Experimental and observational evidence of negative conspecific density dependence in temperate ectomycorrhizal trees

Journal:	*Ecology*
Manuscript ID	ECY21-1156.R1
Wiley - Manuscript type:	Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Jevon, Fiona; Dartmouth College Department of Biological Sciences, Biology De La Cruz, Dayna; Wellesley College, Department of Biological Sciences Lang, Ashley; Indiana University Bloomington, Department of Biological Sciences LaManna, Joseph; Marquette University, Biological Sciences Orwig, David; Harvard University, Harvard Forest Record, Sydne; Bryn Mawr College, Biology Kouba, Paige; University of California Davis, Department of Plant Sciences Ayres, Matthew; Dartmouth College, Biological Sciences Matthes, Jaclyn; Wellesley College, Department of Biological Sciences
Substantive Area:	Community Analysis/Structure/Stability < Community Ecology < Substantive Area
Organism:	Plants, Conifers < Gymnosperms < Plants, Angiosperms < Plants, Gymnosperms < Plants, Fungi (specify type in field below)
Habitat:	Deciduous Forest < Temperate Zone < Terrestrial < Habitat, Coniferous Forest < Temperate Zone < Terrestrial < Habitat
Geographic Area:	Northeast US (CT, MA, ME, NH, NJ, NY, PA, RI, VT) < United States < North America < Geographic Area
Key words/phrases:	conspecific negative density dependence, diversity, saplings, ectomycorrhizae, mycorrhizal fungi, temperate tree seedlings, survival
Abstract:	Conspecific negative density dependence (CNDD) promotes tree species diversity by reducing recruitment near conspecific adults due to biotic feedbacks from herbivores, pathogens, or competitors. While this process is well-described in tropical forests, tests of temperate tree species range from strong positive to strong negative density dependence. To explain this, several studies have suggested that tree species traits may help predict the strength and direction of density dependence: for example, ectomycorrhizal-associated tree species typically exhibit either positive or weaker negative conspecific density dependence. More generally, the strength of density dependence may be

predictably related to other species-specific ecological attributes such as shade tolerance, or the relative local abundance of a species. To test the strength of density dependence and whether it affects seedling community diversity in a temperate forest, we tracked the survival of seedlings of three ectomycorrhizal-associated species experimentally planted beneath conspecific and heterospecific adults on the Prospect Hill tract of the Harvard Forest, in Massachusetts, USA. Experimental seedling survival was always lower under conspecific adults, which increased seedling community diversity in one of six treatments. We compared these results to evidence of CNDD from observed sapling survival patterns of 28 species over approximately 8 years in an adjacent 35-hectare forest plot. We tested whether species-specific estimates of CNDD were associated with mycorrhizal association, shade tolerance, and local abundance. We found evidence of significant, negative conspecific density dependence (CNDD) in 23 of 28 species, and positive conspecific density dependence in two species. Contrary to our expectations, ectomycorrhizal-associated species generally exhibited stronger (e.g. more negative) CNDD than arbuscular mycorrhizal-associated species. CNDD was also stronger in more shade tolerant species but was not associated with local abundance. Conspecific adult trees often have a negative influence on seedling survival in temperate forests, particularly for tree species with certain traits. Here we found strong experimental and observational evidence that ectomycorrhizal-associating species consistently exhibit CNDD. Moreover, similarities in the relative strength of density dependence from experiments and observations of sapling mortality suggest a mechanistic link between negative effects of conspecific adults on seedling and sapling survival and local tree species distributions.

ECY21-1156: Revision 1
Running Head: CNDD in ECM trees

Correspondence to:
Fiona V. Jevon
Yale School of the Environment
Yale University
195 Prospect Street
New Haven, CT 06511, United States
fiona.jevon@yale.edu

Title (max 120 char):

Experimental and observational evidence of negative conspecific density dependence in temperate ectomycorrhizal trees

Authors: Fiona V. Jevon[1,2], Dayna De La Cruz[3], Joseph A. LaManna[4], Ashley K. Lang[5], David A. Orwig[6], Sydne Record[7], Paige V. Kouba[8], Matthew P. Ayres[1], Jaclyn Hatala Matthes[2]

Author affiliations:
[1]Department of Biological Sciences, Dartmouth College, Hanover NH 03755, United States
[2]Current affiliation: Yale School of the Environment, Yale University, New Haven, CT 06511, United States
[3]Department of Biological Sciences, Wellesley College, Wellesley, MA 02481, USA
[4]Department of Biological Sciences, Marquette University, Milwaukee, WI, 53201 USA
[5]Department of Biological Sciences, Indiana University, Bloomington, IN, 47405 USA
[6]Harvard Forest, Harvard University, Petersham, MA 01366, USA
[7]Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania 19010 USA
[8]Department of Plant Sciences, University of California, Davis, CA, 95616 USA

Open Research Statement:

Data are provided as private-for-peer review: all data and code can be found in the public repository at https://data.mendeley.com/datasets/ws3cdn28n8/4 (3). The only exception is data associated the second seedling census: this dataset is still undergoing moderation but will be posted alongside the first census at https://harvardforest1.fas.harvard.edu/exist/apps/datasets/showData.html?id=HF253 (4). However, code for all analyses described here can be found in the public repository at https://data.mendeley.com/datasets/ws3cdn28n8/4 (6).

Abstract

Conspecific negative density dependence (CNDD) promotes tree species diversity by reducing recruitment near conspecific adults due to biotic feedbacks from herbivores, pathogens, or competitors. While this process is well-described in tropical forests, tests of temperate tree species range from strong positive to strong negative density dependence. To explain this, several studies have suggested that tree species traits may help predict the strength and direction of density dependence: for example, ectomycorrhizal-associated tree species typically exhibit either positive or weaker negative conspecific density dependence. More generally, the strength of density dependence may be predictably related to other species-specific ecological attributes such as shade tolerance, or the relative local abundance of a species. To test the strength of density dependence and whether it affects seedling community diversity in a temperate forest, we tracked the survival of seedlings of three ectomycorrhizal-associated species experimentally planted beneath conspecific and heterospecific adults on the Prospect Hill tract of the Harvard Forest, in Massachusetts, USA. Experimental seedling survival was always lower under conspecific adults, which increased seedling community diversity in one of six treatments. We compared these results to evidence of CNDD from observed sapling survival patterns of 28 species over approximately 8 years in an adjacent 35-hectare forest plot. We tested whether species-specific estimates of CNDD were associated with mycorrhizal association, shade tolerance, and local abundance. We found evidence of significant, negative conspecific density dependence (CNDD) in 23 of 28 species, and positive conspecific density dependence in two species. Contrary to our expectations, ectomycorrhizal-associated species generally exhibited stronger (e.g. more negative) CNDD than arbuscular mycorrhizal- associated species. CNDD was also stronger in more shade tolerant species but was not associated with local abundance.

Conspecific adult trees often have a negative influence on seedling survival in temperate forests, particularly for tree species with certain traits. Here we found strong experimental and observational evidence that ectomycorrhizal-associating species consistently exhibit CNDD. Moreover, similarities in the relative strength of density dependence from experiments and observations of sapling mortality suggest a mechanistic link between negative effects of conspecific adults on seedling and sapling survival and local tree species distributions.

Keywords: conspecific negative density dependence, diversity, saplings, temperate tree seedlings, mycorrhizal fungi, ectomycorrhizae, shade tolerance

Introduction

Conspecific negative density dependence (CNDD), whereby population growth rates decline with increasing population density due to a negative feedback on recruitment or survival, is a critical mechanism that can support local tree diversity (Chesson 2000). One example of CNDD is known as the Janzen-Connell hypothesis. This hypothesis suggests that specialized enemies, such as herbivores or pathogens, reduce the recruitment of offspring nearby parent trees in highly diverse tropical forests (Janzen 1970; Connell 1971). This process hinders the formation and preservation of monodominant stands, and the resulting spatial distribution of trees supports the coexistence of many species. It is broadly assumed that CNDD can operate as a stabilizing mechanism to support diverse communities; however, very few studies of density dependence have quantified the effects of CNDD on diversity. Theoretical work (Chesson 2000) and observational studies (LaManna et al. 2017; Johnson et al. 2012) have linked higher species diversity to stronger CNDD, but experimental evidence of this link remains rare (Bagchi et al.

2014; Levine and HilleRisLambers 2009). In particular, few studies show whether and how CNDD can produce greater diversity over ecological time.

Strong CNDD is pervasive in the tropics (Comita et al. 2014; Terborgh 2012), making it an attractive potential driver of latitudinal patterns of tree diversity. However, there is also increasing support for CNDD as a mechanism that influences tree communities in temperate forests (Johnson et al. 2014, 2012; McCarthy-Neumann and Kobe 2010; Ramage et al. 2017; Jiang et al. 2020, 2021). While this work illustrates the potential for CNDD to drive population dynamics in temperate systems, there is wide variation in the strength of CNDD among tree species (Bennett et al. 2017; Johnson et al. 2014) and along environmental gradients (LaManna et al. 2016; Smith and Reynolds 2015).

Plant functional traits provide one potential means for predicting differences in the strength of density dependence among species (Bennett et al. 2017; Brown et al. 2019; Jia et al. 2020). In particular, functional traits that confer resistance to attack from herbivores or pathogens, the primary drivers of CNDD, may be associated with the strength of density dependence. For tree species, the type of mycorrhizal association, either arbuscular (AM) or ectomycorrhizal (ECM), has been associated with patterns in CNDD. In previous studies, ectomycorrhizal-associated tree species tend to exhibit weaker or even positive density dependence (Bennett et al. 2017; Jiang et al. 2020, 2021; Chen et al. 2019; Qin et al. 2021), possibly due to the greater protection from soil pathogens that the fungi confer to their tree host (Corrales et al. 2016; Bennett et al. 2017). Indeed, recent work suggests that while both AM and ECM fungal networks may partially counteract conspecific negative density dependent mortality, ECM fungi may be more effective than AM fungi at countering the mortality agents which typically drive CNDD patterns (Liang et al. 2021). However, in other studies the strength of CNDD was similar in AM and ECM associated tree species (Jia et al. 2020).

In addition to mycorrhizal association, shade tolerance has been associated with species-specific patterns in CNDD; shade tolerant tree species are less susceptible to microbial attack due to conservative life history strategies, suggesting that they should exhibit weaker CNDD (McCarthy-Neumann and Kobe 2008; Song et al. 2021). However, shade tolerant species are also more likely to be infected by necrotrophic fungal pathogens, which kill their hosts and feed on the decaying tissue, while shade intolerant species are more likely to be infected by biotrophs, which feed on live tissue without killing their hosts (García-Guzmán and Heil 2014). Indeed, low light areas of the forest where shade tolerant seedlings are likely to be found typically contain higher pathogen loads (Augspurger and Kelly 1984). Therefore, despite their conservative allocation strategy, shade tolerant species may be *more* affected by CNDD, if the pathogens driving density dependence are more abundant and virulent. Indeed, the few explicit tests of how shade tolerance relates to CNDD in temperate forests are conflicting: shade tolerant species may be more (Jia et al. 2020) or less (Brown et al. 2019) likely to exhibit CNDD than shade intolerant species within temperate forests.

Additionally, temperate species do not always follow the same patterns as tropical species: in the tropics, rare species typically exhibit stronger CNDD, which helps maintain diverse communities with many rare species (Xu, Wang, and Yu 2015; Comita et al. 2010; Mangan et al. 2010). In temperate forests, the pattern between CNDD and local abundance is less clear: while some studies show that rare species exhibit stronger negative density feedbacks (Johnson et al. 2012), others show the opposite pattern, with more abundant species exhibiting stronger CNDD (Zhu et al. 2015; LaManna et al. 2016). Quantifying the strength of CNDD across multiple co-occurring species can help to detangle sources of variability in CNDD studies and is needed to generalize the importance of CNDD in temperate, as well as tropical, forests. In this study we used a combination of experimental and observational approaches to ask how prevalent CNDD is in a temperate forest, with particular emphasis on whether ectomycorrhizal-associated species commonly exhibit CNDD. We used a seedling planting experiment to test whether the species identity (conspecific versus heterospecific) of neighboring mature trees influences seedling survival in ectomycorrhizal-associating tree species, and whether CNDD effects on ECM seedling mortality alter seedling community diversity. We then used the survival of naturally occurring saplings to quantify the strength of CNDD across 28 co-occurring woody species. Finally, we asked whether mycorrhizal type, shade tolerance, and local abundance predict variation in the strength of CNDD among species.

