, and R. J. Norby. 2011. Litterfall $^{15}N$ abundance indicates declining soil nitrogen availability in a free-air $CO_2$ enrichment experiment. Ecology 92:133-139.

Gattinger, A. 1887. The Tennessee flora; with special reference to the flora of Nashville. The author, Nashville, Tenn.

Gavier-Pizarro, G. I., V. C. Radeloff, S. I. Stewart, C. D. Huebner, and N. S. Keuler. 2010. Housing is positively associated with invasive exotic plant species richness in New England, USA. Ecological Applications 20:1913-1925.

Gee, G. W. B., J.M. 1986. Particle-size analysis. Pages 383–411 Methods of soil analysis. American Society of Agronomy, Madison, Wisconsin, USA.

Gholz, H. L., D. A. Wedin, S. M. Smitherman, M. E. Harmon, and W. J. Parton. 2000. Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. Global Change Biology 6:751-765.

Gleason, H. A. 1926. The Individualistic Concept of the Plant Association. Bulletin of the Torrey Botanical Club 53:7-26.

Gomez-Aparicio, L. and C. D. Canham. 2008. Neighborhood models of the effects of invasive tree species on ecosystem processes. Ecological Monographs 78:69-86.

Gomez-Aparicio, L. and C. D. Canham. 2008. Neighbourhood analyses of the allelopathic effects of the invasive tree *Ailanthus altissima* in temperate forests. Journal of ecology 96:447-458.

Grandy, A., R. Sinsabaugh, J. Neff, M. Stursova, and D. Zak. 2008. Nitrogen deposition effects on soil organic matter chemistry are linked to variation in enzymes, ecosystems and size fractions. Biogeochemistry 91:37-49.

Grotkopp, E., M. Rejmanek, and T. L. Rost. 2002. Toward a causal explanation of plant invasiveness: Seedling growth and life-history strategies of 29 pine (*Pinus*) species. American Naturalist 159:396-419.

Guesewell, S., G. Jakobs, and E. Weber. 2006. Native and introduced populations of *Solidago gigantea* differ in shoot production but not in leaf traits or litter decomposition. Functional Ecology 20:575-584.

Hall, M. C., P. Stiling, D. C. Moon, B. G. Drake, and M. D. Hunter. 2006. Elevated $CO_2$ increases the long-term decomposition rate of *Quercus myrtifolia* leaf litter. Global Change Biology 12:568-577.

Handley, R. J., T. Steinger, U. A. Treier, and H. Muller-Scharer. 2008. Testing the evolution of increased competitive ability (EICA) hypothesis in a novel framework. Ecology 89:407-417.

Hattenschwiler, S. and P. M. Vitousek. 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends in Ecology & Evolution 15:238-243.

He, W. M., Y. L. Feng, W. M. Ridenour, G. C. Thelen, J. L. Pollock, A. Diaconu, and R. M. Callaway. 2009. Novel weapons and invasion: biogeographic differences in the competitive effects of *Centaurea maculosa* and its root exudate (+/-)-catechin. Oecologia 159:803-815.

Heisey, R. M. and T. K. Heisey. 2003. Herbicidal effects under field conditions of *Ailanthus altissima* bark extract, which contains ailanthone. Plant and Soil 256:85-99.

Hendershot, W. H., H. Lalande, and M. Duquette. 1993. Soil reaction and exchangeable acidity. Pages 141–145 in M. R. Carter, editor. Soil sampling and methods of analysis. Lewis Publishers, Boca Raton, Florida, USA.

Hinz, H. L. and M. Schwarzlaender. 2004. Comparing invasive plants from their native and exotic range: What can we learn for biological Control? Weed Technology 18:1533-1541.

Hobbie, S. E. 2008. Nitrogen effects on decomposition: A five-year experiment in eight temperate sites. Ecology 89:2633-2644.

Hofmockel, K. S., D. R. Zak, and C. B. Blackwood. 2007. Does atmospheric $NO_3^-$ deposition alter the abundance and activity of ligninolytic fungi in forest soils? Ecosystems 10:1278-1286.

Hood, G. M. 2010. PopTools. http://www.poptools.org/

Hoorens, B., R. Aerts, and M. Stroetenga. 2003. Is there a trade-off between the plant's growth response to elevated $CO_2$ and subsequent litter decomposability? Oikos 103:17-30.

