PLANT-SOIL FEEDBACKS OF *CASTANEA DENTATA* REINTRODUCTION

by

ERIN MARAY COUGHLIN

(Under the Direction of Nina Wurzburger)

ABSTRACT

Plant interactions with soil biota result in plant-soil feedbacks, which have effects on plant growth and survival and broader community-level consequences. These interactions should be considered in restoration and species reintroduction, yet little work has integrated this perspective. Here we investigate the ecological consequences and management requirements of hybrid *Castanea dentata* reintroduction by studying plant-soil feedbacks of this species and other forest dominants. We conducted a fully reciprocal greenhouse experiment testing the effect of species-specific soil inoculum on seedling growth and survival. Our results suggest that *C. dentata* and hybrids have similar belowground interactions and are regulated by negative plant-soil feedbacks. Our results suggest that *Q. alba* and *L. tulipifera*-dominated forests provide favorable planting conditions for hybrids, and we predict that reintroduction may promote *Q. alba*. We conclude that plant-soil feedbacks are vital processes in forest ecosystems and should be incorporated into management plans for species reintroduction.

INDEX WORDS: plant-soil feedback, microbial assemblage, American chestnut, species reintroduction, applied ecology

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ERIN MARAY COUGHLIN

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ERIN MARAY COUGHLIN

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Major Professor:	Nina Wurzburger
Committee:	Richard Shefferson
	Richard Lankau
	Stacy Clark

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
August 2015

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CHAPTER 1

INTRODUCTION

Introduced pests and pathogens continue to alter the composition of eastern temperate forests (Fisher, Henk et al. 2012, Boyd, Freer-Smith et al. 2013). In response, there is interest in creating resistant hybrids or transgenic forms of threatened species with the goal of reintroduction to the native range (Merkle, Andrade et al. 2006, Sniezko 2010). Several breeding programs of this nature are underway, such as those for *Juglans cinerea* L. (White walnut) (Michler, Pijut et al. 2006), *Ulmus americana* L. (American elm) (Newhouse, Schrodt et al. 2007) and *Castanea dentata* (Marsh.) Borkh. (American chestnut) (Anagnostakis 2012). However, reintroductions of these species have not yet been implemented on a large scale. Given the novelty of this type of management action, the effectiveness and the long-term ecological consequences of hybrid reintroduction are unclear.

A consideration of soil microorganisms is critical in the context of restoration and reintroduction. Microbial effects on plants can fall along a spectrum from direct positive (mutualistic symbionts (Smith and Read 2008)) to negative (antagonistic pathogens (Mills and Bever 1998)) associations, as well as numerous indirect effects from decomposers and nutrient-cycling organisms (Bardgett and Wardle 2010). Since plants promote microbial communities in species-specific ways, “feedbacks” can manifest in the survival and growth of plants (Bezemer, Lawson et al. 2006, Bardgett and Wardle

2010, Bever, Platt et al. 2012). These plant-soil feedbacks have broader consequences on the composition of plant communities, species diversity, coexistence and plant productivity (Reynolds, Packer et al. 2003, Bonanomi, Giannino et al. 2005, Kardol, Cornips et al. 2007, van der Heijden, Bardgett et al. 2008). Therefore, in the context of restoration, plant-soil interactions are critical to consider (Eviner and Hawkes 2008, van der Putten, Bardgett et al. 2013), as the composition of existing microbial communities has the potential to promote or suppress newly introduced species. Species reintroductions will likely generate new, species-specific effects on soil microbes, however, we have limited information from which to guide efforts and virtually no work has been done to integrate this perspective into reintroduction efforts.