Materials and methods:

Site Description

This study took place on the Prospect Hill tract of the Harvard Forest (HF) located in Petersham, Massachusetts. This forest is in the northern hardwood-hemlock-white-pine transition zone (42.530°'N, 72.190°'W, 300 m elevation above sea level). The mean annual temperature and precipitation are 7.1°C and 1066 mm, respectively. For the observational part of our study, we utilized the 35-hectare HF ForestGEO plot where every woody stem >1 cm diameter at breast height (DBH) has been identified to species, tagged, geolocated and its diameter was measured (Orwig et al. 2022). The experimental portion of our study took place in plots that are adjacent to the ForestGEO plot (Appendix 1: Figure S1).

Experimental Methods

We selected three ectomycorrhizal species: *Pinus resinosa, Quercus rubra* and *Pinus strobus* which represent a range in other plant traits, including shade tolerance(Niinemets and Valladares 2006). All three species are common at our study site, although the population of *Pinus resinosa* were planted there in the early 1900s and now consist of both planted and naturally regenerated individuals. In the forest adjacent to the mapped ForestGEO plot, we located 30 experimental plots (Appendix 1: Figure S1). Each circular plot had a diameter of 20 m and was centered on a focal *Pinus resinosa, Quercus rubra*, or *Pinus strobus* with a DBH greater than 28cm such that there were ten plots centered on each of the three species. We chose locations where none of the other study species occurred: e.g., in a plot centered on a *Q. rubra* tree, there were no *Pinus resinosa* or *Pinus strobus*. We used these plots to plant seedlings of each of the three species beneath both conspecific and heterospecific "adult" trees (defined as trees with a DBH greater than 28 cm).

In addition to these three species, we also planted *Picea abies* seedlings. *Picea abies* are present at this site but not native to the area. Like *Pinus resinosa*, *Picea abies* was commonly planted in the early 1900s and now naturally regenerate at this site. We included this species in the seedling planting primarily to increase the diversity of our planted seedling communities. *Picea abies* was chosen as it was available from the same nursery as the three experimental species, was a similar size and age to the other three species, associates with the same type of mycorrhizal fungi, and is a common species at this study site (Table 1). We purchased bare-root seedlings in May 2019 which were grown outdoors at the New Hampshire State Nursery in Boscawen, NH. *Picea abies* and *Quercus rubra* seedlings were two years old at planting while *Pinus strobus* and *Pinus resinosa* were three years old.

Experimental planting

Within each plot, we constructed two subplots, 1 m x 1 m, into which the seedlings were planted. Subplots were located approximately 1 meter apart and within 2 meters of the base of the trunk of the focal tree, and subplot type ("even" or "uneven") was randomly assigned. In each subplot, we planted 20 seedlings. To test whether CNDD promotes diversity, and whether the effects of CNDD on diversity were dependent on the initial diversity of the community, in one subplot ("Even"), we planted 5 individuals of each species. In the other ("Uneven"), we planted 11 individuals of the same species as the focal tree (conspecifics), and 3 of each of the heterospecific species. Thus, the two subplots had the same total number of seedlings, but the conspecific seedling was either at the same density as each of the heterospecifics, or at a much higher density, as would be more likely under natural regeneration conditions. We used a standard, randomized planting design such that the spatial arrangement of conspecific seedlings relative to heterospecific seedlings was consistent across all subplots of the same type (Supplementary Fig 2). All 1200 seedlings were planted between May 31st and June 7th, 2019.

Before planting, each subplot was cleared of aboveground stems greater than 20cm in height with hand clippers, so that competition with herbaceous plants and ferns was minimized across all plots. Resprouts from clipped vegetation, primarily ferns, were rare and were re-clipped when they emerged. Next, leaf litter was removed and set aside. A wooden 1m^2 frame with grid lines at 20cm intervals, creating a 5x5 grid, was then placed on the ground to serve as the planting guide (Appendix 1: Figure S2). Using an auger (7.6 cm diameter), we dug holes in 20 locations in each subplot. Removed soil was placed on a tarp and homogenized. The individually tagged seedlings were then planted and covered by the homogenized soil. Throughout the planting process, the roots of the seedlings were kept moist with water in spray bottles. After the 20 seedlings were planted, the subplot was flagged, watered, and re-covered by leaf litter.

Within each 10-m radius plot, we also identified and measured the diameter of each tree > 2.5 cm DBH as well as the distance of each stem to the seedling subplots. To account for possible differences in light environment that could influence seedling survival, we took a hemispherical photo using a fisheye lens from the center of each plot to capture the light environment. Photographs were taken between 07:30 and 09:30 am to avoid overexposure. Images were analyzed with WINSCANOPY (Regent Instruments Inc., Quebec, Canada) to calculate the gap fraction (a metric of canopy openness) of each of the 30 plots.

Seedlings were tracked individually throughout the summer. After all seedlings had been in the ground for two weeks, seedlings were censused for survival and their initial heights were measured to account for any differences in survival that were due to variation in initial seedling size. Seedlings were censused again after an additional 10 weeks for their final survival status. Seedlings were presumed dead if their needles had all turned brown (conifers) and if they had no remaining leaves (*Q. rubra*). We continued to monitor all seedlings, regardless of status, for the full 12 weeks of the experiment.

Statistical analysis of experimental seedling mortality

We removed 17 individual seedlings from the analysis (1 *P. strobus*, 13 *Q. rubra* and 3 *P. resinosa*) that died within the first two weeks, presumably due to transplant shock rather than as a result of our experimental treatments. For each seedling species, we calculated the overall odds ratio of survival under conspecific and heterospecific focal adults, such that an odds ratio < 1 indicates a lower chance of survival beneath a conspecific adult. We then fit a binomial mixed model using the R package lme4 (Bates, Maechler, Bolker, & Walker, 2015) to predict seedling survival as a function of the adult species identity of the plot (based on whether the focal tree in the plot was a conspecific or heterospecific), initial seedling height, subplot type (even or uneven) and canopy gap fraction, with the plot as a random effect. For the *P. abies* seedlings, we fit the same model without the adult identity variable as all seedlings were growing in heterospecific plots.

To account for possible effects of both the focal tree in each plot (our experimental treatment), and also the effects of neighboring trees, we used the full 314-m2 plot to calculate a neighborhood competition index (NCI) following Canham, LePage, and Coates (2004) and modified as in other similar analyses (Zhang et al. 2017; Bai et al. 2012; Magee et al. 2020). For each plot, we calculated NCI values to compare the effects of conspecific and heterospecific adults within 10 meters as follows:

$$\mathrm{NCI}_{\mathrm{conspecific}} = \Sigma \frac{DBHconspecific}{distance} \qquad \text{eq.1}$$

$$\mathrm{NCI}_{\mathrm{heterospecific}} = \Sigma \frac{DBHheterospecific}{distance} \qquad \text{eq. 2}$$

We then used the results of the first set of models (using the identity of the focal adult tree as our treatment) to inform which parameters to include in the second set (including the NCI as our treatment), including any parameter from the first model set with $p < 0.1$. We ran a second set of binomial mixed models that did not include adult identity, but did include $\mathrm{NCI}_{\mathrm{conspecific}}$, $\mathrm{NCI}_{\mathrm{heterospecific}}$, and any of the parameters identified from model set 1 with plot ID as a random variable.

Finally, we tested whether the observed seedling mortality affected the diversity of the seedling community. To do this, we calculated the overall survival of each seedling subplot. We then simulated random mortality at that level for each subplot, by randomly assigning a survival code to each seedling until the overall observed mortality of the plot was met. We ran this simulation 1000 times for each subplot. As all our experimental plots were planted with the same species richness (4), we used the Shannon's Equitability Index (J) (Begon, Harper and Townsend 1996):

$$J = \frac{-\sum_{i=1}^{S} p_i * log(p_i)}{log(SR)} \qquad \text{eq. 3}$$

where p_i is the proportion of each species and SR is the species richness. We used this metric to assess whether the diversity we observed at the end of our experiment differed from what the diversity would be if mortality had occurred randomly with respect to species identity of the seedlings.

Observational sapling survival

To determine whether naturally regenerating saplings exhibited a similar pattern to those we experimentally planted, as well as to quantify density dependence in a wider variety of naturally occurring saplings, we used the subset of the Harvard Forest ForestGeo plot that has been re-censused as of 2021. The first census occurred from June 2010 through January 2014. The re-census occurred from May 2018 to September 2019. Individual trees were revisited and their survival status was recorded.

Using the initial census, we calculated the same metrics of neighborhood competition as for our experimental plots (eq.1 and eq. 2) for each individual stem in the forest. We used a maximum distance of 20 m. We only assessed patterns for species with more than 10 individual saplings and more than 20 individual adults. To account for differences in average size of each species as well as maximize the number of species included in our analysis, we defined individuals as saplings if their DBH was less than the median DBH of that species, up to a maximum cutoff of 12.7 cm DBH, and individuals greater than the median as "adults" (Table 1). To confirm that our results were not sensitive to the choice of methods, we re-ran the CNDD estimation using 15m maximum distance to neighbors and 25 m maximum distance to neighbors. We also re-ran the CNDD estimation using an alternative method to distinguish between saplings and adults: using a DBH cutoff of either 3cm, 8cm or 12.7cm for species with a median DBH less than 5cm, greater than 5 but less than 12.7cm, and greater than 12.7cm respectively, as a common concern with CNDD analyses is that the distinction can be arbitrary (Detto et al. 2019). Results of from these alternative analyses can be found in Appendix 1: Figure S3.

To calculate the overall effect of neighborhood on sapling mortality, we fit a generalized linear mixed-effects model (GLMM) with binomial errors and a complementary log–log link to assess the relative importance of factors determining individual sapling mortality using the R package lme4 (Bates et al. 2015). To account for differences in the length of time between the two censuses for individual trees, we used a log(time) offset of the number of years between the two censuses (range: 5.25 - 9.5 years) for each individual stem as in Johnson et al. (2017). The diameter at breast height (DBH) of the sapling at the first census was included as a covariate(Johnson et al. 2017). Both NCI_{con} and NCI_{het} were also included in the model to account for the overall effects of competition as well as the specific effects of conspecific neighbors. All three variables were scaled by subtracting the overall mean and dividing by two standard deviations (Gelman 2008). Species was included as a random effect, and both NCI_{con} and NCI_{het} were estimated with random slopes for each species. The model coefficients for each species of NCI_{con} and NCI_{het} were used to estimate $CNDD_{coeff}$, such that $CNDD_{coeff}$ was the difference between the model coefficient for NCI_{con} and NCI_{het}.

To further account for the possibility that our $CNDD_{coeff}$ estimate could be produced by underlying spatial or other factors not accounted for in this simple statistical model, we additionally performed null model testing (LaManna, Mangan, and Myers 2021). Using the same model structure as above, we recalculated values of NCI_{con} and NCI_{het} for each sapling after randomizing the observed proportion of the total NCI that was conspecific: effectively keeping the total neighborhood density the same as the observed, but randomizing the density of conspecific neighbors. We also kept the observed values of DBH, and the mortality outcome. We re-ran this null model 1000 times, and estimated $CNDD_{null}$ as the difference between the mean NCI_{con} and NCI_{het} coefficients using the same model structure as described above. We then calculated the final estimate of CNDD for each species ($CNDD_{est}$) as follows

$$CNDD_{est} = -(CNDD_{coeff} - CNDD_{null}) \qquad \text{eq. 4}$$

where $CNDD_{coeff}$ is the estimate based on the difference between the model coefficients for NCI_{con} and NCI_{het} using the observed data, and $CNDD_{null}$ is the estimate based on the difference between the model coefficients for NCI_{con} and NCI_{het} using the randomized NCI_{con}. The negative sign is to make the estimate more interpretable: as all the models were run as hazard functions, with survival coded as zero and mortality coded as one, taking the inverse of the estimate mreans that a negative $CNDD_{est}$ indicates a species exhibited stronger CNDD, while a positive $CNDD_{est}$ indicates that greater conspecific adult density was associated with a higher likelihood of survival. For each species, we took the mean across the 1000 iterations of the null model randomization, and we considered our estimate of CNDD significant if the 95% confidence interval of $CNDD_{est}$ did not cross 0. We also confirmed that the mean null model coefficients were significantly different from the coefficients based on the observed values for each species using a paired t-test. Full visualization of the null model distribution for each species relative to $CNDD_{obs}$ can be found in Appendix 1: Figure S4.

