


RESEARCH ARTICLE

Plant–soil feedbacks and the introduction of *Castanea* (chestnut) hybrids to eastern North American forests

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The reintroduction of disease-resistant hybrids is a commonly proposed solution to the introduction of pathogens and pests that weaken or eliminate native plant species. Plant interactions with soil biota result in plant–soil feedbacks (PSFs), which have consequences for individual plant growth and survival as well as broader community-level processes, such as species diversity and coexistence. Because of their importance, species reintroduction should consider these interactions, yet little work has integrated this perspective. Here, we investigate the effects of hybrid *Castanea* (chestnut) reintroduction on PSFs and how these mechanisms may influence the recruitment of other species in contemporary forests. We also examine how blight-resistant *Castanea* hybrids perform in the soil conditions of contemporary forests and we compare their belowground interactions with those of *Castanea dentata*. We conducted a reciprocal greenhouse experiment testing the effect of species-specific soil inoculum on the growth and survival of *C. dentata*, *Castanea* hybrids, and other forest dominants. Our results suggest that *C. dentata* and hybrids had similar belowground interactions and were regulated by negative PSFs, meaning soil microbial communities reduced conspecific growth and survival. These negative PSFs may involve the presence of the non-native pathogen *Phytophthora cinnamomi*. Soil inoculum of *C. dentata* and *Castanea* hybrids had similar effects on heterospecific growth, suggesting *Castanea* restoration will have neutral effects on natural regeneration in restoration plantings. We conclude that *Castanea* hybrids may fill a similar belowground niche to their parent species, and that site selection, screening for soil pathogens, and site planting density will be important to restoration.

Key words: forest dominants, hybrid chestnut, microbial assemblage, negative plant–soil feedback, species reintroduction

Implications for Practice

- *Castanea* hybrids had similar interactions with soil biota to their parent species, *Castanea dentata*, and may fill a similar belowground niche.
- Non-native pathogen presence and planting density in restoration sites will be important to *Castanea* hybrid growth and survival.
- Microbial assemblages of reintroduced *Castanea* may have little to no effect on the growth of other dominant forest species.

Introduction

Introduced pests and pathogens continue to alter the composition of eastern temperate forests of the United States (Fisher et al. 2012; Boyd 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 (Snieszko 2006; Merkle et al. 2007; Newhouse et al. 2014). Several programs of this nature are underway, such as those for *Juglans cinerea* L. (butternut or white walnut) (Michler et al. 2006) and the formerly widespread *Ulmus americana* L. (American elm) (Newhouse et al. 2007) and *Castanea*

dentata (Marsh.) Borkh. (American chestnut) (Collins et al. 2017). However, because of regulatory challenges and lack of complete resistance in many hybrids, reintroductions of these species have not yet been implemented on a large scale. Given the present rarity of this type of management action, the effectiveness and the long-term ecological consequences of hybrid reintroduction are unclear.

Castanea dentata, once a dominant canopy tree of eastern forests, experienced widespread mortality in the early 20th century due to an introduced airborne 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 that rarely reaches a

Author contributions: EMC, RPS, NW conceived and designed the research; EMC performed the research; EMC analyzed the data; SLK contributed field site access; EMC, RPS, SLK, NW wrote and edited the manuscript.

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doi: 10.1111/rec.13326

Supporting information at:

<http://onlinelibrary.wiley.com/doi/10.1111/rec.13326/supinfo>

reproductive stage (Paillet 2002). Prior to chestnut blight, *C. dentata* was a foundation species because of its high abundance and important stabilizing effect on ecological conditions for other species (Ellison et al. 2005). Because of its dominance, loss of *C. dentata* resulted in large changes in nutrient cycling (Ellison et al. 2005), forest resource pulses and availability (Diamond et al. 2000; Dalgleish & Swihart 2012), and forest community composition (Elliott & Swank 2008). The importance of *C. dentata* has motivated nonprofit organizations, academic institutions, and state and federal agencies to attempt to restore the species to its native range by producing hybrids bred or genetically modified for blight resistance (Steiner et al. 2017).