Castanea dentata, once a dominant canopy tree of Eastern forests, experienced widespread mortality in the early 20th century due to an introduced Asian fungal blight (causal agent *Cryphonectria parasitica* (Murrill) Barr) (Hepting 1974, Paillet 2002). The species is now considered functionally extinct, existing only as an understory shrub (Paillet 2002). Prior to chestnut blight, *C. dentata* was a foundation species because of its important stabilizing effect on ecological conditions for other species (Ellison, Bank et al. 2005). *C. dentata* was extremely abundant, making up approximately 25-50% of trees across much of its range (Russell 1987), and produced reliable annual mast crops, which provided an important source of food for wildlife (Gilland, Keiffer et al. 2012). Because of its dominance, loss of *C. dentata* resulted in large changes in nutrient cycling (Ellison, Bank et al. 2005), forest resource pulses and availability (Diamond, Giles et al. 2000, Dalgleish and Swihart 2012), and forest community composition

(Elliott and Swank 2008). In addition, *C. dentata* was a prized timer species because it produced attractive, highly rot-resistant wood (Walker 1999, Youngs 2000). The ecological and economic importance of *C. dentata* has motivated non-profit organizations and federal agencies to attempt to restore the species to its native range by creating blight-resistant hybrids.

The restoration of *Castanea dentata* is perhaps the largest and most developed project involving reintroduction of disease-resistant hybrids, and it is considered a model for plant species reintroduction (Jacobs, Dalgleish et al. 2013). Since the early 1980's, The American Chestnut Foundation (TACF) has been developing blight-resistant *Castanea* hybrids through backcross breeding, which uses *Castanea mollissima* Blume (Chinese chestnut) as the initial source of blight resistance and results in hybrids that are approximately 15/16 *Castanea dentata* in their genetic make-up (Anagnostakis 2012). Throughout the breeding process, trees are selected for their level of blight resistance and *C. dentata*-like appearance and growth form (Diskin, Steiner et al. 2006). TACF has made significant efforts to create blight-resistant hybrids and large-scale restoration of the species is imminent.

The prospect of large-scale restoration of C. dentata raises questions about how the hybrid will perform in contemporary forests and how, in turn, the forest community will respond to its introduction. Understanding the ecology of *Castanea* hybrids from a plant-soil feedback perspective will be critical for restoration efforts because existing evidence points to the importance of soil biota on C. dentate growth. For example, *C. dentata* is particularly sensitive to the soil oomycete pathogen *Phytophthora cinnamomi*

Rands, which resulted in significant reduction of the species in the Southeastern portion of the range prior to chestnut blight (Crandall, Gravatt et al. 1945). However, *C. dentata* also associates with a large number of ectomycorrhizal fungi (Palmer, Lindner et al. 2008), which are beneficial symbionts that exchange water and macronutrients for plant photosynthate (Smith and Read 2008). Members of the Fagaceae family are dependently mycorrhizal (Smith and Read 2008), therefore associations with mycorrhizal fungi are most likely vital to the establishment and growth of *C. dentata*.

These biotic interactions determine many community and ecosystem-level consequences of reintroduction because they regulate plant species abundance and distribution. Many temperate species are characterized by pathogen-driven, negative plant-soil feedbacks, or soil conditioning effects that are antagonistic to conspecific growth (Mills and Bever 1998, Packer and Clay 2003, McCarthy-Neumann and Ibanez 2013). These negative plant-soil feedbacks play an important role in maintaining plant community diversity and coexistence of competitors (Bever 2003, Bonanomi, Giannino et al. 2005). Indirect reciprocal positive interactions can occur when two competing species exhibit negative plant-soil feedbacks, resulting in species codominance (Bonanomi, Giannino et al. 2005). Prior to chestnut blight, *C. dentata* codominated the canopy with *Quercus* spp. throughout much of its range (Paillet 2002), a dynamic that may have been driven in part by negative plant-soil feedbacks. Since its demise, *Quercus*, *Carya* and *Acer* species replaced *C. dentata* throughout most of Eastern forests *(Woods and Shanks 1959, Stephenson 1986, van de Gevel, Hart et al. 2012)*. As a result, plant-soil feedbacks throughout Appalachian forests have undoubtedly changed since the introduction of chestnut blight. Current ecological knowledge of *C. dentata* and *Castanea* hybrids is needed to predict the consequences of reintroduction and ecological niche of hybrids in modern forests (Jacobs, Dalgleish et al. 2013). This leads us to ask—how will *Castanea* hybrids perform in the soil conditions of modern forests, and are their below-ground interactions similar to the parent species, *C. dentata*? Furthermore, how will *Castanea* hybrid reintroductions affect the soil microbial community, and what subsequent effect will this have on the recruitment of other forest species?