Finally, we compared this species level estimate of CNDD at this site to species level plant traits. We assigned each species a dominant mycorrhizal association based on values from

FungalRoot (Soudzilovskaia et al. 2020) and a shade tolerance value based on Niinemets and Valladares (2006). We then used a linear model to estimate the effects of mycorrhizal association, shade tolerance, and local abundance (log transformed) on CNDD_{est}. Because the species in this study varied dramatically in their typical and mature size, and because AM species were generally smaller (Table 1), we also included the median DBH of the species as a predictor in the model. To ensure that our results were robust to model structure, we also iteratively checked all possible two-way interactions of the four species level predictors (mycorrhizal association, shade tolerance, local abundance and median DBH); none were significant, so we report the results of the additive model. All analyses we performed in R version 4.0.5.

Results

Experimental seedling survival

All seedling species had higher survival rates when planted beneath heterospecific trees than when planted under conspecific trees (Figure 1, Table 2). *Quercus rubra*, which had the lowest overall mortality, also exhibited the least difference in survival (88.5% under conspecifics, 96.7% under heterospecifics, odds ratio: 0.91). *Pinus resinosa* had the highest overall mortality, with only 3.8% seedling survival beneath conspecifics and 15.4% beneath heterospecifics (odds ratio 0.25). *Pinus strobus* showed the greatest absolute difference in survival between conspecifics (73.9%) and heterospecifics (94.4%) and the strongest effect of the identity of the neighboring adult tree (Table 2).

Results were qualitatively similar when comparing the density of adult trees in the surrounding community, as measured by NCI, to seedling survival. All four species exhibited declining survival with increasing conspecific adult density (NCI_{con}), but all but *Q. rubra* also exhibited declining survival in response to increasing heterospecific density (NCI_{het}) as well

(Table 3). When comparing the NCI_{con} coefficient estimates and standard errors with those of NCI_{het}, only *P. strobus* exhibited CNDD: in other words, the coefficient estimate for the negative effect of conspecific neighbors did not overlap with the weaker negative effect of heterospecific neighbors (Table 3).

None of the seedling species exhibited survival differences between the even and unevenly planted plots (Table 2). In the evenly planted seedling subplots, which began the experiment with perfectly even communities (J=1), observed mortality caused a decline in seedling diversity; however, this decline in diversity was indistinguishable from simulated random mortality (Figure 2a). The seedling diversity in the unevenly planted subplots either stayed the same or, in the case of the unevenly planted subplots beneath *P. resinosa* adults, substantially increased (Figure 3b). In those plots, the seedling community had changed from an equitability index (J) of 0.85 (uneven) to 0.98 at the end of the experiment- almost perfectly even, and far higher than the diversity predicted if the mortality had been random. These locations experienced the highest total mortality (59.7%), and also the highest difference between conspecific seedling mortality (96%) and heterospecific seedling mortality (12.6%). Experimental seedling survival was also affected by factors other than the local neighborhood. For example, *Pinus strobus* and *Pinus resinosa* seedlings that were initially taller had slightly lower survival. *Picea abies* was the only species whose survival increased with increasing light availability as measured by the gap fraction (Table 2).

Observed sapling survival

Overall, the probability of sapling survival in our observational CNDD analysis decreased with increasing local density of conspecifics (Table 4). Sapling survival was also positively associated with sapling DBH and negatively associated with local density of heterospecifics; however, the effect of these drivers was considerably lower than the effect of conspecific neighbors (Table 4). The strength of CNDD varied substantially among species. In twenty- three of the twenty- eight species used in our analysis, sapling survival decreased significantly with increasing conspecific density, whereas in two species sapling survival increased with increasing conspecific density (Figure 3).

Tree species that typically associate with ECM fungi were much more likely to exhibit CNDD than those that typically associate with AM fungi (Figure 3B, Table 5); 12 of the 13 ECM-associated species in this analysis exhibited significant CNDD. In contrast, 4 of the 15 AM-associated species either did not exhibit significant CNDD or instead were more likely to survive when growing in areas with greater densities of conspecifics (e.g. had significantly positive estimates of CNDD). AM-associated saplings also had a somewhat lower overall survival (61%) than ECM-associated saplings (72%). Although there are a similar number of AM and ECM associating species at this site, ECM species tend to have higher abundance and overall about 66% of saplings are ECM-associating species. In addition, the AM-associating species at this site tend to be smaller (Table 1). However, the median DBH of the species was not associated with stronger CNDD (Table 5).

Shade tolerance was also a significant predictor of CNDD at the species level, such that more shade tolerant species generally exhibited stronger, more negative CNDD than shade intolerant speceis (Figure 4). In contrast, local abundance was not a significant predictor of the strength of CNDD (Figure 4).

CNDD estimated in naturally occurring saplings generally aligned with the results from the seedling experiment. *Quercus rubra*, which showed the weakest response to the nearby conspecific adults (Table 2) and no response to neighborhood conspecific density (Table 3) in the seedling experiment, showed no significant evidence of CNDD at the sapling stage (Figure 3). *Pinus strobus*, which exhibited the strongest response to nearby conspecific adults (Table 2) and neighborhood conspecific densities (Table 3) in the experimental data, showed strong evidence of CNDD in the analysis of observed sapling survival (Figure 3). *Pinus resinosa,* which exhibited intermediate CNDD in the seedling experiment, was also intermediate in the observed sapling analysis.

Discussion

CNDD and mycorrhizal associations

The results of both our field experiment and our analysis of sapling survival indicate that sapling mortality is higher beneath conspecific adults in this system. In particular, the agreement between these two separate analyses for the three tree species common to the two studies provides strong evidence of CNDD in this temperate forest, particularly in ectomycorrhizal trees. Indeed, the apparent ubiquity of CNDD among ectomycorrhizal species in this forest came as a surprise, as previous work has found weaker or positive patterns of density dependence among ECM associating trees (Bennett et al. 2017; Jiang et al. 2020, 2021; Brown et al. 2019). Notably, however, several of these studies found these patterns primarily in plant growth rates rather than in survival (Bennett et al. 2017; Brown et al. 2019). Given that there can be intraspecific tradeoffs between survival and growth rates (Seiwa 2007), it is possible that CNDD estimates based on growth and survival may differ within a species (Brown et al, 2019). In addition, the details of the species included in this study may differ from those in other locations. At this site, while there is a similar number of species that associate with AM and ECM fungi, ECM-associating trees are much more abundant. Thus, the availability of mutualists for AM-associating species is likely lower and/or patchier, and recent evidence shows that AM colonization is greater on plants that grow in stands where other AM-associating plants are more abundant (Grünfeld et al. 2020). This could have the result that regeneration near to conspecifics comes with greater access to appropriate mutualists, which could dampen the negative effects typically associated with proximity to conspecific adults for AM-associating species (Liang et al. 2021). In contrast, mutualist availability is likely ubiquitous for ECM-associating species, as ECM species are well distributed at this site. Finally, estimates of species level CNDD in one context may not generalize, as there is increasing evidence that the strength of CNDD can change due to variation in the presence of large mammals (Murphy and Comita 2021), climate (Liu and He 2021), and along environmental gradients (Brown, White, and Peet 2021; LaManna et al. 2016; Magee et al. 2020; Record et al. 2016).

CNDD and shade tolerance

We also found evidence that shade tolerance predicted the strength of CNDD: shade tolerant species tended to show somewhat greater CNDD, although the effect was strongly driven by a single species (*Picea abies*). This is consistent with some (Jia et al. 2020; García-Guzmán and Heil 2014) but not other (Brown et al. 2019) studies on shade tolerance and CNDD. This discrepancy may be because there are conflicting pressures on trees which result in no clear net effect. Shade tolerant species typically have more conservative life history strategies, which might make them less sensitive to CNDD (McCarthy-Neumann and Kobe 2008; Song et al. 2021). However, shade intolerant species are less likely to be limited by conspecific-associating fungal pathogens, and more likely to be limited by herbivorous insects or pathogens with weaker effects on mortality, perhaps making them less likely to exhibit CNDD (Jia et al. 2020; García-Guzmán and Heil 2014). Taken together with results from this study, it seems that shade tolerance may not have a consistent effect on strength of CNDD in temperate forests.

CNDD and other species characteristics

Local abundance is a strong driver of CNDD in tropical trees, where rare species typically exhibit the strongest CNDD (Comita et al. 2010). In temperate forests, however, the results have been mixed, with some analyses indicating that rare species exhibit stronger CNDD (Johnson et al. 2012; Seiwa et al. 2019) and others finding that more abundant species exhibit stronger CNDD (K. Zhu et al. 2015). Indeed the effect of local abundance may also interact with other characteristics. For example, rare AM species typically exhibit stronger CNDD than common AM species, but the same may not hold true for ECM species (Jiang et al. 2020, 2021). In this study we found that local abundance had no apparent effect on the strength of CNDD, overall or interacting with mycorrhizal type. This may be important for understanding the effects of CNDD on diversity in this forest: theoretical work suggests that if variation among species in CNDD is correlated with abundance, with stronger CNDD for rare species, then diversity may not be maintained. However, if CNDD is unrelated to abundance, as in our study here, or even stronger for common species, then CNDD may help promote the maintenance of diversity (Stump and Comita 2018).

Additional plant characteristics may also influence species susceptibility to CNDD. For example, species with larger seeds may be more tolerant to conspecific neighbors as their seeds act as carbohydrate stores, insuring them against biomass losses to herbivores and pathogens (Lebrija-Trejos et al. 2016; Seiwa et al. 2019). This may help explain why the large-seeded *Q. rubra* exhibited either weak or no CNDD in both the experiment and the observational study; however, the effect of cotyledon reserves is likely larger at younger ages. Notably, naturally occurring *Q. rubra* seedlings at this site do exhibit CNDD, but the effect decreases as the size of the seedling increases (Jevon et al. 2020), which is consistent with what we found here (that the evidence of CNDD was stronger in the experiment, which assessed much smaller individuals) The apparent weakening of CNDD in this species as individuals increase in size in also consistent with previous work suggesting that CNDD is often strongest at the seed to seedling transition or at younger age classes (Zhu et al. 2015). We note that as we assessed large seedlings (2 and 3 years old) in the experiment, and saplings (up to 12.7cm DBH) in the observational analysis, the overall results of this study are likely conservative with respect to how many species exhibit CNDD and the apparent strength of the effect relative to the true strength of CNDD that is experienced by trees in this forest.

CNDD and diversity

The change in diversity in one out of six of our experimental treatments also illustrates how CNDD mortality can affect community diversity (Figure 3). Importantly, it illustrates that alone, differences in mortality in seedlings growing near conspecific and heterospecific adults is not enough to generate greater diversity. The overall mortality must also be relatively high, and the initial diversity low, to result in meaningful changes to seedling community diversity. This is consistent with conceptual models suggesting that overall differences in mortality rates among life stages, alone or in combination with non-random mortality, can be an important determinant of community diversity (Green, Harms, and Connell 2014). The significant increase in diversity in even one treatment during this short-term experiment provides clear evidence that CNDD can act as a mechanism to support local diversity, particularly in systems or situations with high mortality.

Conclusions

Based on these patterns, we suggest that there is strong evidence for CNDD in temperate tree species. Our results also suggest that tree species associated with ectomycorrhizal fungi exhibit CNDD, which runs counter to previous studies (Bennett et al. 2017; Jiang et al. 2020, 2021; Brown et al. 2019). This suggests caution when generalizing about how plant traits predict CNDD. Instead, integrating information about multiple plant characteristics, as well as the environmental context, will help to better predict species-level patterns in CNDD. We found experimentally that CNDD is capable of increasing seedling community diversity. However, theoretical work suggests that, in some cases, interspecific variation in the strength of CNDD decreases its ability to promote coexistence (Stump and Comita 2018), and results from this study and others show large variation in the strength of CNDD among co-occurring species. Therefore, although evidence of CNDD in temperate forests is accumulating, the consequences for diversity remain poorly understood.

Acknowledgements

The authors thank our funding sources, including the Smithsonian Institute and CTFS ForestGEO for supporting the mapped plot, the National Science Foundation's LTER program (DEB 06-20443, DEB 12-37491, DEB 18-32210), Harvard University, the Harvard Forest REU program (National Science Foundation DBI-1459519 & DBI-1950364 to Record), the National Science Foundation (award number 1638406 to Matthes and DEB-2024903 to LaManna), the National Aeronautic and Space Administration (award number 20-BIODIV20-0024 to Record), and the New England Botanical Club Graduate Student Research Award. This work would not have been possible without the 22 technicians who collected the adult tree census data in the CTFS-ForestGEO plot. The authors would also like to thank Manisha Patel and everyone involved in the Harvard Forest REU program for supporting this project.

