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ECOSPHERE

Seedling survival declines with increasing conspecific density in a common temperate tree

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Abstract. Feedbacks between plants and their soil microbial communities often drive negative density dependence in rare, tropical tree species, but their importance to common, temperate trees remains unclear. Additionally, whether negative density dependence is driven by natural enemies (e.g., soil pathogens) or by high densities of seedlings has rarely been assessed. Density dependence may also depend on seedling size, as smaller and/or younger seedlings may be more susceptible to mortality agents. We monitored seedlings of *Quercus rubra*, a common, canopy-dominant temperate tree, to investigate how the density of neighboring adults and seedlings influenced their survival over two years. We assessed how the soil microbial community influenced seedling survival by growing seedlings in a glasshouse inoculated with soil collected from beneath conspecific and heterospecific mature trees. In the field, seedling survival was lower in areas with high densities of mature conspecifics but was unrelated to either conspecific or heterospecific seedling density. Smaller seedlings were also more sensitive than larger seedlings to neighboring adult conspecifics. In the glasshouse, seedlings grown with soil from beneath a conspecific adult had a higher mortality rate than seedlings grown with soil from beneath heterospecific adults or sterilized soil, suggesting that soil microbial communities drive the patterns of mortality in the field. These results illustrate the importance of negative density-dependent feedbacks resulting from the soil microbial community in a common and ecologically important temperate tree species.

Key words: Janzen–Connell hypothesis; plant–soil feedbacks; *Quercus rubra*; temperate forests; tree seedlings.

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Introduction

The Janzen–Connell hypothesis is one well-supported mechanism for the maintenance of tree species richness in tropical forests. This negative feedback between trees and their offspring results in the greatest recruitment of seedlings at an intermediate distance from their parent tree, due to the trade-off between dispersal distance and density-dependent pathogens and seed predators (Janzen 1970, Connell 1971). The distance dependence model of Janzen–Connell fits into a more general model of conspecific-negative density dependence

(CNDD), whereby population growth rates decrease at higher densities due to natural enemies and/or intraspecific competition. In forests, CNDD is typically measured as a decrease in the survival of seedlings growing in areas with higher densities of conspecifics.

Conspecific-negative density dependence is a widespread phenomenon that may interact with species abundances in a variety of ways to increase forest diversity (Harms et al. 2000, Terborgh 2012, Comita et al. 2014, LaManna et al. 2017). For example, the seedlings of common species may be more likely to encounter conspecifics and be targets of natural enemies, resulting in lower recruitment, while rare species, because of their rarity, may have fewer specialist natural enemies, and therefore greater recruitment. This would present a stabilizing pressure on both common and rare populations, and promote diversity (Chesson 2000). However, there is increasing evidence that in some systems, rare species exhibit stronger CNDD, perhaps as a result of negative density-dependent interactions keeping their density on the landscape low (Comita et al. 2010, Mangan et al. 2010, Johnson et al. 2012).

Many studies have shown that tropical seedlings of various species exhibit CNDD patterns, with seedling survival decreasing at shorter distances to a conspecific tree or at higher densities of nearby conspecific trees (Terborgh 2012, Comita et al. 2014). In tropical forests, a combination of soil pathogens and insect herbivores is largely responsible for patterns of density or distance-dependent seedling mortality (Bagchi et al. 2014). In particular, soil pathogen populations tend to increase with tree density, driving declines in seedling survival (Liang et al. 2016). Experiments that manipulated the soil microbial communities using fungicide (Liu et al. 2015) or in the glasshouse (Petermann et al. 2008, Mangan et al. 2010) repeatedly found that eliminating the soil microbial communities associated with an adult plant removed the effect of the adult on seedling survival.

An additional mechanism that may drive negative density-dependent patterns of seedling mortality in forests is high conspecific seedling density (Lu et al. 2015, Johnson et al. 2017). Seed rain patterns typically result in high seed densities close to the adult that produces them (Nathan and Muller-Landau 2000). This may produce high densities of conspecific seedlings near adult trees (Lambers and Clark 2003, Kuang et al. 2017), which may also reduce seedling survival due either to competition for resources (Tyler and D'Antonio 1995), or because high seedling densities attract more herbivores and pathogens (Alvarez-Loayza and Terborgh 2011). Whether greater seedling mortality beneath conspecific adults observed in natural populations is due to the effects of natural enemies drawn to conspecific adults or due to the density of conspecific seedlings beneath adults has not been adequately addressed (Comita et al. 2014).