The restoration of *Castanea dentata* is perhaps the largest and most advanced reintroduction project involving disease-resistant hybrids, and it is considered a model for plant species reintroduction (Jacobs et al. 2013). For nearly 100 years, public and private programs have been breeding for blight resistance (Anagnostakis 2012), and most recently, *Castanea* hybrids produced through a backcross breeding technique (Burnham et al. 1986) are being tested in forest field trials (Clark et al. 2014, 2016, 2019). This method uses Asian chestnut species, most notably *Castanea mollissima* Blume (Chinese chestnut), as the initial source of blight resistance. After subsequent backcrosses to *C. dentata*, followed by intercrossing, hybrids are theoretically 15/16 *C. dentata* in their genetic make-up, but maintain *C. mollissima* genes for blight resistance (Burnham et al. 1986; Anagnostakis 2012). Throughout the breeding process, trees are selected for their level of blight resistance and *C. dentata*-like growth form and phenotypic characteristics (Diskin et al. 2006; Cipollini et al. 2017). Hybrids are therefore expected to be functionally equivalent to native *C. dentata* in all traits except blight resistance. However, because selection for *C. dentata* traits focused entirely on aboveground characteristics, we do not if hybrids are similar in their belowground traits. More recently, researchers have generated transgenic *C. dentata* with enhanced blight resistance through transformation of an oxalate oxidase gene from wheat, but this material is not yet federally approved for release (Newhouse et al. 2014). The reintroduction of *C. dentata* will depend on the success of these programs in producing *Castanea* hybrids with durable blight resistance that can compete successfully in the wild with negligible ecological impacts.

To effectively manage and predict the outcomes of *Castanea* reintroduction, it will be important to consider how hybrids interact with soil microorganisms, and subsequently, how these effects will impact the broader forest community. Direct microbial effects on plants can fall along a spectrum from positive mutualistic symbionts (Smith & Read 2008) to negative antagonistic (Mills & Bever 1998) associations with pathogens, and there are also numerous indirect effects from decomposers and nutrient-cycling organisms (Bardgett & Wardle 2010). Plants alter the composition of soil microbial communities in species-specific ways, and “feedbacks” can manifest through the subsequent effects of plant-conditioned microbial communities on the survival and growth of both conspecific and heterospecific plant species (Bezemer et al. 2006; Bardgett & Wardle 2010; Bever et al. 2012).

These plant–soil feedbacks (PSFs) have broad consequences on the composition of plant communities, species diversity,

coexistence, and plant productivity (Bonanomi et al. 2005; van der Heijden et al. 2008; Mack et al. 2019). Many temperate species are characterized by pathogen-driven negative PSFs, or soil conditioning effects that are antagonistic to conspecific growth (Mills & Bever 1998; Packer & Clay 2003; McCarthy-Neumann & Ibáñez 2013). These negative PSFs play an important role in maintaining plant community diversity and coexistence of competitors (Bever 2003; Bonanomi et al. 2005; Mack et al. 2019). Indirect reciprocal positive interactions can occur when two competing species exhibit negative PSFs, resulting in species codominance (Bonanomi et al. 2005). As such, biotic interactions help determine community and ecosystem-level consequences of reintroduction because they regulate plant species abundance and distribution. In the context of restoration, plant–soil interactions are critical to consider (Eviner & Hawkes 2008; van der Putten et al. 2013), as the composition of existing microbial communities has the potential to promote or suppress newly introduced species. Species reintroductions will also generate new, species-specific effects on soil microbes; however, little work has been done to integrate this perspective into reintroduction efforts. Here, we examine how PSFs could inform the reintroduction and management of hybrid *Castanea dentata*.

The prospect of large-scale restoration of *C. dentata* raises questions about how the hybrid will perform in contemporary forests and, in turn, how the forest community will respond to its introduction. Few studies have examined the ecology of hybrids in comparison with their parent species, and of those studies, most have focused on aboveground interactions, such as those with animals (Blythe et al. 2015; Goldspiel et al. 2019). Of those studies examining belowground interactions, even fewer have focused on those with soil biota. Understanding the ecology of *Castanea* hybrids from a PSF perspective will be critical for restoration efforts because (1) existing evidence points to the importance of soil biota on *C. dentata* growth and (2) little is known about how the belowground interactions of hybrids compared to those of the parent species. Interactions with pathogens are important to PSF dynamics because they can drive negative feedbacks. *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 et al. 1945). Hybrids can also be sensitive to this pathogen because blight-resistance breeding did not select for resistance to *Phytophthora*. *C. dentata* also associates with a large number of ectomycorrhizal fungi (Palmer et al. 2008; Stephenson et al. 2017), which are beneficial symbionts that exchange water and macronutrients for plant photosynthate (Smith & Read 2008). Members of the Fagaceae family are dependently mycorrhizal (Smith & Read 2008) and mycorrhizal colonization is associated with higher growth rates in *C. dentata* (Bauman et al. 2017). These mutualisms play an important role in PSFs because they can drive positive feedbacks. Associations with mycorrhizal fungi were likely vital to the establishment and growth of *C. dentata* and will be important in the reestablishment of hybrids, yet we do not currently know if *C. dentata* and hybrids form similar mycorrhizal

associations. Prior to chestnut blight, *C. dentata* co-occurred with *Quercus* (oak) and *Carya* (hickory) species (Braun 1947; Paillet 2002), a dynamic that may have been driven by negative PSFs. Following the loss of chestnut, forest community composition changed, with *Quercus*, *Carya*, *Acer* (maple), and *Pinus strobus* L. (white pine) species replacing *C. dentata* throughout most eastern forests (Frothingham 1924; Woods & Shanks 1959; Stephenson 1986). In addition, fire suppression during the 20th century has led to a shift from fire-tolerant to shade-tolerant, fire-sensitive plant communities through the positive feedback cycle of mesophication (Nowacki & Abrams 2008). As a result, the dynamics of PSFs in eastern forests have undoubtedly changed since the functional loss of *C. dentata*.

