

Plasticity and biotic interactions mediate plant persistence in a changing world

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ABSTRACT

Anthropogenic global change is occurring today at a faster rate and larger scale than ever before. Understanding how plants will respond to such large-scale disturbance is critical for biodiversity conservation, yet the ecological and evolutionary mechanisms underlying these responses remain poorly understood. In this thesis, I investigated the mechanisms underlying plant response to two major drivers of global change, climate change and the widespread mortality of foundation species. First, I examined genetic and plastic plant trait responses to climatic variation using elevation gradients, which serve as space-for-time substitutions for climate change. Through field observations in three populations of the North American shrub *Rhododendron maximum* (rosebay rhododendron), I found that while several traits respond significantly to elevation, these trait responses typically occur in some, but not all, populations. A common garden experiment indicated that trait variation within and among populations was driven by plasticity and genetic divergence, respectively. These findings suggest that plasticity can be a viable climate change response, although the magnitude of this plasticity will likely differ among genetically distinct populations. Next, I examined whether plant-soil biota interactions and/or light variation associated with foundation tree decline mediate the expansion of *R. maximum* in southeastern US forests where *Tsuga canadensis* (eastern hemlock), a dominant foundation tree species, is declining due to non-native insect invasion. Using a controlled inoculation experiment, I found that, in high light (matching infested *T. canadensis* crowns), *R. maximum* seedling performance was highest in *T. canadensis*-conditioned soils, medial in *R. maximum*-conditioned soils, and lowest in interspace soils. Genomic sequencing indicated that such variation in performance can be attributed to variation in mycorrhizal and saprotrophic soil fungal guilds. In low light (matching healthy *T. canadensis* crowns), however, soil inoculation did not affect plant performance and plants performed worse on average. These findings suggest interactions with soil biota can act synergistically with altered light environments to mediate species' responses to widespread foundation tree mortality, providing evidence for a novel

mechanism of plant response to major disturbance. Overall, my work suggests that plant persistence in a changing world will depend on multiple mechanisms, including plasticity, genetic differentiation, and biotic interactions.

TABLE OF CONTENTS

INTRODUCTION	1
Global Change: Causes, Consequences, and Mechanisms of Response	1
References	4
CHAPTER I: Trait variation along elevation gradients in a dominant woody shrub is population-specific and driven by plasticity	7
Abstract	8
Introduction	9
Materials and Methods	12
Results	17
Discussion	19
Acknowledgments	23
References	25
Appendix	30
CHAPTER II: Plant-soil feedbacks mediate shrub expansion in declining forests, but only in the right light	39
Abstract	40
Introduction	41
Materials and Methods	44
Results	53
Discussion	56
Acknowledgments	62
References	63
Appendix	71
CONCLUSION AND FUTURE DIRECTIONS	79
VITA	81

LIST OF TABLES

Table I.1. Site characteristics for each of three <i>Rhododendron maximum</i> populations sampled in this study, including the number of <i>R. maximum</i> individuals sampled ($N_{\text{individual}}$), gradient aspect, the range of elevation, and the soil taxonomic classes present along each gradient.....	30
Table I.2. Statistics for linear models incorporating elevation (Elev), population (Pop), and their interaction as fixed effects, predicting variation in five climatic, edaphic, and topographic variables (mean annual temperature (MAT), annual precipitation (AP), topographical slope (Slope), soil nitrogen (N) content, and soil carbon:nitrogen (C:N) ratio) along elevation in three natural <i>R. maximum</i> populations.....	31
Table I.3. Statistics for linear models, incorporating population (Pop), elevation (Elev), and their interaction as fixed effects, of seven functional trait values along elevation at three field locations containing sampled <i>Rhododendron maximum</i> populations ($N = 90$ sampled <i>R. maximum</i> individuals).....	32
Table I.4. Statistics for linear mixed effects models, incorporating population of origin (Pop), elevation of origin (Elev), and their interaction as fixed effects, and individual identity (i.e., sampled individual in field population) as a random effect, of seven functional trait values measured on 16-month old <i>Rhododendron maximum</i> cuttings in a common garden.....	33
Table II.5. Chi-square (X^2) values and associated degrees of freedom (DF) for linear models predicting variation in six growth response variables measured on <i>Rhododendron maximum</i> seedlings grown for 11 months in soil inoculations sourced from beneath conspecific <i>R. maximum</i> patches, declining <i>T. canadensis</i> trees, or interspace locations, in either high or low light.....	71
Table II.6. Chi-square (X^2) values and associated degrees of freedom (DF) for linear models predicting variation in six fungal community traits across experimental soil subsamples ($N = 48$) sourced from beneath <i>Rhododendron maximum</i> patches, declining <i>Tsuga canadensis</i> trees, or interspace locations.....	72
Table II.7. Standardized partial correlation coefficients (β), associated standard error (SE), and chi-square (X^2) values with associated degrees of freedom (DF) indicating effects of five predictor variables, the relative abundance of mycorrhizal, saprotrophic, and pathogenic fungi, and fungal species richness, on <i>Rhododendron maximum</i> seedling total biomass ($N = 48$).....	73

LIST OF FIGURES

Figure I.1. <i>Rhododendron maximum</i> elevation gradient and common garden sites are situated throughout the South Central Appalachian region of the <i>R. maximum</i> range (shown in green).	34
Figure I.2. Variation in five environmental variables along elevation in three <i>Rhododendron maximum</i> populations (North Carolina [NC]; Tennessee [TN]; Virginia [VA]).	35
Figure I.3. The effect of elevation on traits related to growth and timing (internode diameter, internode length, shoot length, leaf bud break phenology) in <i>Rhododendron maximum</i> measured at the individual level in natural field populations along three elevation gradients (A-D) and the effect of source elevation on mean $\pm$ SE trait values measured at the replicated cutting level in a common garden (E-H).	36
Figure I.4. The effect of elevation on leaf-level traits (leaf area, leaf dry mass, specific leaf area [SLA], leaf nitrogen [N] content) in <i>Rhododendron maximum</i> measured at the individual level in natural field populations along three elevation gradients (A-D) and the effect of source elevation on mean $\pm$ SE trait values measured at the replicated cutting level in a common garden (E-H).	37
Figure I.5. Population means of three traits (internode diameter, leaf area, and leaf dry mass) differ across <i>Rhododendron maximum</i> populations (North Carolina, NC; Tennessee, TN; Virginia, VA) in both field (A-C) and common garden environments (D-F).	38
Figure II.6. Field sampling locations, field sampling design, and experimental design for <i>Rhododendron maximum</i> inoculation experiment.	74
Figure II.7. Variation in <i>Rhododendron maximum</i> total, shoot, and root biomass across soil inoculum sources and light environments.	75
Figure II.8. Variation in <i>Rhododendron maximum</i> total shoot, and root biomass visualized as feedbacks, simulating movement from home (<i>R. maximum</i> -conditioned) into away (<i>Tsuga canadensis</i> -conditioned or interspace) soils.	76
Figure II.9. Mean ($\pm$ SE) relative abundance of total mycorrhizal (ericoid + ectomycorrhizal) fungi, ericoid mycorrhizal fungi, ectomycorrhizal fungi, saprotrophic fungi, pathogenic fungi, and fungal species richness across soil inocula sourced from three paired locations.	77
Figure II.10. Relationships between <i>Rhododendron maximum</i> seedling total biomass following an 11-month soil inoculation experiment and the relative abundance of mycorrhizal, saprotrophic, and pathogenic fungi, and fungal species richness.	78

LIST OF ATTACHMENTS

Attachment 1. Supporting Information for Chapter 2 (Figure S1, Figure S2, and Appendix S1)	Attachment1.pdf
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INTRODUCTION

Global Change: Causes, Consequences, and Mechanisms of Response

Through atmospheric CO₂ inputs and associated climatic changes, worldwide introductions of non-native species, habitat conversion, and nitrogen deposition, human activity is causing species extinctions, shuffling species distributions and phenotypes, and bringing about novel ecological and evolutionary interactions in nearly every ecosystem and every region of the globe (Kareiva *et al.* 1993; Sala *et al.* 2000; Zavaleta *et al.* 2003; MEA 2005; Parmesan 2006; Tylianakas *et al.* 2008). Temperature increases of ~0.6°C in the 20th century (Jones *et al.* 2001) have altered the seasonal timing of flowering plants and pollinators, creating ecological mismatches that can lead to the extinction of one or both species (Kiers *et al.* 2010; Rafferty & Ives 2010). At the same time, non-native insect pest and pathogen outbreaks damage some 35 million hectares of forests each year and are leading to the demise of many once common and abundant foundation tree species (Ellison *et al.* 2005; FAO 2010). As some species wholly disappear from ecosystems, others may cope with a changing world by dispersing into favorable habitats or by undergoing rapid phenotypic or genetic change. Thus, species are faced with the possibility of interacting with novel environments and organisms, which may have both ecological and evolutionary consequences (Jump & Peñuelas 2005; Bellard *et al.* 2012). A major frontier for plant ecology and global change biology therefore lies in understanding the extent and relative importance of various ecological and evolutionary mechanisms that comprise a species' 'adaptive capacity' (Nicotra *et al.* 2015) and enable plant species to persist through rapid environmental change.

Genetic and phenotypic change may enable many long-lived plant species to persist through rapid climatic change (Jump & Peñuelas 2005; Nicotra *et al.* 2010; Hoffmann & Sgró 2011). Evolutionary processes can occur across relatively short, ecologically relevant time scales (Thompson 1998; Schoener 2011; Hendry 2016), making standing genetic variation and evolutionary change potentially viable means for plants to persist *in situ* as climatic regimes fluctuate (Bell & Gonzales 2009; Hoffmann &

Sgró 2011). Moreover, phenotypic plasticity, the ability of a single genetic individual to express a range of physical traits, may be key for plants to alter resource allocation or seasonal timing at time scales ranging from days to years (Nicotra *et al.* 2015). It is necessary to make the distinction between genetic and phenotypic change to understand and predict potential plant responses to climate change, yet empirical evidence for the extent and potential variability of each mechanism is lacking for many plant species (Gienapp *et al.* 2007).

Interactions between a plant and other organisms can also determine that plant's persistence in a changing world (Bardgett & Wardle 2010; Van der Putten *et al.* 2016). In particular, as plant species are lost or gained from ecosystems via global change-induced extinction or migration, plants may interact with novel microbial communities belowground, which can profoundly impact plant performance. For example, range-expanding plant species may experience enemy release from soil pathogens as they track a changing climate upwards along elevation or latitude, enabling them to outperform local species (Van Grunsven *et al.* 2007; Engelkes *et al.* 2008). Alternatively, plant invasions may fail if adequate mutualistic soil fungi are lacking in the introduced range (Nuñez *et al.* 2009). While there is increasing awareness of the importance of plant-soil interactions for plant community development and response to environmental change (Ehrenfeld *et al.* 2005; Bardgett & Wardle 2010; Van der Putten *et al.* 2013, 2016), many questions remain unanswered (Van der Putten *et al.* 2016). For example, how might plant-soil interactions mediate plant responses to the loss of a single species from a system, such as a common foundation tree species? Further, only a handful of studies have tested for the context-dependency of plant-soil interactions on other relevant abiotic gradients (Hoeksema *et al.* 2010; McCarthy-Neumann & Ibáñez 2012, 2013; Smith & Reynolds 2015).

My thesis is focused on understanding the ecological and evolutionary mechanisms driving plant responses to two major contemporary changes impacting forests globally: climatic change and the decline of foundation tree species. Using field observations, greenhouse experiments, and genomic analysis, I investigated the

mechanisms through which a dominant North American forest species, *R. maximum*, responds to these two drivers of environmental change.

In Chapter 1, I used field observations paired with a common garden experiment to investigate genetic- and plastic-based trait responses to elevation gradients, which serve as space-for-time climate change proxies, in three populations of the dominant North American shrub *Rhododendron maximum* (rosebay rhododendron). I found that several traits respond strongly to elevation, primarily through phenotypic plasticity; however, trait responses were highly variable among populations, which were themselves genetically differentiated. This chapter emphasizes the importance of sampling multiple populations to avoid over- or underestimating potential climate change response and suggests that plasticity may be a viable climate change response for certain plant species, including *R. maximum*.

In Chapter 2, I used a greenhouse inoculation experiment and genomic analysis to test whether plant-soil interactions mediate *R. maximum* expansion in southeastern forests where *Tsuga canadensis* (eastern hemlock), a dominant foundation tree species, is declining due to non-native insect invasion. I inoculated *R. maximum* seedlings with soils conditioned by either *T. canadensis*, *R. maximum*, or neither species, compared soil fungal communities among these soil sources, and replicated inoculations in high and low light environments that match the understory environment of dead and healthy *T. canadensis* stands, respectively. I found that *R. maximum* performance was highest in *T. canadensis*-conditioned soils, medial in *R. maximum*-conditioned soils, and lowest in soils conditioned by neither species due in part to soil fungal community differentiation. Importantly, these soil effects were evident in high light environments but not low light environments, where plants performed worse on average. This work suggests that biotic interactions belowground act synergistically with concurrent abiotic disturbances aboveground to enable *R. maximum* expansion into declining *T. canadensis* forests and provides a framework for future work to examine whether such mechanisms drive plant responses to foundation tree loss from forest systems globally.

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CHAPTER I:
TRAIT VARIATION ALONG ELEVATION GRADIENTS IN A
DOMINANT WOODY SHRUB IS POPULATION-SPECIFIC AND
DRIVEN BY PLASTICITY

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Abstract

Elevation gradients are frequently used as space-for-time substitutions to infer species' trait responses to climate change. However, studies rarely investigate whether trait responses to elevation are widespread or population-specific within a species. Moreover, the relative genetic and plastic contributions to such trait responses may not be well understood. Here, we examine plant trait variation across individuals of the dominant woody shrub, *Rhododendron maximum*, along elevation gradients in three populations in the South Central Appalachian Mountains, USA, in both field and common garden environments. We ask the following: (1) do plant traits vary along elevation? (2) do trait responses to elevation differ across populations, and if so, why? and (3) does genetic differentiation or phenotypic plasticity drive trait variation within and among populations? We found that internode length, shoot length, leaf dry mass, and leaf area varied along elevation, but that these responses were generally unique to one population, suggesting that trait responses to environmental gradients may be population-specific. A common garden experiment identified no genetic basis to trait variation along elevation of origin but detected population-level genetic differentiation in traits, suggesting that plasticity drives local trait differentiation while genetic variation drives regional differentiation. Our findings highlight the importance of examining multiple

locations in future elevation studies and indicate that, for a given plant species, the magnitude of trait responses to global climate change may vary across populations.

Introduction

Contemporary climate change is altering the availability of resources and habitats critical to plant performance (Parmesan and Yohe 2003). In a rapidly changing climate, the ability of a plant species to acclimate via phenotypic plasticity or undergo genetic change will play a key role in that species' persistence (Walther *et al.* 2002; Franks *et al.* 2014; Nicotra *et al.* 2015). Examining how a plant species' traits respond phenotypically or genetically to existing climatic gradients is therefore critical for understanding and predicting whether and how plants may persist *in situ* despite a changing climate (Chevin and Lande 2010; Nicotra *et al.* 2010; Anderson and Gezon 2015).

Phenotypic plasticity and genetic change have inherently unique characteristics and limitations with respect to potential climate change response. Plasticity is recognized for its potential key role in short-term adaptive responses to rapid environmental change because it can allow a plant to both maximize fitness under optimal conditions and tolerate stressful environments in suboptimal conditions (Gianoli 2004; Hendry 2016). Plasticity may also be advantageous at local spatial scales where genetic differentiation may be hindered by gene flow (Kawecki & Ebert 2004; Hamann *et al.* 2016). However, plasticity typically does not produce phenotypic spectra as extreme as those produced by genetic change, which may therefore provide long-term rescue from escalating climate change (DeWitt *et al.* 1998; Gienapp *et al.* 2008). Because genetic responses are typically slow relative to plastic responses, however, adaptive evolution may be most effective when climatic change is gradual and below a critical threshold (Lynch and Lande 1993; Aitken *et al.* 2008).