Seedling survival is also a function of several other important factors, which may interact with mortality caused by density-dependent processes. For example, seedlings typically exhibit greater overall mortality (Walters and Reich 1996) and stronger effects of CNDD (McCarthy-Neumann and Ibáñez 2013) in low light conditions. Recently, studies have suggested that the response of seedlings to density-dependent effects may also depend on the type of mycorrhizal fungi with which they associate, as ectomycorrhizal species typically exhibit less negative, or even positive, effects from neighboring conspecifics (Bennett et al. 2017, Kadowaki et al. 2018, Jiang et al. 2020, Liang et al. 2020). Seedling size may also play a role: Smaller seedlings are likely younger, and seedlings are most vulnerable to herbivory and infection from pathogens in their earliest stages of development (Kitajima and Fenner 1992). Small seedlings may also be more vulnerable regardless of age due to smaller carbohydrate reserves with which to recover from biomass loss (Jones and Sharitz 1998, Baraloto et al. 2005). Larger seedlings are typically from larger seeds with greater carbohydrate reserves, making them more resilient to density-dependent mortality (Song et al. 2018, Seiwa et al. 2019).

In this study, we examined the effects of neighboring conspecific and heterospecific adults on the survival of *Quercus rubra* seedlings. Previous work has shown both positive (Dickie et al. 2002, Bennett et al. 2017, Dyderski and Jagodziński 2019) and negative (Crow 1992, Beckage and Clark 2003, Ibáñez and McCarthy-Neumann 2016) density dependences in *Q. rubra.* In addition, *Q. rubra* is a masting species with heavy seeds that limit dispersal distance (Sork 1984,

Jagodziński et al. 2018); therefore, it exhibits large variation in the density of young seedlings. In addition to being a common and ecologically important tree (Cavender-Bares 2016), these attributes make *Q. rubra* an ideal species to test the importance of both adult neighborhood and seedling density to seedling survival.

We combined field observations with a glasshouse study in order to test both the survival pattern of seedlings in a natural population and two potential mechanisms driving that pattern. Specifically, we tested whether *Q. rubra* seedling survival declines with increasing neighborhood density of conspecific adults. While seedlings may respond to increases in overall densities of adults, CNDD requires that conspecific adults exert a greater pressure than heterospecific adults; therefore, we also tested the relationship between heterospecific adult density and seedling survival. Patterns of density-dependent mortality could be driven by either high densities of conspecific seedlings in areas with high densities of conspecific adults or effects from the adults themselves, most likely species-specific natural enemies. Therefore, we tested whether adult neighborhood density predicts seedling density and if greater seedling density increases seedling mortality. Additionally, the sensitivity of seedlings to these mortality agents may be greater for smaller seedlings; therefore, we tested whether seedling height interacts with conspecific neighborhood density to explain variation in individual-level survival. Finally, we used a glasshouse experiment to further test whether seedling density and/or natural enemies in the soil microbial community associated with a conspecific adult drives seedling mortality.

Materials and Methods

Site description

This study took place at the Center for Tropical Forest Science Forest Global Earth Observatory (CTFS-ForestGEO) plot at the Harvard Forest in central Massachusetts, USA., in the northern hardwood-hemlock-white-pine transition zone (42°31′48″ N, 72°11′24″ W, 300 m elevation above sea level). At Harvard Forest, the mean annual temperature and precipitation are 7.1°C and 1066 mm, respectively. The 35-ha ForestGEO plot was fully mapped by 2014, with every woody stem >1 cm diameter at breast height (DBH) identified to species, tagged, geolocated, and its diameter was measured (Orwig et al. 2014, Anderson-Teixeira et al. 2015). The plot contained >116,000 stems of 51 woody species. *Q. rubra* is the seventh most abundant woody species in the plot in terms of live stem number (n = 4407), but second only to *Tsuga canadensis* in terms of basal area (9.57 m^2/ha) and contributes disproportionately to local carbon storage and uptake (Urbanski et al. 2007, Eisen and Barker-Plotkin 2015).