Current ecological knowledge of *C. dentata* and *Castanea* hybrids is needed to predict the consequences of reintroduction and ecological niche of hybrids in contemporary forests (Jacobs et al. 2013). Also, to our knowledge, no studies have compared PSFs of hybrids to their parent species. This comparison is important to our understanding of the ecological functioning of hybrids and if it differs from that of *C. dentata*. If the direction and strength of hybrids' belowground interactions are not significantly different from those of the parent species, this would support the postulation that hybrid reintroduction could effectively restore ecological functioning of chestnut in contemporary forests. This leads us to ask how will *Castanea* hybrids perform in the soil conditions of contemporary forests, and are their belowground interactions similar to those of the parent species, *C. dentata*? Furthermore, how will *Castanea* hybrid reintroduction affect PSFs, and what subsequent effect will this have on the recruitment of other forest species?

To address these questions, we investigated the direction and strength of PSFs for *C. dentata*, *Castanea* hybrids, and current forest dominants *Quercus alba* L. (white oak), *Liriodendron tulipifera* L. (tulip poplar), and *Pinus strobus*. Using field-collected soils as inocula, we conducted a reciprocal greenhouse experiment to examine the effects of species-specific inoculum on tree growth and mortality. Given their close genetic relatedness and selection for *C. dentata* traits during hybrid breeding, we expected that (1) *C. dentata* and *Castanea* hybrid PSFs would be similar in their direction and strength. We also predicted that (2) *Q. alba* and *P. strobus* would produce soil most favorable to chestnut growth because these species, like *Castanea*, are ectomycorrhizal and may promote mutualists specific to *Castanea*. In contrast, *L. tulipifera* forms arbuscular mycorrhizae and may not promote favorable mutualists for *Castanea*. Thus, we predicted that (3) *Q. alba* and *P. strobus* seedlings would have the higher growth in *Castanea* spp., *Q. alba*, and *P. strobus* soils than in *L. tulipifera* soil.

Methods

Site Description and Soil Collection

We collected soil inoculum in May and June 2014 from two forest sites containing experimental *Castanea* restoration plantings (VA and NC) and one natural, unrestored forest site (GA) in the Central Appalachian Broadleaf Forest Province in the Southern

Appalachian Mountains (Clark et al. 2016, 2019) (elevation: 840–1,050 m above sea level) (Fig. S1). *Castanea dentata* and hybrid *Castanea* seedlings (BC3F3, described by Hebard 2006) were planted at the VA and NC sites following a shelterwood with reserve regeneration harvest in 2009. The residual canopies of these forests were dominated by *Quercus* spp., with minor components of *Liriodendron tulipifera* and *Pinus strobus*, while recent regeneration consists primarily of *L. tulipifera* and *Betula lenta* L. (sweet birch), with minor components of *Acer rubrum* L. (red maple). Soils at our sites were primarily fine-loamy, mesic Typic Hapludults (VA and NC) and fine, kaolinitic, Kanhapludults (GA) (USDA National Cooperative Soil Survey).

At each site, we collected soil inocula from beneath three to four of the largest planted *C. dentata* and *Castanea* hybrids and native *P. strobus*, *Q. alba*, and *L. tulipifera* at the site in order to capture species-specific microbial assemblages. Not all species were present at every site, but each species was present in at least two sites. Inoculum soil samples (0–15 cm) were collected using a hand trowel from three locations beneath the canopy of each tree, which on average consisted of 25% O horizon and 75% A horizon by depth. We homogenized soil by species within sites, but soils remained separated across sites. Soils were stored at 4°C for up to 2.5 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 m) (Fig. S1). We used field collected soils to emulate the structure and composition of soil that our study species would experience in situ. 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 it with sand and peat (1:1:2 sand, peat, mixed field soil) and steam sterilized for 45 minutes.

Plant Growth Experiment

We conducted a reciprocal transplant experiment with five tree species: *Castanea dentata*, *Castanea* hybrid (BC3F3, described by Hebard 2006), *P. strobus*, *Q. alba*, and *L. tulipifera*. *C. dentata*, and *Castanea* hybrids used in our experiment were each collected from three seed lots in fall 2013 and provided to us by The American Chestnut Foundation. Each tree species received soil inoculum treatments from all species as well as a sterile control treatment in which we did not add inoculum. These latter three species were selected for this experiment because of their abundance across the southeastern range of *C. dentata*. Sample size was 10 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. Non-*Castanea* seeds were procured from Sheffield's Seed Company.