Natural climatic gradients associated with elevation are frequently used as spatial substitutions to infer potential trait responses to temporal climate change (Fukami and Wardle 2005; Körner 2007; Sundqvist *et al.* 2013; Read *et al.* 2014). These gradients encompass spatial variation in climatic factors including temperature, precipitation, and

growing season length and should thus present strong environmental or selective pressures on plant traits that mirror contemporary climate change pressures (Dunne *et al.* 2004). Moreover, natural climatic gradients capture temporal scales (i.e., multiple plant generations) that are typically difficult to capture in experimental climate manipulations, allowing insight into both long-term and short-term responses (Grinnell 1924; Dunne *et al.* 2004; Fukami and Wardle 2005; Sundqvist *et al.* 2013).

Performance-related foliar, morphological, and phenological plant traits are highly sensitive to climatic environment (Medeiros and Ward 2013; Pratt and Mooney 2013) and may therefore genetically or phenotypically ‘track’ climatic variation associated with elevation (Whittaker 1956; Fukami and Wardle 2005; Sundqvist *et al.* 2013; reviewed in Read *et al.* 2014). For example, plants at higher elevations have lower growth rates, smaller and thicker leaves, higher leaf nutrient content per unit area, later bud break, and earlier senescing phenology (Clausen *et al.* 1940; Oleksyn *et al.* 1998; Vitasse *et al.* 2009; Bresson *et al.* 2011; Read *et al.* 2014). Common garden experiments, which identify the genetic basis of *in situ* trait variation by exposing plants sourced from different elevations to a single environment, have attributed such trait responses to genetic differentiation, plasticity, or both mechanisms (Hoffmann *et al.* 2009; Anderson & Gezon 2015; reviewed in Read *et al.* 2014).

The use of natural spatial gradients as climate change proxies may be confounded by fine-scale environmental heterogeneity, covarying abiotic or biotic factors, or historical site attributes of the gradient (Dunne *et al.* 2004; Primack *et al.* 2009; De Frenne *et al.* 2013; Lenoir *et al.* 2013; Kooyers *et al.* 2015). The magnitude of trait responses to climatic factors along elevation may thus vary across locations, yet most studies to date have examined trait responses to elevation at a single location. For example, in a recent meta-analysis of leaf trait responses to elevation within and among species (Read *et al.* 2014), 75% of studies examining intraspecific trait responses did so at only one site. Further, few studies have explicitly asked whether, and why, trait responses to elevation are or are not consistent across multiple populations or locations (but see Morecroft *et al.* 1992; Kooyers *et al.* 2015). Thus, to understand whether patterns

of trait variation are widespread or idiosyncratic within a species and to ensure accurate ecological and evolutionary inferences, it is critical to study trait variation and potential confounding environmental factors along elevation at multiple locations.

Here, we examined genetic- and plastic-based trait variation along elevation in three geographically distinct populations of *Rhododendron maximum*, a dominant native shrub of eastern North America. We selected *R. maximum* because it is geographically widespread, occurs from 0-1800 m a.s.l., and often forms continuous populations across steep elevation gradients (Gleason and Cronquist 1991). Specifically, we addressed three questions: (1) do plant traits vary along elevation gradients? (2) do trait responses to elevation differ among populations, and if so, why? and (3) does genetic divergence or phenotypic plasticity drive trait variation within and among populations? To address the first question, we examined variation in eight quantitative leaf, stem, and phenology traits (internode diameter, internode length, shoot length, leaf area, leaf dry mass, specific leaf area [SLA], leaf nitrogen [N] content, leaf bud break phenology) in three *R. maximum* populations occurring along geographically distinct elevation gradients. We predicted that plant traits become increasingly ‘conservative’ at higher elevations in response to colder temperatures and shorter growing seasons (i.e., shorter and smaller internodes and shoots, smaller and lighter leaves, lower SLA and leaf N content, later bud break). We then addressed the second question by exploring the relative importance of five climatic, edaphic, and topographic variables on trait variation within and among populations and predicted that the magnitude of trait responses to elevation varies across sites due to environmental differentiation and/or population genetic differentiation. To address the third question, we planted and measured traits on replicate cuttings of the individuals sampled in natural field populations in a common garden. Consistent with past theoretical and empirical work (Kawecki & Ebert 2004; Hamann *et al.* 2016), we predicted that local trait variation (i.e., along elevation within each population) is driven by plasticity, while regional variation (i.e., among populations) is driven by genetic differentiation.

Materials and Methods

Focal species and study sites

Rhododendron maximum (Ericaceae) is a long-lived, evergreen woody shrub widely distributed throughout eastern North America (Gleason and Cronquist 1991). It is a dominant forest understory component across millions of hectares of temperate forest in South Central Appalachia, USA (Clinton 2004), the broad mountain region in which the study sites are located (Fig. I.1, all tables and figures are located in the appendix).

We selected elevation gradients at three locations along which to examine environmental and *R. maximum* trait variation based on the following criteria: (1) a steep elevation gradient (>400 m total elevation change) that presents a gradient of mean annual temperature falling within the range of regional climate change projections for the next ~100 years (+1 to +5°C, scenarios RCP2.6 and RCP8.5; IPCC 2014), (2) a continuous *R. maximum* population across the gradient, and (3) a north- to northeast-facing aspect, to minimize environmental and trait variability due to exposure. The three sites are located in: (a) George Washington and Jefferson National Forest, Virginia (36.6900°N, 81.6364°W; hereafter, ‘VA population’); (b) Cherokee National Forest, Tennessee (36.1413°N, 82.2756°W; hereafter, ‘TN population’); and (c) Pisgah National Forest, North Carolina (35.7230°N, 82.2461°W; hereafter, ‘NC population’) (Fig. I.1). Collectively, sites represent a total elevation gradient of ~800 m. All three sites were established as National Forest within four years of one another (1916-1920; USFS 1973). Site descriptions are provided in Table I.1.

Environmental gradient quantification

To examine potential environmental drivers of *R. maximum* trait variation within and among sites, we collected data on climatic, edaphic, and topographic variation. Using GPS coordinates obtained at each individual sampling location (Oregon 650t, Garmin, Olathe, KS, USA), we extracted elevation above sea level and slope from digital elevation models in ArcMap 10.1 (Esri, Redlands, CA, USA) and interpolated mean

annual temperature (MAT) and annual precipitation (AP) data, representative of 1960-1990 conditions, from the WorldClim database (Hijmans *et al.* 2005) at the highest resolution available (30 arc-seconds).

To characterize edaphic conditions in each population, we measured total soil N and soil carbon:nitrogen (C:N) ratio along each gradient. We collected and pooled three soil samples (2x10 cm) at each of ten elevation levels along each transect ($n = 30$ total soil samples) and quantified total soil N and C:N using dynamic flash combustion elemental analysis (Flash Elemental Analyzer 1112, Thermo Fisher Scientific, Waltham, MA, USA).

Trait variation in natural field populations

We collected trait data in late July through early August 2014 during the *R. maximum* growing season. Along each gradient, we sampled three *R. maximum* individuals at each of ten elevation intervals of approximately 50 meters, for a total of 30 sampled individuals per site ($N = 3$ individuals x 10 elevation intervals x 3 sites = 90 total individuals). Sampled *R. maximum* individuals were separated by >40 m to minimize the probability of sampling siblings or clones.

We assessed stem morphology in the field by measuring internode and elongating shoot length and internode diameter of two outer canopy stems per individual. Internode length and diameter were averaged across the three fully developed internodes directly below the elongating shoot. To assess leaf traits, we harvested the two most recently produced but fully expanded leaves from an outer canopy leaf whorl to assess leaf area, leaf dry mass, SLA, and leaf N content. Field fresh leaves were stored at 0°C and transported to the lab, then scanned to calculate total fresh leaf area (WinFOLIA 2011a, Regent Instruments, Canada). Leaf dry mass was recorded after leaves were oven-dried at 70°C for 72 h. Specific leaf area was calculated as leaf area/leaf dry mass. Leaf area, dry mass, and SLA were averaged at the individual level. Dried leaves were ground to a fine powder (8000D Mixer/Mill, SPEX SamplePrep, Metuchen, NJ, USA) and analyzed for

total leaf N content with dynamic flash combustion elemental analysis (FlashEA® 1112, Thermo Scientific, Waltham, MA, USA).

Genetic-based quantitative trait variation in common garden

To estimate the genetic contributions to *R. maximum* trait variation measured in the field, we established a common garden at the University of Tennessee, Knoxville, Tennessee, USA (Fig. I.1; 35.9579°N, 83.9248°W; elevation 281 m; MAT 14.4°C; AP 125.7 cm). During field sampling in late July through early August 2014, we harvested ten terminal shoot cuttings (~15 cm in length) from each *R. maximum* individual that we sampled ($N = 900$ cuttings). The cuttings were kept moist and transported at 0°C to a greenhouse, where we scored the lower 5 cm of each cutting using a razor, dipped the scored section in a root-inducing growth hormone (Hormodin 3, Olympic Horticultural Products, Mainland, PA, USA) and planted each cutting in potting media consisting of equal parts peat moss and perlite. We removed all but three terminal leaves and uniformly cut the remaining three leaves to 5 cm length to minimize evaporative moisture loss and encourage root growth. After a six-month rooting period on a mist bed (misted with tap water every 15 minutes) we transplanted all living, rooted cuttings ($N = 504$) into individual, randomized 1-gallon pots filled with the same peat-perlite mixture in an outdoor common garden. Cuttings were watered to field capacity and given biweekly fertilizer treatments (200 ppm of Peters Professional 21-7-7 Acid Special, Everris NA, Dublin, OH, USA) during the growing season to minimize non-genetic (maternal-like) effects associated with clone cuttings (Roach and Wulff 1987). To further minimize maternal-like effects, we grew cuttings in a common environment for 16 months before measuring traits on new growth (since cutting) only. By exposing individuals from diverse environments to a single, common environment, common gardens minimize environmentally-induced plasticity and effectively expose the genetic basis of complex, quantitative trait phenotypes (Clausen *et al.* 1940; Dunne *et al.* 2004; Fukami and Wardle 2005; Anderson *et al.* 2014; De Villemereuil *et al.* 2016).

In December 2015, after 16 months of growth in common conditions, we measured average internode diameter, shoot length, and internode length and sampled one fully mature leaf per cutting to quantify leaf area, dry leaf mass, and specific leaf area on all surviving cuttings ($n = 151, 185$, and 168 cuttings sourced from NC, TN, and VA transects, respectively) to quantify genetic-based trait variation. We additionally measured spring leaf bud break (flushing) phenology throughout the growing season as the earliest day on which leaf bud scales opened and any emerging new leaf was visible; these data were collected every second day continually until all cuttings had flushed. Sample sizes vary among traits measured in the common garden (i.e., shoot length, but not internode length, could be measured on plants that did not produce multiple new nodes); this information is provided in Table I.4.

Statistical analysis

Environmental gradient characterization: To explore whether the three *R. maximum* populations experience similar climatic, edaphic, and topographic gradients along elevation, we built linear models including population (NC, TN, or VA), elevation (m), and the interaction between population and elevation as fixed effects. Separate models were built for MAT, AP, slope, soil N, and soil C:N. Each variable was transformed prior to analysis as needed to increase conformance to normality. We calculated ANOVA tables using partial sums of squares and assessed significance at $\alpha = 0.05$ using F statistics. We calculated standardized beta coefficients for elevation based on correlations between trait and elevation z-scores (obtained by scaling and centering untransformed data on zero).

Trait variation in natural field populations: To quantify patterns of within-population trait variation (along elevation) and among-population variation, we used linear models implemented in R 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria). We included population (NC, TN, or VA), elevation (m), and the interaction between population and elevation as fixed effects. We included population as a fixed effect because we are specifically interested in the variation in trait responses to elevation

explained by population. Separate models were built for each trait measured in the field, and traits were transformed prior to analysis as needed to increase conformance to normality. We calculated ANOVA tables using partial sums of squares, and significance was assessed at $\alpha = 0.05$ using F statistics. We calculated standardized beta coefficients for elevation based on correlations between trait and elevation z-scores.

Genetic-based quantitative trait variation in common garden: To quantify patterns of genetic-based trait differentiation along elevation and among populations, we used a maximum likelihood, mixed modeling approach implemented with the R package lme4 (Bates *et al.* 2015). Source elevation (m), source population (NC, TN, or VA), and their interaction were included as fixed effects. Because each field-sampled individual was represented by replicated cuttings in the common garden, individual identity was included as a random effect. Again, separate models were built for each trait, and traits were transformed prior to analysis as needed. Chi-square tests were performed to determine significance, assessed at $\alpha = 0.05$, for each fixed effect. Standardized beta coefficients were calculated as for field traits, described above.

Environmental contributions to trait variation: To examine the relative importance of climatic, edaphic, and topographic factors in driving significant trait variation along elevation, we used multiple regression analysis. Specifically, we built models predicting variation in each trait using MAT, AP, slope, soil N, and soil C:N as fixed effects. We analyzed traits that exhibited significant variation along elevation in field or common garden environments in the trait analyses above. If significant population x elevation effects were detected in the trait analyses (and/or an overall elevation effect was driven by a single population), we conducted analyses only for those populations exhibiting significant responses to elevation. Traits were transformed prior to analysis as needed to increase conformance to normality.

Results

Environmental gradient characterization

We detected significant effects of elevation and population, but not their interaction, on MAT and slope (Table I.2), indicating that mean values of these variables differ across sites but that sites present similar changes in MAT and slope. On average, MAT decreases 1.9°C (Fig. I.2a) and slope increases 18 degrees (Fig. I.2c) along increasing elevation across sites. We found significant main and interactive effects of population and elevation on AP and soil N (Table I.2), indicating that mean values of these variables, as well as the magnitude of their change along elevation, differ by population. Due to the significant interaction, we ran separate population-specific models for each variable (using elevation as a fixed effect). We found that AP increases on average 28, 17.2, and 13.4 cm in the NC, TN, and VA populations, respectively (Fig. I.2b). Soil N increases by 0.76% and 1.1% N with elevation in the NC and TN populations, respectively, and does not vary significantly with elevation in the VA population (Fig. I.2d). Finally, we detected a significant effect of population, but not elevation, on soil C:N (Table I.2), indicating that soil C:N differs on average across populations but does not vary significantly with elevation (Fig. I.2e).

Trait variation in natural field populations

The interaction between population and elevation was significant for internode length and shoot length (Table I.3) and approached significance for leaf dry mass ($p = 0.053$), indicating that the magnitude of response to elevation in these traits varies by population. Thus, for these traits, we ran separate models for each population (using elevation as a fixed effect) to examine how trait values varied along elevation at each location. Internode length, shoot length, and leaf dry mass significantly decreased with elevation in the NC population only ($R^2 = 0.56, 0.54, 0.33$; $F_{1,28} = 14.94, 19.77, 13.94$; $p < 0.0001, 0.0001, 0.001$; respectively), with internodes on average 3-fold shorter, shoots 5-fold shorter, and leaves 20% lighter (i.e., lower mass) at highest elevations relative to

lowest elevations (Figs. I.3b, c; 4b). We detected a significant main effect of elevation, but no elevation-by-population effect, for leaf area (Table I.3), with leaves becoming smaller with increasing elevation (Fig. I.4a). However, elevation explained relatively little variation in this trait ($R^2 = 0.06$), and we thus explored whether a single population was driving this effect. We found that leaf area decreased significantly in the NC population only ($R^2 = 0.22$, $F_{1,28} = 9.36$, $p = 0.005$), with leaves on average 14% smaller at highest elevations relative to lowest elevations. We identified no main or interactive effect of elevation on internode diameter, SLA, or leaf N content (Figs. I.3a; I.4c, d).

We found a significant effect of population on internode diameter, leaf area, and leaf dry mass (Table I.3), indicating population-level differentiation in mean phenotypic values of these traits. Individuals in the NC population had, on average, 12% and 8% smaller internode diameters, 22% and 13% less leaf area, and 22% and 17% lighter leaves than plants in the TN and VA populations, respectively (Fig. I.5a, b, c). We detected no main or interactive effect of population on SLA or leaf N content (Table I.3).

Genetic-based quantitative trait variation in common garden

Significant effects of elevation present in field populations for internode length, shoot length, leaf dry mass, and leaf area were absent in the common garden (Table I.4), indicating no genetic basis to clinal trait variation in these traits. However, we detected an overall significant, negative effect of source elevation on SLA in the common garden (Table I.4, Fig. I.4g), although source elevation explained very little variation in this trait ($R^2 = 0.02$). We found no significant main or interactive effect of source elevation on common garden values of any other trait (Table I.4; Figs. I.3, I.4).