Field survival analysis

During the summer of 2017, we established 133 one-m^2 seedling plots in a gridded pattern within the ForestGEO plot (Fig. 1, Data S1). We defined a seedling as any woody plant with a DBH <1 cm. While heterospecific seedlings varied in size, all *Q. rubra* seedlings that we encountered were <32 cm tall. During year 1, each seedling in every plot was identified and its stem tagged such that each seedling could be uniquely identified in subsequent censuses, which were performed in summers of 2018 and 2019. Only *Q. rubra* seedlings tagged in 2017, which followed a *Q. rubra* mast year (Clark 2019), were used in the field survival analysis (n = 308): New recruits from years 2 and 3, both non-mast years, were

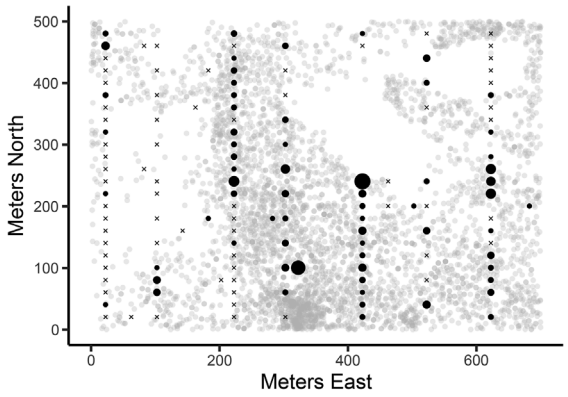

Fig. 1. Seedling plot locations at the study site. Crosses indicate plots that contained no*Quercus rubra*-seedlings in 2017. Filled black circles indicate plots with*Q. rubra*seedlings in 2017, sized by the abundance of*Q. rubra*seedlings, which ranged from 1 to 42. Gray circles indicate individual*Q. rubra*trees >1 cm DBH.

excluded, as there were so few individuals (22 in 2018 and 12 in 2019).

Susceptibility to mortality agents changes with seedling age as woody plants become less vulnerable as their cell walls thicken and lignification occurs (Kitajima and Fenner 1992, Rodriguez-Molina et al. 2002). For the majority of our *Q. rubra* seedling data (i.e., individuals tagged in 2017), age is unknown. We thus measured the height of each seedling, as height likely accounts for some variation in seedling age, as well as variation in seed size and growth rate. The height of the seedlings we tagged in 2017 ranged from 1.2 to 30.5 cm, with an average height of 10.7, while the 34 new recruits from 2018 and 2019 ranged from 1 to 22.5 cm with an average height of 9.2 cm. We therefore believe that many of the seedlings we tagged in 2017, particularly the smaller statured ones, were new germinants.

To assess the importance of neighboring adult trees on survival, we calculated a Neighborhood Competition Index (NCI) for each seedling following (Canham et al. 2004) and modified as in Bai et al. (2012) and Zhang et al. (2017). For each seedling plot, we calculated NCI values to compare the effects of conspecific (*Q. rubra*) and heterospecific (all other species) adults as follows:

$$\mathrm{NCI}_{\mathrm{con}} = \sum \frac{\mathrm{DBH\,conspecifics}}{\mathrm{distance}},$$

$$\mathrm{NCI}_{\mathrm{het}} = \sum \frac{\mathrm{DBH\,heterospecifics}}{\mathrm{distance}}.$$

We used a maximum distance of 20 m as this was the minimum distance between our seedling plots and the edge of the ForestGEO plot, and previous work has shown that tree–tree interactions generally disappear at greater distances (Wang et al. 2010). We checked that our results were not sensitive to the choice of 20 m by fitting all models using alternate maximum distances of 15 and 25 m. To test our hypothesis that seedling survival was driven by the density of seedlings, we used counts of either conspecific or heterospecific seedlings within the 1-m^2 plot in 2017.