Prior to planting, we stratified seeds for at least 6 weeks. *C. dentata*, *Castanea* hybrids, and *Q. alba* seeds were weighed and planted in 1:1 peat and sand in 8.25" cone-tainers and grown in a growth chamber for 5 weeks. We randomly assigned seedlings to a soil treatment, then transplanted into 2-L tree pots containing 97.5% sterilized matrix soil and 2.5% soil inoculum by volume and transferred to a greenhouse. Seedlings were arranged using a randomized block design. At transplant, we measured basal diameter of *Castanea* species and *Q. alba* to estimate initial biomass through allometry. Due to the longer length of time required for *P. strobus* stratification, we directly seeded these species in the greenhouse into inoculated 2-L pots and thinned to the largest seedling after 4 weeks. Pots were allowed to dry between waterings, and received water two to three times per week. Seedlings were harvested after 5 months, and root and shoot biomass of each seedling was cleaned and dried for at least 72 hours at 60°C and weighed separately.

We estimated total initial biomass, or biomass at planting, of seedlings using allometric regression with basal diameter as the predictor (Nelson et al. 1999). Logarithmic transformation was used for *Q. alba* seedlings to meet assumptions of normality (Baskerville 1972). Final total biomass of dead seedlings was considered zero if it was visually confirmed that seedling mortality resulted from soil pathogen infection (i.e. black roots or brown staining inside stem). The seedling was eliminated from analysis if the cause of mortality was unknown or clearly resulted from an aboveground problem, e.g. white fly damage. Since multiple *P. strobus* seeds were directly planted then thinned, initial biomass was considered 0.

Data Analysis

For each species, we analyzed the effect of soil treatment (predictor) on growth (response), which we calculated as final dry biomass—estimated initial dry biomass, using linear models. We tested seed weight as a covariate and inoculum collection site as a random effect. We used the Akaike information criterion (AIC) to determine the best fitting models, and the best-fit model was tested for normality. We selected the model with the lowest AIC but gave preference to a model with a higher AIC within two AIC units of the lowest AIC value if it had fewer parameters. We log-transformed growth data for *P. strobus* to meet assumptions of normality. Mixed-effects models were tested using maximum likelihood. We also conducted a type II

Table 1. Results of the type II ANOVA for models with growth and mortality as response variables. Asterisks indicate p values ≤ 0.05 . CADE, *Castanea dentata*; CAHY, *Castanea* hybrid; PIST, *Pinus strobus*; QUAL, *Quercus alba*.

Species	Growth			Mortality		
	df	F value	p value	df	χ^2	p value
CADE	4	2.642	0.049*	4	9.348	0.053
CAHY	4	3.660	0.012*	4	6.786	0.148
QUAL	5	2.701	0.040*	—	—	—
PIST	5	1.980	0.587	—	—	—

analysis of variance (ANOVA) to determine significance of soil treatment using R package *car* 3.0-7 (Fox & Weisberg 2019). We used generalized linear hypothesis testing to conduct post hoc comparisons using R package *multcomp* 1.4–12 (Hothorn et al. 2008). We compared seedling growth responses to specific soil treatments within and between species using Tukey's honestly significant difference (HSD). To test hypothesis 1, we contrasted growth of *C. dentata* and *Castanea* hybrids in the same soil treatments, as well as contrasted growth response across soil treatment within these two species. To test hypotheses 2 and 3, we contrasted growth response to soil treatment within each species. With the same package, we assessed differences between conspecific and heterospecific soil treatments within tree species with another post hoc comparison by contrasting conspecific soil treatments with the combined heterospecific treatments for a given tree species.

We analyzed the effect of soil treatment (predictor) on mortality (response) 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. All analyses were conducted in R 3.6.2 (R Core Team 2019).

Results

The linear model with growth as the response and soil treatment as the predictor variable had the best fit of all models tested (Table S1). Neither seed weight as a covariate, nor site as a random effect improved model fit. Overall, *Castanea dentata* and the *Castanea* hybrid were similar in how they conditioned soils and responded to species-specific microbial assemblages. We found that *C. dentata* and *Castanea* hybrid seedlings had higher growth in heterospecific soils relative to conspecific soils, and mortality only occurred in *C. dentata* and *Castanea* hybrid seedlings. Soil inoculum did not have a significant effect on the growth of *Quercus alba* and *Pinus strobus*.

Hypothesis 1: *C. dentata* and *Castanea* hybrid PSFs would be similar in their direction and strength.