Source population had a significant effect on leaf area and leaf dry mass (Table I.4), indicating a genetic basis to population-level differences in these traits. Trait differentiation among populations qualitatively reflected patterns we found in field populations, with common garden plants from the North Carolina population having, on average, 12% and 17% less leaf area, and 6% and 9% lighter leaves than plants from the Tennessee and Virginia populations, respectively (Fig. I.5e, f). We identified a

marginally significant effect of source population on internode diameter ($p = 0.061$), although differentiation in this trait did not qualitatively reflect patterns we observed in the field (Fig. I.5a, d). We identified no significant main or interactive effect of source population on internode length, shoot length, SLA, or leaf bud break in the common garden (Table I.4), indicating absence of genetic differentiation among populations for these traits.

Environmental contributions to trait variation

Multiple regression analysis indicated that variation in MAT alone explains significant variation in internode length ($R^2 = 0.42$, $F_{1,25} = 10.68$; $p = 0.003$) and shoot length ($R^2 = 0.45$, $F_{1,25} = 10.71$; $p = 0.003$) in the NC field population, whereas variation in soil N explains significant variation in leaf dry mass in this field population ($R^2 = 0.24$, $F_{1,25} = 4.34$; $p = 0.05$). Although we detected a significant response of leaf area to elevation in the field that appears to be driven by the NC population, the environmental variables we analyzed do not explain significant variation in this trait overall or in the NC population individually ($p > 0.05$, all variables). Similarly, no environmental variable explained significant variation in common garden SLA values ($p > 0.05$, all variables).

Discussion

Elevation gradients are commonly used to examine intraspecific trait variation in the context of climate change. However, studies have rarely examined whether environmental gradients and/or trait responses to these gradients are consistent across sites. Moreover, the relative genetic and plastic contributions to trait clines along elevation are unknown for many species. In this study, we found that traits in the dominant woody shrub *R. maximum* often did not respond to elevation and that the trait variation we did observe was typically population-specific. These findings suggest that trait responses to environmental variation in one population may not reflect a species' general response, underscoring the importance of sampling multiple locations. Finally, a

paired common garden experiment suggested that plasticity drives trait responses to environmental variation locally (i.e., along elevation within a population), while genetic divergence drives trait differentiation at coarser spatial scales (i.e., across populations).

Trait responses to elevation exist but differ across populations

In partial support of our expectations, four of seven traits exhibited “conservative” responses to increasing elevation (Cordell *et al.* 1998; Pérez-Harguindeguy *et al.* 2013) in natural *R. maximum* populations. However, these trait responses were generally restricted to the NC population, and three of seven traits exhibited no significant response to elevation, suggesting that the effect of environmental gradients along elevation can be variable and population-specific. This could be because the climatic, edaphic, and topographic gradients we examined appear to covary along elevation most consistently in the NC population. Additionally, the gradient in annual precipitation was about 63% and 109% steeper in the NC population than the VA and TN populations, respectively. Thus, plants in this population could be experiencing a relatively consistent set of environmental pressures along elevation and/or a stronger moisture gradient, leading to stronger clinal trait responses. Edaphic gradients along elevation appear to be less consistent in the VA and TN populations, which could cause a countergradient effect (Conover and Present 1990; Conover and Schultz 1995) and ‘noise’ in clinal trait responses to climatic variation.

Fine-grained spatial variation in such factors as topography, surface hydrology, canopy cover, or wind effects, can be particularly important for understory plant species (Geiger and Aron 2003; Graae *et al.* 2012; De Frenne *et al.* 2013; Lenoir *et al.* 2013; Opedal *et al.* 2015) and may account for substantial trait plasticity (Anderson and Gezon 2015). Moreover, variation in biotic interactions, including those with herbivores, pathogens, and mutualists, can strongly influence trait phenotypes (Gross *et al.* 2009; Bardgett & Wardle 2010). Thus, *R. maximum* traits in this study may be responding to a mosaic of fine-scale abiotic and biotic variation in the understory, rather than to a single, linear gradient along elevation. Such fine-grained environmental heterogeneity may

ultimately prove beneficial for understory plant species such as *R. maximum* in the context of climate change, creating micro-refugia where species might persist locally despite increasingly unfavorable conditions overall (Lenoir *et al.* 2013). Future work examining trait variation with respect to microclimatic conditions specific to the understory (i.e., understory light level, soil moisture, and temperature) as well as biotic interactions could provide further insight into the extent of potential climate change buffering for understory species like *R. maximum*.

We have shown that climatic and/or edaphic gradients can differ even across sites that occur in the same region, have similar land-use histories, and contain similar plant communities, and that these differences may lead to population-specific trait responses to elevation. However, most studies examining intraspecific plant trait responses to elevation, including 75% of those reviewed by Read *et al.* (2014), have not done so across multiple sites. Among studies that examined multiple populations, population-specific effects of elevation on at least some traits appear common (i.e., Morecroft *et al.* 1992; Byars *et al.* 2007; Premoli and Brewer 2007; Hoffmann *et al.* 2009; Gonzalo-Turpin and Hazard 2009; Fajardo and Piper 2011; Kooyers *et al.* 2015). For example, Kooyers and co-authors (2015) found that the directional effect of elevation on four of ten traits in *Mimulus guttatus* was reversed with increasing latitude due to variation in growing season length, temperature, and seasonal water availability across sites. The general lack of replication in the field, coupled with evidence from our study and others for population-specific trait responses, underscores the need for ongoing examination of the consistency, or lack thereof, of trait responses to elevation among populations.

Plasticity within, genetic divergence among sites

Consistent with our expectations, trait variation along elevation within natural populations did not persist in the common garden, indicating a lack of genetic basis to trait variation at small spatial scales. These findings corroborate previous work demonstrating extensive plasticity in foliar and growth-related traits of *R. maximum* (Nilsen 1986; Nilsen and Bao 1988) and other *Rhododendron* species (Hébert *et al.* 2011;

Niinemets *et al.* 2003) to light, moisture, or temperature manipulations. Phenotypic plasticity can be adaptive in spatially or temporally heterogeneous environments by buffering performance and enabling plants to persist through large environmental shifts and/or to colonize novel environments (Moran 1992; Ernande and Dieckmann 2004; Lind and Johansson 2007; Chevin and Lande 2010; Baythavong 2011; Hendry 2016).

Rhododendron maximum populations in the southern Appalachian region have both persisted and increased in frequency and growth following several regional forest disturbances, including heavy logging and *Castanea dentata* (American chestnut) blight (Boring *et al.* 1981; Plocher and Carvell 1987; Dobbs and Parker 2004, Elliott and Vose 2012), and, more recently, non-native insect invasion causing widespread mortality of *Tsuga canadensis* (eastern hemlock; Ford *et al.* 2012). Thus, it is possible that *R. maximum* populations in this region have evolved increased plasticity in response to past environmental heterogeneity and frequent, severe disturbance events. Regardless of its evolutionary source, however, the phenotypic plasticity we observed along elevation will likely enable *R. maximum* to persist through future temporal climate change.

The most likely explanation for the lack of genetic differentiation within populations along elevation may be high gene flow (Kawecki & Ebert 2004).

Rhododendron maximum seeds are wind-mediated or passively dispersed (Hille Ris Lambers *et al.* 2005), and pollination is accomplished primarily by bees (Romancier 1971). Thus, assuming no phenological barriers to reproduction, these two processes may generate sufficient gene flow within populations to effectively hinder adaptive genetic divergence from occurring along environmental gradients associated with elevation (Savolainen *et al.* 2007; Kremer *et al.* 2014).

While we found little evidence for genetic differentiation within populations, results from the common garden suggest that populations may be genetically differentiated from one another in at least two traits (leaf area and leaf dry mass). These findings are broadly consistent with theoretical expectations that genetic differentiation should occur with increasing geographic distance between populations, as the result of increasing environmental differentiation and/or genetic isolation (Kawecki & Ebert 2004;

Baythavong 2011). Specifically, the NC population appears to be most differentiated from the VA and TN populations, suggesting that the former population may be locally adapted to a unique set of climatic (i.e., steep precipitation gradient) and edaphic conditions. Future work involving reciprocal transplants both within (from high to low elevation and *vice versa*) and among populations could elucidate the relative importance of local adaptation and/or adaptive plasticity at varying spatial scales in this species (Kawecki & Ebert 2004; Blanquart *et al.* 2013). Additionally, molecular analyses to characterize the genetic makeup of these populations could complement the current study by providing further insight into the various evolutionary processes (i.e., gene flow, drift, selection, or plasticity) underlying trait variation in this system.

Conclusions

Our findings suggest that multiple populations within a species may not exhibit a single, universal response to climatic variation, highlighting the importance of sampling multiple populations to avoid over- or underestimating the breadth of possible trait responses. Further, our results indicate phenotypic plasticity will likely play an important role in allowing *R. maximum* to persist locally despite a changing climate. Ongoing work to identify the relative roles of climatic, edaphic, and biotic environments driving understory plant trait variation could provide insight into why trait patterns do or do not exist along environmental gradients and elucidate the extent to which plants will respond to, or be buffered from, global climate change. Finally, we suggest that a deeper level of sophistication in elevation studies, including replicated observations across multiple populations, will allow us to continue drawing robust climate change inferences from natural spatial gradients.

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Appendix

Table I.1. Site characteristics for each of three *Rhododendron maximum* populations sampled in this study, including the number of *R. maximum* individuals sampled ($N_{\text{individual}}$), gradient aspect, the range of elevation, and the soil taxonomic classes present along each gradient.

Soil taxonomic class was extracted from the USDA-NRCS Web Soil Survey database (<http://websoilsurvey.nrcs.usda.gov/app/>).

<i>Transect</i>	<i>N_{individual}</i>	<i>Aspect</i>	<i>Elevation (m)</i>	<i>Soil taxonomic class</i>
North Carolina	30	N	895-1584	Humic Dystrudepts, Humic Hapludults, Oxyaquic Humedepts, Typic Dystrudepts, Typic Humedepts
Tennessee	30	NE	842-1467	Humic Dystrudepts, Lithic Humedepts, Typic Dystrudepts, Typic Hapludults
Virginia	30	NE	816-1250	Fluventic Dystrudepts, Typic Hapludults

Table I.2. Statistics for linear models incorporating elevation (Elev), population (Pop), and their interaction as fixed effects, predicting variation in five climatic, edaphic, and topographic variables (mean annual temperature (MAT), annual precipitation (AP), topographical slope (Slope), soil nitrogen (N) content, and soil carbon:nitrogen (C:N) ratio) along elevation in three natural *R. maximum* populations.

Variation in these environmental variables was examined for potential importance on elevational trait variation. F-values (*F*) and associated degrees of freedom are displayed for each effect; standardized beta coefficients (β) and standard error (*SE*) are shown for elevation. Beta coefficients of elevation are not shown for those traits on which a significant population x elevation interaction effect was detected; see Results section for population-specific effects of elevation on these variables. Significant ($\alpha < 0.05$) F-values are shown in bold; statistical significance is denoted by the following: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

<i>Environmental Variable</i>	<i>Effect</i>				
	<i>Pop</i>	<i>Elev</i>			
	<i>F</i> _{5,84}	β	<i>SE</i>	<i>F</i> _{5,84}	<i>F</i> _{5,84}
MAT (°C)	125.11****	-1.04	0.04	1329.16****	0.23
AP (cm)	1052.82****	-	-	1186.66****	5.18**
Slope (degrees)	4.65*	0.36	0.15	25.30****	0.54
Soil N content (%)	7.37**	-	-	23.03****	4.20*
Soil C:N ratio	3.57*	0.25	0.16	0.34	2.82

Table I.3. Statistics for linear models, incorporating population (Pop), elevation (Elev), and their interaction as fixed effects, of seven functional trait values along elevation at three field locations containing sampled *Rhododendron maximum* populations ($N = 90$ sampled *R. maximum* individuals).

F-values (F) and associated degrees of freedom (DF) are displayed for each effect; standardized beta coefficients (β) and standard error (SE) are shown for elevation. Beta coefficients of elevation are not shown for those traits on which a significant population x elevation interaction effect was detected; see Results section for population-specific effects of elevation for these traits. Significant ($p < 0.05$) F-values are shown in bold; level of statistical significance is denoted by the following: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), **** ($p < 0.0001$).

<i>Field trait</i>	<i>Effect</i>				
	<i>Pop</i>	<i>Elev</i>			<i>Pop*Elev</i>
	<i>F</i>_{2,84}	β	<i>SE</i>	<i>F</i>_{1,84}	<i>F</i>_{2,84}
Internode diameter (mm)	10.21***	-0.23	0.15	1.39	0.26
Internode length (cm)	2.21	-	-	17.11***	6.59**
Shoot length (cm)	0.02	-	-	16.49***	7.09**
Leaf area (mm ²)	16.07***	-0.41	0.14	9.79**	1.15
Leaf dry mass (mg)	12.51***	-0.42	0.14	3.63	3.05
Specific leaf area (mm ² /mg)	2.11	0.02	0.17	0.96	0.57
Leaf nitrogen content (%)	2.34	-0.09	0.17	0.13	0.50

Table I.4. Statistics for linear mixed effects models, incorporating population of origin (Pop), elevation of origin (Elev), and their interaction as fixed effects, and individual identity (i.e., sampled individual in field population) as a random effect, of seven functional trait values measured on 16-month old *Rhododendron maximum* cuttings in a common garden.

Sample size (N , number of cuttings measured) for each trait is shown. Chi-square (X^2) values and associated degrees of freedom are shown for each effect; standardized beta coefficients (β) and standard error (SE) are shown for elevation. Significant ($p < 0.05$) F-values are shown in bold; level of statistical significance is denoted by the following: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), **** ($p < 0.0001$).

Common garden trait	N	Effect				
		Pop $X^2_{DF=2}$	Elev β	SE	$X^2_{DF=1}$	Pop*Elev $X^2_{DF=2}$
Internode diameter (mm)	153	5.30	0.01	0.14	2.03	1.32
Internode length (cm)	150	1.93	-0.38	0.14	1.84	4.38
Shoot length (cm)	323	1.08	-0.14	0.13	0.48	0.52
Leaf area (mm ²)	317	8.53*	-0.10	0.13	0.09	3.20
Leaf dry mass (mg)	317	6.81*	-0.04	0.13	0.18	2.22
Specific leaf area (mm ² /mg)	314	0.28	-0.15	0.12	5.78*	0.03
Bud break (Julian day)	315	1.588	-0.07	0.13	0.830	0.486

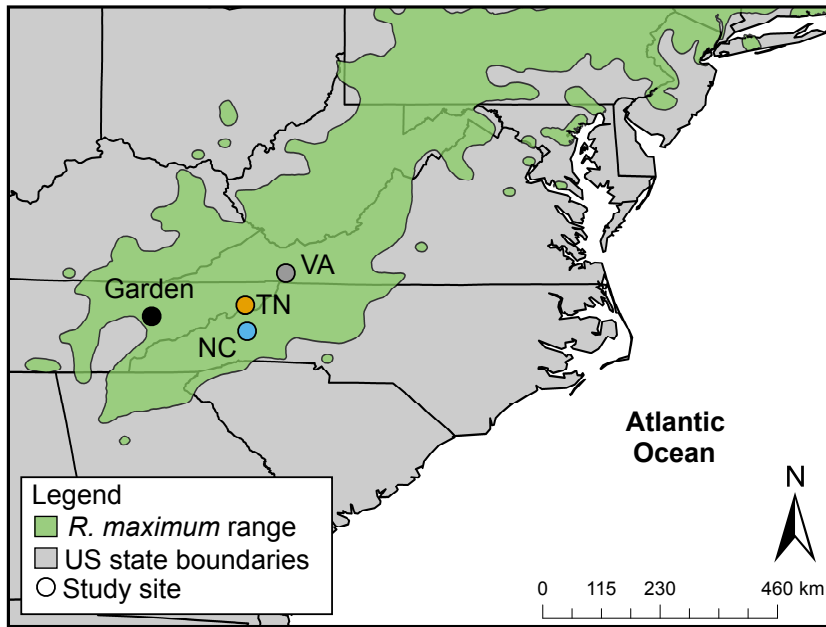


Figure I.1. *Rhododendron maximum* elevation gradient and common garden sites are situated throughout the South Central Appalachian region of the *R. maximum* range (shown in green).