We tested our first hypothesis by modeling the proportion of seedlings in each plot that survived from 2017 to 2018 and from 2018 to 2019 as a function of $\mathrm{NCI}_{\mathrm{con}}$, $\mathrm{NCI}_{\mathrm{het}}$, conspecific seedling abundance, heterospecific seedling abundance, and year using a quasibinomial model, as many plots had few seedlings, and therefore, many outcomes were 0 or 1. Seedling abundances were natural log-transformed to meet the model assumption of linearity. We tested the hypothesis that conspecific seedling abundance would increase with increasing conspecific adult density by log-transforming the seedling abundance counts in 2017 and in 2018 and testing for a correlation with $\mathrm{NCI}_{\mathrm{con}}$. Given the results of the first set of models, we used a binomial mixed model using the R package lme4 (Bates et al. 2015) to test our third hypothesis that smaller *Q. rubra* seedlings are more sensitive to mortality driven by neighboring conspecific adults $\mathrm{NCI}_{\mathrm{con}}$. In this model, we also included fixed effects of $\mathrm{NCI}_{\mathrm{het}}$ and year as additional predictors, and seedling plot as a random effect. All analyses were performed using R version 4.0.0.

Glasshouse experiment

In addition to tracking the survival of *Q. rubra* seedlings in the field, we used a glasshouse experiment to test the hypothesis that the soil microbial community associated with different mature trees could influence seedling survival, as well as to experimentally test the effect of seedling density on survival. We collected >500 acorns from the Harvard Forest in November of 2016. We removed non-viable acorns and cold stratified the remaining acorns by incubating them at 4°C for 4.5 months. In April 2017, we retested the acorns for viability and removed the non-viable ones. Acorns were weighed and placed in individual containers with a wet paper towel and placed in a growth chamber (Percival Scientific, Model I-35VL, Perry, Iowa, USA) at 20°C using 12-h light/dark cycles to germinate. After 2 weeks, the acorns were surface-sterilized with a 2% bleach solution, rinsed with deionized water, and planted on 20–21 April 2017.

One week prior to planting, we collected fresh soil from the ForestGEO plot from within 1 m of the bole of one large adult each of *Q. rubra*, *Betula lenta*, *T. canadensis*, *Pinus strobus*, and *Fagus grandifolia*. Although using soil from only one individual adult tree of each species limits the scope of inference for this glasshouse study, this approach is preferable to the typical plant–soil feedback

approach of collecting soil from beneath multiple individuals of each species and pooling them to use for inoculum, which can lead to significant over-estimation of plant–soil feedback effects (Rinella and Reinhart 2018). While the ideal approach would be to use field replicate adults of each species in laboratory replicated pots, this approach was not feasible in our case: Instead, we took the conservative approach of using a single adult representative of each species. The four heterospecifics represent common ectomycorrhizal trees that co-occur with *Q. rubra* in this area. Tree species that associate with ectomycorrhizal fungi typically exhibit weaker CNDD (Brown et al. 2019, Jiang et al. 2020) or sometimes exhibit positive effects on their nearby seedlings by sharing a common mycelial network (Teste et al. 2009, Liang et al. 2020). Additionally, the strength and direction of feedbacks for an individual species can differ depending on whether the heterospecifics are of the same or different mycorrhizal type (Kadowaki et al. 2018). As our site is dominated by trees that associate with ectomycorrhizal fungi (>80% of basal area), we chose to limit the glasshouse study to only ectomycorrhizal- associated heterospecifics to decrease the variation in seedling survival that might be due to mycorrhizal type.

The soil included significant root and rhizosphere matter, as it was collected from the top 5 cm of the Oa horizon. To ensure that microbial associates from the rhizosphere were included in the treatments, the soil was not sieved but cut into small pieces (~2 cm^3) that were intermixed and gently homogenized. Half the soil was sterilized by autoclaving (Consolidated Stills & Sterilizers, Model 6030, Billerica, Massachusetts, USA) for 90 min on a gravity cycle at 121°C. Successfully germinated acorns were planted either singly or in sets of three in pots with 250 g of a sterile 2:1 mixture of peat moss and sand, and combined with 25 g of either sterilized or unsterilized soil from beneath one of the five mature tree species. The goal of using only a small amount of field-collected soil was to isolate the microbial community as a potential driver of survival, and minimize potential differences in soil chemistry or texture. Pots were tapered rectangles, with a top cross section of 8.9 × 8.9 cm, a height of 12.7 cm, and a total volume of 1006 cm^3.