In contrasting growth response to soil treatments within species, we observed strong negative PSFs in both *C. dentata* and *Castanea* hybrids. The effect of soil treatment on growth was significant in *C. dentata* and *Castanea* hybrids (Table 1). *Castanea* spp. growth was lower in *Castanea* soil in contrast to other species' soils (Fig. 1). Furthermore, *C. dentata* and *Castanea* hybrids had significantly higher growth (2.6x and 1.7x higher, respectively) in combined heterospecific soils relative to conspecific soils (Fig. 2). Growth of both *P. strobus* and *Q. alba* were not significantly different in *C. dentata* versus *Castanea* hybrid soils (*P. strobus*: $p = 0.718$; *Q. alba*: $p = 0.998$), suggesting that *C. dentata* and *Castanea* hybrids have similar conditioning effects on soil microbial assemblages.

When contrasting growth response to the same soil treatment across *C. dentata* and *Castanea* hybrid seedlings, treatments had

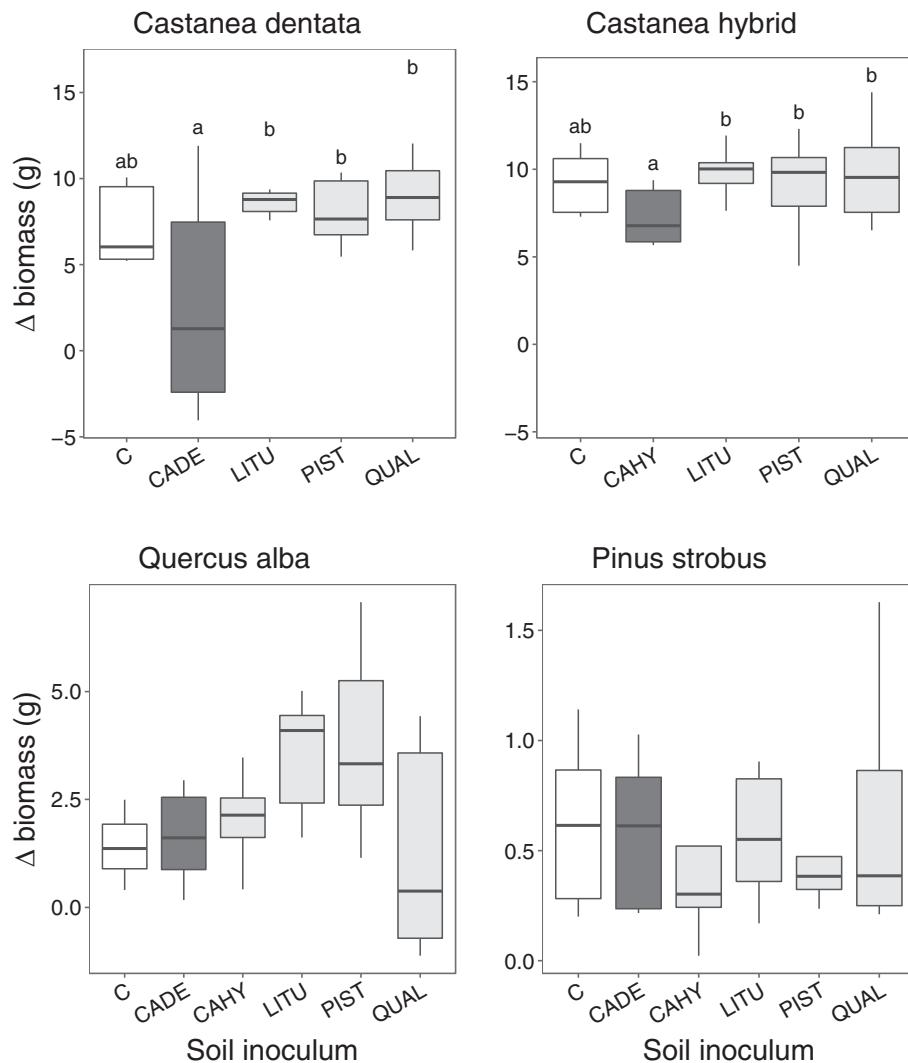


Figure 1. The effect of soil treatment on growth, or change in total biomass (g). Soil treatment has a significant effect on growth in *Castanea dentata*, *Castanea* hybrids, and *Quercus alba*. Letters indicate statistically significant groups for *C. dentata* and *Castanea* hybrids based on Tukey's HSD ($\alpha = 0.05$). Tukey's HSD test did not reveal any significant differences between contrasting soil treatments of *Q. alba*. Bar colors within each panel represent soil treatment type received by seedlings: sterile control (white), conspecific (dark gray), and heterospecific (light gray). C, control; CADE, *Castanea dentata*; CAHY, *Castanea* hybrid; LITU, *Liriodendron tulipifera*; PIST, *Pinus strobus*; and QUAL, *Quercus alba*.

a similar effect on growth of *C. dentata* and hybrid seedlings (Fig. 1). Growth of *C. dentata* in each soil treatment was not significantly different from that of *Castanea* hybrids in the same soils (conspecific [*Castanea*] soil: $p = 1.000$; *Liriodendron tulipifera*: $p = 0.482$; *P. strobus*: $p = 0.458$; *Q. alba*: $p = 0.552$; sterile control: $p = 0.997$).