We sought to determine 1) if *Castanea dentata* and *Castanea* hybrids have similar growth responses to assemblages of microbes, 2) which dominant tree species promote microbial assemblages that are favorable for hybrid *Castanea* growth, and 3) how dominant forest trees will subsequently respond to the soil microbial communities that hybrid *Castanea* promote. In order to address these questions, we investigated the direction and strength of plant-soil feedbacks for *C. dentata*, *Castanea* hybrids and current forest dominants, *Quercus alba* L. (white oak), *Liriodendron tulipifera* L. (tulip poplar) and *Pinus strobus* L. (white pine). Using field-collected soils as inoculum, we conducted a fully reciprocal greenhouse experiment to examine the effects of species-specific inoculum on tree growth and mortality. Seedlings were grown in sterile matrix soil, inoculated with species-specific soils collected from three southeastern forest sites and harvested after five months.

Given their close genetic relatedness and selection for *C. dentata* traits during hybrid breeding, we expected that *C. dentata* and *Castanea* hybrids would have similar plant-soil feedbacks. We predicted that *Q. alba and P. strobus* would produce soil most favorable to chestnut growth because these species, like *Castanea,* are ectomycorrhizal and will likely promote mutualists specific to *Castanea*. *L. tulipifera* forms arbuscular mycorrhizae and may not promote favorable mutualists for *Castanea*. Following that logic, we expected that *Q. alba* and *P. strobus* seedlings would have the greatest growth in *Castanea* spp. soils relative to other tree species.

CHAPTER 2

METHODS

Site Description and Soil Collection

We collected soil inoculum in May and June 2014 from three forest sites containing experimental *Castanea* restoration plantings in the Blue Ridge region of the Southern Appalachian Mountains (elevation=800-1,000 meters) (*Figure 1.1*). *Castanea dentata* and hybrid *Castanea* seedlings were planted at these sites following a shelter-wood cut in 2009. The canopies of these forests are dominated by *Quercu*s spp., *L. tulipifera* and *P. strobus*, while recent regeneration consists primarily of *L. tulipifera* and *Acer rubrum* L. (red maple). Soils are primarily fine-loamy, mesic Typic Hapludults (VA and NC) and Fine, kaolinitic, Kanhapludults (GA) (USDA National Cooperative Soil Survey).

At each site, we collected soils from at least three individual trees of each species in order to capture species-specific microbial assemblages. Soil samples (0-15 cm) were collected using a hand trowel from three locations beneath the canopy of each tree, which consisted of ~ 25% O horizon and 75% A horizon by depth. We homogenized soil by species within sites, but soils were maintained separately across sites. Soils were stored at 4°C for approximately 2-3 weeks until use.

For the matrix potting medium, we collected field soil from three sites within the Whitehall experimental forest in Athens, GA (elevation=200 meters) (*Figure 1.1*).

Whitehall forest soils are primarily Fine, kaolinitic, thermic Typic Kanhapludults (USDA National Cooperative Soil Survey). Soils were collected from areas dominated by *Pinus, Quercus* and *Carya* spp to a depth of ~ 25 cm. We combined soil from all sites and mixed with sand and peat (1:1:2 sand, peat, mixed field soil) and steam sterilized for 45 minutes.

Plant Growth Experiment

We conducted a fully reciprocal plant-soil feedback experiment with five tree species (*Castanea dentata*, *Castanea* hybrid (BC3F3, 15/16 *C. dentata*), *Pinus strobus*, *Quercus alba* and *Liriodendron tulipifera*) in which each tree species received soil inoculum treatments from all species as well as a sterile control treatment. These latter three species were selected for this experiment because of their abundance at our chestnut planting field sites and across the Southeastern range of *C. dentata*. Sample size was ten seedlings per soil treatment (with the exception of *Q. alba* seedlings, which was six per treatment due to lower germination rate). *C. dentata* and *Castanea* hybrids did not receive soil treatments from one another because this interaction was not relevant to our study. We removed *L. tulipifera* seedlings from our analysis because of insufficient replication due to low germination, but this species remained in our analysis as a soil treatment.