For our experimental treatments, we planted a total of 380 acorns in 20 treatments. In each pot, we planted either a single acorn (low density treatment) or three acorns (high-density treatment). Pots were then inoculated with either fresh or sterilized soil from beneath each adult tree. We planted 14 replicate pots of each soil treatment with a single acorn and eight replicate pots for each soil treatment with three acorns.

Acorns in the high-density treatment were planted in a triangle, equidistant from one another, while those growing singly were planted in the middle of the pot. We placed the pots with all treatments intermixed on a wide table in the center of a glasshouse and watered them twice weekly. Once a week, we haphazardly rearranged the pots around the bench to minimize possible microclimate effects. To minimize the effects of light limitation, a common mortality agent for seedlings (Lin et al. 2014), all seedlings received direct light throughout the experiment, far in excess of typical light levels in the forest understory. After 20 weeks, we scored the seedlings as either alive or dead.

We first tested for the effects of the density treatment using a two-sample *t*-test, using the proportion of seedlings that survived in each pot as the response variable in the three acorn pots. We restricted the analysis of the soil treatment effect to pots that began with three seedlings because all seedlings planted singly survived. We took the mean of the proportion of seedlings surviving in each pot in each soil treatment. We then used linear contrasts to test whether the heterospecific soil treatments ($n = 4$) and the sterilized treatments ($n = 5$) each differed from the mean of the fresh *Q. rubra* treatment, to test whether the seedlings growing with a conspecific soil microbial community had lower survival than those receiving heterospecific or sterilized soil microbial communities. All analyses were performed in R version 4.0.0.

Results

Field observations

Overall we found 308 individual *Q. rubra* seedlings in 66 plots in 2017. *Q. rubra* seedling abundance ranged from 1 to 47 individuals/m^2 with a mean of 4.7 individuals/m^2 and a median of 2 individuals/m^2. Heterospecific seedling abundance

ranged from 0 to 48 individuals/m^2, with a mean of 7.5 individuals/m^2 and a median of 4 individuals/m^2. The most abundant heterospecific seedlings in these plots were *Acer rubrum*. We found an additional 22 *Q. rubra* seedlings in 2018 and an additional 12 *Q. rubra* seedlings in 2019 that were not included in subsequent analyses. Survival was remarkably consistent between the two years: of the 308 *Q. rubra* individuals tagged in 2017, 198 (64%) survived to 2018 while 130 (62%) of those survived the second year. Initial heights of the seedlings in 2017 ranged from 1.2 to 30.5 cm.

Plot-level survival declined significantly with an increasing density of conspecific adults (NCI_{con}; Table 1). Survival also declined slightly with increasing density of heterospecific adults (NCI_{het}); however, the effect was small and only marginally significant. These relationships were not sensitive to the choice of maximum distance used in the NCI calculations (Appendix S1: Tables S1, S2). Seedling survival was also not sensitive to the density of either conspecific or heterospecific seedlings within the 1 × 1 m plot (Table 1). The abundance of *Q. rubra* seedlings in 2017 was not related to the density of conspecific adults (Pearson's rho = 0.1, $t = 0.84$, df = 63, $P = 0.406$), nor was the abundance of *Q. rubra* seedlings in 2018 (Pearson's rho: −0.07, $t = -0.50$, df = 52, $P = 0.6192$; Fig. 2).

Individual seedling survival declined strongly and significantly with increasing NCI_{con}, such that about 80% of seedlings survived in plots with low NCI_{con}, but only about 40% of seedlings survived in plots with high NCI_{con} (Table 2). Again, this relationship was not sensitive to the choice of maximum distance used in the NCI calculations (Appendix S1: Tables S3, S4). The effect of NCI_{con} depended on the initial height of the seedling, with larger seedlings relatively unaffected by NCI_{con}, and smaller seedlings exhibiting overall lower survival and greater sensitivity to increasing conspecific adult densities (Fig. 3). Survival also declined slightly with increasing NCI_{het}, but the effect was much smaller than that