In addition, mortality was only observed in *C. dentata* and *Castanea* hybrid seedlings (Table 2), and almost exclusively in conspecific soil treatments. Roots of all trees included in the mortality analysis had symptoms of infection consistent with disease caused by *Phytophthora cinnamomi*, an oomycete soil pathogen to which *Castanea* is particularly susceptible. Eight *C. dentata* seedlings experienced mortality. Forty percent of mortality occurred in conspecific 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, species-specific soil effects were not statistically significant in *Castanea* hybrids and marginally significant in *C. dentata* (Table 1).

Hypothesis 2: *Q. alba* and *P. strobus* would produce soil most favorable to chestnut growth.

We expected that *Castanea* growth would be highest in *Q. alba* and *P. strobus* soils because these species, like *Castanea*, are ectomycorrhizal and would promote beneficial mutualists specific to *Castanea*. The growth of *C. dentata* and *Castanea* hybrid seedlings was highest in heterospecific soils (Fig. 1), but when contrasting growth response to soil treatments within species, there were not significant differences in growth across the

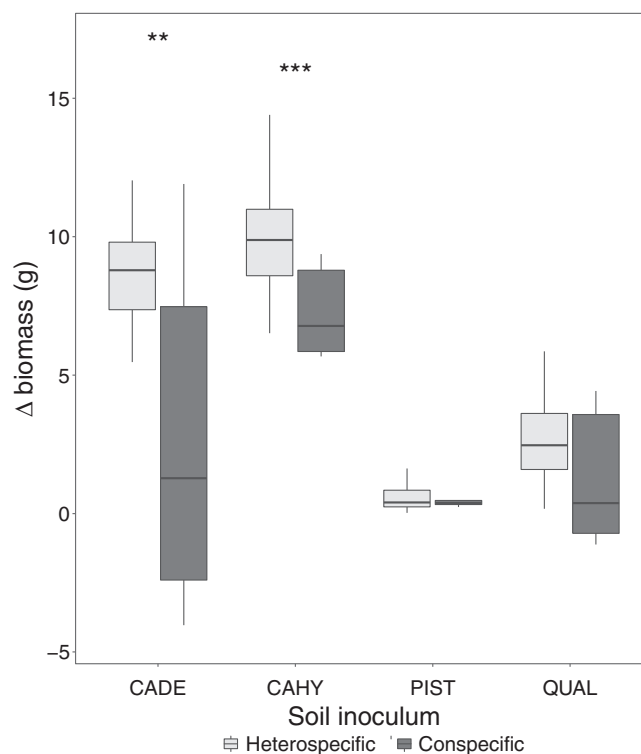


Figure 2. Conspecific versus heterospecific soil origin contrasts of growth, or change in biomass (g), of *Castanea dentata* (CADE), *Castanea* hybrids (CAHY), *Pinus strobus* (PIST), and *Quercus alba* (QUAL). Outliers are represented by points above and below box plots. As indicated by asterisks, for which number indicates level of significance, *Castanea* spp. have significantly higher growth in heterospecific soils relative to conspecific (*C. dentata*: $p = 0.002$, *Castanea* hybrids: $p \leq 0.001$).

three heterospecific soil treatments (*L. tulipifera*, *P. strobus*, and *Q. alba*) in either *C. dentata* or hybrid seedlings.

Hypothesis 3: *Q. alba* and *P. strobus* seedlings would have the greatest growth in *Castanea* spp. soils relative to other tree species.

Soil inoculum had a significant effect on the growth of *Q. alba*, but not on the growth of *P. strobus* (Table 1, Fig. 1). *Q. alba* seedlings had 170% higher growth in *L. tulipifera* and 190% higher growth in *P. strobus* soil than in their own soil.

Table 2. Percent mortality of *Castanea dentata* and *Castanea* hybrid seedlings in each soil treatment. No *Quercus alba* or *Pinus strobus* seedlings experienced mortality. C, control; CADE, *Castanea dentata*; CAHY, *Castanea* hybrid; LITU, *Liriodendron tulipifera*; PIST, *Pinus strobus*; QUAL, *Quercus alba*.

Soil treatment	Species	
	CADE	CAHY
CADE	40%	—
CAHY	—	10%
QUAL	10%	—
PIST	—	—
LITU	—	—
C	10%	—

Although the results of the ANOVA indicate that soil treatment has a significant effect on *Q. alba* growth, a post hoc Tukey's HSD test did not reveal any significant differences in growth response to contrasted soil treatments (Fig. 1).