Locations of the North Carolina (NC, blue symbol), Tennessee (TN, yellow symbol), and Virginia (VA, grey symbol) field sites and the University of Tennessee common garden (Garden, black symbol) are shown.

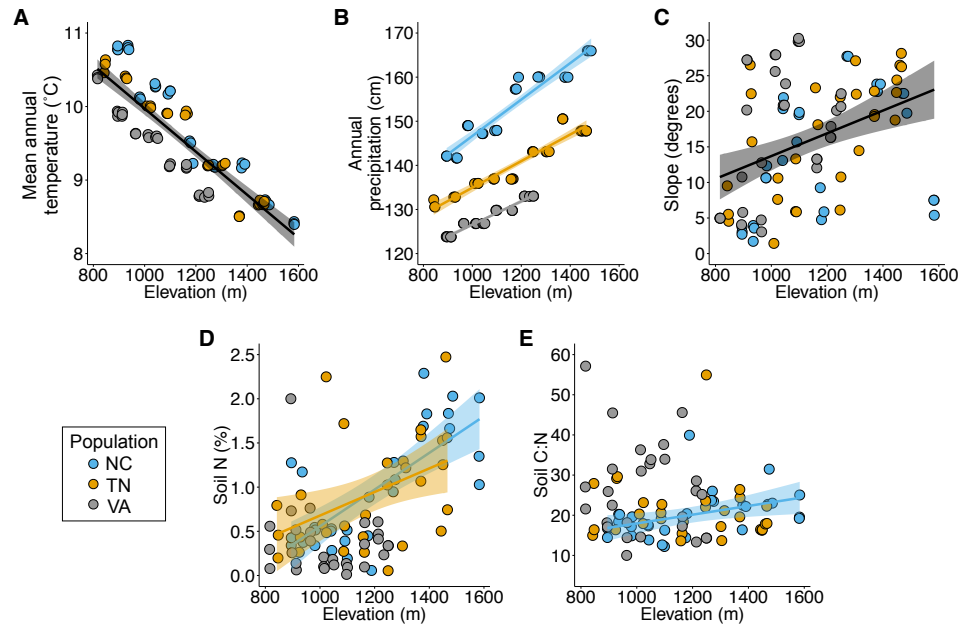


Figure I.2. Variation in five environmental variables along elevation in three *Rhododendron maximum* populations (North Carolina [NC]; Tennessee [TN]; Virginia [VA]).

Mean annual temperature (panel A) and topographic slope (panel C) consistently decrease and increase, respectively, along elevation in each population; annual precipitation (panel B) and soil nitrogen (N) (panel D) increase at different rates across the three populations; and soil carbon:nitrogen ratio (C:N) (panel E) does not vary linearly with elevation. A solid black line indicates a significant ($p < 0.05$) effect of elevation across populations (no population-by-elevation interaction). When statistical models detected significant or marginally significant population-by-elevation interactions, colored regression lines corresponding only to those populations in which traits varied significantly ($p < 0.05$) along elevation are shown (NC, blue; TN, yellow; VA, grey). The shaded region around each line represents the 95% confidence interval for that regression.

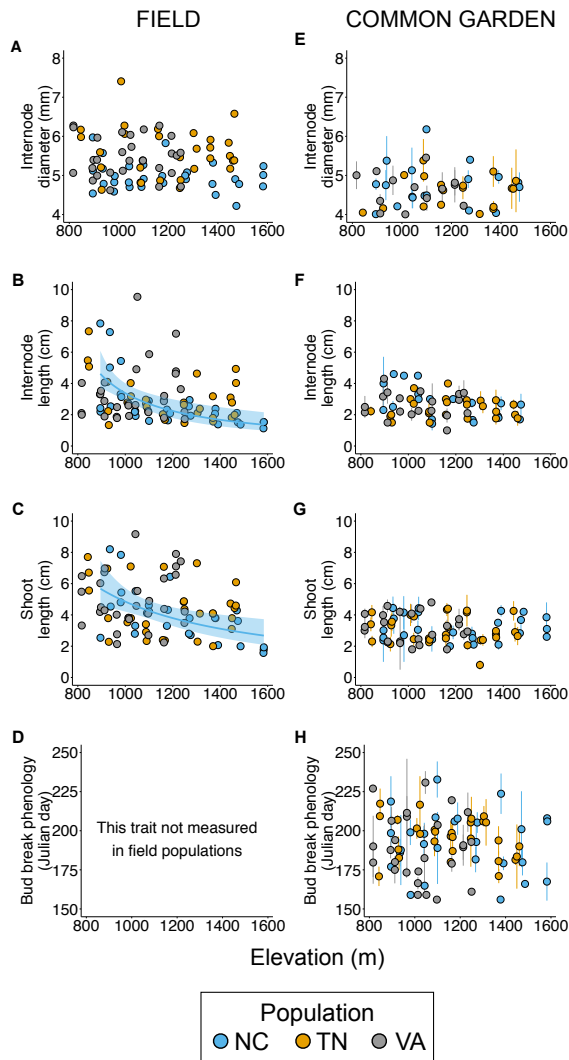


Figure I.3. The effect of elevation on traits related to growth and timing (internode diameter, internode length, shoot length, leaf bud break phenology) in *Rhododendron maximum* measured at the individual level in natural field populations along three elevation gradients (A-D) and the effect of source elevation on mean $\pm$ SE trait values measured at the replicated cutting level in a common garden (E-H).

Populations are represented by each of three colors (North Carolina, blue; Tennessee, yellow; Virginia, grey). A solid black line indicates a significant ($p < 0.05$) effect of elevation across populations (no population-by-elevation interaction). When statistical models detected significant or marginally significant population-by-elevation interactions, colored regression lines corresponding only to those populations in which traits varied significantly ($p < 0.05$) along elevation are shown. The shaded region around each line represents the 95% confident interval for that regression.

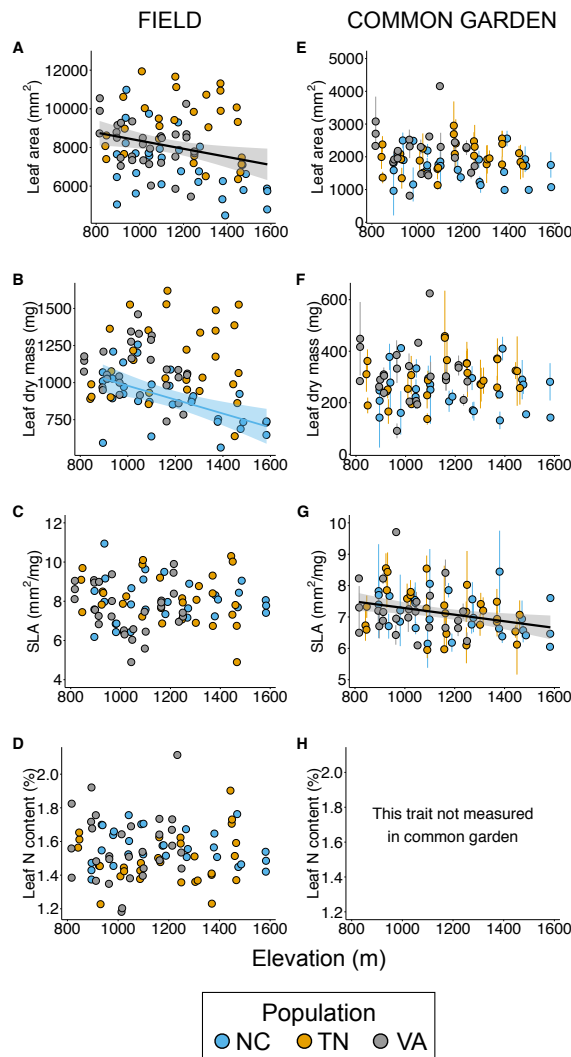


Figure I.4. The effect of elevation on leaf-level traits (leaf area, leaf dry mass, specific leaf area [SLA], leaf nitrogen [N] content) in *Rhododendron maximum* measured at the individual level in natural field populations along three elevation gradients (A-D) and the effect of source elevation on mean $\pm$ SE trait values measured at the replicated cutting level in a common garden (E-H) Populations are represented by each of three colors (North Carolina, blue; Tennessee, yellow; Virginia, grey). A solid black line indicates a significant ($p < 0.05$) effect of elevation across populations (no population-by-elevation interaction). When statistical models detected significant or marginally significant population-by-elevation interactions, colored regression lines corresponding only to those populations in which traits varied significantly ($p < 0.05$) along elevation are shown. The shaded region around each line represents the 95% confident interval for that regression.

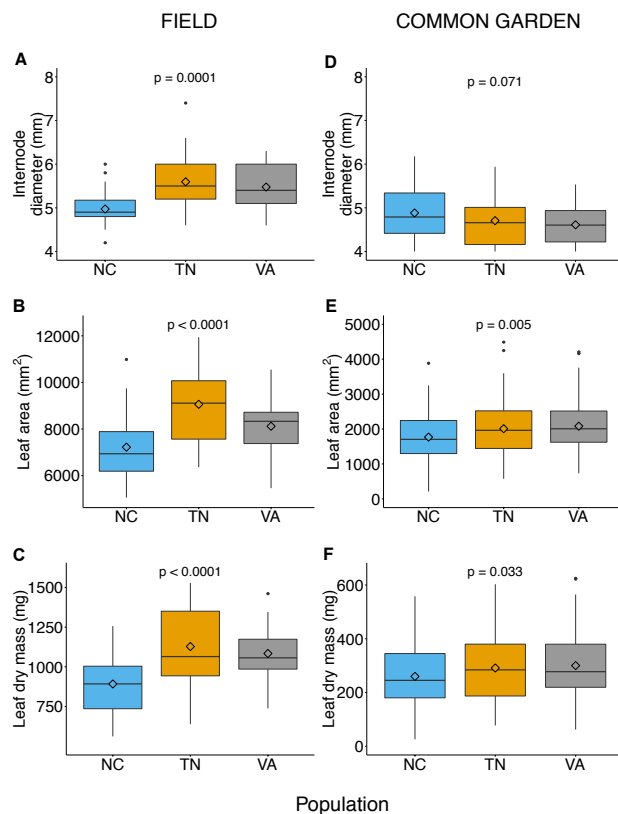


Figure I.5. Population means of three traits (internode diameter, leaf area, and leaf dry mass) differ across *Rhododendron maximum* populations (North Carolina, NC; Tennessee, TN; Virginia, VA) in both field (A-C) and common garden environments (D-F).

Bold horizontal lines within each box represent the median of the data; boxes extend to the upper and lower quartile; whiskers extend from each box to the minimum and maximum values of the data, excluding outliers; outliers are shown by black points above or below whiskers. Within each plot, significance of the effect of population is shown.

CHAPTER II:
PLANT-SOIL FEEDBACKS MEDIATE SHRUB EXPANSION IN
DECLINING FORESTS, BUT ONLY IN THE RIGHT LIGHT

This chapter was written as a manuscript for publication and can be cited as:

Pfennigwerth, A.A., Van Nuland, M., Bailey, J.K., and Schweitzer, J.A. Plant soil-feedbacks mediate shrub expansion in declining forests, but only in the right light. *In revision.*

Throughout the manuscript the term “we” refers to myself and the coauthors listed above. My primary contributions to this manuscript include (1) selection of topic and design of experimental set-up, (2) identification of study locations, (3) collection of plant and soil samples in field and greenhouse 2013-2016, (4) analysis of data, (5) writing the manuscript. M.V.N. managed the fungal OTU pipeline. J.A.S. and J.K.B. advised in the development of the research, and M.V.N., J.A.S., and J.K.B. offered revisions to the manuscript.

Abstract

1. Contemporary global change, including the widespread mortality of foundation tree species, is altering ecosystems and plant communities at unprecedented rates. Plant-soil interactions drive myriad community dynamics, and we hypothesized such interactions may be an important driver of succession following the loss of foundation tree species.
2. We examined whether plant-soil biota interactions, in the context of a putatively important light gradient associated with foundation tree decline, mediate the expansion of *Rhododendron maximum* in southeastern US forests where *Tsuga canadensis* (eastern hemlock), a dominant foundation tree species, is in decline. Using an 11-month, controlled inoculation experiment paired with Illumina sequencing, we tested the following hypotheses: (1) Relative to conspecific (*R. maximum*-conditioned) soils, *R. maximum* seedlings have higher performance in soils conditioned by *T. canadensis* and lower performance in interspace soils (conditioned by neither *T. canadensis* nor *R. maximum*) due to variation in soil fungal biota, and (2) responses in plant performance to soil inoculation differ in high and low light (matching environments under infested and uninfested *T. canadensis* crown, respectively).
3. Supporting our first hypothesis, we found that *R. maximum* seedling performance was highest in *T. canadensis*-conditioned soils, medial in *R. maximum*-conditioned soils, and lowest in interspace soils. Mechanistically, soils conditioned by *T. canadensis* and *R.*

maximum had more mycorrhizal fungi, less saprotrophic fungi, and were less species-rich than interspace soils, and variation in these community traits predicted substantial variation in *R. maximum* seedling biomass. However, in support of our second hypothesis, soil effects on plant performance were evident in high light only; in low light, soil inoculation did not affect plant performance and plants performed worse on average.

4. Synthesis. Our findings suggest interactions with soil biota act synergistically with altered abiotic environments to mediate species responses to widespread foundation tree mortality, providing evidence for a novel mechanism of plant response to major disturbance. Examining plant-soil interactions in the context of relevant abiotic gradients can therefore enhance our understanding, predictions, and management of community development processes following major forest disturbance.

Introduction

Anthropogenic disturbance is a major force structuring plant communities (Kareiva, Kingsolver & Huey 1993; Sala *et al.* 2000; Tylianakis *et al.* 2008) and is now occurring across the globe at a faster rate and larger scale than ever before (MEA 2005). One important form of contemporary disturbance is the global decline of foundation tree species, which is often driven by the introduction of non-native pests and pathogens (Liebhold *et al.* 1995; Simberloff 2000; Lovett *et al.* 2006; Brasier 2008). These non-native introductions pose one of the greatest threats to forests worldwide, damaging nearly 35 million hectares of forested land each year and often fundamentally altering community composition and ecosystem function (FAO 2010). However, despite their widespread nature and profound consequences, we lack a strong understanding of the specific mechanisms driving plant community responses to these widespread mortality events, which in turn limits our ability to predict, plan for, and manage future forest structure and function following major disturbance events.

More than two decades of research suggests that interactions between plants and soil biota can strongly alter plant community development (Van der Putten, Van Dijk & Peters 1993; De Deyn *et al.* 2003; Kardol, Bezemer & Van der Putten 2006) and

community responses to global change (Ehrenfeld, Ravit & Elgersma 2005; Bardgett & Wardle 2010; Van der Putten *et al.* 2016); however, the role of plant-soil interactions remains largely unexplored in systems experiencing disturbances that lead to the loss of foundation species. Plants, through variation in traits, impact soil biota as well as physical and chemical properties that can lead to unique communities of beneficial and pathogenic soil biota associated with individual plants (Bever 1994; Ehrenfeld, Ravit & Elgersma 2005; Van Nuland *et al.* 2016). These soil communities can in turn differentially alter the performance of that or other plant species growing in that soil, creating a plant-soil feedback (Bever 2002; Klironomis 2002; Batten, Scow & Espeland 2008; Van der Putten *et al.* 2013). Feedback effects can persist as soil legacies, influencing plant performance years and even centuries after the causal plant species has been removed (Kardol *et al.* 2007; Grman & Suding 2010; Kulmatiski & Beard 2011; Hamman & Hawkes 2013). The role of plant-soil feedbacks and soil legacies in plant community succession has been repeatedly demonstrated (i.e., De Deyn *et al.* 2003; Kardol, Bezemer & Van der Putten 2006; Kardol *et al.* 2007; Kulmatiski *et al.* 2008; Bauer, Mack & Bever 2015), yet this research has focused largely on succession in open systems, namely ex-arable fields, grasslands, and heathlands, and rarely in forested systems (but see Packer & Clay 2003; Rudgers *et al.* 2007). Moreover, the role of plant-soil feedbacks in succession following the loss of a single, dominant species has not been explored in any system to our knowledge (Van der Putten *et al.* 2016).