References

Augspurger, Carol K., and Colleen K. Kelly. 1984. “Pathogen Mortality of Tropical Tree Seedlings: Experimental Studies of the Effects of Dispersal Distance, Seedling Density, and Light Conditions.” *Oecologia* 61 (2): 211–17.

Bagchi, Robert, Rachel E. Gallery, Sofia Gripenberg, Sarah J. Gurr, Lakshmi Narayan, Claire E. Addis, Robert P. Freckleton, and Owen T. Lewis. 2014. “Pathogens and Insect Herbivores Drive Rainforest Plant Diversity and Composition.” *Nature* 506 (7486): 85–88.

Bai, Xuejiao, Simon A. Queenborough, Xugao Wang, Jian Zhang, Buhang Li, Zuoqiang Yuan, Dingliang Xing, Fei Lin, Ji Ye, and Zhanqing Hao. 2012. “Effects of Local Biotic Neighbors and Habitat Heterogeneity on Tree and Shrub Seedling Survival in an Old-Growth Temperate Forest.” *Oecologia* 170 (3): 755–65.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. “Fitting Linear Mixed-Effects Models Using lme4.” *Journal of Statistical Software* 67 (1): 1–48.

Bennett, Jonathan A., Hafiz Maherali, Kurt O. Reinhart, Ylva Lekberg, Miranda M. Hart, and John Klironomos. 2017. “Plant-Soil Feedbacks and Mycorrhizal Type Influence Temperate Forest Population Dynamics.” *Science* 355 (6321): 181–84.

Brown, Alissa J., Christopher J. Payne, Peter S. White, and Robert K. Peet. 2019. “Shade Tolerance and Mycorrhizal Type May Influence Sapling Susceptibility to Conspecific Negative Density Dependence.” Edited by Pierre Mariotte. *The Journal of Ecology* 4 (July): 103.

Brown, Alissa J., Peter S. White, and Robert K. Peet. 2021. “Environmental Context Alters the Magnitude of Conspecific Negative Density Dependence in a Temperate Forest.” *Ecosphere* 12 (3). https://doi.org/10.1002/ecs2.3406.

Canham, Charles D., Philip T. LePage, and K. Dave Coates. 2004. "A Neighborhood Analysis of Canopy Tree Competition: Effects of Shading versus Crowding." *Canadian Journal of Forest Research. Journal Canadien de La Recherche Forestiere* 34 (4): 778–87.

Chen, Lei, Nathan G. Swenson, Niuniu Ji, Xiangcheng Mi, Haibao Ren, Liangdong Guo, and Keping Ma. 2019. "Differential Soil Fungus Accumulation and Density Dependence of Trees in a Subtropical Forest." *Science* 366 (6461): 124–28.

Chesson, Peter. 2000. "Mechanisms of Maintenance of Species Diversity." *Annual Review of Ecology and Systematics*. https://doi.org/10.1146/annurev.ecolsys.31.1.343.

Comita, Liza S., Helene C. Muller-Landau, Salomón Aguilar, and Stephen P. Hubbell. 2010. "Asymmetric Density Dependence Shapes Species Abundances in a Tropical Tree Community." *Science* 329 (5989): 330–32.

Comita, Liza S., Simon A. Queenborough, Stephen J. Murphy, Jenalle L. Eck, Kaiyang Xu, Meghna Krishnadas, Noelle Beckman, and Yan Zhu. 2014. "Testing Predictions of the Janzen--Connell Hypothesis: A Meta-Analysis of Experimental Evidence for Distance-and Density-Dependent Seed and Seedling Survival." *The Journal of Ecology* 102 (4): 845–56.

Connell, J. H. 1971. "On the Role of Natural Enemies in Preventing Competitive Exclusion in Some Marine Animals and in Rain Forests." In *Dynamics of Populations*, edited by P. J. Den Boer G. R. Gradwell, 298–312. Center for Agricultural Publishing and Documentation.

Corrales, Adriana, Scott A. Mangan, Benjamin L. Turner, and James W. Dalling. 2016. "An Ectomycorrhizal Nitrogen Economy Facilitates Monodominance in a Neotropical Forest." *Ecology Letters* 19 (4): 383–92.

Detto, Matteo, Marco D. Visser, S. Joseph Wright, and Stephen W. Pacala. 2019. "Bias in the Detection of Negative Density Dependence in Plant Communities." *Ecology Letters* 22

(11): 1923–39.

García-Guzmán, Graciela, and Martin Heil. 2014. “Life Histories of Hosts and Pathogens Predict Patterns in Tropical Fungal Plant Diseases.” *The New Phytologist* 201 (4): 1106–20.

Gelman, Andrew. 2008. “Scaling Regression Inputs by Dividing by Two Standard Deviations.” *Statistics in Medicine* 27 (15): 2865–73.

Green, Peter T., Kyle E. Harms, and Joseph H. Connell. 2014. “Nonrandom, Diversifying Processes Are Disproportionately Strong in the Smallest Size Classes of a Tropical Forest.” *Proceedings of the National Academy of Sciences of the United States of America* 111 (52): 18649–54.

Grünfeld, Leonie, Monika Wulf, Matthias C. Rillig, Annette Manntschke, and Stavros D. Veresoglou. 2020. “Neighbours of Arbuscular-Mycorrhiza Associating Trees Are Colonized More Extensively by Arbuscular Mycorrhizal Fungi than Their Conspecifics in Ectomycorrhiza Dominated Stands.” *The New Phytologist* 227 (1): 10–13.

Janzen, Daniel H. 1970. “Herbivores and the Number of Tree Species in Tropical Forests.” *The American Naturalist* 104 (940): 501–28.

Jevon, Fiona V., Sydne Record, John Grady, Ashley K. Lang, David A. Orwig, Matthew P. Ayres, and Jaclyn Hatala Matthes. 2020. “Seedling Survival Declines with Increasing Conspecific Density in a Common Temperate Tree.” *Ecosphere* 11 (11): e03292.

Jiang, Feng, James A. Lutz, Qingxi Guo, Zhanqing Hao, Xugao Wang, Gregory S. Gilbert, Zikun Mao, et al. 2021. “Mycorrhizal Type Influences Plant Density Dependence and Species Richness across 15 Temperate Forests.” *Ecology* 102 (3): e03259.

Jiang, Feng, Kai Zhu, Marc W. Cadotte, and Guangze Jin. 2020. “Tree Mycorrhizal Type Mediates the Strength of Negative Density Dependence in Temperate Forests.” *The Journal* *of Ecology*, May. https://doi.org/10.1111/1365-2745.13413.

Jia, Shihong, Xugao Wang, Zuoqiang Yuan, Fei Lin, Ji Ye, Guigang Lin, Zhanqing Hao, and Robert Bagchi. 2020. “Tree Species Traits Affect Which Natural Enemies Drive the Janzen-Connell Effect in a Temperate Forest.” *Nature Communications* 11 (1): 286.

Johnson, Daniel J., Wesley T. Beaulieu, James D. Bever, and Keith Clay. 2012. “Conspecific Negative Density Dependence and Forest Diversity.” *Science* 336 (6083): 904–7.

Johnson, Daniel J., Norman A. Bourg, Robert Howe, William J. McShea, Amy Wolf, and Keith Clay. 2014. “Conspecific Negative Density-Dependent Mortality and the Structure of Temperate Forests.” *Ecology* 95 (9): 2493–2503.

Johnson, Daniel J., Richard Condit, Stephen P. Hubbell, and Liza S. Comita. 2017. “Abiotic Niche Partitioning and Negative Density Dependence Drive Tree Seedling Survival in a Tropical Forest.” *Proceedings of the Royal Society B: Biological Sciences* 284 (1869): 20172210.

LaManna, Joseph A., Scott A. Mangan, Alfonso Alonso, Norman A. Bourg, Warren Y. Brockelman, Sarayudh Bunyavejchewin, Li-Wan Chang, et al. 2017. “Plant Diversity Increases with the Strength of Negative Density Dependence at the Global Scale.” *Science* 356 (6345): 1389–92.

LaManna, Joseph A., Scott A. Mangan, and Jonathan A. Myers. 2021. “Conspecific Negative Density Dependence and Why Its Study Should Not Be Abandoned.” *Ecosphere* 12 (1). https://doi.org/10.1002/ecs2.3322.

LaManna, Joseph A., Maranda L. Walton, Benjamin L. Turner, and Jonathan A. Myers. 2016. “Negative Density Dependence Is Stronger in Resource-Rich Environments and Diversifies Communities When Stronger for Common but Not Rare Species.” Edited by Marcel

Rejmanek. *Ecology Letters* 19 (6): 657–67.

Lebrija-Trejos, Edwin, Peter B. Reich, Andres Hernández, and S. Joseph Wright. 2016. "Species with Greater Seed Mass Are More Tolerant of Conspecific Neighbours: A Key Driver of Early Survival and Future Abundances in a Tropical Forest." *Ecology Letters* 19 (9): 1071–80.

Levine, Jonathan M., and Janneke HilleRisLambers. 2009. "The Importance of Niches for the Maintenance of Species Diversity." *Nature* 461 (7261): 254–57.

Liang, Minxia, Liuqing Shi, David F. R. P. Burslem, David Johnson, Miao Fang, Xinyi Zhang, and Shixiao Yu. 2021. "Soil Fungal Networks Moderate Density-Dependent Survival and Growth of Seedlings." *The New Phytologist*, no. nph.17237 (January). https://doi.org/10.1111/nph.17237.

Liu, Yu, and Fangliang He. 2021. "Warming Intensifies Soil Pathogen Negative Feedback on a Temperate Tree." *The New Phytologist*, April. https://doi.org/10.1111/nph.17409.

Magee, Lukas, Amy Wolf, Robert Howe, Jonathan Schubbe, Kari Hagenow, and Benjamin Turner. 2020. "Density Dependence and Habitat Heterogeneity Regulate Seedling Survival in a North American Temperate Forest." *Forest Ecology and Management*, November, 118722.

Mangan, Scott A., Stefan A. Schnitzer, Edward A. Herre, Keenan M. L. Mack, Mariana C. Valencia, Evelyn I. Sanchez, and James D. Bever. 2010. "Negative Plant-Soil Feedback Predicts Tree-Species Relative Abundance in a Tropical Forest." *Nature* 466 (7307): 752–55.

McCarthy-Neumann, Sarah, and Richard K. Kobe. 2008. "Tolerance of Soil Pathogens Co-Varies with Shade Tolerance across Species of Tropical Tree Seedlings." *Ecology* 89 (7):

1883–92.

———. 2010. “Conspecific and Heterospecific Plant-Soil Feedbacks Influence Survivorship and Growth of Temperate Tree Seedlings.” *The Journal of Ecology* 98 (2): 408–18.

Murphy, Stephen J., and Liza S. Comita. 2021. “Large Mammalian Herbivores Contribute to Conspecific Negative Density Dependence in a Temperate Forest.” *The Journal of Ecology* 109 (3): 1194–1209.

Niinemets, Ülo, and Fernando Valladares. 2006. “Tolerance to Shade, Drought, and Waterlogging of Temperate Northern Hemisphere Trees and Shrubs.” *Ecological Monographs* 76 (4): 521–47.

Orwig, David A., Jason A. Aylward, Hannah L. Buckley, Bradley S. Case, and Aaron M. Ellison. 2022. “Land-Use History Impacts Spatial Patterns and Composition of Woody Plant Species across a 35-Hectare Temperate Forest Plot.” *PeerJ* 10 (January): e12693.

Qin, Jianghuan, Yan Geng, Xiaoyu Li, Chunyu Zhang, Xiuhai Zhao, and Klaus von Gadow. 2021. “Mycorrhizal Type and Soil Pathogenic Fungi Mediate Tree Survival and Density Dependence in a Temperate Forest.” *Forest Ecology and Management* 496 (September): 119459.

Ramage, Benjamin S., Daniel J. Johnson, Erika Gonzalez-Akre, William J. McShea, Kristina J. Anderson-Teixeira, Norman A. Bourg, and Keith Clay. 2017. “Sapling Growth Rates Reveal Conspecific Negative Density Dependence in a Temperate Forest.” *Ecology and Evolution* 7 (19): 7661–71.