Discussion

We conducted a PSF study with the goal of inferring how plant–microbial interactions could influence the ability of *Castanea* hybrids to reestablish in eastern forests and how these communities would be influenced by this reintroduction. Our findings suggest that *Castanea dentata* and *Castanea* hybrids have similar PSFs. We found support for our first hypothesis as *C. dentata* and *Castanea* hybrids had similar directionality and strength of responses in growth to species-specific assemblages of microbes, and both exhibited significantly lower growth in conspecific soil than heterospecific soils. Contrary to our second hypothesis, we found that hybrid *Castanea* growth was not significantly different in *Quercus alba*, *Liriodendron tulipifera*, and *Pinus strobus* soil treatments. Lastly, our study suggests that the hybrid *Castanea* soil microbial assemblages may have little effect on the growth of current forest dominants. Contrary to our third hypothesis, we found no effect of soil treatment on *P. strobus*, and no significant differences between contrasted soil treatments for *Q. alba*, which suggests that species-specific soil microbial assemblages could be less important to *Q. alba* and *P. strobus* growth than we expected, particularly relative to *Castanea*.

Our findings have encouraging implications for meeting the goals of hybrid reintroduction. One major objective of species restoration, and particularly in the restoration of a foundational species like *C. dentata*, is to restore the species' ecological function. This objective requires that the interactions of the reintroduced hybrid with biotic organisms and the abiotic environment reflect those of the parent species (Jacobs et al. 2013). We found that *C. dentata* and *Castanea* hybrids responded in a similar pattern to soil inoculum treatments. Both species experienced significantly lower growth in conspecific soil relative to the sterile control and hetero-specific soil, indicating negative PSFs, which suggests that reintroduced *Castanea* hybrids may fill a similar belowground ecological niche to their parent species. Historically, in old growth stands, *C. dentata* rarely grew in monoculture (Frothingam 1924) and often codominated with *Quercus* and *Carya* species (Paillet 2002). The negative PSFs that we observed in *Castanea* could have promoted coexistence with these historical competitors.

Interactions with soil microbes appear to be important to the growth and survival of *Castanea* hybrids, and we can use information about their PSFs to inform management of hybrid *Castanea* reintroduction. Because *Castanea* seedlings exhibit negative PSFs, it will be essential to consider both the soil conditioning history of reintroduction sites and the density of planted hybrids within sites. Soil conditioning effects on microbial assemblages remain in soils even after plant removal (Kardol et al. 2007). Therefore, in forest restoration sites, soil conditioning by preexisting tree species may affect the growth and survival of planted *Castanea* hybrids. It may be especially important in regeneration harvests, such as shelterwoods or clearcuts, where some or all overstory species have been removed, to have a record of which species previously occurred (and in what abundance) in those sites. Specifically, preexisting wild *C. dentata* root sprouts in restoration sites 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. Most plant species experience conspecific negative density dependence, meaning proximity to adult individuals negatively affects offspring growth and survival (Johnson et al. 2012). Negative PSFs play an important role in driving these density-dependent effects because they limit conspecific growth (McCarthy-Neumann & Ibáñez 2013). The negative PSFs that we have observed in *Castanea* hybrids suggest that higher planting density could increase the risk seedling mortality in restoration sites.

The negative PSFs we have observed in *C. dentata*, and to a lesser extent, *Q. alba*, are consistent with the historical coexistence of these species. However, this feedback may be further exacerbated by the introduction of the soil pathogen *Phytophthora cinnamomi*. This oomycete pathogen is a growing management concern in eastern U.S. forests as it 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; Balci et al. 2007). *Phytophthora* is widespread in the southern and central portion of *C. dentata*'s range and is predicted to expand northward with climate change (Burgess et al. 2017). Members of the Fagaceae family are susceptible to *P. cinnamomi*, with the *Quercus* genus having moderate susceptibility, and the *Castanea* genus being highly susceptible (Crandall et al. 1945; McConnell et al. 2015). Because *C. dentata* possesses no natural resistance to the pathogen and

breeding of blight-resistant hybrids did not previously select for *Phytophthora* resistance, many hybrids are also susceptible (Anagnostakis 2001; Jacobs 2007). Although we did not test our seedling roots for *Phytophthora* colonization, patterns of mortality in our greenhouse experiment as well as previous samples (Clark unpublished data) from our planting sites suggest that the pathogen may be directly involved in the negative PSFs of *Castanea*. In two of our field sites, the presence of *P. cinnamomi* was confirmed outside and within the planting areas, and since 2009, mortality of *Castanea* spp. planted at our sites has resulted primarily from *P. cinnamomi* infection, particularly at one site (Clark et al. 2014). In our experiment, mortality of *Castanea* was highest in conspecific soil treatments, and of those that died, conspecific deaths were isolated to soil inoculum from the one field site in which *P. cinnamomi* had been confirmed. Given this evidence, we suspect that *P. cinnamomi* is involved in the negative PSF that we observed in *C. dentata* and *Castanea* hybrids because the pathogen can directly influence seedling growth and survival. Given the increasing pervasiveness of *P. cinnamomi*, these pathogen-driven negative PSFs in *Castanea* hybrids could pose concerns for the effectiveness of restoration by reducing postintroduction survival and limiting population growth. Careful site selection, soil testing, and low planting densities may be necessary to reduce mortality of reintroduced hybrids.