Although plant-soil interactions contribute to plant community dynamics, net outcomes of plant-soil feedbacks may depend on environmental context (Wardle *et al.* 2004; Van der Putten *et al.* 2016). For example, plant-soil feedbacks in forested ecosystems can be affected by light environment (McCarthy-Neumann & Ibáñez 2012). Light environment may influence net plant-soil feedback effects by altering soil moisture, nutrient, or pathogen levels and/or by causing plants to alter carbon allocation to mutualists or to defensive compound production (Smith & Reynolds 2015). With few exceptions, however (Hoeksema *et al.* 2010; McCarthy-Neumann & Ibáñez 2012, 2013; Smith & Reynolds 2015), the context-dependency of plant-soil feedbacks across

biologically relevant abiotic gradients and the implications of such context-dependency for plant community development remains, in general, poorly understood (Van der Putten *et al.* 2016).

Here, we conduct a controlled soil inoculation experiment to assess the role of biotic soil legacy effects along with concurrent changes in light environment on plant responses to foundation tree mortality. We use the widespread mortality of one of the most dominant foundation tree species in eastern North American forests, *Tsuga canadensis* (L.) Carr. (Pinaceae) (eastern hemlock), owing to the invasion of a non-native pest, *Adelges tsugae* Annand (hemlock woolly adelgid), as a model system to address this question. Since its initial introduction to the US about 65 years ago (Stoetzel 2002), the hemlock woolly adelgid has invaded at least 45% of the *T. canadensis* range (Morin *et al.* 2011). Following *T. canadensis* mortality in the southern portion of its range, the native woody shrub *Rhododendron maximum* L. (rosebay rhododendron) increases in growth and dominance up to 2.6-fold (Ford *et al.* 2012) and is expected to be the primary species to replace *T. canadensis* when present (Ellison *et al.* 2005; Ford & Vose 2007; Elliott & Vose 2010; Spaulding & Rieske 2010; Ford *et al.* 2012; Krapfl, Holzmüller & Jenkins 2012; Brantley, Ford & Vose 2013). At least two mechanisms may explain this expansion. First, *R. maximum* may capitalize on *T. canadensis* soil legacies in the form of beneficial fungal (including mycorrhizal) communities. Alternatively, the species may capitalize on increased light availability following *T. canadensis* crown defoliation (Elliott & Vose 2012; Ford *et al.* 2012). To the best of our knowledge, neither mechanism, nor potential interactions between the two, have been empirically assessed.

In this study, we address the following hypotheses: (1) relative to conspecific, *R. maximum*-conditioned soils, *R. maximum* seedling performance is enhanced in *T. canadensis*-conditioned soils and reduced in interspace soils (outside the influence of both *R. maximum* and *T. canadensis*) due to variation in soil fungal biota, and (2) growth responses to soil inoculation varies with light environment. To test our hypotheses, we (1) assessed *R. maximum* seedling growth and biomass after 11 months of growth in soils inoculated with either *T. canadensis*-conditioned, *R. maximum*-conditioned, or interspace

soils; (2) sequenced and compared soil fungal communities from each soil source and examined correlations between fungal community traits and seedling biomass; and (3) assessed the effect of light environment by replicating inoculation treatments in low and high light conditions matching healthy and dying *T. canadensis* canopies, respectively. To the best of our knowledge, our study is the first to examine the role of plant-soil feedbacks in the context of a putatively important abiotic gradient for plant community development following foundation species mortality.

Materials and Methods

Study system

Tsuga canadensis, one of the most historically dominant foundation tree species in eastern North American forests (historical range ~228 million ha), is in decline across its range because of the invasion of the non-native hemlock woolly adelgid. The adelgid was first detected in the US in 1951 (Stoetzel 2002) and, as of 2010, has invaded 45% of the *T. canadensis* range (Morin *et al.* 2011). *Tsuga canadensis* has no known resistance to the adelgid, and individuals typically die within 4-15 years of infestation (Ellison *et al.* 2005; Orwig, Foster & Mausel 2002). Recent research indicates that, following *T. canadensis* mortality in the southern portion of its range, *R. maximum*, when present, increases in growth and dominance up to 2.6-fold (Ford *et al.* 2012).

Rhododendron maximum is a native evergreen woody shrub that occurs in forest understories throughout eastern North America (Little 1977; Wofford 1989; Gleason & Cronquist 1991). It is particularly abundant in the Southern Appalachian Mountains (Woods & Shanks 1959; Ford *et al.* 2012) where it commonly exceeds densities of 5,000 stems/ha (Clinton, Boring & Swank 1994). In addition to its contemporary expansion in declining *T. canadensis* forests (Ford *et al.* 2012), *R. maximum* has expanded in response to previous disturbances including the blight-induced mortality of the once dominant *Castanea dentata* (American chestnut), extensive logging, and possibly fire suppression (McGee & Smith 1967; Plocher & Carvell 1987; Baker & Van Lear 1998; Dobbs & Parker 2004; Elliott & Vose 2012).

Rhododendron maximum expansion in declining *T. canadensis* forests may be driven in part by one or a combination of the following: (1) the ability to capitalize on biotic soil legacies or (2) the ability to capitalize on increased light availability resulting from *T. canadensis* defoliation. First, by associating with mutualistic ericoid mycorrhizal (ERM) fungi (Wurzburger & Hendrick 2007, 2009) that are highly saprotrophic and aid in survival under harsh environmental conditions (Johnson *et al.* 2013), *R. maximum* can effectively extract nutrients from the extremely acidic, nutrient-poor, organic soils characteristic of both *R. maximum* thickets (Jaiswal, Jayasinghe & Kuhnert 2012) and *T. canadensis* stands (Jenkins, Aber & Canham 1999; Ellison *et al.* 2005). Importantly, while *R. maximum* is thought to form exclusive ERM associations (Wurzburger & Hendrick 2007, 2009), *T. canadensis* is thought to associate exclusively with ectomycorrhizal (ECM) fungi (O'Brien, Gomola & Horton 2011). However, recent molecular evidence shows that many fungi commonly classified as ECM can be isolated from the roots of ERM host plants, and *vice versa* (Bougoure *et al.* 2007; Collier & Bidartondo 2009; Grelet *et al.* 2010; Villarreal-Ruiz *et al.* 2012), raising the possibility that *R. maximum* forms mutualisms with *T. canadensis*-conditioned ECM communities during expansion. Alternatively, despite its shade-tolerance, *R. maximum* may require canopy openings to spread (Plocher & Carvell 1987). Expansion may therefore be driven in part by positive plant-soil feedbacks in *T. canadensis* soils, by increased light availability following *T. canadensis* crown defoliation (Elliott & Vose 2012; Ford *et al.* 2012), or by a combination of both mechanisms.

Greenhouse inoculation experiment

To assess our first hypothesis regarding the role of biotic soil legacies in promoting *R. maximum* expansion, we grew *R. maximum* seedlings in three types of soil inoculation, quantifying the fungal communities from each of these soils at experiment's end to determine differences in fungal associations. The three soil treatments were: (1) *T. canadensis*-conditioned inoculum, (2) *R. maximum*-conditioned inoculum, and (3) interspace inoculum (Fig. II.6c, all tables and figures are located in the appendix). These

three biotic soil environments simulate, respectively: (1) directional expansion into declining *T. canadensis* stands, (2) no expansion; i.e., persistence in current location, or (3) random/non-directional expansion into areas occupied by neither *T. canadensis* nor *R. maximum* (Fig. II.6b). To examine our second hypothesis regarding the role of altered light environments, we replicated all soil inoculation combinations in low and high light conditions, representative of healthy, uninfested *T. canadensis* crowns or *T. canadensis* crowns with severe adelgid-induced dieback, respectively (Fig. II.6c).

We collected soils and *R. maximum* seeds from three locations in the southern portion of the *T. canadensis* range in southeastern USA (Fig. II.6a): George Washington and Jefferson National Forest, Virginia (36.6900°N, 81.6364°W); Cherokee National Forest, Tennessee (36.1413°N, 82.2756°W); and Pisgah National Forest, North Carolina (35.7230°N, 82.2461°W). To capture environmental and plant genetic variation that could potentially cause variation in plant-soil feedbacks, we sourced soils and *R. maximum* seeds from ten sampling sites across an approximately 580-m north-facing elevational gradient at each of the three locations. Soils at these locations consist of Dystrudepts, Hapludults, and Humudepts. Each gradient encompasses, on average, +2°C change in mean annual temperature, and about -20 cm change in annual precipitation from high to low elevation.

In October 2013, we collected mature seedpods from 10 *R. maximum* individuals (i.e., seed families), each approximately 50 m in elevation apart, along each elevational gradient ($N = 10 \text{ individuals} \times 3 \text{ gradients} = 30 \text{ seed families}$). Seedpods were transported to the lab and stored at 0°C (Olson 1974). In January 2014, seeds were carefully harvested from dry seedpods, sown, and germinated in flats containing a sterile 50:50 mixture of peat moss and vermiculite, and watered continuously from below. After seedling establishment (May 2014), similar-sized individuals were transplanted in sets of three into Roottrainer pots (Stuewe and Sons, Inc., Tangent, OR, USA) in a randomized block design. After 2 weeks, seedlings were thinned to one plant per pot. During May-August 2014, seedlings were given biweekly 200 ppm treatments of fertilizer (N:P:K ratio of 21:7:7; Peters Professional® Acid Special, Everris NA Inc., Dublin, OH, USA).

Fertilizer treatments were terminated 2 weeks before soils were inoculated with experimental soil communities.

During the third week of July 2014, we revisited the three field sites and collected soils at locations corresponding to sampled *R. maximum* seed families ($N = 30$ sites). At each location, we collected soil samples from three adjacent locations ≥ 5 m apart: (1) beneath a declining or dead *T. canadensis* individual; (2) beneath a paired *R. maximum* individual; and (3) a paired interspace forest soil location ($N = 30$ sites $\times$ 3 soil sources = 90 unique soil samples; Fig. II.6b). Declining or dead *T. canadensis* individuals were trees determined to have crown defoliation of 50-100% based on visual estimates of percentage of defoliation (to the nearest 10%) of each sampled tree. To avoid any potential influence of focal species, interspace sampling locations were ≥ 5 m from the nearest *T. canadensis* and *R. maximum* individuals and were typically sparsely occupied by graminoid or forb species, which associate with arbuscular mycorrhizal fungi (Read 1991). For each soil sample, we collected and homogenized three 2×10 cm soil cores in plastic zipper bags. We immediately transported and stored samples in the laboratory at 0°C . Each soil sample was kept as an un-pooled inoculum source at the seed family ($N = 30$) and soil source ($N = 3$; *T. canadensis*-conditioned, *R. maximum*-conditioned, or interspace) level to maintain a one-to-one correspondence between field units and greenhouse containers and to conserve site-to-site soil biota variation (Reinhart & Rinella 2016).

During the first week of September 2014, *R. maximum* seedlings were transplanted into 13×9 cm pots containing a sterile 50:50 mixture of peat moss and vermiculite (filled to 75% maximum capacity) and inoculated with 1 teaspoon (5 cm^3) of either *T. canadensis*-conditioned, *R. maximum*-conditioned, or interspace soil collected at the corresponding seed family location. Because the sterile peat moss/vermiculite background soil represented $\sim 99\%$ total soil volume in each pot, potential abiotic effects of the soil inocula (see Fig. S1 in Attachment 1) are minimized (Troelstra *et al.* 2001; Kardol, Bezemer & Van der Putten 2006; Carvalho *et al.* 2010). Four replicates of each seed family $\times$ soil source combination were then placed in a high light treatment and four

in a low light treatment. The low light treatment was achieved by draping 80% shade cloth over a 0.6-m tall PVC frame built on top of the greenhouse bench; the high light treatment received ambient light. The low light environment matches the environment under a healthy, uninfested hemlock canopy and received on average $35 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR) while the high light treatment matches the environment under a severely declining or dead hemlock canopy and received on average $575 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (Canham *et al.* 1994; Hadley 2000). Plants were watered as needed (~4 days/week) and allowed to grow for 11 months in a glasshouse at the University of Tennessee, Knoxville, TN, USA. Total sample size was 720 pots (30 seed families $\times$ 3 soil sources $\times$ 2 light environments $\times$ 4 replicates; Fig. II.6).

Plant data collection

To examine potential soil or light effects on *R. maximum* phenology, we recorded date of leaf bud break (flushing) for each seedling throughout spring 2015, following winter dormancy. We considered date of leaf bud break to be the earliest day on which leaf bud scales opened, revealing a new leaf. We collected these data every other day until all plants had flushed new leaves.

After 11 months of growth in experimental conditions, we destructively harvested plants to measure biomass and specific leaf area (SLA). We collected two leaves per plant for SLA and collected the remaining shoot biomass by clipping each plant stem at the soil level. Loose soil was shaken from roots, then roots were washed with water over 2 mm, then 0.5 mm sieves to remove all soil while retaining fine roots. Leaves were stored at 0°C and scanned to calculate total fresh leaf area (WinFOLIA 2011a, Regent Instruments, Canada), oven-dried at 70°C for 48 h, then weighed for dry leaf mass. Specific leaf area was calculated by dividing fresh leaf area by dry leaf mass and averaged at the individual level. Aboveground (shoot) and belowground (root) biomass components were oven-dried at 70°C for 48 hours then weighed. To obtain total shoot biomass, we added total dry leaf mass and remaining shoot biomass; to obtain total plant biomass, we summed total shoot and root biomass. Finally, to assess potential differences

in biomass allocation, we calculated root:shoot ratio by dividing root biomass by shoot biomass.

Plant-soil feedback calculations

To visualize how expansion into (1) *T. canadensis*-conditioned ‘away’ soils versus (2) interspace ‘away’ soils affects *R. maximum* performance relative to persistence in *R. maximum*-conditioned ‘home’ soils, we calculated two pairwise plant-soil feedback ratios. Because we are particularly interested in visualizing differences in the effects of two away-soil environments on plant performance, we use an “away vs. home” feedback approach rather than the more conventional “home vs. away” approach (Bever 1994), such that positive and negative feedback values correspond to net advantages and disadvantages, respectively, in away soils (*T. canadensis*-conditioned or interspace) relative to ‘home’ soils. We calculated the first feedback (hereafter, ‘Hem:Rhodo feedback’) as $\ln(\text{biomass of plants in } T. \text{ canadensis-conditioned soil} / \text{biomass in } R. \text{ maximum-conditioned soil})$, which represents the change in performance when *R. maximum* moves from home soils to *T. canadensis* away soils. We calculated the second feedback (hereafter ‘Inter:Rhodo feedback’) as $\ln(\text{biomass of plants in interspace soil} / \text{biomass in } R. \text{ maximum-conditioned soil})$, which represents the change in performance when *R. maximum* moves from home soils to interspace away soils. Log-transformation provides feedback scores that are symmetrical around zero (i.e., the no-effect point or neutral feedback; Brinkman *et al.* 2010), providing an unbiased visualization of the relative effects of inoculation. Due to random mortality during the experiment, we averaged biomass of surviving seedlings at the seed family $\times$ soil source $\times$ light environment level prior to calculating each feedback.

Soil fungal sequencing and bioinformatics

To assess soil biotic differences and determine whether *R. maximum* seedlings experienced unique fungal communities in each soil inoculation under the experimental growing conditions, we sequenced fungal DNA from a subsample of experimental soils

from the high light treatment. We collected a ~5 mL soil subsample from each pot during destructive harvest, pooled replicates at the seed family $\times$ soil source $\times$ light environment level, and immediately froze all pooled samples at -20°C. We then extracted DNA from paired *T. canadensis*, *R. maximum*, and interspace soils from each of 16 randomly selected seed families ($N = 3$ soil sources $\times$ 16 seed families = 48 DNA samples) per the manufacturer's protocol (PowerSoil® DNA Isolation Kit, MO BIO Laboratories, Carlsbad, CA, USA). We immediately froze samples at -20°C, verified DNA amplification via PCR on a subsample of each extraction, then shipped remaining frozen DNA extractions to Northern Arizona University for Illumina sequencing.