Record, Sydne, Richard K. Kobe, Corine F. Vriesendorp, and Andrew O. Finley. 2016. “Seedling Survival Responses to Conspecific Density, Soil Nutrients, and Irradiance Vary with Age in a Tropical Forest.” *Ecology* 97 (9): 2406–15.

Seiwa, Kenji. 2007. “Trade-Offs between Seedling Growth and Survival in Deciduous Broadleaved Trees in a Temperate Forest.” *Annals of Botany* 99 (3): 537–44.

Seiwa, Kenji, Kazuhiko Masaka, Miki Konno, and Susumu Iwamoto. 2019. “Role of Seed Size and Relative Abundance in Conspecific Negative Distance-Dependent Seedling Mortality for Eight Tree Species in a Temperate Forest” 453 (117537). https://doi.org/10.1016/j.foreco.2019.117537.

Smith, Lauren M., and Heather L. Reynolds. 2015. “Plant–soil Feedbacks Shift from Negative to Positive with Decreasing Light in Forest Understory Species.” *Ecology* 96 (9): 2523–32.

Song, Xiaoyang, Jie Yang, Min Cao, Luxiang Lin, Zhenhua Sun, Handong Wen, and Nathan G. Swenson. 2021. “Traits Mediate a Trade-off in Seedling Growth Response to Light and Conspecific Density in a Diverse Subtropical Forest.” *The Journal of Ecology* 109 (2): 703–13.

Soudzilovskaia, Nadejda A., Stijn Vaessen, Milagros Barcelo, Jinhong He, Saleh Rahimlou, Kessy Abarenkov, Mark C. Brundrett, Sofia I. F. Gomes, Vincent Merckx, and Leho Tedersoo. 2020. “FungalRoot: Global Online Database of Plant Mycorrhizal Associations.” *The New Phytologist* 227 (3): 955–66.

Stump, Simon Maccracken, and Liza S. Comita. 2018. “Interspecific Variation in Conspecific Negative Density Dependence Can Make Species Less Likely to Coexist.” *Ecology Letters* 21 (10): 1541–51.

Terborgh, John. 2012. “Enemies Maintain Hyperdiverse Tropical Forests.” *The American Naturalist* 179 (3): 303–14.

Xu, Meng, Yongfan Wang, and Shixiao Yu. 2015. “Conspecific Negative Density Dependence Decreases with Increasing Species Abundance.” https://doi.org/10.1890/ES15-00144.1.

Zhang, Zhaochen, Michael J. Papaik, Xugao Wang, Zhanqing Hao, Ji Ye, Fei Lin, and Zuoqiang Yuan. 2017. "The Effect of Tree Size, Neighborhood Competition and Environment on Tree Growth in an Old-Growth Temperate Forest." *Journal of Plant Ecology* 10 (6): 970–80.

Zhu, Kai, Christopher W. Woodall, Joao V. D. Monteiro, and James S. Clark. 2015. "Prevalence and Strength of Density-Dependent Tree Recruitment." *Ecology* 96 (9): 2319–27.

Zhu, Yan, Liza S. Comita, Stephen P. Hubbell, and Keping Ma. 2015. "Conspecific and Phylogenetic Density-Dependent Survival Differs across Life Stages in a Tropical Forest." Edited by Richard Shefferson. *The Journal of Ecology* 103 (4): 957–66.

Table 1. Characteristics of species included in the analysis of CNDD for saplings from two censuses. Species used in the planting experiment are in bold. AM: species that typically associate with arbuscular mycorrhizae; ECM: species that typically associate with ectomycorrhizae*. The operational cutoff between saplings and adults was the median DBH for unshaded species and 12.7 cm for shaded species.

Species	Total abundance	Mycorrhizal association	Median DBH	Species code
Viburnum dentatum	53	AM	1.1	Vibude
Viburnum lantanoides	96	AM	1.2	Vibual
Viburnum cassinoides	1846	AM	1.3	Vibuca
Lindera benzoin	83	AM	1.4	Lindbe
Ilex verticillata	1266	AM	1.5	Ilexve
Amelanchier laevis	354	AM	1.4	Amella
Crataegus spp.	259	AM	1.5	Cratsp
Acer pennsylvanicum	425	AM	1.8	Acerpe
Nemopanthus mucronatus	377	AM	1.8	Nemomu
Castanea dentata	1020	ECM	2.2	Castde
Hamamelis virginiana	3578	AM	2.45	Hamavi
Fagus grandifolia	4362	ECM	3.0	Fagugr
Sorbus americana	74	AM	3.25	Sorbam
Tsuga canadensis	24,222	ECM	5.4	Tsugca
Betula alleghaniensis	5015	ECM	5.6	Betula
Betula lenta	1545	ECM	8.6	Betule
Betula populifolia	123	ECM	9.5	Betupo
Fraxinus americana	197	AM	10.1	Fraxam
Nyssa sylvatica	193	AM	10.6	Nysssy
Acer rubrum	12,967	AM	11.9	Acerru
Prunus serotina	266	AM	13.3	Prunse
Betula papyrifera	590	ECM	15.35	Betupa
Picea abies	911	ECM	16.5	Piceab
Picea rubens	106	ECM	18.15	Piceru
Pinus strobus	2149	ECM	22.1	Pinust

Quercus rubra	4407	ECM	28.6	Querru
Quercus velutina	227	ECM	30.4	Querve
Pinus resinosa	789	ECM	32.8	Pinure

*Mycorrhizal associations determined according to the FungalRoot database (Soudzilovskaia et al. 2020)

Table 2. Coefficient estimates (standard error in parentheses) from binomial mixed effects model of seedling survival for each of the four seedling species. In all models, plot was included as a random effect. * indicates p< 0.05, †indicates p < 0.1.

Seedling species	Conspecific adult	Seedling height	Gap Fraction	Subplot type (uneven)
Pinus resinosa	-1.96 (0.86)*	-0.10 (0.04)*	0.18 (0.12)	0.25 (0.46)
Quercus rubra	-1.38 (0.57)*	-0.01 (0.03)	0.00 (0.09)	0.07 (0.47)
Pinus strobus	-2.76 (1.09)*	-0.06 (0.03)*	-0.11 (0.17)	-0.25 (0.41)
Picea abies	na	-0.05 (0.04)	0.22 (0.13)†	-0.43 (0.38)

Table 3. Coefficient estimates (standard error in parentheses) from binomial mixed effects model of survival for each of the four experimental seedling species using the quantitative variables NCI_{con} and NCI_{het}. In all models, plot was included as a random effect. * indicates $p < 0.05$, †indicates $p < 0.1$.

Seedling species	NCI_{con}	NCI_{het}	Seedling height	Gap Fraction	Subplot type (uneven)
Pinus resinosa	-0.032 (0.013)*	-0.016 (0.015)	-0.097 (0.042)*	0.145 (0.120)	0.282 (0.463)
Quercus rubra	-0.015 (0.015)	0.005 (0.010)	-0.009 (0.031)	-0.045 (0.098)	0.029 (0.469)
Pinus strobus	-0.061 (0.021)*	-0.022 (0.016)	-0.061 (0.030)*	-0.105 (0.152)	-0.283 (0.407)
Picea abies	-0.021 (0.029)	-0.023 (0.012)†	-0.042 (0.040)	0.170 (0.118)	-0.426 (0.379)

Table 4. Overall standardized coefficient estimates from GLMM predicting sapling mortality as a function of initial size and local tree community. $NCI_{con}$and NCI_{het} represent local densities of conspecific trees, and heterospecific trees, respectively. Species was included as a random effect with NCI_{con} and NCI_{het} both estimated with a random slope for each species. Individual random effects coefficients for each species can be found in Appendix 1: Table S1.

Predictor	Estimate (SE)	P
(intercept)	-1.867 (0.249)	<0.001
DBH	-0.805 (0.036)	<0.001
NCI_{Het}	-0.106 (0.147)	0.468
NCI_{Con}	0.948 (0.271)	<0.001
Random Effects		
σ^2	1.64	
$\tau_{00\ species}$	1.41	
$\tau_{11\ species\ *\ NCIcon}$	1.20	
$\tau_{11\ species\ *\ NCIhet}$	0.40	
ICC	0.46	
$N_{species}$	28	
Observations	24250	
Marginal R^2 / Conditional R^2	0.121 / 0.527	

Table 5. Coefficient estimates from linear model predicting the strength of the species level estimate of CNDD as a function of four species level characteristics: mycorrhizal association, shade tolerance, local abundance and median DBH of the species at this site.

Predictor	Estimate (SE)	P
(Intercept)	-0.250 (1.00)	0.805
Mycorrhizal type (ECM)	-1.111 (0.482)	0.033
Shade tolerance	-0.540 (0.240)	0.036
Median DBH	0.001 (0.025)	0.965
Log(abundance)	0.228 (0.130)	0.095

Overall model $R^2_{adjusted} = 0.257$

Figure captions

Figure 1. Plot level survival of each of the four seedling species when planted beneath a conspecific (pale green) or a heterospecific (dark green) adult tree. Seedlings from both subplots in each 20m diameter plot are included in each point, such that each point represents survival of 40 planted seedlings. N = 30 plots for each species.

Figure 2. Mean Shannon's equitability index (J), a metric of community evenness, at the end of the experiment (green) relative to 1000 model simulations of equivalent level of mortality occurring randomly (black). Values for seedling subplots that were planted with equal numbers of each seedling species (A) and subplots that were planted with the conspecific seedling dominating (B), averaged across the 10 replicated adult trees. Error bars represent 95% confidence intervals. Dashed lines represent Shannon's equitability index of the community as it was initially planted.

Figure 3. Estimates of conspecific negative density dependence (CNDD) for saplings of 28 tree species at the Harvard Forest (A). Error bars represent the total range of estimates using 1000 iterations of the null model (see statistical methods section for details). Blue points represent species that typically associate with arbuscular mycorrhiza (AM), green points represent species that typically associate with ectomycorrhizae (ECM). B) Boxplot of all estimates by mycorrhizal type, showing the significant difference between AM tree species and ECM tree species. Species used in the seedling experiment are in bold. Species codes as in Table 1.

Figure 4. Estimates of conspecific negative density dependence (CNDD) for saplings of 28 tree species at the Harvard Forest as a function of shade tolerance (A) and local abundance (B). Blue points represent species that typically associate with arbuscular mycorrhiza (AM), green points represent species that typically associate with ectomycorrhizae (ECM). Gray line in A) illustrates significant negative relationship between shade tolerance and CNDD (see Table 5). Vertical error bars represent 95% confidence intervals (see statistical methods section for details). Horizontal error bars in A) are standard error from Niinemets and Valladares (2006). Note that *Crataegus sp., Nemopanthus mucronatuso*, *Viburnum lantanoides* and *Viburnum cassinoides* are missing from a), as there was no estimate of shade tolerance available.