Phytophthora cinnamomi-induced mortality and species declines can also alter the dynamics of PSFs in forest ecosystems more indirectly on a community scale. Gómez-Aparicio et al. (2017) found that *P. cinnamomi*-induced loss of *Quercus suber* altered the direction and strength PSFs of other tree species in Mediterranean *Quercus* forests through changes to the abiotic and biotic soil conditions. They found that the direction in which *P. cinnamomi*-induced loss modified PSFs (whether they became more positive or negative) was not uniform across the landscape and depended on canopy openness. Since *P. cinnamomi*'s introduction to North America preceded that of chestnut blight by at least 75 years (Crandall & Gravatt 1967; Anagnostakis 2012), it is likely that *P. cinnamomi*-induced declines in *C. dentata* were indirectly altering the PSFs of eastern U.S. forests before the introduction of blight. Thus, it is possible that the direction and strength of *C. dentata* PSFs were different (perhaps less negative or neutral) prior to the introduction of *P. cinnamomi*. In contemporary eastern forests, we might expect to observe variation across the landscape in the effects of *P. cinnamomi* on PSFs of dominant canopy species because there is significant heterogeneity in geography, abiotic and biotic soil attributes, and plant community composition.

A final implication of this study is that change to the soil microflora caused by *Castanea* hybrid reintroduction may not have a strong effect on the growth of the forest dominants we examined. *Castanea* reintroduction may not result in large changes to dominant tree community composition of mixed hardwood forests in the Blue Ridge region. However, our study only examined a small number of tree species common in this region, and we used an ex situ experiment. Thus, it is possible that, in situ, *Castanea* soils could affect the growth of the forest dominants we studied, as well as other dominant species, such as *Acer rubrum* or *Betula lenta*. Also, *Castanea* reintroduction could influence the current forest community in ways that we did not test for, e.g. direct competition or abiotically mediated

PSFs, such as those driven by pH and nutrient quality of litter. Additionally, future work examining the composition and ecology of the soil microbial communities involved in these PSFs would allow us to better understand what mechanisms are driving patterns of growth. Genetic analysis of microbial taxa would confirm the presence of pathogens, such as *Phytophthora*, or specialized mutualists, and help us determine which microbial species might be involved in the PSFs we observed.

PSFs can vary considerably across spatial and temporal scales due to variation in climate, soil biogeochemistry, local adaptation, and a combination of these factors. For example, the availability of soil nutrients can influence the direction and strength of PSFs by altering the resource exchange economy between plants and microbial symbionts (Revillini et al. 2016). Broad-scale variation in nutrient availability across the geographic range can lead to local adaptation in these plant-microbial interactions. In this study, we did not use local seed sources relative to the soil collection locations, and therefore cannot draw any conclusions about local adaptation of these PSFs. Future work should examine PSFs across the species range and incorporate local seed sources to broaden our understanding of the ecological impacts of reintroduction.

Our findings provide novel insights into how PSFs may affect management involving native tree species reintroduction, and we argue that PSFs are valuable in guiding species reintroduction plans. We have found that hybrid *Castanea* exhibit negative feedbacks, which leads us to three specific conclusions about management strategies and implications for reintroduction. We have concluded that (1) *Castanea* hybrids may fill a similar belowground niche to their parent species, (2) pathogen presence and planting densities in restoration sites are important to *Castanea* growth and survival, and (3) microbial assemblages of reintroduced *Castanea* may have little effect on the growth of heterospecifics. Our findings are useful to *Castanea* hybrid reintroduction and management in southeastern forests, and we argue that similar approaches could be used in other study systems and geographic locations, as well as with transgenic *Castanea*. This approach could benefit other native plant species reintroductions by improving our understanding of regionally specific management requirements and the potential ecological impacts of reintroduction.

Acknowledgments

This study was supported by The Odum School of Ecology, The Garden Club of Georgia (GCG), and National Garden Clubs, Inc. (NGC). The American Chestnut Foundation (TACF) provided *C. dentata* and hybrid *Castanea* seeds for our experiment. We thank R. Lankau, J. Johnson, J. Minucci, B. Walker, and M. Patillo for their assistance with this research. We thank S. Sheth, R. Wooliver, A. Querns, E. Vtipil, and S. Flake for their constructive comments on the manuscript.

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Supporting Information

The following information may be found in the online version of this article:

Figure S1. Collection sites of inoculum and matrix soils used in our PSF greenhouse study.

Table S1. Model selection for each species.