Fungal amplicons were generated in a two-step process (Berry *et al.* 2011) for the ITS2 region using 5.8s_Fun/ITS4_Fun primers. We processed the resultant ITS amplicon data using akutils (<https://github.com/alk224/akutils-v1.2>), which includes modifications to a QIIME 1.9.1 workflow (Caporaso *et al.* 2010). Such modifications are necessary since it has been shown that default QIIME settings can significantly overestimate microbial diversity (Krohn *et al.* 2016). Prior to sample processing, we trimmed primers using the “akutils strip_primers” command, and identified and removed PhiX Control using the “akutils phix_filtering” command (Krohn 2016). We then joined paired end reads with the “akutils join_paired_reads” command, which uses fastq-join from ea-utils (Aronesty 2011). Because the ITS region is a size-polymorphic locus, we trimmed the raw joined data to a uniform 220 bp before demultiplexing. The trimmed joined data was demultiplexed with the “split_libraries_fastq.py” script in QIIME and underwent stricter minimum quality thresholds of q20 ($q = 19$), 0-3 low quality base calls allowed ($r = 1-3$), and each read required to be at least 95% high quality ($p = 0.95$) (Krohn *et al.* 2016). We used vsearch 1.1.1 (Rognes *et al.* 2015) to remove chimeras against the Gold database (<http://drive5.com/uchime/gold.fa>). Demultiplexing and quality filtering resulted in a total of 404,701 high-quality reads (average per sample = $7,494 \pm 234$ SE). We used the “akutils pick_otus” command for operational taxonomic unit (OTU) picking and taxonomy assignment ($N = 63$ unique fungal OTUs). Specifically, we used the prefix_suffix OTU picker in QIIME to dereplicate sequences on the first 100 bases.

OTUs were picked at 97% similarity using the open reference picker UCLUST (Edgar 2010) and taxonomy assignment was performed with BLAST (Altschul *et al.* 1990) against the UNITE nucleotide database (Abarenkov *et al.* 2010). To quantify the relative abundance of fungal taxa within and among samples (i.e., the percentage of an OTU occurring in a sample relative to all other OTUs in that sample, such that the sum of percentages equals 100% per sample), we used output from the “akutils core_diversity” command that incorporated an OTU table having been filtered at the Bokulich threshold (0.005% by biom table; Bokulich *et al.* 2013). See Appendix S1 in Attachment 1 for full sequencing methods.

We assigned fungal OTUs a guild or lifestyle status using the FUNGuild database (Nguyen *et al.* 2016) of fungal taxa with known or suspected ecological functions. Because FUNGuild assigns guilds at the genus level, we excluded OTUs in our dataset that were resolved above the genus level. For analyses, we assigned taxa to one of six functional guilds: plant pathogenic fungi, ericoid mycorrhizal fungi, ectomycorrhizal fungi, saprotrophic fungi, ‘other’ (including lichen forming, animal pathogenic fungi, etc.), or undetermined (those OTUs that were not identified to genus or below or whose genus was not represented in the FUNGuild database). Taxa identified as ‘other’ ($N = 6$ OTUs) and ‘undetermined’ ($N = 27$ OTUs) were excluded from further guild-based analyses. We considered only FUNGuild assignments with a confidence score of ‘probable’ or ‘highly probable’ and classified taxa with assignment scores below these as undetermined (Lankau & Keymer 2016).

Statistical analysis

To test hypotheses regarding the role of soil legacies, altered light environments, and their interaction on *R. maximum* seedling performance in the context of *T. canadensis* mortality, we built linear mixed-effects models in R v. 3.2.1 (R Core Team 2015). We built models using the ‘lmer’ function in the R package lme4 (Bates *et al.* 2015), which uses restricted maximum likelihood. We built separate models for each response variable measured, including total biomass, shoot biomass, root biomass, root-to-shoot ratio, leaf

bud break phenology, and SLA. To increase conformance to normality, we square root-transformed belowground biomass and log-transformed root-to-shoot ratio, leaf phenology, and SLA. For each model, we included soil source (*T. canadensis*-conditioned, *R. maximum*-conditioned, or interspace), light environment (high or low), and their interaction as fixed effects and, to account for the blocked nature of our field sampling design, seed family and population as random effects. We performed Chi-square tests to determine significance, assessed at $\alpha = 0.05$, for each fixed effect, and we calculated post-hoc Tukey's pairwise differences for growth responses to soil sources in high and low light.

To determine whether the plant-soil feedbacks we calculated, which represent the effect on *R. maximum* performance of movement into either *T. canadensis*-conditioned soils or interspace soils, were different from each other, we built linear models predicting total, shoot, and root biomass feedback values in high and low light, with feedback type (Hem:Rhodo or Inter:Rhodo) as a fixed effect. We calculated post-hoc Tukey's pairwise differences to compare the two types of feedbacks. To determine whether feedbacks were non-neutral (i.e., significant from zero), we conducted one-sample t-tests.

To test our hypothesis that soil fungal communities differ among soil sources and that such differences explain *R. maximum* growth responses to soil inoculation, we examined variation in soil fungal community traits (fungal species richness and the relative abundance of five functional guilds: ericoid mycorrhizal fungi, ectomycorrhizal fungi, total mycorrhizal fungi (ERM + ECM), saprotrophic fungi, and pathogenic fungi). To examine whether fungal community traits varied significantly across soil sources, we built linear mixed effects models, including soil source as a fixed effect and seed family and population as random effects. To increase conformance to normality, we log-transformed richness and mycorrhizal fungal abundance and square root-transformed pathogenic fungal abundance prior to analysis. Finally, to test variation in fungal community traits predicted *R. maximum* performance, we used multiple regression analysis. We built a linear mixed effects model to predict variation in total seedling biomass (for those plants grown in high light only) that included fungal richness and the

relative abundances of total mycorrhizal fungi (summing the relative abundances of ericoid + ectomycorrhizal fungi to avoid collinearity effects), saprotrophic fungi, and pathogenic fungi as fixed effects and soil source, seed family, and population as random effects. Prior to analysis, we log-transformed total biomass to increase conformance to normality.

Results

Plant growth responses to soil and light

In support of our hypotheses, we detected significant effects of soil source, light environment, and their interaction on total biomass (Table II.5). Because the interaction between soil source and light environment was significant, we ran separate models to examine how soil inoculation effects differed in low and high light. Soil source effects were significant in high ($X^2 = 12.636$, $p = 0.002$, Fig. II.7a) but not low ($X^2 = 0.420$, $p = 0.811$, Fig. II.7b) light environments. In high light, seedlings grown with *T. canadensis*-conditioned inoculum accumulated, on average, 16% more total biomass than those grown with interspace inoculum (Fig. II.7a); total biomass production in *R. maximum* soils did not differ from that in either interspace or *T. canadensis* soils (Fig. II.7a). Across soils, plants accumulated on average 1.7 times more total biomass in high versus low light.

We detected significant effects of soil source, light environment, and a marginal ($p = 0.058$) interaction effect on shoot biomass (Table II.5). Because the interaction between soil source and light environment trended towards significance, we ran two separate models to examine how light environment might affect shoot biomass responses to soil source. Consistent with patterns in total biomass, soil inoculum source had a significant effect on shoot biomass in high light only (high light: $X^2 = 16.809$, $p = 0.0002$, Fig. II.7c; low light: $X^2 = 1.182$, $p = 0.554$, Fig. II.7d). In high light, seedlings grown with *R. maximum* and *T. canadensis* inoculum accumulated similar amounts of shoot biomass and, respectively, 14% and 21% more shoot biomass than those grown with interspace

inoculum, on average (Fig. II.7c). Across soils, plants accumulated on average 1.4 times more shoot biomass in high versus low light (compare Figs. II.7c and d).

We detected significant effects of light environment on root biomass, root-to-shoot ratio, leaf bud break phenology and SLA (Table II.5). Plants grown in high light accumulated, on average, about 3.1 times more root biomass (Fig. II.7e, f), had 54% lower root-to-shoot ratios (see Fig. S2a, b in Attachment 1), broke bud 30 days earlier (see Fig. S2c, d in Attachment 1), and had 29% lower SLA (see Fig. S2e, f in Attachment 1) than in plants grown low light. Soil source trended towards significantly altering leaf bud break phenology (Table II.5; $p = 0.080$); plants grown in *T. canadensis* and *R. maximum* soils broke bud on average 3.5 and 6.9 days later than in interspace soil, respectively (see Fig. S2c in Attachment 1). We detected no main or interactive effect of soil source on root biomass, root-to-shoot ratio, or SLA (Table II.5; Figs. II.7e, f; see Fig. S2 in Attachment 1).

Plant-soil feedbacks

In high light, we detected a significant effect of feedback type (Hem:Rhodo or Inter:Rhodo) on total ($F_{1,44} = 4.701, p = 0.0356$) and shoot ($F_{1,43} = 5.918, p = 0.0192$) feedbacks, but not root feedbacks ($F_{1,46} = 1.970, p = 0.167$). Post-hoc comparisons indicated that, in high light, Hem:Rhodo and Inter:Rhodo feedbacks were significantly different for total and shoot biomass but not root biomass (Figs. II.8a, c, e). Hem:Rhodo feedbacks trended towards being positive for total ($t_{21} = 1.537, p = 0.139$) and shoot ($t_{20} = 1.272, p = 0.218$) biomass, but did not differ significantly from zero (Figs. II.8a, c). Inter:Rhodo feedbacks were negative for total ($t_{23} = -1.532, p = 0.139$) and shoot ($t_{23} = -2.368, p = 0.027$) biomass, though only the latter differed significantly from zero (Fig. II.8a, c). Hem:Rhodo ($t_{22} = 1.039, p = 0.310$) and Inter:Rhodo ($t_{24} = -0.974, p = 0.340$) root biomass feedbacks were neutral (Fig. II.8e).

In low light, we detected no significant effect of feedback type on total ($F_{1,35} = 0.091, p = 0.765$), shoot ($F_{1,35} = 0.165, p = 0.687$), or root ($F_{1,36} = 0.109, p = 0.743$) feedbacks, and post-hoc comparisons indicated no significant difference between

Hem:Rhodo and Inter:Rhodo feedbacks for any biomass measurement in low light (Figs. 3b, d, f). Hem:Rhodo total ($t_{20} = -1.117$, $p = 0.277$), shoot ($t_{19} = 1.164$, $p = 0.259$), and root ($t_{21} = 0.035$, $p = 0.972$) feedbacks were not significantly different from zero, nor were Inter:Rhodo total ($t_{15} = 0.332$, $p = 0.744$), shoot ($t_{16} = 0.332$, $p = 0.745$), and root ($t_{15} = 0.349$, $p = 0.7321$) feedbacks (Figs. II.8b, d, f).

Fungal community

In support of hypothesis 1, we detected a significant effect of soil source on the relative abundance of total mycorrhizal (comprising 7 operational taxonomic units [OTUs]), ericoid mycorrhizal (ERM, 4 OTUs), ectomycorrhizal (ECM, 3 OTUs) and saprotrophic fungi (20 OTUs), but not pathogenic fungi (3 OTUs) (Table II.6). Mycorrhizal fungi were on average 37% and 33% more abundant in *T. canadensis* and *R. maximum* soils than in interspace soils, respectively (Fig. II.9a). This pattern was reflected in both the relative abundance of ERM and ECM fungi. Ericoid mycorrhizal fungi were, on average 33% and 30% more abundant in *T. canadensis*-conditioned and *R. maximum*-conditioned soils than in interspace soils, respectively (Fig. II.9b). Similarly, ECM fungi were 123% and 101% more abundant in *T. canadensis*-conditioned and *R. maximum*-conditioned soils than in interspace soils on average (Fig. II.9c). On the contrary, saprotrophic fungi were about 34% and 23% less abundant in *T. canadensis* and *R. maximum* soils than in interspace soils (Fig. II.9d). Additionally, we detected significant variation in fungal species richness among soil sources (Table II.6) wherein soils conditioned by *T. canadensis* contained, on average, 10% fewer species than did interspace soils (Fig. II.9f). We detected no difference in the relative abundance of pathogenic fungi across soils, and this guild was rare in general ($\leq 1\%$ relative abundance/sample) (Fig. II.9e).

Multiple regression analysis further supported hypothesis 1 by identifying mycorrhizal and saprotrophic fungi as important predictors of *R. maximum* seedling performance. We detected a significant effect of the relative abundance of mycorrhizal and saprotrophic fungi, but not the relative abundance of pathogenic fungi or fungal

richness, on total *R. maximum* seedling biomass (Table II.7). Collectively, variation in the abundance of mycorrhizal and saprotrophic fungal guilds explained ~32% of variation in *R. maximum* seedling total biomass. For each 10% increase in mycorrhizal fungal abundance, total biomass increases $15 \pm 6\%$ (mean $\pm$ SE) (Fig. II.10a). Conversely, for each 10% increase in saprotrophic fungal abundance, total biomass decreases $39 \pm 10\%$ (Fig. II.10b). We also detected a marginally significant effect of fungal richness on total biomass (Table II.7, $p = 0.053$), with a 10% increase in fungal richness resulting in a $17 \pm 9\%$ decrease in biomass (Fig. II.5d).

Discussion

Our work examining plant-soil interactions in declining forests is one of the first to demonstrate that biotic interactions belowground interact with abiotic disturbances aboveground to drive plant responses to major disturbance. Specifically, we have demonstrated that biotic plant-soil feedbacks, in conjunction with concurrent changes in light environment, likely facilitate the expansion of the woody shrub *R. maximum* in southeastern US forests experiencing widespread mortality of the dominant foundation tree species *T. canadensis*. Overall, our study provides broad support to previous work suggesting that *R. maximum* will continue to expand as *T. canadensis* is lost (Ford *et al.* 2012), opens the soil ‘black box’ by identifying the potential putative players driving plant-soil feedback effects, and suggests that synergistic interactions between belowground biotic and aboveground abiotic mechanisms can facilitate species’ responses to major forest disturbances.

Plant-soil feedbacks mediate plant responses to major disturbance

Our findings that, in high light environments, seedlings performed best in *T. canadensis* soils (simulating directional expansion into declining *T. canadensis* stands), medially in *R. maximum* soils (simulating persistence in conspecific soils), and worst in interspace soils (simulating random expansion), support our first hypothesis and suggest that *R. maximum* could take advantage of novel soil and light environments in declining

T. canadensis stands. Specifically, soil biota may enable *R. maximum* persistence in existing conspecific thickets, facilitate expansion into areas previously inhabited by *T. canadensis* trees, and prevent expansion into areas that have not been inhabited by *T. canadensis* and *R. maximum*.

Our findings that *R. maximum* performance is enhanced in declining *T. canadensis* environments contrast with previous work with *Quercus rubra*, a common replacement species in northeastern *T. canadensis* forests. Lewis *et al.* (2008) demonstrated that *Q. rubra* accumulated significantly *less* biomass and had significantly *lower* ectomycorrhizal colonization rates when grown in declining *T. canadensis* stands versus congeneric stands. Altered interactions with soil biota, including mycorrhizal fungi, may therefore hasten the expansion of certain species (i.e., *R. maximum*), but slow the expansion of others (i.e., *Q. rubra*) as *T. canadensis* disappears from eastern forests. Previous work has also shown that ectomycorrhizal colonization of *Q. rubra* is reduced in *R. maximum* thickets relative to open forests (Walker *et al.* 1999) and that regeneration and survival of many hardwood tree species is lower in *R. maximum* thickets than other forest locations (Clinton & Vose 1996; Nilsen *et al.* 1999; Walker *et al.* 1999; Beckage *et al.* 2000; Nilsen *et al.* 2001; Lei *et al.* 2002). This may be due to the inhibitory effects of *R. maximum* soil conditioning on tree and/or ectomycorrhizal growth (Clinton & Vose 1996; Nilsen *et al.* 2001), reduced light availability under *R. maximum* thickets (Nilsen *et al.* 2001; Lei *et al.* 2002), and/or spatial competition from dense *R. maximum* root mats (Walker, Miller & Horton 2005). Our work builds on these studies in suggesting that soil biotic interactions may enable *R. maximum* to expand more effectively than hardwood tree species into declining *T. canadensis* stands, persist in such novel environments, and prevent heterospecific recruitment into those areas.