Huang, W., E. Siemann, G. S. Wheeler, J. W. Zou, J. Carrillo, and J. Q. Ding. 2010. Resource allocation to defence and growth are driven by different responses to generalist and specialist herbivory in an invasive plant. Journal of Ecology 98:1157-1167.

Hull-Sanders, H. M., R. Clare, R. H. Johnson, and G. A. Meyer. 2007. Evaluation of the evolution of increased competitive ability (EICA) hypothesis: Loss of defense against generalist but not specialist herbivores. Journal of Chemical Ecology 33:781-799.

Hunt, H. W., E. R. Ingham, D. C. Coleman, E. T. Elliott, and C. P. P. Reid. 1988. Nitrogen limitation of production and decomposition in prairie, mountain meadow, and pine forest. Ecology 69:1009-1016.

Hutchinson, G. E. 1957. Population studies—animal ecology and demography—concluding remarks. Cold Spring Harbor Symposia on Quantitative Biology 22:415-427.

Inderjit, J. L. Pollock, T. R. Seastedt, R. M. Callaway, and J. Kaur. 2008. Allelopathy and plant invasions: traditional, congeneric, and bio-geographical approaches. Biological Invasions 10:875-890.

Iversen, C. M., T. D. Hooker, A. T. Classen, and R. J. Norby. 2011. Net mineralization of N at deeper soil depths as a potential mechanism for sustained forest production under elevated $CO_2$. Global Change Biology 17:1130-1139.

Iversen, C. M., J. K. Keller, C. T. Garten, and R. J. Norby. 2012. Soil carbon and nitrogen cycling and storage throughout the soil profile in a sweetgum plantation after 11 years of $CO_2$ enrichment. Global Change Biology 18:1684-1697.

Iversen, C. M., J. Ledford, and R. J. Norby. 2008. $CO_2$ enrichment increases carbon and nitrogen input from fine roots in a deciduous forest. New Phytologist 179:837-847.

Iversen, C. M. and R. J. Norby. 2008. Nitrogen limitation in a sweetgum plantation: implications for carbon allocation and storage. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 38:1021-1032.

Jacob, M., K. Viedenz, A. Polle, and F. M. Thomas. 2010. Leaf litter decomposition in temperate deciduous forest stands with a decreasing fraction of beech (*Fagus sylvatica*). Oecologia 164:1083-1094.

Janssens, I. A., W. Dieleman, S. Luyssaert, J. A. Subke, M. Reichstein, R. Ceulemans, P. Ciais, A. J. Dolman, J. Grace, G. Matteucci, D. Papale, S. L. Piao, E. D. Schulze, J. Tang, and B. E. Law. 2010. Reduction of forest soil respiration in response to nitrogen deposition. Nature Geoscience 3:315-322.

Johnson, N. C., G. W. T. Wilson, M. A. Bowker, J. A. Wilson, and R. M. Miller. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proceedings of the National Academy of Sciences of the United States of America 107:2093-2098.

Johnson, R. H., H. M. Hull-Sanders, and G. A. Meyer. 2007. Comparison of foliar terpenes between native and invasive *Solidago gigantea*. Biochemical Systematics and Ecology 35:821-830.

Jongejans, E., K. Shea, O. Skarpaas, D. Kelly, A. W. Sheppard, and T. L. Woodburn. 2008. Dispersal and demography contributions to population spread of *Carduus nutans* in its native and invaded ranges. Journal of ecology 96:687-697.

Joshi, J. and K. Vrieling. 2005. The enemy release and EICA hypothesis revisited: incorporating the fundamental difference between specialist and generalist herbivores. Ecology Letters 8:704-714.

Kanerva, T., A. Palojarvi, K. Ramo, and S. Manninen. 2008. Changes in soil microbial community structure under elevated tropospheric $O^-_3$ and $CO_2$. Soil Biology & Biochemistry 40:2502-2510.

Kawakami, T., T. J. Morgan, J. B. Nippert, T. W. Ocheltree, R. Keith, P. Dhakal, and M. C. Ungerer. Natural selection drives clinal life history patterns in the perennial sunflower species, *Helianthus maximiliani.* Molecular Ecology 20:2318-2328.

Keane, R. M. and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17:164-170.

Keller, S. R. and D. R. Taylor. 2008. History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. Ecology Letters 11:852-866.