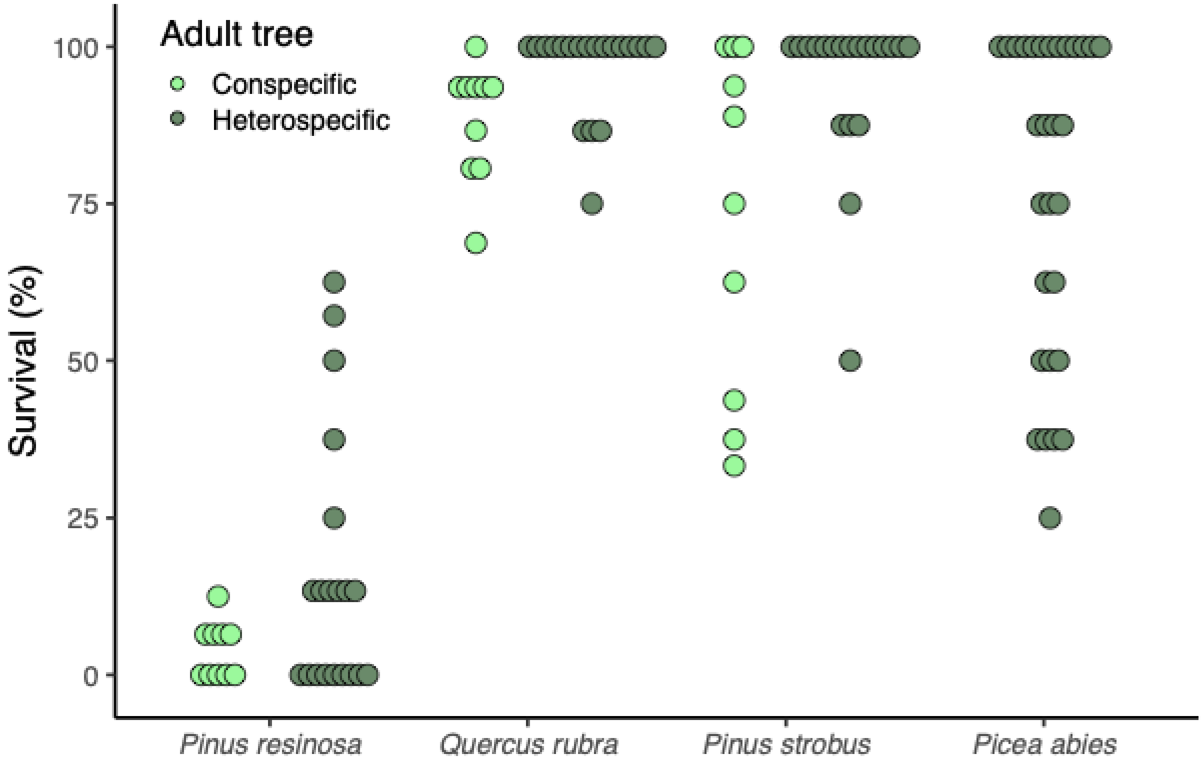


Figure 1. Plot level survival of each of the four seedling species when planted beneath a conspecific (pale green) or a heterospecific (dark green) adult tree. Seedlings from both subplots in each 20m diameter plot are included in each point, such that each point represents survival of 40 planted seedlings. N = 30 plots for each species.

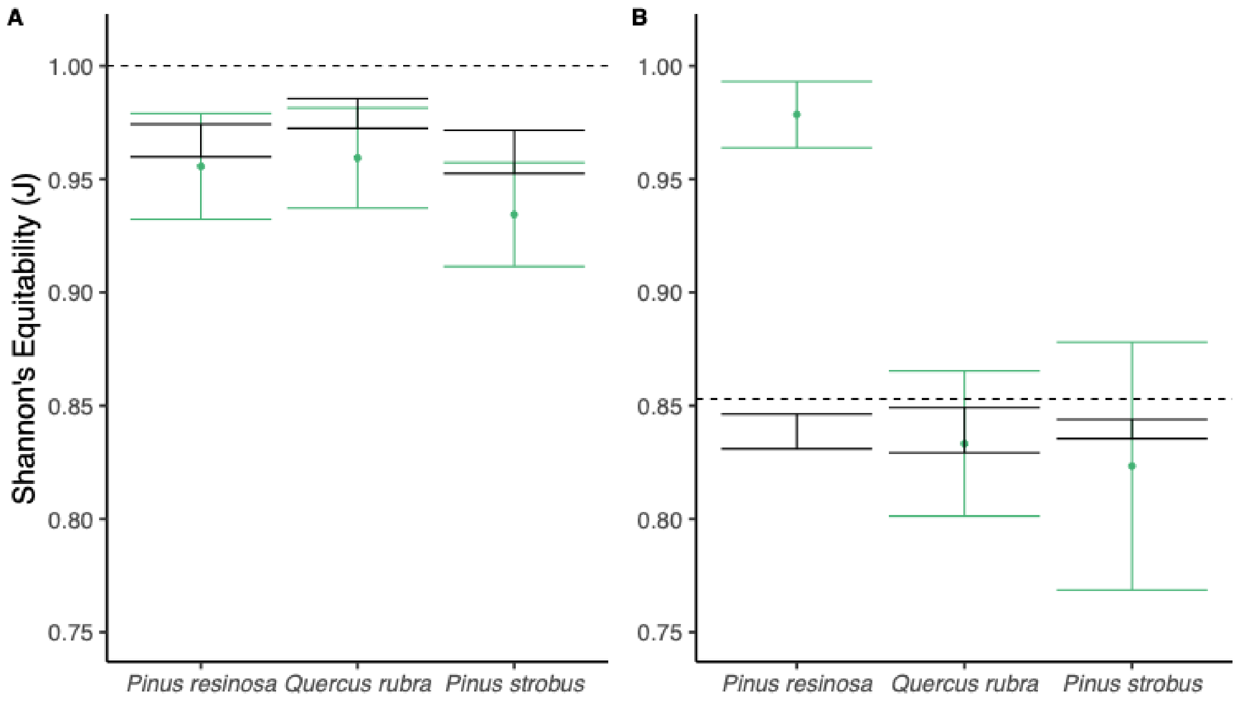
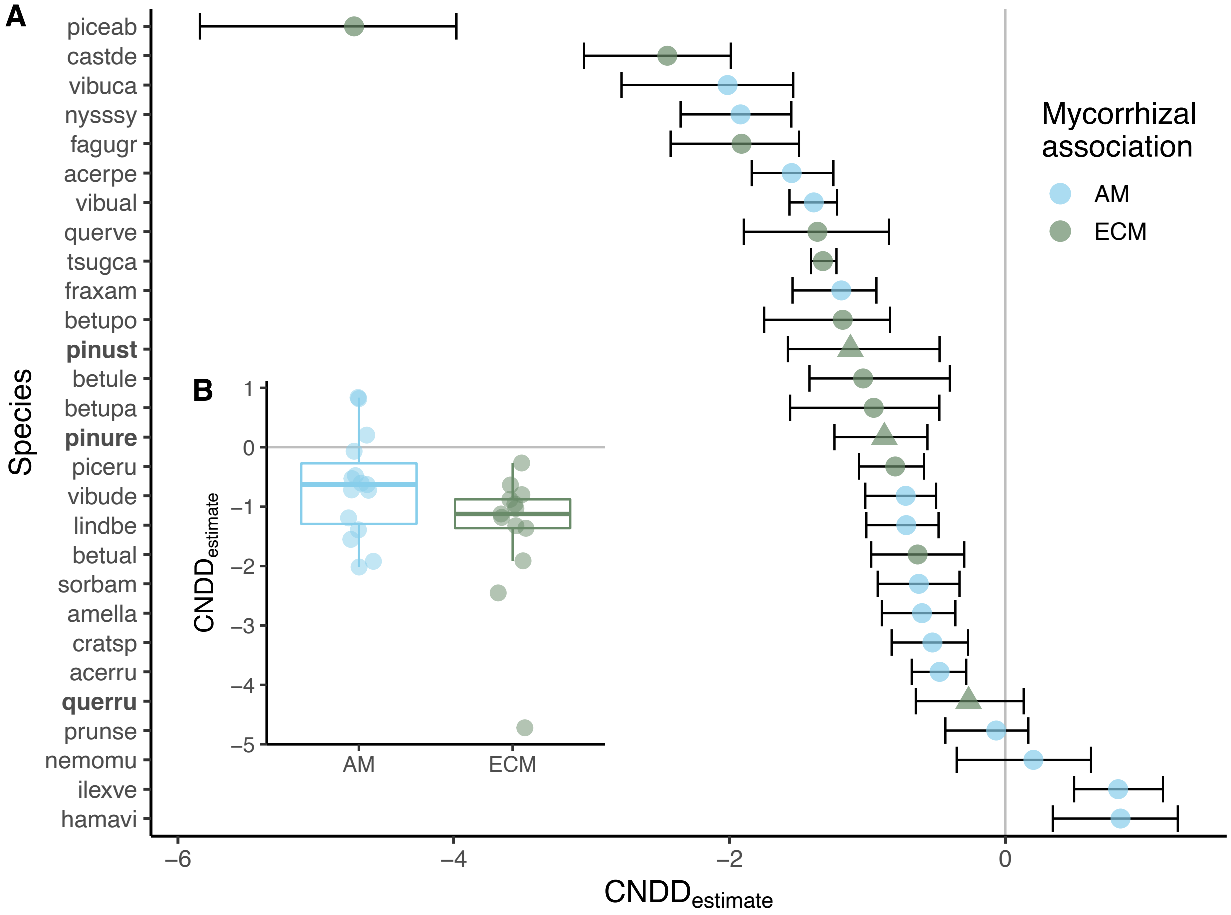


Figure 2. Mean Shannon's equitability index (J), a metric of community evenness, at the end of the experiment (green) relative to 1000 model simulations of equivalent level of mortality occurring randomly (black). Values for seedling subplots that were planted with equal numbers of each seedling species (A) and subplots that were planted with the conspecific seedling dominating (B), averaged across the 10 replicated adult trees. Error bars represent 95% confidence intervals. Dashed lines represent Shannon's equitability index of the community as it was initially planted.

827



828

829 **Figure 3.** Estimates of conspecific negative density dependence (CNDD) for saplings of 28 tree 830 species at the Harvard Forest (A). Error bars represent the total range of estimates using 1000 831 iterations of the null model (see statistical methods section for details). Blue points represent 832 species that typically associate with arbuscular mycorrhiza (AM), green points represent species 833 that typically associate with ectomycorrhizae (ECM). B) Boxplot of all estimates by mycorrhizal 834 type, showing the significant difference between AM tree species and ECM tree species. Species 835 used in the seedling experiment are in bold. Species codes as in Table 1.

836

837

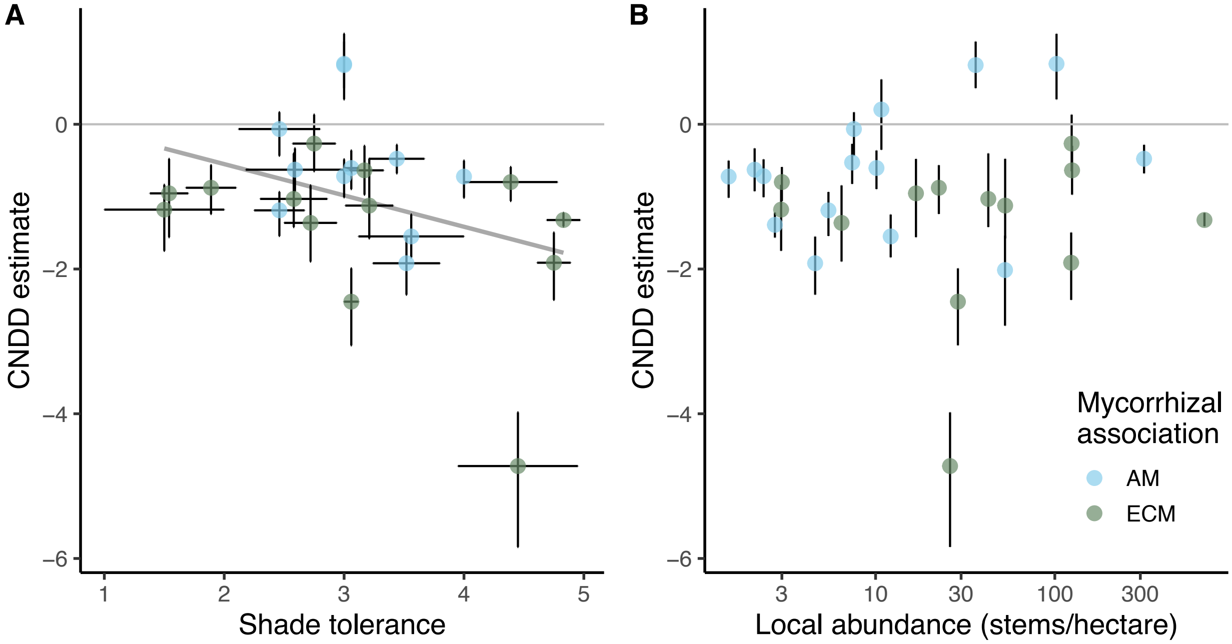


Figure 4. Estimates of conspecific negative density dependence (CNDD) for saplings of 28 tree species at the Harvard Forest as a function of shade tolerance (A) and local abundance (B). Blue points represent species that typically associate with arbuscular mycorrhiza (AM), green points represent species that typically associate with ectomycorrhizae (ECM). Gray line in A) illustrates significant negative relationship between shade tolerance and CNDD (see Table 5). Vertical error bars represent 95% confidence intervals (see statistical methods section for details). Horizontal error bars in A) are standard error from Niinemets and Valladares (2006). Note that *Crataegus sp., Nemopanthus mucronatuso*, *Viburnum lantanoides* and *Viburnum cassinoides* are missing from a), as there was no estimate of shade tolerance available.