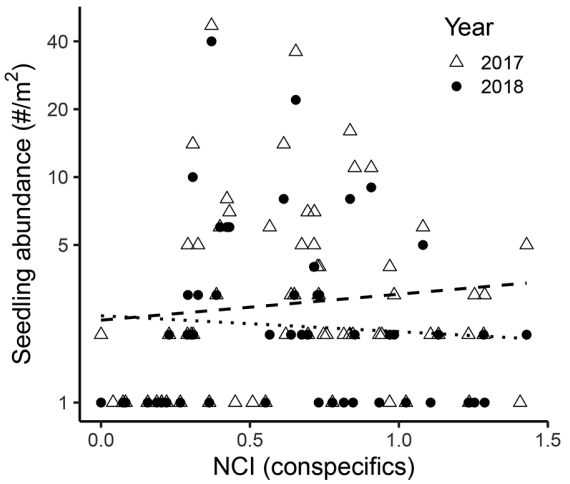

Fig. 2. *Quercus rubra* seedling abundances in 2017 and 2018 were unrelated to the neighborhood competition index (NCI). The dashed line and open triangles represent 2017, and the dotted line and closed circles represent 2018. Note the log scale on the *y*-axis.

Table 1. Parameters for the quasibinomial model predicting plot-level *Quercus rubra* seedling survival as a function of adult neighborhood.

Predictors	Estimate (standard error)	*P* value
$NCI_{conspecific}$	−1.237 (0.530)	0.021*
$NCI_{heterospecific}$	−0.379 (0.220)	$0.088^{m.s.}$
Year	−0.027 (0.311)	0.387
ln(*Q. rubra* seedling abundance + 1)	0.084 (0.224)	0.708
ln(heterospecific seedling abundance + 1)	−0.055 (0.183)	0.765

Notes: Note that seedling abundances are from the year prior to the survival census and are natural log-transformed. The year estimate shows the effect on survival in 2019 relative to 2018. Significance levels are indicated as * when $P < 0.05$, *m.s.* when $P < 0.1$.

Table 2. Parameters for the binomial mixed effect model predicting individual *Quercus rubra* seedling survival as a function of the adult neighborhood.

Fixed effects	Estimate (standard error)	*P* value
Intercept	1.829 (0.948)	0.054
$NCI_{conspecific}$	**−3.044 (1.087)**	**0.005***
$NCI_{heterospecific}$	−0.348 (0.181)	$0.055^{m.s.}$
Height	0.032 (0.056)	0.564
Year	−0.409 (0.210)	$0.052^{m.s.}$
Height × $NCI_{conspecific}$	**0.174 (0.088)**	**0.048***

Notes: Seedling plot was included as a random effect. The year estimate shows the effect on survival in 2019 relative to 2018. Parameters with a significant ($P < 0.05$) effect on *Q. rubra* seedling survival are indicated in bold, with * when $P < 0.05$, *m.s.* when $P < 0.1$.

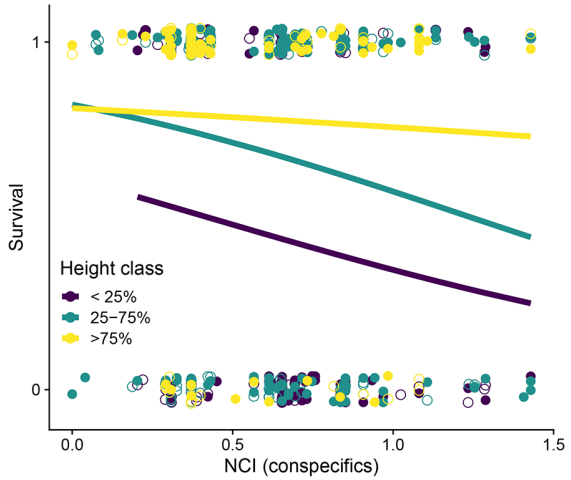

Fig. 3. Survival of *Quercus rubra* seedlings across the range of conspecific adult densities. Each point represents an individual seedling, jittered vertically for clarity. Colors indicate the seedling size class, as determined by their height in the year prior to the survival census: purple, lower height quartile; blue, middle two height quartiles; yellow, upper height quartile. Lines show the best-fit logistic models for each seedling size class. Closed points represent seedling survival status in 2018, and open points represent survival status in 2019.

of NCI_{con} and was only marginally significant (Table 2).