King, J. S., K. S. Pregitzer, D. R. Zak, W. E. Holmes, and K. Schmidt. 2005. Fine root chemistry and decomposition in model communities of north-temperate tree species show little response to elevated atmospheric $CO_2$ and varying soil resource availability. Oecologia 146:318-328.

Klironomos, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67-70.

Knops, J. M. H., S. Naeemw, and P. B. Reich. 2007. The impact of elevated $CO_2$, increased nitrogen availability and biodiversity on plant tissue quality and decomposition. Global Change Biology 13:1960-1971.

Knorr, M., S. D. Frey, and P. S. Curtis. 2005. Nitrogen additions and litter decomposition: A meta-analysis. Ecology 86:3252-3257.

Koricheva, J., H. Nykanen, and E. Gianoli. 2004. Meta-analysis of trade-offs among plant antiherbivore defenses: Are plants jacks-of-all-trades, masters of all? American Naturalist 163:E64-E75.

Kowarik, I. and I. Saumel. 2007. Biological flora of Central Europe: *Ailanthus altissima* (Mill.) Swingle. Perspectives in Plant Ecology Evolution and Systematics 8:207-237.

Kulmatiski, A., K. H. Beard, J. R. Stevens, and S. M. Cobbold. 2008. Plant-soil feedbacks: a meta-analytical review. Ecology Letters 11:980-992.

Lajeunesse, M. J. 2010. Achieving synthesis with meta-analysis by combining and comparing all available studies. Ecology 91:2561-2564.

Lankau, R. A. 2007. Specialist and generalist herbivores exert opposing selection on a chemical defense. New Phytologist 175:176-184.

Lee, C. E. 2002. Evolutionary genetics of invasive species. Trends in Ecology & Evolution 17:386-391.

Levine, J. M., P. B. Adler, and S. G. Yelenik. 2004. A meta-analysis of biotic resistance to exotic plant invasions. Ecology Letters 7:975-989.

Linhart, Y. B. and M. C. Grant. 1996. Evolutionary significance of local genetic differentiation in plants. Annual Review of Ecology and Systematics 27:237-277.

Liu, L. L. and T. L. Greaver. 2010. A global perspective on belowground carbon dynamics under nitrogen enrichment. Ecology Letters 13:819-828.

Liu, L. L., J. S. King, F. L. Booker, C. P. Giardina, H. L. Allen, and S. J. Hu. 2009. Enhanced litter input rather than changes in litter chemistry drive soil carbon and nitrogen cycles under elevated $CO_2$: a microcosm study. Global Change Biology 15:441-453.

Liu, L. L., J. S. King, and C. P. Giardina. 2007. Effects of elevated atmospheric $CO_2$ and tropospheric $O^-_3$ on nutrient dynamics: decomposition of leaf litter in trembling aspen and paper birch communities. Plant and Soil 299:65-82.

Lumley, T. 2009. Leaps: regression subset selection. http://cran.r-project.org/web/packages/leaps/index.html.

Macel, M., C. S. Lawson, S. R. Mortimer, M. Smilauerova, A. Bischoff, L. Cremieux, J. Dolezal, A. R. Edwards, V. Lanta, T. M. Bezemer, W. H. van der Putten, J. M. Igual, C. Rodriguez-Barrueco, H. Mueller-Schaerer, and T. Steinger. 2007. Climate vs. soil factors in local adaptation of two common plant species. Ecology 88:424-433.

Madritch, M. D. and B. J. Cardinale. 2007. Impacts of tree species diversity on litter decomposition in northern temperate forests of Wisconsin, USA: a multi-site experiment along a latitudinal gradient. Plant and Soil 292:147-159.

Madritch, M. D. and R. L. Lindroth. 2011. Soil microbial communities adapt to genetic variation in leaf litter inputs. Oikos 120:1696-1704.

Manel, S., M. K. Schwartz, G. Luikart, and P. Taberlet. 2003. Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology & Evolution 18:189-197.

Marchetto, K. M., E. Jongejans, K. Shea, and S. A. Isard. 2010. Plant spatial arrangement affects projected invasion speeds of two invasive thistles. Oikos 119:1462-1468.

Maron, J. L., S. C. Elmendorf, and M. Vila. 2007. Contrasting plant physiological adaptation to climate in the native and introduced range of *Hypericum perforatum*. Evolution 61:1912-1924.