Appendix S1

Supplement to: *Experimental and observational evidence of negative conspecific density dependence in temperate ectomycorrhizal trees*

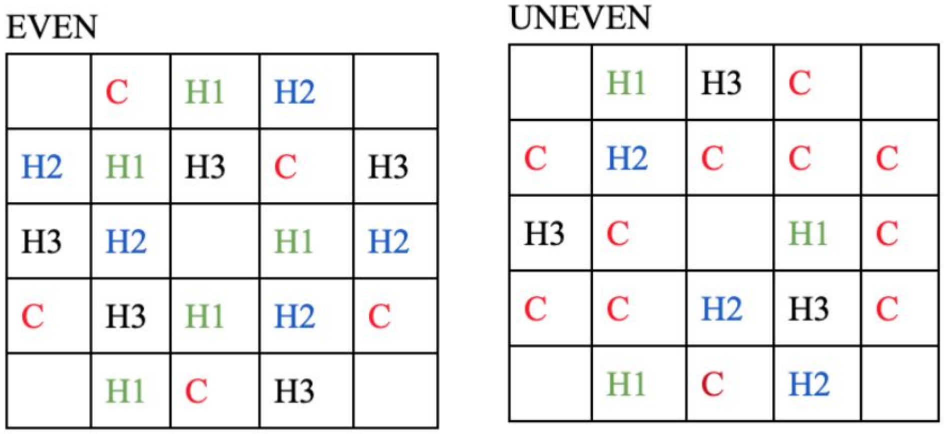
Fiona V. Jevon[1,2], Dayna De La Cruz[3], Joseph A. LaManna[4], Ashley K. Lang[5], David A. Orwig[6], Sydne Record[7], Paige V. Kouba[8], Matthew P. Ayres[1], Jaclyn Hatala Matthes[2]

[1]Department of Biological Sciences, Dartmouth College, Hanover NH 03755, United States
[2]Current affiliation: Yale School of the Environment, Yale University, New Haven, CT 06511, United States
[3]Department of Biological Sciences, Wellesley College, Wellesley, MA 02481, USA
[4]Department of Biological Sciences, Marquette University, Milwaukee, WI, 53201 USA
[5]Department of Biological Sciences, Indiana University, Bloomington, IN, 47405 USA
[6]Harvard Forest, Harvard University, Petersham, MA 01366, USA
[7]Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania 19010 USA
[8]Department of Plant Sciences, University of California, Davis, CA, 95616 USA

Table S1. Standardized coefficient estimates from GLMM predicting sapling mortality as a function of initial size (DBH) and local tree community. $NCI_{con}$and NCI_{het} represent local densities of conspecific trees, and heterospecific trees, respectively. Species was included as a random effect with a random intercept, and NCI_{con} and NCI_{het} were both estimated with a random slope for each species. Note that as the response variable is seedling mortality, positive values of coefficients represent effects that increase likelihood of mortality and negative values of coefficients represent effects that increase likelihood of survival (e.g. DBH). Species abbreviations are as in Table 1.

Species	Intercept	DBH	NCI_{con}	NCI_{het}
acerpe	-2.317	-0.805	1.547	0.170
acerru	-2.638	-0.805	0.382	-0.065
amella	-2.260	-0.805	0.644	-0.220
betual	-2.716	-0.805	0.842	0.297
betule	-2.848	-0.805	1.317	0.730
betupa	-1.164	-0.805	0.492	-0.664
betupo	-0.165	-0.805	0.806	0.046
castde	-1.068	-0.805	2.068	-0.762
cratsp	-2.518	-0.805	0.656	-0.048
fagugr	-3.122	-0.805	1.279	-0.869
fraxam	-1.506	-0.805	1.120	-0.287
hamavi	-3.581	-0.805	-0.782	-0.062
ilexve	-2.886	-0.805	-0.403	0.406
lindbe	-2.062	-0.805	0.682	-0.242
nemomu	-2.220	-0.805	-0.246	-0.266
nysssy	-1.604	-0.805	1.406	-0.777
piceab	1.092	-0.805	3.896	-1.444
piceru	-1.438	-0.805	0.645	-0.184
pinure	-0.410	-0.805	0.641	0.044
pinust	-1.637	-0.805	1.440	1.249
prunse	-1.835	-0.805	0.089	0.186
querru	-1.641	-0.805	0.302	0.074
querve	-1.575	-0.805	1.307	0.277
sorbam	-2.444	-0.805	0.737	0.036
tsugca	-4.120	-0.805	1.583	0.325
vibual	-2.341	-0.805	1.363	-0.374
vibuca	-0.160	-0.805	1.727	-0.555
vibude	-1.360	-0.805	0.646	0.222

Figure S1. Location of the 35 hectare ForestGEO plot (gray rectangle), where censuses took place, relative to our thirty experimental plots at the Harvard Forest. Open circles represent experimental plots, with the color corresponding to the identity of the adult beneath which the seedlings were planted. White circles: *Pinus resinosa*, Blue circles: *Pinus strobus*, Orange circles: *Quercus rubra*.



EVEN

	C	H1	H2	
H2	H1	H3	C	H3
H3	H2		H1	H2
C	H3	H1	H2	C
	H1	C	H3	

UNEVEN

	H1	H3	C	
C	H2	C	C	C
H3	C		H1	C
C	C	H2	H3	C
	H1	C	H2	

Figure S2. Planting diagrams for the seedling subplots. Red C indicates location of conspecific seedling. H1, H2 and H3 indicate locations of the three heterospecific seedlings. At each plot, species were randomly assigned to be heterospecific 1, 2 or 3.

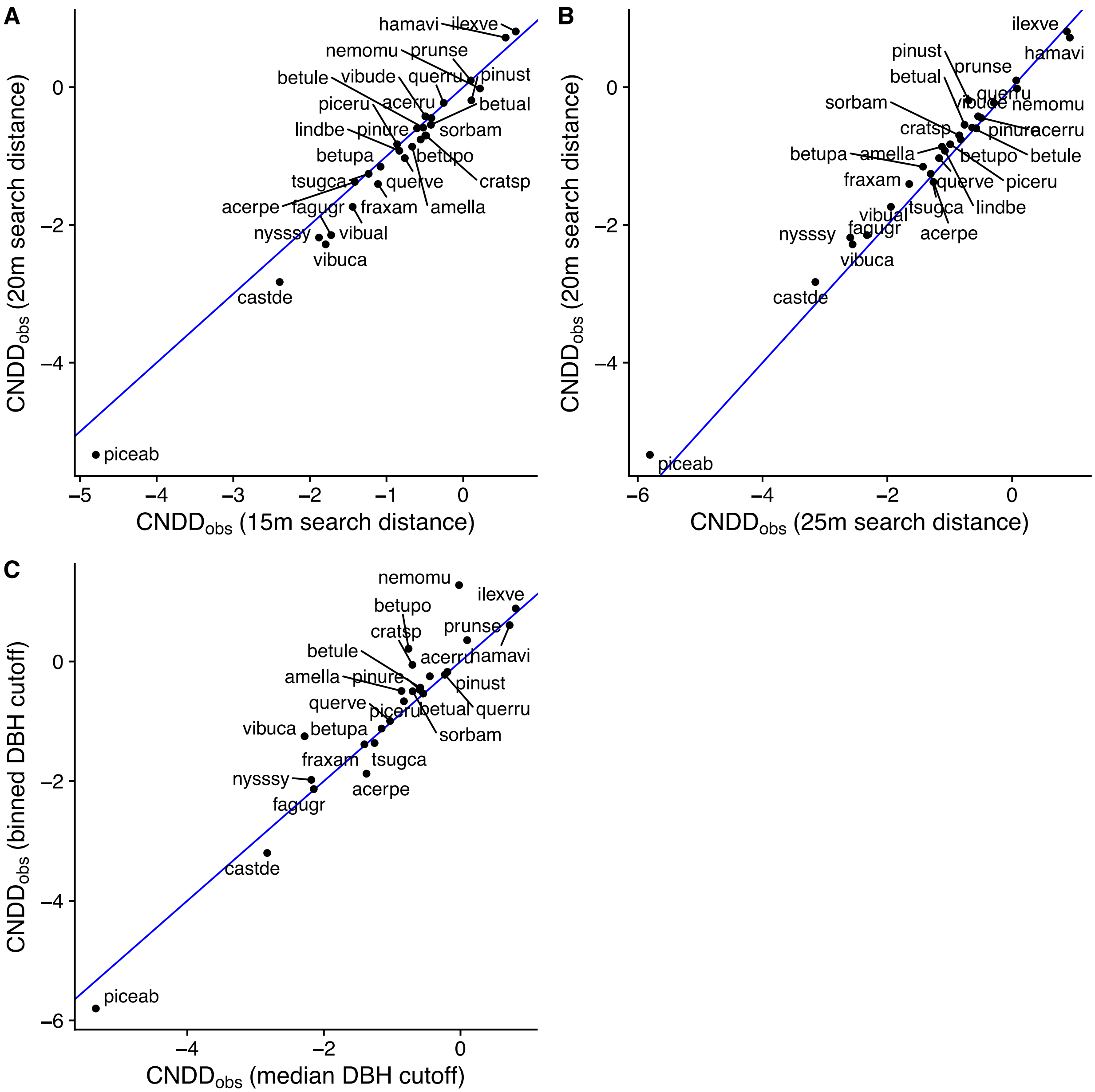


Figure S3. Species level estimates of CNDD from observed data calculated using different methods arrive at similar estimates. A) Maximum distance of included neighbors for estimates were 15m and 20m away from focal saplings, DBH cutoff between saplings and adults was the median DBH of the species, unless that was greater than 12.7cm in which case 12.7cm was used. B) Maximum distance of included neighbors for estimates were 25m and 20m away from focal saplings, DBH cutoff between saplings and adults was the median DBH of the species, unless that was greater than 12.7cm in which case 12.7cm was used. C) Maximum distance of included neighbors for estimates were 20m, DBH cutoff between saplings and adults was the median DBH of the species unless that was greater than 12.7cm in which case 12.7cm was used (median DBH) or classified by binning into either 5cm, 8cm or 12.7cm based on the median DBH of the species. Blue line indicates 1:1. Species abbreviations are as in Table 1.