Glasshouse experiment

Quercus rubra seedlings in the glasshouse survived at much higher rates than in the field: Overall, 92% of the seedlings grown survived the 20-week experiment. Across all soil treatments, survival varied significantly between the seedlings growing singly (100% survival) and the seedlings growing in pots with three individuals (87.5% survival; $t = -4.226$, $P < 0.001$). Seedling survival in the three seedling pots differed between the soil treatments, such that the treatment with the lowest survival (62.5%) was the fresh *Q. rubra* treatment, while the next lowest treatment had 79% survival (sterilized *P. strobus* treatment) and survival in other treatments ranged from 88% to 100%. Survival in pots with fresh *Q. rubra* soil was thus much lower than pots with either fresh heterospecific soil ($t = -2.245$, $P = 0.026$) or sterilized soil ($t = -2.037$, $P = 0.043$; Fig. 4).

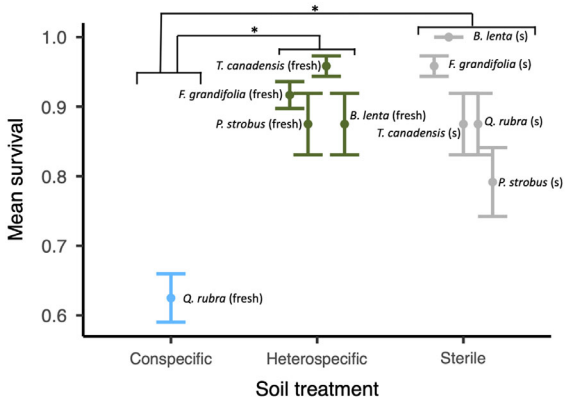

Fig. 4. The mean values of pot-level survival in three acorn pots by soil treatment. Tree species of soil origin are indicated in the labels adjacent to mean values, where (fresh) indicates fresh inocula and (s) indicates sterilized inocula. Each point represents a mean of eight pots, each with three seedlings, and error bars are standard errors of the mean. Significant differences ($P < 0.05$) between treatment groups according to linear contrasts are indicated by *.

Discussion

We observed that *Q. rubra* seedlings growing in the field exhibit conspecific-negative density dependence in that they are less likely to survive in areas with a higher abundance of adult *Q. rubra* trees. Importantly, we found that the effect of conspecific trees was much stronger (more negative) than that of heterospecific trees, which were marginally significant in our models. This suggests that the effects of the adult neighborhood on seedling survival were due not just to competition for light or resources from nearby adult trees, but from species-specific density-dependent processes. Our results concur with some previous work on *Q. rubra* seedlings that suggest survival and growth are reduced when seedlings grow in sites with higher *Q. rubra* basal area (Crow 1992, Beckage and Clark 2003). Our findings that support the existence of adult conspecific-negative density dependence are also in agreement with previous work showing that temperate tree species in the eastern USA typically exhibit negative density dependence in seedling survival (Johnson et al. 2012).

We hypothesized that one species-specific density-dependent process that could drive higher

seedling mortality at higher adult densities was greater conspecific seedling abundance. Surprisingly, the abundance of *Q. rubra* seedlings was unrelated to our metric of conspecific adult neighborhood (NCI_{con}). This may be due to a combination of dispersal, seed predation, and abiotic factors that had a greater effect on seedling abundance than the local density of adults. For example, the seedling plot with the greatest abundance of *Q. rubra* seedlings occurred near the edge of a light gap, caused by a beaver swamp in the area. Although anecdotal, this suggests that the light environment, as well as other abiotic factors, may play a more important role in seedling abundance than the density of nearby adults.

In addition, the abundance of *Q. rubra* seedlings was not a significant driver of seedling mortality in the field. While some studies have found a strong effect of conspecific seedling density on survival (Lu et al. 2015, Johnson et al. 2017, Dyderski and Jagodziński 2019), others have not (Alvarez-Loayza and Terborgh 2011, Bai et al. 2012) and meta-analysis suggests that these effects are rare (Moles and Westoby 2004). The lack of effect of both conspecific and heterospecific seedling densities on seedling survival suggests that seedlings are more sensitive to the effects of adult trees in this study, suggesting that neither resource competition among seedlings nor high seedling densities attracting natural enemies are driving seedling mortality. In addition to the effects of neighboring adults and their associated soil pathogens, light environment (Walters and Reich 1996), soil nutrient availability (Schreeg et al. 2005), herbivory (Myster and McCarthy 1989, Augspurger and Kitajima 1992, Moles and Westoby 2004), and microclimate (Germino et al. 2002) are all drivers of variation in seedling mortality. Together, the lack of relationship between adult density and seedling densities, as well as the lack of relationship between seedling densities and survival, strongly suggests that seedling density is not responsible for the increase in seedling mortality at higher conspecific adult densities.