Maron, J. L., M. Vila, and J. Arnason. 2004. Loss of enemy resistance among introduced populations of St. John's Wort (*Hypericum perforatum*). Ecology 85:3243-3253.

Maron, J. L., M. Vila, R. Bommarco, S. Elmendorf, and P. Beardsley. 2004. Rapid evolution of an invasive plant. Ecological Monographs 74:261-280.

McCarthy, H. R., R. Oren, K. H. Johnsen, A. Gallet-Budynek, S. G. Pritchard, C. W. Cook, S. L. LaDeau, R. B. Jackson, and A. C. Finzi. 2010. Re-assessment of plant carbon dynamics at the Duke free-air $CO_2$ enrichment site: interactions of atmospheric $CO_2$ with nitrogen and water availability over stand development. New Phytologist 185:514-528.

McDowell, S. C. L. 2002. Photosynthetic characteristics of invasive and noninvasive species of *Rubus* (Rosaceae). American Journal of Botany 89:1431-1438.

McKenney, J. L., M. G. Cripps, W. J. Price, H. L. Hinz, and M. Schwarzlander. 2007. No difference in competitive ability between invasive North American and native European *Lepidium draba* populations. Plant Ecology 193:293-303.

Meyer, G., R. Clare, and E. Weber. 2005. An experimental test of the evolution of increased competitive ability hypothesis in goldenrod, *Solidago gigantea*. Oecologia 144:299-307.

Montague, J. L., S. C. H. Barrett, and C. G. Eckert. 2008. Re-establishment of clinal variation in flowering time among introduced populations of purple loosestrife (*Lythrum salicaria*, Lythraceae). Journal of Evolutionary Biology 21:234-245.

Muller, C. and N. Martens. 2005. Testing predictions of the 'evolution of increased competitive ability' hypothesis for an invasive crucifer. Evolutionary Ecology 19:533-550.

Muller-Scharer, H., U. Schaffner, and T. Steinger. 2004. Evolution in invasive plants: implications for biological control. Trends in Ecology & Evolution 19:417-422.

Murren, C. J., L. Douglass, A. Gibson, and M. R. Dudash. 2006. Individual and combined effects of Ca/Mg ratio and water on trait expression in *Mimulus guttatus*. Ecology 87:2591-2602.

Norby, R. J., Iversen C.M., Tharp M.L. 2008. ORNL FACE Nitrogen Concentrations: Leaves, Litter, Fine Roots, Wood. Carbon Dioxide Information Analysis Center. http://cdiac.ornl.gov/

Norby, R. J., Iversen C.M., Childs J., Tharp M.L. 2010. ORNL Net Primary Productivity Data. Carbon Dioxide Information Analysis Center. http://cdiac.ornl.gov/

Norby, R. J., M. F. Cotrufo, P. Ineson, E. G. O'Neill, and J. G. Canadell. 2001. Elevated $CO_2$, litter chemistry, and decomposition: a synthesis. Oecologia 127:153-165.

Norby, R. J. and C. M. Iversen. 2006. Nitrogen uptake, distribution, turnover, and efficiency of use in a $CO_2$-enriched sweetgum forest. Ecology 87:5-14.

Norby, R. J., J. D. Sholtis, C. A. Gunderson, and S. S. Jawdy. 2003. Leaf dynamics of a deciduous forest canopy: no response to elevated $CO_2$. Oecologia 136:574-584.

Norby, R. J., D. E. Todd, J. Fults, and D. W. Johnson. 2001. Allometric determination of tree growth in a $CO_2$-enriched sweetgum stand. New Phytologist 150:477-487.

Norby, R. J., J. M. Warren, C. M. Iversen, B. E. Medlyn, and R. E. McMurtrie. 2010. $CO_2$ enhancement of forest productivity constrained by limited nitrogen availability. Proceedings of the National Academy of Sciences of the United States of America 107:19368-19373.

Nunez, M. A., T. R. Horton, and D. Simberloff. 2009. Lack of belowground mutualisms hinders Pinaceae invasions. Ecology 90:2352-2359.

Nunez, M. A. and K. A. Medley. 2011. Pine invasions: climate predicts invasion success; something else predicts failure. Diversity and Distributions 17:703-713.

Ohsawa, T. and Y. Ide. 2008. Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. Global Ecology and Biogeography 17:152-163.