C. dentata, *Castanea* hybrids and *Q. alba* seeds were planted in 1:1 peat and sand and grown in a growth chamber for 5 weeks. Seedlings were then transplanted into 2 L tree pots containing 97.5% sterilized matrix soil and 2.5% soil inoculum by volume. We determined height and basal diameter in order to estimate initial biomass with allometric relationships. *P. strobus* and *L. tulipifera* seeds required a longer stratification period and were direct seeded into inoculated two-liter pots and thinned after 4 weeks. Pots were allowed to dry between waterings, and received water approximately 2-3 times per week. Seedlings were harvested after 5 months of growth in treatment soils, and root and shoot biomass of each seedling was cleaned and dried for at least 72 hours at 60°C and weighed separately.

Data Analysis

We determined the effect of soil inoculum treatment on seedlings as the relative growth response (RGR), which is the effect of a species-specific inoculum on seedling growth relative to the sterile control. We calculated RGR as $\left(\frac{G_{ij} - \mu_{ic}}{\mu_{ic}}\right) * 100$, where G_{ij} is the growth of a replicate of species *i* receiving soil treatment *j*, and μ_{ic} is the average growth of the control treatment of species *i*. To calculate growth, we estimated initial biomass of seedlings using allometric regression with basal diameter as the predictor (Nelson, Mesquita et al. 1999). Logarithmic regression was used for Q. alba seedlings to meet assumptions of normality (Baskerville 1972). Final biomass of dead seedlings was considered zero if it was confirmed that seedling mortality resulted from soil pathogen infection. The seedling was eliminated from analysis if the cause of mortality was unknown or clearly resulted from an above-ground problem, for example herbivory.

All analyses were conducted in R 3.1.1 for Mac (R Core Team 2014). We analyzed the effect of soil treatment on RGR and final root and shoot biomass of seedlings using mixed effects models in R package *lme4* with site as a random effect and seed weight as a covariate (Bates, Maechler et al. 2014). We used AIC to determine the best fitting models, and the best-fit model was tested for normality. We also conducted an analysis of deviance using type II Wald chi-square tests to determine significance of fixed effects using R package *car* (Fox and Weisberg 2011). Since multiple *P. strobus* seeds were directly planted then thinned, we did not include the seed weight covariate in our model for that species. We used generalized linear hypothesis testing to conduct post-hoc comparisons using R package *multcomp* (Hothorn, Bretz et al. 2008). We compared seedling growth responses to specific soil treatments within and between species using Tukey's HSD. We assessed differences between conspecific and overall heterospecific soil treatments within tree species using "home versus away" contrasts. In these contrasts, "home" soil was the conspecific soil treatment and "away" was all heterospecific treatments combined for a given tree species.

We analyzed the effect of soil treatment on mortality using a generalized linear model with a binomial distribution. A "1" was assigned to seedlings that died, while a "0" was assigned to seedlings that survived. We conducted an analysis of deviance using type II Wald chi-square tests in R package *car* to determine significance of soil treatment.