Soil fungi as drivers of plant-soil feedbacks

While numerous studies have inferred the role of soil biota in driving plant-soil feedback effects on plant performance, few have identified the microbes responsible for such effects (but see Keymer & Lankau 2016). Here, we identified two fungal guilds that

appear to be responsible for the effects we observed. First, mycorrhizal fungi, which were most abundant in *T. canadensis*- and *R. maximum*-conditioned soils, scaled positively with *R. maximum* seedling biomass. While it is not surprising that soils inhabited by ectomycorrhizal (ECM) and ericoid mycorrhizal (ERM) host plants, respectively, contained more mycorrhizal fungi than interspace soils, it is less intuitive that these soils contained similar, elevated quantities of both types of mycorrhizal fungi. These findings corroborate recent molecular work that has amplified fungi commonly classified as ERM from ECM plant roots, and *vice versa* (Bougoure *et al.* 2007; Collier & Bidartondo 2009; Grelet *et al.* 2010; Villarreal-Ruiz *et al.* 2012) and suggest that these two types of mycorrhizal fungi may be less host-specific than traditionally thought (i.e., Smith & Read 2008). Thus, *R. maximum* may readily form associations with either mycorrhizal type to effectively extract nitrogen and phosphorus from organic soils, thus performing equally well in both soils conditioned by conspecifics and by *T. canadensis*.

Moreover, we found that increasing abundance of saprotrophs, which were more abundant in interspace soils than in *T. canadensis*-conditioned soils, negatively influenced *R. maximum* biomass. Free-living saprotrophic fungi may share fundamental decomposition niches with mycorrhizal fungi and compete with plant roots and mycorrhizal fungi for N in N-limited systems (Gadgil & Gadgil 1971; Nordin, Schmidt & Schaver 2004; Bödeker *et al.* 2016), including temperate forests (van der Heijden, Bardgett & van Straalen 2008). Thus, a mycorrhizal release from belowground competition with functionally similar saprotrophic fungi could explain the increased performance in conspecific and *T. canadensis*-conditioned soils than interspace soils. Future competition experiments would provide insight into this potential mechanism.

Negative feedbacks with soil pathogens in conspecific soils are expected to facilitate species dispersal into heterospecific soils (Kulmatiski *et al.* 2008; Mangan *et al.* 2010; Van der Putten *et al.* 2013). Several studies have demonstrated that such ‘pathogen release’ drives the successful movement of non-native (Reinhart *et al.* 2003; Callaway *et al.* 2004) or range-expanding (Van Grunsven *et al.* 2007; Engelkes *et al.* 2008; McCarthy-Neumann & Ibáñez 2012) species into novel environments. However, we

found that pathogenic fungi were rare in general (generally <1% relative abundance), equally abundant across soil sources, and did not significantly affect *R. maximum* performance, suggesting that pathogen release was not an important mechanism driving the feedbacks we observed.

Finally, we focused *a priori* hypotheses and sequencing efforts on soil fungi because of the established importance of mycorrhizal associations for both *R. maximum* (Wurzburger & Hendrick 2007, 2009) and *T. canadensis* (O'Brien, Gomola & Horton 2011) and because this soil ecosystem has many characteristics to suggest it is dominated by fungi rather than bacteria (i.e., it is dominated by slow growing plant species with low leaf litter quality and soils are well-developed, acidic, organic matter-rich, and nutrient-poor; van der Heijden, Bardgett & van Straalen 2008). Still, soil nutrient status (see Fig. S1 in Attachment 1) and/or the composition of bacteria, protozoa, or archaea communities may have been changed through our inoculations. However, as we (1) added soil inocula at a rate of approximately 1:99 dilution (99% sterile background potting mix), which is more than an order of magnitude more dilute than those of previous studies examining biotic plant-soil feedback (Kardol, Bezemer & Van der Putten 2006; Carvalho *et al.* 2010), and (2) consistently fertilized plants leading up to the start of the experiment, abiotic differences should have been strongly minimized (Kardol, Bezemer & Van der Putten 2006). Moreover, as we detected differentiation in biologically relevant fungal guilds among soil sources that explained substantial (~32%) variation in *R. maximum* performance, our findings suggest that, while non-fungal soil taxa may be partially responsible for the plant-soil feedback effects we observed, soil fungi are responsible for a considerable portion of feedback effects we observed and the expansion of *R. maximum* in declining *T. canadensis* forests.

Context-dependency of plant-soil feedback effects

Light environment had a strong, significant effect on the six growth responses we measured in our greenhouse experiment. Increased light availability from adelgid-induced *T. canadensis* crown defoliation thus likely contributes to *R. maximum* expansion

in declining *T. canadensis* forests, supporting previous predictions (Ford *et al.* 2012; Krapfl, Holzmüller & Jenkins 2012). Given *R. maximum* is heavily shade tolerant (Ford *et al.* 2012), this strong positive response to increased light availability was unexpected. However, our findings provide empirical support to anecdotal evidence that *R. maximum* may spread and form new thickets most effectively after canopy openings are created (Plocher & Carvell 1987). In other words, while *R. maximum* can tolerate extremely low light levels, including those of healthy *T. canadensis* understories (Canham *et al.* 1994; Hadley 2000), the species does not necessarily prefer such conditions and may opportunistically capitalize on canopy openings when they occur.

Importantly, inoculation effects were evident in high light only, suggesting that increased light availability enhances the net positive effects of plant-soil biota interactions beneath declining *T. canadensis* trees, leading to *R. maximum* expansion into these areas. Across all soil-light combinations, seedlings performed best in conditions that match the belowground and aboveground environments into which *R. maximum* would move while expanding in declining *T. canadensis* stands: *T. canadensis*-conditioned soil communities and increased light availability. Given that pathogen susceptibility is generally higher in low light environments (i.e., dense forest understories) relative to higher light environments (i.e., canopy gaps) (Augspurger & Kelley 1984; Reinhart *et al.* 2010; McCarthy-Neumann & Ibáñez 2012), it is possible that detrimental pathogenic fungi played a disproportionately large role on seedling performance in low light treatments, outweighing effects of beneficial soil biota (i.e., mycorrhizal fungi) that contributed to plant-soil feedbacks in high light. While mortality rates throughout the experiment were low, mortality was slightly more frequent in low light (2.9%) than in high light (0.9%), suggesting that increased pathogenic activity could have occurred in low light. However, because we focused our sequencing efforts on soils from high light treatments, where we observed strong soil source effects, further experimentation would be needed to address fully the level of fungal community differentiation along this light gradient. Alternatively, shading can reduce mycorrhizal biomass and root colonization (Shi *et al.* 2014; Konvalinková *et al.* 2015) and cause plant

growth responses to mycorrhizal colonization to become neutral or negative because of the potentially high cost of carbon (C) investment for the plant under low light (photosynthetically-limiting) conditions (Johnson *et al.* 2015; Konvalinková & Jansa 2016). Thus, beneficial effects of mycorrhizal fungal colonization on *R. maximum* performance may have been dampened in low light environments.

Conclusions and Implications

The landscape-level mortality of foundation tree species, such as *T. canadensis* in eastern North American forests, is and will continue to have widespread, dramatic, and cascading effects on future plant communities and ecosystem function. In the southern portion of the *T. canadensis* range, the native shrub *R. maximum* is already expanding in response to *T. canadensis* decline, yet the mechanisms underlying this response have been poorly studied. Our findings support and provide a mechanistic explanation for previous predictions that, when present, *R. maximum* will be the primary species to recruit into declining *T. canadensis* stands (Ellison *et al.* 2005; Ford *et al.* 2012). Interactions with soil biota may enable certain members of the plant community to replace declining foundation trees. As soil inoculation has been shown to be an effective approach for steering plant community succession in certain systems, including grasslands (Grman & Suding 2010; Kardol & Wardle 2010; Wubs *et al.* 2016), we invite future work to build upon our study by testing the feasibility and efficacy of soil inoculation as a tool to guide forest succession towards specific restoration goals that may or may not include *R. maximum*. We have also demonstrated that identifying the microbes responsible for plant-soil feedback effects on plant performance is both possible and insightful. As next-generation sequencing methods become more accessible and less expensive, we encourage ecologists to continue advancing plant-soil interaction research by using these tools and developing appropriate genomic analyses to answer basic and applied questions in this field. Finally, we are, to our knowledge, the first to demonstrate that plant-soil feedbacks mediate species' response the loss of a single species and one of the first to provide compelling evidence for the context dependency of plant-soil

feedbacks. We have identified a dual mechanism, involving both above- and belowground processes, that may allow some species to capitalize on disturbance and dominate succession following widespread tree mortality events. Our work provides a framework to explore similar mechanisms in other systems from which foundation species are being lost and informs predictions and management actions regarding future forests in the face of ongoing global change.

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Appendix

Table II.5. Chi-square (X^2) values and associated degrees of freedom (DF) for linear models predicting variation in six growth response variables measured on *Rhododendron maximum* seedlings grown for 11 months in soil inoculations sourced from beneath conspecific *R. maximum* patches, declining *T. canadensis* trees, or interspace locations, in either high or low light.

Models incorporated experimental soil inoculum source (Soil), light environment (Light), and their interaction as fixed effects and *R. maximum* seed family and site (North Carolina, Tennessee, or Virginia) as random effects. Significant ($\alpha < 0.05$) effects are shown in bold; statistical significance is denoted by the following: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), **** ($p < 0.0001$).

		<i>Growth response</i>					
		Total biomass	Shoot biomass	Root biomass	Root-to-Shoot	Leaf phenology	Specific leaf area
<i>Effect</i>	<i>DF</i>	X^2	X^2	X^2	X^2	X^2	X^2
<i>Soil</i>	2	11.01**	15.65***	3.18	1.46	5.04	0.21
<i>Light</i>	1	309.80****	149.11****	483.74****	445.32****	146.13****	205.87****
<i>Soil</i> × <i>Light</i>	2	7.45*	5.70	5.27	2.82	0.12	2.17

Table II.6. Chi-square (X^2) values and associated degrees of freedom (DF) for linear models predicting variation in six fungal community traits across experimental soil subsamples ($N = 48$) sourced from beneath *Rhododendron maximum* patches, declining *Tsuga canadensis* trees, or interspace locations.

Models incorporate experimental soil inoculum source (Soil source) as a fixed effect and *R. maximum* seed family and site (North Carolina, Tennessee, or Virginia) as random effects. Significant ($\alpha < 0.05$) effects are shown in bold; statistical significance is denoted by the following: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), **** ($p < 0.0001$).

Community trait response	Soil source effect
	$X^2_{DF=2}$
Functional guild relative abundances	
Mycorrhizal fungi (7 OTUs)	10.175**
Ericoid mycorrhizal fungi (4 OTUs)	6.289*
Ectomycorrhizal fungi (3 OTUs)	6.456*
Saprotrophic fungi (20 OTUs)	8.2018*
Plant pathogenic fungi (3 OTUs)	0.0873
Fungal species richness	6.7442*

Table II.7. Standardized partial correlation coefficients (β), associated standard error (SE), and chi-square (X^2) values with associated degrees of freedom (DF) indicating effects of five predictor variables, the relative abundance of mycorrhizal, saprotrophic, and pathogenic fungi, and fungal species richness, on *Rhododendron maximum* seedling total biomass ($N = 48$).

Rhododendron maximum seed family and site (North Carolina, Tennessee, or Virginia) were included as random effects. Significant ($\alpha < 0.05$) sources of variation are shown in bold; statistical significance is denoted by the following: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), **** ($p < 0.0001$).

<i>Predictor variable</i>	<i>Total biomass response</i>		
	β	<i>SE</i>	$X^2_{DF=1}$
Mycorrhizal abundance	0.22	0.12	5.31*
Saprotrophic abundance	-0.38	0.12	14.20***
Pathogenic abundance	-0.09	0.10	0.10
Fungal species richness	-0.18	0.14	3.75

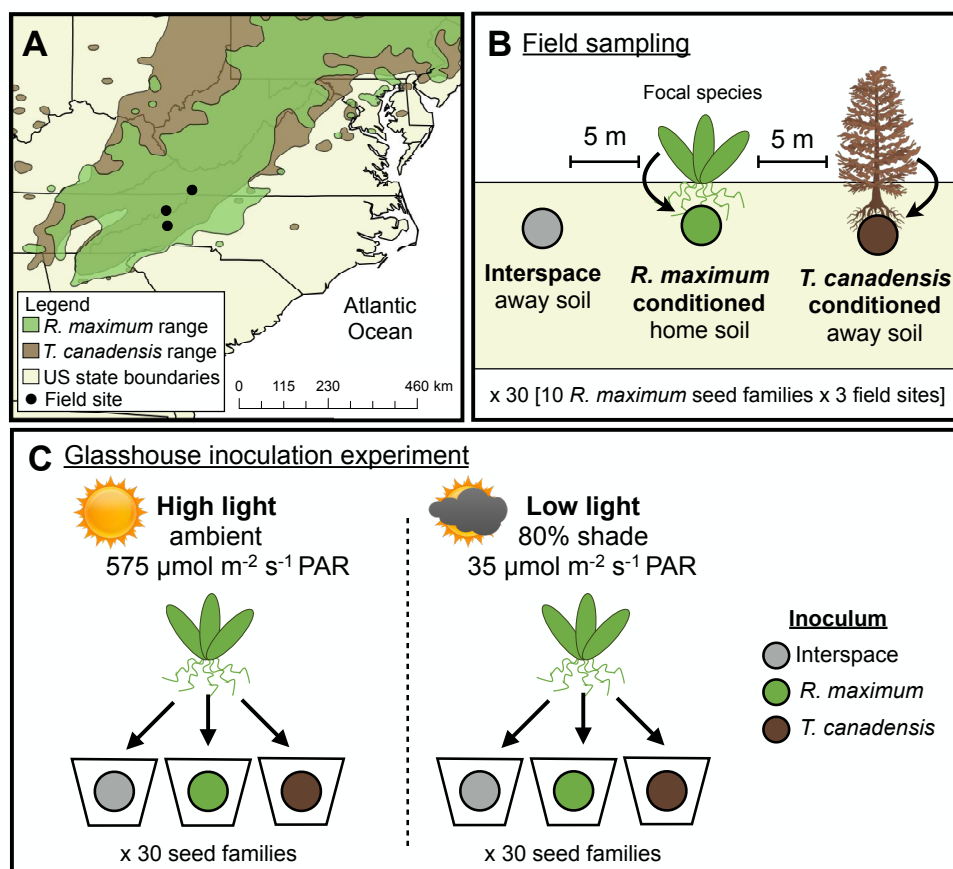


Figure II.6. Field sampling locations, field sampling design, and experimental design for *Rhododendron maximum* inoculation experiment.

(A) Soil inocula and *R. maximum* seeds were collected from three field sites (indicated by black dots) in the southern portions of the *R. maximum* and *Tsuga canadensis* geographic ranges, indicated by green and brown shading, respectively. (B) Soils and *R. maximum* seeds were collected in a paired design at 10 *R. maximum* seed family locations at each of 3 field sites ($N = 30$ seed families). At each seed family, three types of soil were collected: *R. maximum*-conditioned soil, collected directly beneath the *R. maximum* individual; a paired interspace soil, ≥ 5 m from *R. maximum* and *T. canadensis* individuals; and a paired *T. canadensis*-conditioned soil, collected directly beneath a *T. canadensis* individual ≥ 5 m from *R. maximum* individuals and the interspace sampling location. (C) Field-sourced seeds and soils were used in an 11-month glasshouse inoculation experiment. Seedlings of each of 30 seed families were grown in soils containing either interspace, *R. maximum*-conditioned, or *T. canadensis*-conditioned inoculum sourced from the corresponding individual's field sampling location. Inoculation treatments were replicated in both high ($\sim 575 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation [PAR]) and low ($\sim 35 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) light, which match, respectively, infested or uninfested *T. canadensis* crowns ($N = 30$ seed families $\times$ 3 soil inoculum sources $\times$ 2 light treatments $\times$ 4 replicates = 720 total pots).