Olsson, K. and J. Agren. 2002. Latitudinal population differentiation in phenology, life history and flower morphology in the perennial herb *Lythrum salicaria*. Journal of Evolutionary Biology 15:983-996.

Pan, J. J., B. Widner, D. Ammerman, and R. E. Drenovsky. 2010. Plant community and tissue chemistry responses to fertilizer and litter nutrient manipulations in a temperate grassland. Plant Ecology 206:139-150.

Pattison, R. R., G. Goldstein, and A. Ares. 1998. Growth, biomass allocation and photosynthesis of invasive and native Hawaiian rainforest species. Oecologia 117:449-459.

Piculell, B. J., J. D. Hoeksema, and J. N. Thompson. 2008. Interactions of biotic and abiotic environmental factors in an ectomycorrhizal symbiosis, and the potential for selection mosaics. Bmc Biology 6.

Pregitzer, C., J. Bailey, S. Hart, and J. Schweitzer. 2010. Soils as agents of selection: feedbacks between plants and soils alter seedling survival and performance. Evolutionary Ecology 24:1045-1059.

Rapo, C., H. Muller-Scharer, K. Vrieling, and U. Schaffner. Is there rapid evolutionary response in introduced populations of tansy ragwort, *Jacobaea vulgaris*, when exposed to biological control? Evolutionary Ecology 24:1081-1099.

Ridenour, W. M., J. M. Vivanco, Y. L. Feng, J. Horiuchi, and R. M. Callaway. 2008. No evidence for trade-offs: *Centaurea* plants from America are better competitors and defenders. Ecological Monographs 78:369-386.

Riggs, J. S., Tharp M.L., Norby R.J. 2010. ORNL FACE Weather Data. Carbon Dioxide Information Analysis Center. http://cdiac.ornl.gov/

Rosenthal, R. 1979. The file drawer problem and tolerance for null results. Psychological Bulletin 86:638-641.

Rout, M. E. and R. M. Callaway. 2009. An Invasive Plant Paradox. Science 324:734-735.

Saltonstall, K. 2002. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. Proceedings of the National Academy of Sciences of the United States of America 99:2445-2449.

Schall, M. J. and D. D. Davis. 2009. *Ailanthus altissima* wilt and mortality: Etiology. Plant Disease 93:747-751.

Schweitzer, J. A., J. K. Bailey, D. G. Fischer, C. J. Leroy, E. V. Lonsdorf, T. G. Whitham, and S. C. Hart. 2008. Plant-soil-microorganism interactions: Heritable relationship between plant genotype and associated soil microorganisms. Ecology 89:773-781.

Schweitzer, J. A., J. K. Bailey, S. C. Hart, G. M. Wimp, S. K. Chapman, and T. G. Whitham. 2005. The interaction of plant genotype and herbivory decelerate leaf litter decomposition and alter nutrient dynamics. Oikos 110:133-145.

Schweitzer, J. A., J. K. Bailey, B. J. Rehill, G. D. Martinsen, S. C. Hart, R. L. Lindroth, P. Keim, and T. G. Whitham. 2004. Genetically based trait in a dominant tree affects ecosystem processes. Ecology Letters 7:127-134.

Seifert, E. K., J. D. Bever, and J. L. Maron. 2009. Evidence for the evolution of reduced mycorrhizal dependence during plant invasion. Ecology 90:1055-1062.

Siemann, E. and W. E. Rogers. 2001. Genetic differences in growth of an invasive tree species. Ecology Letters 4:514-518.

Silvertown, J. and R. Law. 1987. Do plants need niches—some recent developments in plant community ecology. Trends in Ecology & Evolution 2:24-26.

Simons, A. M. 2003. Invasive aliens and sampling bias. Ecology Letters 6:278-280.

Sinsabaugh, R. L. 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology & Biochemistry 42:391-404.

Sinsabaugh, R. L., M. M. Carreiro, and D. A. Repert. 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60:1-24.

Sinsabaugh, R. L., C. L. Lauber, M. N. Weintraub, B. Ahmed, S. D. Allison, C. Crenshaw, A. R. Contosta, D. Cusack, S. Frey, M. E. Gallo, T. B. Gartner, S. E. Hobbie, K. Holland, B. L. Keeler, J. S. Powers, M. Stursova, C. Takacs-Vesbach, M. P. Waldrop, M. D. Wallenstein, D. R. Zak, and L. H. Zeglin. 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11:1252-1264.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787-792.