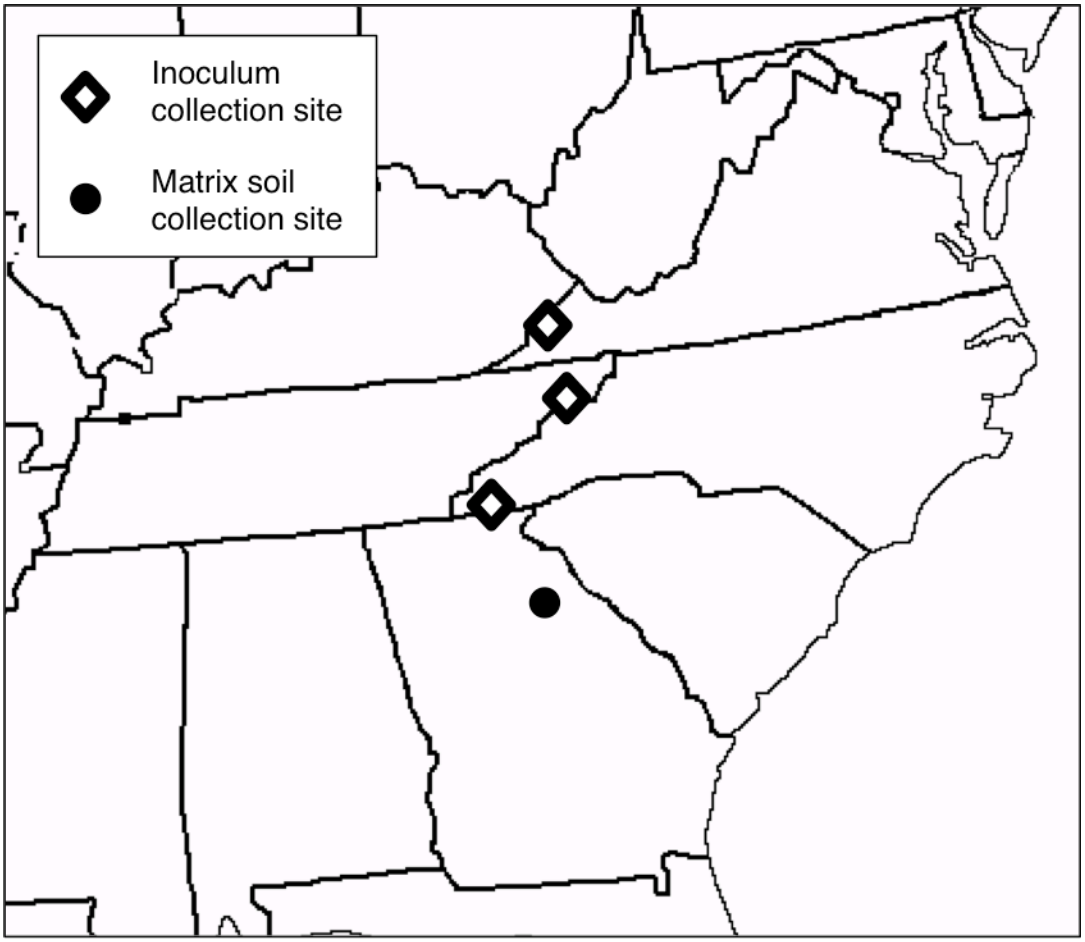

Figure 1.1: Collection sites of inoculum and matrix soils used in our plant-soil feedback greenhouse study.

CHAPTER 3

RESULTS

Overall, we found that *Castanea* seedlings had a higher relative growth response in heterospecific soils relative to conspecific soils and mortality only occurred in *Castanea* seedlings. We found that *C. dentata* and *Castanea* hybrids are similar in how they condition soils and respond to species-specific microbial assemblages. Soil inoculum did not have a significant effect on the growth of *Q. alba* and *L. tulipifera*.

C. dentata and *Castanea* hybrid seedlings had similar patterns in their relative growth response (RGR) to soil treatments (*Figure 2.2*), suggesting that these species are ecologically similar in their belowground interactions. RGRs of *C. dentata* and hybrids to each treatment were not significantly different ("home" (*Castanea)* soil: P=1.000, *L. tulipifera*: P=0.482, *P. strobus*: P=0.458, *Q. alba*: P=0.552, sterile control: P=0.997). Additionally, patterns of other species' growth responses were similar in *C. dentata* and *Castanea* hybrid soil treatments. Relative growth responses of both *P. strobus* and *Q. alba* were not significantly different in *C. dentata* versus *Castanea* hybrid soils (*P. strobus*: P=0.983, *Q. alba*: P=1.000), suggesting that *C. dentata* and *Castanea* hybrids have similar conditioning effects on soil microbial assemblages.

We observed strong negative feedbacks in both *C. dentata* and *Castanea* hybrids. The effect of soil treatment on relative growth response was significant in *C. dentata* and highly significant in *Castanea* hybrids (P=0.043, P=0.009 respectively).

Castanea spp. growth was lower in *Castanea* soil in contrast to other species' soils (*Figure 2.2*). Furthermore, *C. dentata* and *Castanea* hybrids had a significantly higher RGR in combined "away" soils relative to "home" soils (*P* = 0.002, *P* = 0.0002 respectively) (*Figure 2.1*), suggesting that *Castanea* species condition soils in a way that is antagonistic to conspecific growth.