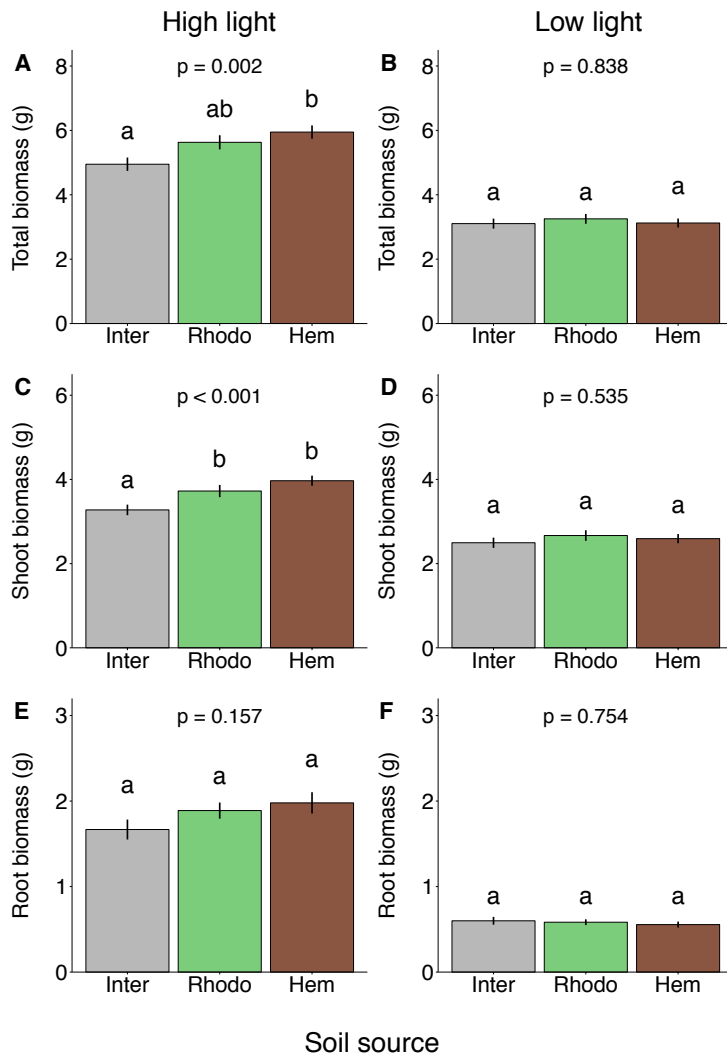


Figure II.7. Variation in *Rhododendron maximum* total, shoot, and root biomass across soil inoculum sources and light environments.

Shown are mean ($\pm$ SE) total biomass (a, b), shoot biomass (c, d), and root biomass (e, f), of *R. maximum* seedlings subjected to either high or low light environments and grown with soil inoculations from one of three paired locations: interspace areas without *T. canadensis* or *R. maximum* individuals (i.e., Inter, grey bars), beneath *R. maximum* individuals (i.e., Rhodo, green bars), or beneath declining *T. canadensis* trees (i.e., Hem, brown bars). The significance of the soil source effect on each biomass category in each light treatment is shown. Within each panel, bars that do not share letters are significantly different from each other (Tukey test $p < 0.05$). See Fig. S2 in Attachment 1 for variation in additional traits (root:shoot ratio, leaf phenology, specific leaf area) across soil and light environments.

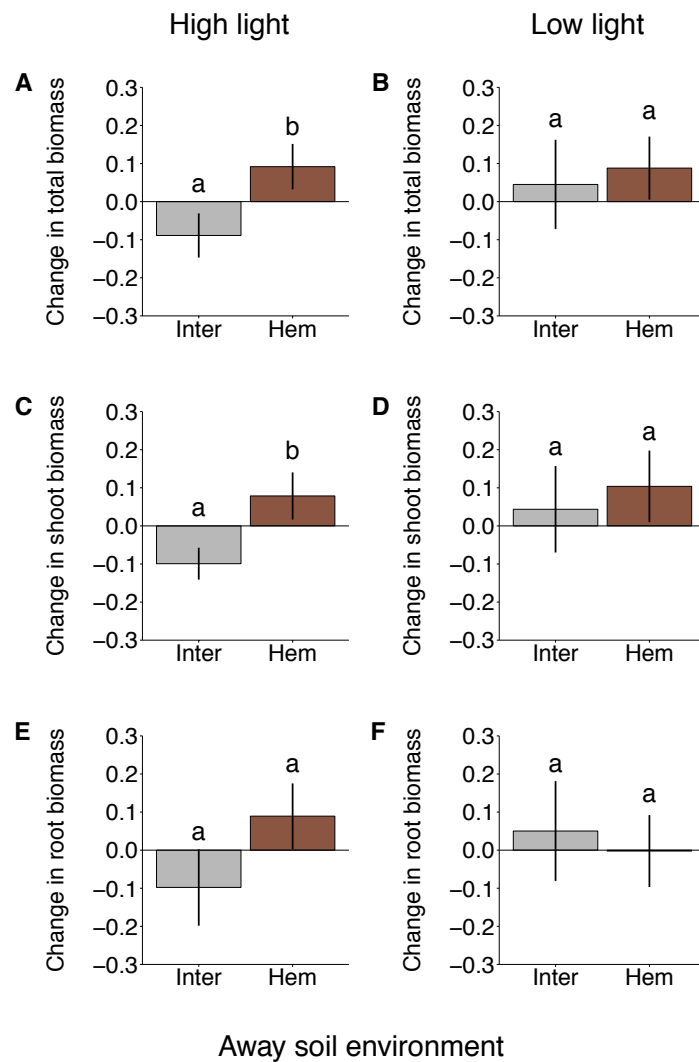


Figure II.8. Variation in *Rhododendron maximum* total shoot, and root biomass visualized as feedbacks, simulating movement from home (*R. maximum*-conditioned) into away (*Tsuga canadensis*-conditioned or interspace) soils.

Shown are mean ($\pm$ SE) soil feedbacks of *R. maximum* total (A, B), shoot (C, D), and root biomass (E, F) in *T. canadensis*-conditioned away soils (Hem, brown bars) or interspace away soils (Inter, grey bars) relative to *R. maximum*-conditioned home soils. Feedbacks were calculated as $\ln(\text{biomass}_{\text{away}}/\text{biomass}_{\text{home}})$. Negative feedbacks (A Inter, C Inter) correspond to a net disadvantage in away soils (interspace [Inter] or *T. canadensis*-conditioned [Hem]) relative to home (*R. maximum*-conditioned) soils; positive feedbacks (A Hem, C Hem) correspond to a net benefit on away soils. Within each panel, feedbacks that do not share the same letter are significantly different from each other (Tukey test $p < 0.05$).

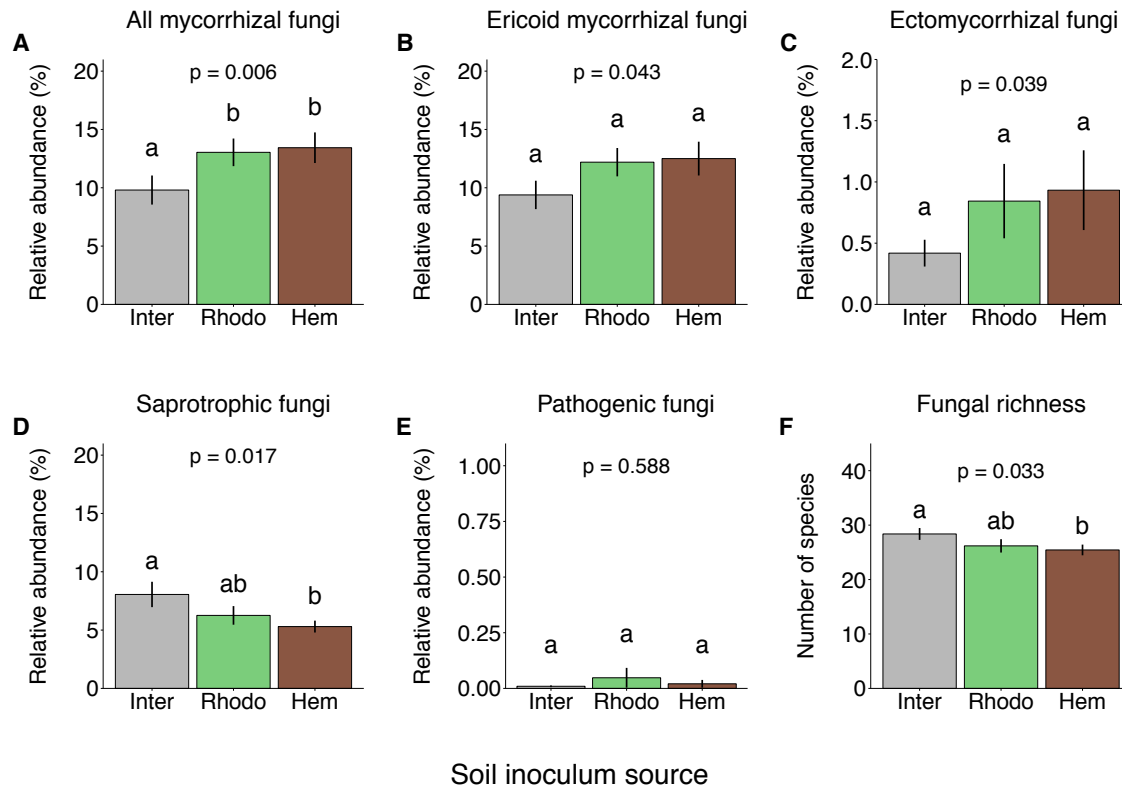


Figure II.9. Mean ($\pm$ SE) relative abundance of total mycorrhizal (ericoid + ectomycorrhizal) fungi, ericoid mycorrhizal fungi, ectomycorrhizal fungi, saprotrophic fungi, pathogenic fungi, and fungal species richness across soil inocula sourced from three paired locations.

Inoculum source locations include interspace areas without *Tsuga canadensis* or *Rhododendron maximum* [Inter, grey bars], beneath *R. maximum* individuals [Rhodo, green bars], or beneath declining *T. canadensis* trees [Hem, brown bars]. Fungal community data is based on a subsample ($N = 48$) of post-experiment soils collected from high light treatments and pooled at the seed family level. The significance of the soil source effect for each community trait is shown. Within each panel, bars that do not share letters are significantly different from each other (Tukey test $p < 0.05$)

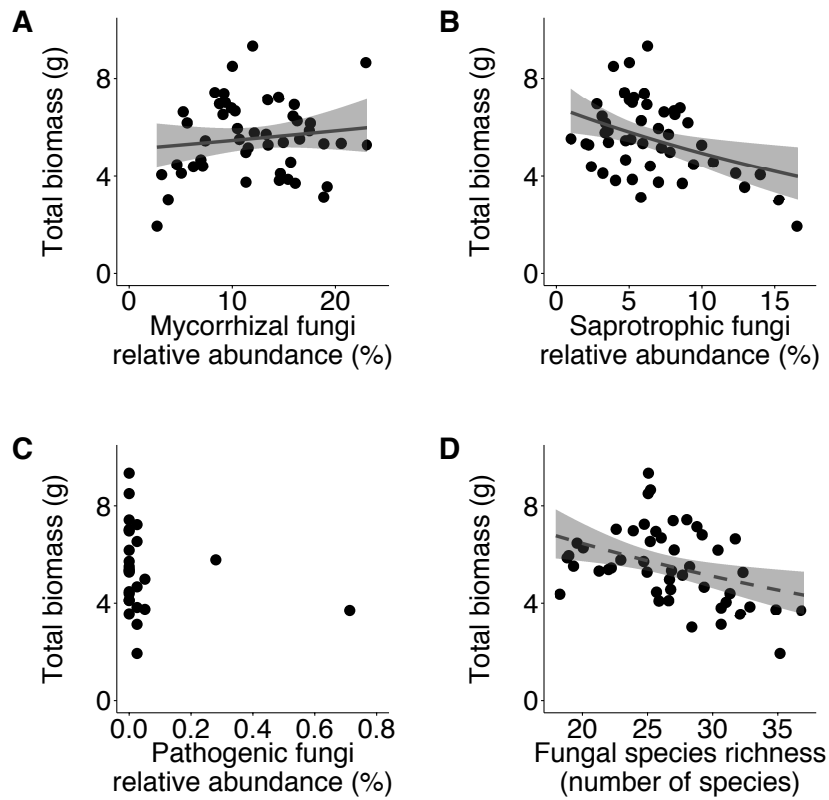


Figure II.10. Relationships between *Rhododendron maximum* seedling total biomass following an 11-month soil inoculation experiment and the relative abundance of mycorrhizal, saprotrophic, and pathogenic fungi, and fungal species richness.

Total biomass data were pooled at the seed family level; soil and biomass samples were sampled per a random fungal sequencing sampling design ($N = 48$ soil samples from 16 seed families). Back-transformed regression lines of best fit $\pm$ 95% confidence intervals are shown for each significant model; solid lines (A, B) represent a significant ($p < 0.05$) effect; dashed lines (D) represent a marginally significant ($p < 0.1$) effect.

CONCLUSION AND FUTURE DIRECTIONS

My thesis investigated the ecological and evolutionary mechanisms that mediate plant persistence under global change using the dominant North American shrub *Rhododendron maximum* as a model system. In Chapter I, I showed that *R. maximum* trait clines along elevation are frequently absent, driven by phenotypic plasticity, and highly population-dependent. In Chapter II, I showed that belowground plant-fungal interactions contribute to *R. maximum* expansion in declining forests but that the outcomes of these interactions may ultimately depend on aboveground abiotic context. Taken collectively, my work suggests that tolerance, acclimation, and biotic interactions contribute to *R. maximum*'s high adaptive capacity for environmental change. This capacity to cope with change could underlie *R. maximum*'s historic response to disturbance (i.e., clear-cut logging, American chestnut decline) and will likely enable *R. maximum* to persist, even expand, in the face of contemporary change, including climate change and *Tsuga canadensis* decline. Finally, my work collectively underscores the context dependent nature of ecological and evolutionary processes that will likely mediate plant responses to global change. Acknowledging and incorporating such context dependency in future research and conservation project designs would likely improve the relevance and effectiveness of each.

Future work could build upon this research in several ways. First, as described in Chapter I, a limited number of studies have examined trait responses to elevation gradients at more than one location. Thus, future work examining when, where, and why plant traits do or do not respond consistently to environmental gradients at different locations would be beneficial. First, formalizing a meta-analysis that (1) examines the frequency of replication in elevation gradient studies and (2) quantifies the level of consistency of trait responses to elevation within replicated studies would provide insight into the general or idiosyncratic nature of plant trait variation along climatic gradients across numerous plant species. Within the *R. maximum* system, future work extending sampling efforts and reciprocal transplants into the northern portion of the species' range,

where the species becomes less dominant and smaller in stature, could provide insight into whether the species is locally adapted to environmental differentiation across its broad geographic distribution.

As demonstrated in Chapter II, the outcomes of plant-soil feedbacks can be dependent on aboveground abiotic context. However, with few exceptions, plant-soil feedback research published to date has generally not tested for the context-dependency of plant-soil feedback effects. Future work should therefore incorporate realistic and relevant abiotic treatments in plant-soil feedback experimental designs to begin to develop a predictive framework of plant-soil interaction outcomes along abiotic gradients in the context of global change (i.e., light availability, nutrient availability, temperature, or precipitation). In the context of *T. canadensis* decline, further work examining the effects of plant-soil feedbacks and light availability on the relative performances of *Acer*, *Betula*, *Fagus*, and *Quercus* species would provide further insight into community responses of *T. canadensis* decline.

VITA

Alix Ann Pfennigwerth was born on April 11, 1989 in Knoxville, Tennessee and graduated from Farragut High School in May 2007. She graduated *summa cum laude* from the University of Tennessee in December 2011 with a B.S. in Biological Sciences and a concentration in Ecology and Evolutionary Biology. From there, Alix worked for a variety of land management, conservation and scientific research organizations including The National Park Service, The Tennessee Invasive Plant Council, The Tennessee Tree Improvement Program, and the Classen and Kwit Labs at the University of Tennessee. In August 2013, Alix enrolled as a graduate student in the Department of Ecology and Evolutionary Biology at the University of Tennessee, Knoxville and completed her thesis in May 2017. Following graduation, she will work as an Ecologist with the U.S. Geological Survey at the Southwest Biological Science Center in Moab, Utah.