Smith, M. D. and A. K. Knapp. 2001. Physiological and morphological traits of exotic, invasive exotic, and native plant species in tallgrass prairie. International Journal of Plant Sciences 162:785-792.

Souza, L., R. T. Belote, P. Kardol, J. F. Weltzin, and R. J. Norby. 2010. $CO_2$ enrichment accelerates successional development of an understory plant community. Journal of Plant Ecology-Uk 3:33-39.

Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at http://websoilsurvey.nrcs.usda.gov.

Stastny, M., U. Schaffner, and E. Elle. 2005. Do vigour of introduced populations and escape from specialist herbivores contribute to invasiveness? Journal of ecology 93:27-37.

Strayer, D. L., V. T. Eviner, J. M. Jeschke, and M. L. Pace. 2006. Understanding the long-term effects of species invasions. Trends in Ecology & Evolution 21:645-651.

Strickland, M. S., C. Lauber, N. Fierer, and M. A. Bradford. 2009. Testing the functional significance of microbial community composition. Ecology 90:441-451.

Strickland, M. S., E. Osburn, C. Lauber, N. Fierer, and M. A. Bradford. 2009. Litter quality is in the eye of the beholder: initial decomposition rates as a function of inoculum characteristics. Functional Ecology 23:627-636.

Stritar, M. L., J. A. Schweitzer, S. C. Hart, and J. K. Bailey. 2010. Introduced ungulate herbivore alters soil processes after fire. Biological Invasions 12:313-324.

Thebaud, C. and D. Simberloff. 2001. Are plants really larger in their introduced ranges? American Naturalist 157:231-236.

Thomas, M. B. and A. M. Reid. 2007. Are exotic natural enemies an effective way of controlling invasive plants? Trends in Ecology & Evolution 22:447-453.

Thompson, J. N. 1997. Evaluating the dynamics of coevolution among geographically structured populations. Ecology 78:1619-1623.

Thompson, J. N. 1999. Specific hypotheses on the geographic mosaic of coevolution. American Naturalist 153:S1-S14.

Treseder, K. K. 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecology Letters 11:1111-1120.

U.S. Department of Agriculture, N. R. C. S. National Soil Survey Handbook, title 430-VI. Available online at http://soils.usda.gov/technical/handbook/.

van der Putten, W. H., C. Vandijk, and B. A. M. Peters. 1993. Plant-specific soil-borne diseases contribute to succession in foredune succession.  Nature 362:53-56.

van Grunsven, R. H. A., W. H. van der Putten, T. M. Bezemer, W. L. M. Tamis, F. Berendse, and E. M. Veenendaal. 2007. Reduced plant-soil feedback of plant species expanding their range as compared to natives. Journal of ecology 95:1050-1057.

van Kleunen, M. and M. Fischer. 2008. Adaptive rather than non-adaptive evolution of *Mimulus guttatus* in its invasive range. Basic and Applied Ecology 9:213-223.

van Kleunen, M. and B. Schmid. 2003. No evidence for an evolutionary increased competitive ability in an invasive plant. Ecology 84:2816-2823.

Vasemagi, A. 2006. The adaptive hypothesis of clinal variation revisited: Single-locus clines as a result of spatially restricted gene flow. Genetics 173:2411-2414.

Vitousek, P. M. 1994. Beyond global warming—ecology and global change. Ecology 75:1861-1876.

Vivanco, L. and A. T. Austin. 2006. Intrinsic effects of species on leaf litter and root decomposition: a comparison of temperate grasses from North and South America. Oecologia 150:97-107.

Voriskova, J., P. Dobiasova, J. Snajdr, D. Vanek, T. Cajthaml, H. Santruckova, and P. Baldrian. 2011. Chemical composition of litter affects the growth and enzyme production by the saprotrophic basidiomycete *Hypholoma fasciculare*. Fungal Ecology 4:417-426.

Wardle, D. A., K. S. Nicholson, M. Ahmed, and A. Rahman. 1994. Interference effects of the invasive plant *Carduus nutans* L. against the nitrogen fixation ability of *Trifolium repens* L. Plant and Soil 163:287-297.