Mortality was only observed in *C. dentata* and *Castanea* hybrid seedlings. Roots of all trees included in the mortality analysis had symptoms of infection by *Phytophthora cinnamomi*, an oomycete soil pathogen to which *Castanea* is particularly susceptible. Eight *C. dentata* seedlings experienced mortality. 40% of mortality occurred in "home" soil, 14% in control soil and 10% in *Q. alba* soil. One *Castanea* hybrid seedling experienced mortality in its own soil. Although the highest mortality occurred in seedlings growing in conspecific soils, this trend was not statistically significant in *Castanea* hybrids (P=0.148) and only marginally significant in *C. dentata* (P=0.053).

Overall, soil inoculum had little to no effect on the relative growth response of *Q. alba* and *P. strobus* (*Figure 2.2*). The effect of soil treatment on relative growth response was significant in *Q. alba* (P=0.032) and *Q. alba* seedlings tended to grow better in *L. tulipifera* and *P. strobus* soil than in their own soil. Although the results of the Wald Chi-Square test indicate that soil treatment has a significant effect on *Q. alba* growth, the Tukey's HSD did not reveal any significant differences between contrasting soil treatments (*Figure 2.2*). *P. strobus* seedling growth and final biomass were not significantly affected by soil treatment (P=0.88), and *P. strobus* seedlings grown in live soil inoculum tended to have lower growth than those in the sterile control treatment.

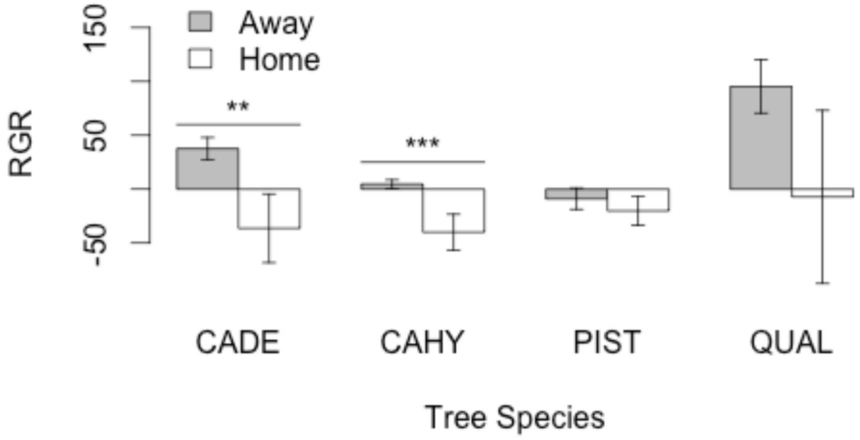

Figure 2.1: Home vs. away soil origin contrasts of relative growth response, or seedling growth relative to the average growth of sterile controls, of *Castanea dentata* (CADE), *Castanea* hybrids (CAHY), *Pinus strobus* (PIST) and *Quercus alba* (QUAL). *Castanea* spp. have significantly higher growth response in heterospecific soils relative to conspecific soils (*C. dentata*: P=0.002, *Castanea* hybrids: P=0.0002). Values represent mean and standard error (SE).