At the individual level, smaller seedlings not only exhibited higher mortality but were also more sensitive to increasing densities of conspecific adults. This may be due to a variety of processes. Firstly, some of the variation in seedling size may represent differences in seedling age, and oak seedlings are most susceptible to fungal infection during early germination (Korstian 1927). Although size is an imperfect proxy for age in young *Q. rubra* (Burns and Honkala 1990), we believe that many of these *Q. rubra* seedlings were likely first-year seedlings at the beginning of this study. In addition to the large acorn crop in 2016 (Clark 2019), the *Q. rubra* seedlings were a similar size to the new recruits we found in subsequent years. *Quercus* sp. seedling growth rates (Pérez-Ramos et al. 2010) and survival (Tecklin and Mc Creary 1991, Gómez 2004, Arosa et al. 2015) are often related to acorn size, so seedling size in this study may represent the available carbohydrate reserves from residual acorns. Larger seedlings are more buffered from density-dependent mortality agents, likely due to their ability to recover from biomass loss (Armstrong and Westoby 1993, Bonfil 1998), in this case from density-dependent mortality agents. This finding is consistent with previous work suggesting that neighborhood effects are greater for younger, smaller seedlings (Bai et al. 2012, Zhu et al. 2015).

In contrast to the field, we observed a strong effect of seedling density on seedling survival in the glasshouse, as the only mortality we observed occurred in pots with three seedlings. Importantly, this suggests that seedling competition may influence survival in extremely high densities often used in glasshouse experiments (including this one), but the threshold for seedling density to reduce survival is higher than is commonly found under field conditions. The effects of seedling density in the glasshouse could not be due to the full suite of possibilities that occur in the field but are much more likely due at least in part to resource competition for light, water, or nutrients, as well as any interactive effects of these factors with our soil treatments.

Among the high-density pots, we also found that the presence of a conspecific soil microbial community was associated with a lower probability of seedling survival, suggesting that the pattern of mortality in the field could have been due to the presence or abundance of soil pathogens in the soil beneath a conspecific adult. Unlike the difference in mortality rates between the single acorn pots and the trios, this difference

occurred among the soil treatments which all had three acorns to begin with. This concurs with many previous studies indicating the importance of the soil microbial community, particularly soil pathogens, for driving seedling survival in a wide variety of systems (Kulmatiski et al. 2008), as well as previous glasshouse work showing soil pathogen-driven CNDD occurring only at higher seedling density (Packer and Clay 2000). Competition for light in the three acorn pots may have also played a role: Previous work has shown that negative plant–soil feedbacks are more likely to occur at low light (McCarthy-Neumann and Ibáñez 2013). The results from the glasshouse experiment have a limited scope of inference: The soil microbial communities came from a single individual adult of each species, and therefore, it is not certain that they represent species-level patterns. However, the congruence between the field mortality patterns (higher mortality at greater conspecifics densities, not due to seedling competition alone) and the results of the glasshouse soil treatments strongly suggest that the soil microbial community associated with conspecific adults is responsible for the density-dependent mortality exhibited in small *Q. rubra* seedlings in this study.

Conclusion

We showed that adult plant–soil feedbacks decreased seedling survival in a common temperate tree, *Q. rubra*, using a combination of field observations and a glasshouse experiment. We also saw that smaller seedlings were more sensitive to the effects of neighboring adult conspecifics. Assessing the importance of plant–soil feedbacks on the distribution of trees in a temperate forest helps us test the generality of conspecific density-dependent mortality beyond the tropics to better understand the underlying mechanisms governing global forest composition.

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Data Availability

All data are available as supplemental material.

Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3292/full