Whitham, T. G., W. P. Young, G. D. Martinsen, C. A. Gehring, J. A. Schweitzer, S. M. Shuster, G. M. Wimp, D. G. Fischer, J. K. Bailey, R. L. Lindroth, S. Woolbright, and C. R. Kuske. 2003. Community and ecosystem genetics: A consequence of the extended phenotype. Ecology 84:559-573.

Whitney, K. D. and C. A. Gabler. 2008. Rapid evolution in introduced species, 'invasive traits' and recipient communities: challenges for predicting invasive potential. Diversity and Distributions 14:569-580.

Wiedenmann, J., A. Baumstark, T. L. Pillen, A. Meinesz, and W. Vogel. 2001. DNA fingerprints of *Caulerpa taxifolia* provide evidence for the introduction of an aquarium strain into the Mediterranean Sea and its close relationship to an Australian population. Marine Biology 138:229-234.

Williams, J. L., H. Auge, and J. L. Maron. 2008. Different gardens, different results: native and introduced populations exhibit contrasting phenotypes across common gardens. Oecologia 157:239-248.

Williamson, M. and A. Fitter. 1996. The varying success of invaders. Ecology 77:1661-1666.

Williamson, M. H. and K. C. Brown. 1986. The analysis and modeling of british invasions. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 314:505-522.

Willis, A. J., M. B. Thomas, and J. H. Lawton. 1999. Is the increased vigour of invasive weeds explained by a trade-off between growth and herbivore resistance? Oecologia 120:632-640.

Wilson, S. D. and D. Tilman. 1993. Plant competition and resource availability in response to disturbance and fertilization. Ecology 74:599-611.

Wolfe, B. E., V. L. Rodgers, K. A. Stinson, and A. Pringle. 2008. The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. Journal of ecology 96:777-783.

Wright, I. J., P. B. Reich, and M. Westoby. 2001. Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. Functional Ecology 15:423-434.

Xiong, S. J. and C. Nilsson. 1999. The effects of plant litter on vegetation: a meta-analysis. Journal of ecology 87:984-994.

Zak, D. R., K. S. Pregitzer, A. J. Burton, I. P. Edwards, and H. Kellner. 2011. Microbial responses to a changing environment: implications for the future functioning of terrestrial ecosystems. Fungal Ecology 4:386-395.

Zak, D. R., K. S. Pregitzer, P. S. Curtis, and W. E. Holmes. 2000. Atmospheric $CO_2$ and the composition and function of soil microbial communities. Ecological Applications 10:47-59.

Zak, D. R., D. B. Ringelberg, K. S. Pregitzer, D. L. Randlett, D. C. White, and P. S. Curtis. 1996. Soil microbial communities beneath *Populus grandidentata* crown under elevated atmospheric $CO_2$. Ecological Applications 6:257-262.

Zhang, D. Y. and X. H. Jiang. 2006. Interactive effects of habitat productivity and herbivore pressure on the evolution of anti-herbivore defense in invasive plant populations. Journal of Theoretical Biology 242:935-940.

Zheng, J. Q., S. J. Han, Y. Wang, C. G. Zhang, and M. H. Li. 2010. Composition and function of microbial communities during the early decomposition stages of foliar litter exposed to elevated $CO_2$ concentrations. European Journal of Soil Science 61:914-925.

Zobel, M. 1997. The relative role of species pools in determining plant species richness. An alternative explanation of species coexistence? Trends in Ecology & Evolution 12:266-269.

Zou, J. W., W. E. Rogers, and E. Siemann. 2008. Increased competitive ability and herbivory tolerance in the invasive plant *Sapium sebiferum*. Biological Invasions 10:291-302

# VITA

Emmi Felker-Quinn attended Smith College, where she majored in Biological Sciences. She graduated in 2006 with a Bachelor of Arts degree and Highest Honors in Biological Sciences for her thesis entitled “H.G. Jesup in western Massachusetts (1863-1876) and the role of non-native species in the flora.” She then worked for Drs. Rich Norby and Aimee Classen as a technician in several ecosystem experiments at Oak Ridge National Laboratory for a year before applying to graduate school. She joined the Department of Ecology and Evolutionary Biology as a graduate student in 2007, under the direction of Drs. Jennifer Schweitzer and Joseph Bailey. She enjoys teaching biology, conducting research in plant and soil ecology, and writing in the third person.