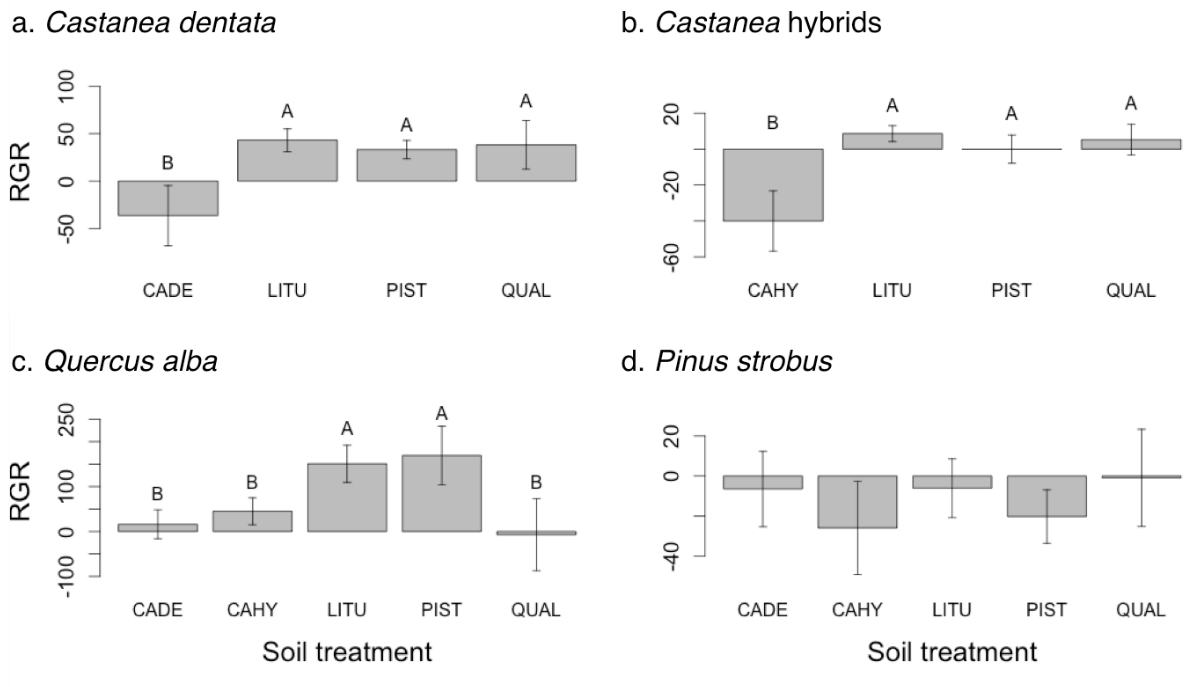

Figure 2.2. The effect of soil treatment on relative growth response (RGR), or seedling growth relative to the average growth of sterile controls, of a) *Castanea dentata* seedlings, b) *Castanea* hybrid seedlings, c) *Quercus alba* and d) *Pinus strobus* seedlings. Soil treatment has a significant effect on growth response in *C. dentata* (P=0.043), *Castanea* hybrids (P=0.009) and *Q. alba* (P=0.032). Values represent mean and standard error (SE). Letters indicate significance groups of Tukey's HSD.

CADE= *Castanea dentata*, CAHY= *Castanea* hybrid, LITU= *Liriodendron tulipifera*, PIST= *Pinus strobus* and QUAL= *Quercus alba*

CHAPTER 4

DISCUSSION

Our study demonstrates that *C. dentata* and *Castanea* hybrids have similar belowground ecological interactions. We expected that *C. dentata* and *Castanea* hybrids would perform similarly in soil treatments because hybrids are 15/16 *C. dentata* in genetic makeup. We found that *C. dentata* and *Castanea* hybrids had similar responses in growth to species-specific assemblages of microbes, which supports our first hypothesis. However, it is not clear from our results which current forest dominants will promote soil microbial assemblages favorable to hybrid *Castanea* growth. We expected that *Q. alba* and *P. strobus* soils would promote hybrid growth because those species, like *Castanea*, are ectomycorrhizal. Contrary to our expectations, our results suggest that hybrid *Castanea* growth was not significantly different in *Q. alba*, *L. tulipifera* and *P. strobus* soil treatments. This could indicate that ectomycorrhizal symbionts are not as important to *Castanea* plant-soil feedbacks as we predicted. It could also suggest that ectomycorrhizal mycelium may be more ubiquitous in our inoculum soils than we expected. Lastly, our study demonstrates that the hybrid *Castanea* soil microbial assemblages have no relative effect on the growth of current forest dominants. We expected that *Castanea* hybrid soils would promote *Q. alba* and *P. strobus* because those species are ectomycorrhizal. Contrary to our expectations, we found little to no effect of soil treatment on *Q. alba* and *P. strobus*. This may suggest that species-specific soil microbial assemblages are less important to *Q. alba* and *P. strobus* than we predicted, particularly relative to *Castanea*.

The results of our study have important inferences for management involving Castanea hybrid reintroduction. The first major inference is that our findings have encouraging implications for meeting the goals of hybrid Castanea breeding and reintroduction. The primary objective of The American Chestnut Foundation's backcross breeding program is to ecologically restore *C. dentata* by reintroducing blight-resistant hybrids (Diskin, Steiner et al. 2006). We found that *C. dentata* and *Castanea* hybrids performed similarly in soil inoculum treatments, suggesting that reintroduced hybrid *Castanea* may fill a similar belowground ecological niche to their parent species.

Second, because interactions with soil microbes are important to the growth and survival of *Castanea* hybrids, we can use information about their plant-soil feedbacks to enlighten management involving hybrid *Castanea* reintroduction. Since we have found that *Castanea* exhibit negative plant-soil feedbacks, it will be essential to consider the soil conditioning history of reintroduction sites as well as the density of planted hybrids within sites. Effects on soil microbial assemblages remain in soils even after plant removal. Therefore in shelter wood or clear-cut stands, soil conditioning by preexisting tree species may affect the growth and survival of planted *Castanea* hybrids. Specifically, preexisting wild *C. dentata* root sprouts may limit hybrid seedling survival because conspecific soil conditioning has a negative effect on *Castanea* growth. Planting density within sites may also be important to seedling survival. High planting density may result in increased seedling mortality because negative feedback, which involves soil conditioning that is antagonistic to conspecific growth, is a density-dependent process. Historically, Castanea grew in nearly monodominant stands (Paillet 2002), which seems contradictory our results demonstrating negative feedbacks and our suggestions regarding hybrid *Castanea* plantings. However, the soil pathogen *Phytophthora* has become considerably more abundant throughout the southern portion of *Castanea*'s range than it was prior to blight, and may play an important role in the negative feedbacks of *Castanea* in modern forests.

Our results suggest that negative feedbacks in *Castanea* may involve *Phythophthora* susceptibility. *Pythophthora cinnamomi* is a growing management concern in Eastern forests as the pathogen is frequently spread through human traffic and planting of infected nursery stock, and it is difficult to eliminate once introduced to an area (Hardham 2005). Members of the Fagaceae family are susceptible to *P. cinnamomi*, with the *Quercus* genus having moderate susceptibility, and the *Castanea* genus being the highly susceptible (Crandall, Gravatt et al. 1945). *C. dentata* possesses no natural resistance to the pathogen and breeding of blight-resistant hybrids did not previously select for *Phytophthora* resistance, therefore many hybrids are susceptible (Anagnostakis 2001, Jacobs 2007). Although we did not test our seedling roots for *Phythophthora*, patterns of mortality in our greenhouse experiment as well as previous tests at our planting sites suggest that the pathogen may be involved in the negative plant-soil feedbacks of *Castanea*. In two of our field sites, the presence of *P. cinnamomi* has been confirmed, and since 2009, mortality of planted *Castanea* spp. in our sites has resulted primarily from *P. cinnamomi* infection *(Clark, Schlarbaum et al. 2014)*. Mortality of *Castanea* was highest in conspecific soil treatments. Of those that died, conspecific deaths were isolated to soil inoculum from one field site in which *Phytophthora cinnamomi* had been confirmed.

Lastly, *Castanea* hybrid reintroduction may not have a significant relative effect on the growth of certain forest dominants in the Southeast. From our results, it is not apparent that *Castanea* hybrid microbial assemblages will have an effect on the growth of forest dominants. *Castanea* reintroduction may not result in large changes to dominant tree community composition of mixed hardwood forests in the Blue Ridge region.

This study is important because it has produced novel findings regarding the application of plant-soil feedbacks to management involving native tree species reintroduction. We have demonstrated that plant-soil feedbacks can be used to make suggestions for species reintroduction plans. We have found that hybrid Castanea exhibit negative feedbacks, which has lead us to specific conclusions about management strategies for reintroduction. Plant-soil feedbacks can vary considerably across spatial and temporal scales due to variation in climate and soil biogeochemistry (Bardgett and Wardle 2010). Since plant-soil feedbacks vary temporally and spatially for a given species, our understanding of the ecological impacts of reintroduction would be strengthened by more long-term, regionally specific research. While our findings are most relevant to *Castanea* hybrid reintroduction in Southeastern forests, our approach/method could be applied to other areas of the native range of Castanea as well as to other native plant reintroductions to understand regionally specific management requirements and ecological consequences of reintroduction.

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