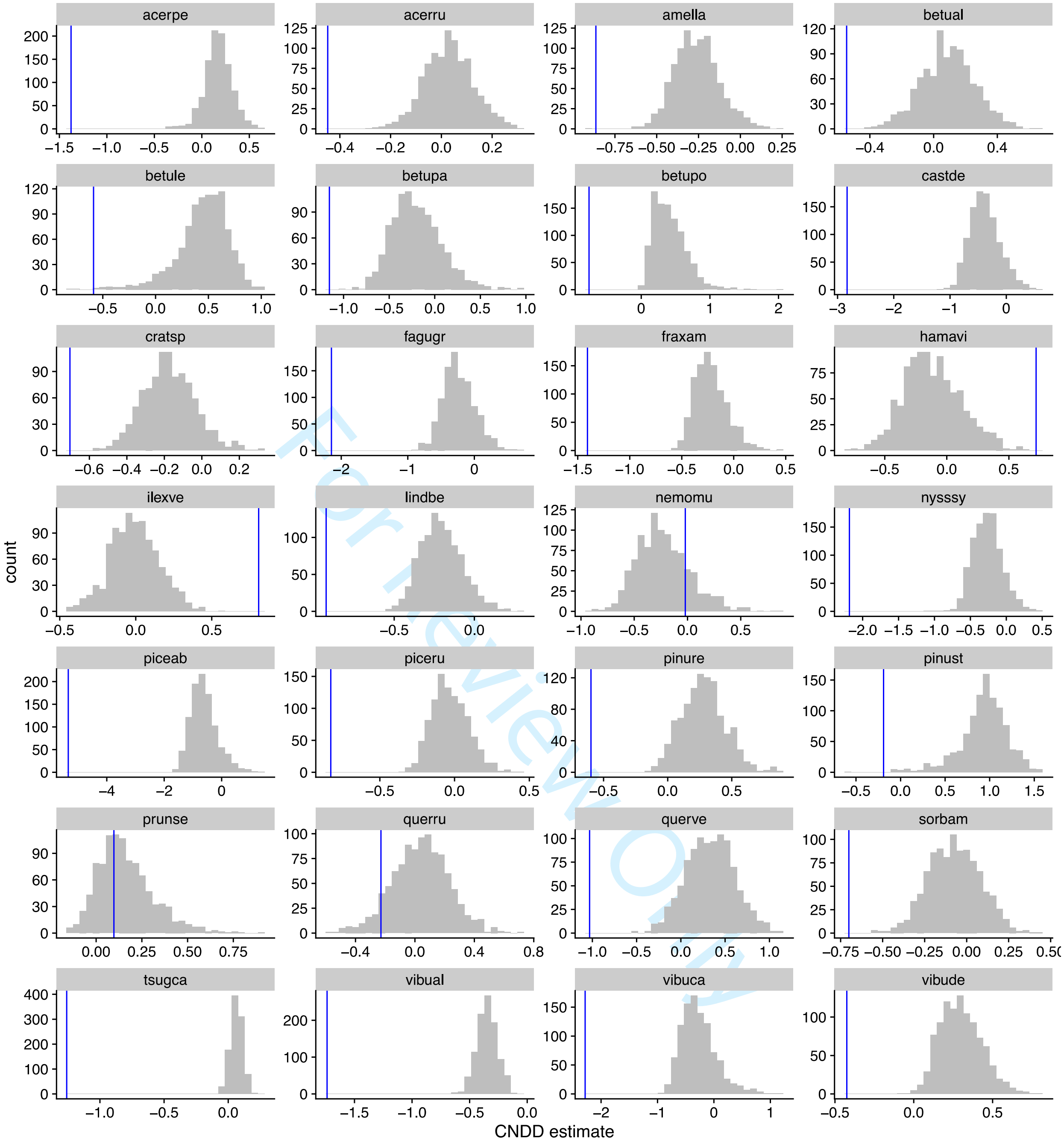


Figure S4. Estimates of CNDD based on observed data relative to the distribution of CNDD estimates from 1000 runs of a null model. Null model randomly redistributed the proportion of the neighborhood that was conspecific based on the observed distribution of conspecific neighborhood densities for that species but kept the total neighborhood density and the DBH of the sapling the same. Blue lines represent CNDD based on observed data, gray histograms represent distribution of 1000 runs of the null model. Species abbreviations are as in Table 1.

ECY21-1156: Revision 1

Running Head: CNDD in ECM trees

Correspondence to:
Fiona V. Jevon
Yale School of the Environment
Yale University
195 Prospect Street
New Haven, CT 06511, United States
fiona.jevon@yale.edu

Title (max 120 char):

Experimental and observational evidence of negative conspecific density dependence in temperate ectomycorrhizal trees

Authors: Fiona V. Jevon[1,2], Dayna De La Cruz[3], Joseph A. LaManna[4], Ashley K. Lang[5], David A. Orwig[6], Sydne Record[7], Paige V. Kouba[8], Matthew P. Ayres[1], Jaclyn Hatala Matthes[2]

Author affiliations:
[1]Department of Biological Sciences, Dartmouth College, Hanover NH 03755, United States
[2]Current affiliation: Yale School of the Environment, Yale University, New Haven, CT 06511, United States
[3]Department of Biological Sciences, Wellesley College, Wellesley, MA 02481, USA
[4]Department of Biological Sciences, Marquette University, Milwaukee, WI, 53201 USA
[5]Department of Biological Sciences, Indiana University, Bloomington, IN, 47405 USA
[6]Harvard Forest, Harvard University, Petersham, MA 01366, USA
[7]Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania 19010 USA
[8]Department of Plant Sciences, University of California, Davis, CA, 95616 USA

Open Research Statement:

Data are provided as private-for-peer review: all data and code can be found in the public repository at ~~https://data.mendeley.com/datasets/ws3cdn28n8/3 (3).~~https://data.mendeley.com/datasets/ws3cdn28n8/4 (3). The only exception is data associated the second seedling census: this dataset is still undergoing moderation but will be posted ~~along side~~alongside the first census at ~~https://harvardforest1.fas.harvard.edu/exist/apps/datasets/showData.html?id=HF253~~https://harvardforest1.fas.harvard.edu/exist/apps/datasets/showData.html?id=HF253 (4). However, code for all analyses described here can be found in the public repository at ~~https://data.mendeley.com/datasets/ws3cdn28n8/3~~https://data.mendeley.com/datasets/ws3cdn28n8/4 (6).

Abstract

Conspecific negative density dependence (CNDD) promotes tree species diversity by reducing recruitment near conspecific adults due to biotic feedbacks from herbivores, pathogens, or ~~competition~~competitors. While this process is well-described in tropical forests, tests of temperate tree species range from strong positive to strong negative density dependence. To explain this, several studies have suggested that tree species traits may help predict the strength and direction of density dependence: for example, ectomycorrhizal-associated tree species typically exhibit either positive or weaker negative ~~or positive~~ conspecific density dependence. More generally, the strength of ~~conspecific~~ density dependence may be predictably related to other species-specific ecological attributes such as shade tolerance, or the relative local abundance of a species. To test the strength of density dependence and whether it affects seedling community diversity in a temperate forest, we tracked the survival of seedlings of three ectomycorrhizal-associated species experimentally planted ~~1200 seedlings of four ectomycorrhizal tree species~~ beneath conspecific and heterospecific adults ~~and tracked their survival and changes to the diversity of~~on the ~~planted seedling community. We then~~Prospect Hill tract of the Harvard Forest, in Massachusetts, USA. Experimental seedling survival was always lower under conspecific adults, which increased seedling community diversity in one of six treatments. We compared these results to evidence of CNDD from observed sapling ~~mortality~~survival patterns of 28 species over approximately 8 years in ~~a~~an adjacent 35-hectare forest plot~~ in Massachusetts, USA.~~ . We ~~also~~ tested whether species-specific estimates of CNDD were associated with mycorrhizal ~~types~~association, shade tolerance, ~~or~~and local abundance ~~predicted~~. We found evidence of significant, negative conspecific density dependence~~.~~

~~Experimental seedling mortality was always higher under conspecific adults, which increased seedling community diversity in one of six treatments.~~ (CNDD) in 23 of 28 species, ~~naturally occurring sapling mortality increased with higher~~and positive conspecific ~~adult densities, indicating CNDD. All 13~~density dependence in two species. Contrary to our expectations, ectomycorrhizal-associated species generally exhibited ~~CNDD, while all 5 of the tree species that exhibited the opposite pattern (with greater sapling survival occurring at higher densities of conspecific adults) were trees species that associate with~~stronger (e.g. more negative) CNDD than arbuscular ~~mycorrhizae. Shade tolerance and local abundance were~~mycorrhizal- associated species. CNDD was also stronger in more shade tolerant species but was not associated with ~~CNDD~~local abundance. Conspecific adult trees often have a negative influence on seedling survival in temperate forests, particularly for tree species with certain traits. Here we found strong experimental and observational evidence that ectomycorrhizal-associating species consistently exhibit CNDD. Moreover, similarities in the relative strength of density dependence from experiments and observations of sapling mortality suggest a mechanistic link between negative effects of conspecific adults on seedling and sapling survival and local tree species distributions.

Keywords: conspecific negative density dependence, diversity, saplings, temperate tree seedlings, mycorrhizal fungi, ectomycorrhizae, shade tolerance

Introduction

Conspecific negative density dependence (CNDD), whereby population growth rates decline with increasing population density due to a negative feedback on recruitment or survival, is a critical mechanism that can support local tree diversity (Chesson 2000). One example of CNDD is known as the Janzen-Connell hypothesis. This hypothesis suggests that specialized enemies, such as herbivores or pathogens, reduce the recruitment of offspring nearby parent trees in highly diverse tropical forests (Janzen 1970~~,~~; Connell 1971). This process hinders the formation and preservation of monodominant stands, and the resulting spatial distribution of trees supports the coexistence of many species. It is broadly assumed that CNDD can operate as a stabilizing mechanism to support diverse communities; however, very few studies of density dependence have quantified the effects of CNDD on diversity. Theoretical work (Chesson 2000) and observational studies (~~Johnson et al. 2012,~~ LaManna et al. 2017; Johnson et al. 2012) have linked higher species diversity to stronger CNDD, but experimental evidence of this link remains rare (Bagchi et al. 2014; Levine and HilleRisLambers 2009~~, Bagchi et al. 2014~~). In particular, few studies show whether and how CNDD can produce greater diversity over ecological time.

Strong CNDD is pervasive in the tropics (~~Terborgh 2012,~~ Comita et al. 2014; Terborgh 2012), making it an attractive potential driver of latitudinal patterns of tree diversity. However, there is also increasing support for CNDD as a mechanism that influences tree communities in temperate forests ~~(McCarthy-Neumann and Kobe 2010, Johnson et al. 2012, 2014, Ramage et al. 2017).~~(Johnson et al. 2014, 2012; McCarthy-Neumann and Kobe 2010; Ramage et al. 2017; Jiang et al. 2020, 2021). While this work illustrates the potential for CNDD to drive population dynamics in temperate systems, there is wide variation in the strength of CNDD among tree species (~~Johnson et al. 2014,~~ Bennett et al. 2017; Johnson et al. 2014) and along environmental gradients (LaManna et al. 2016; Smith and Reynolds 2015~~, LaManna et al. 2016~~).

Plant functional traits provide one potential means for predicting differences in the strength of density dependence among species (Bennett et al. 2017~~,~~; Brown et al. 2019~~,~~; Jia et al.

2020). In particular, functional traits that confer resistance to attack from herbivores or pathogens, the primary drivers of CNDD, may be associated with the strength of density dependence. For tree species, the type of mycorrhizal association, either arbuscular~~-~~ (AM) or ectomycorrhizal~~-~~ (ECM), has been associated with patterns in CNDD. In previous studies, ectomycorrhizal-associated tree species tend to exhibit weaker or even positive density dependence (Bennett et al. 2017~~, Chen et al. 2019,~~; Jiang et al. 2020, 2021~~,~~; Chen et al. 2019; Qin et al. 2021), possibly due to the greater protection from soil pathogens that the fungi confer to their tree host (Corrales et al. 2016~~,~~; Bennett et al. 2017). Indeed, recent work suggests that while both AM and ECM fungal networks may partially counteract conspecific negative density dependent mortality, ECM fungi may be more effective than AM fungi at countering the mortality agents which typically drive CNDD patterns (Liang et al. 2021). However, in other studies the strength of CNDD was similar in AM and ECM associated tree species (Jia et al. 2020).

In addition to mycorrhizal association, shade tolerance has been associated with species-specific patterns in CNDD; shade tolerant tree species are less susceptible to microbial attack due to conservative life history strategies, suggesting that they should exhibit weaker CNDD (McCarthy-Neumann and Kobe 2008~~,~~; Song et al. 2021). However, shade tolerant species are also more likely to be infected by necrotrophic fungal pathogens, which kill their hosts and feed on the decaying tissue, while shade intolerant species are more likely to be infected by biotrophs, which feed on live tissue without killing their hosts (García-Guzmán and Heil 2014). Indeed, low light areas of the forest where shade tolerant seedlings are likely to be found typically contain higher pathogen loads (Augspurger and Kelly 1984). Therefore, ~~in spite of~~despite their conservative allocation strategy, shade tolerant species may be *more* affected by CNDD, if the pathogens driving density dependence are more abundant and virulent. Indeed, the few explicit tests of how shade tolerance relates to CNDD in temperate forests are conflicting: shade tolerant species may be more (Jia et al. 2020) or less (Brown et al. 2019) likely to exhibit CNDD than shade intolerant species within temperate forests.

Additionally, temperate species do not always follow the same patterns as tropical ~~ones~~species: in the tropics, rare species typically exhibit stronger CNDD, which helps maintain diverse communities with many rare species (Xu, Wang, and Yu 2015; Comita et al. 2010~~,~~; Mangan et al. 2010~~, Xu et al. 2015~~). In temperate forests, the pattern between CNDD and local abundance is less clear: while some studies show that rare species exhibit stronger negative density feedbacks (Johnson et al. 2012), others show the opposite pattern, with more abundant species exhibiting stronger CNDD ~~(Zhu et al. 2015).~~(K. Zhu et al. 2015; LaManna et al. 2016). Quantifying the strength of CNDD across multiple co-occurring species can help to detangle sources of variability in CNDD studies and is needed to generalize the importance of CNDD in temperate, as well as tropical, forests.

In this study we used a combination of experimental and observational approaches to ask how prevalent CNDD is in a temperate forest, with particular emphasis on whether ectomycorrhizal-associated species commonly exhibit CNDD. We used a seedling planting experiment to test whether the species identity (conspecific versus heterospecific) of neighboring mature trees influences seedling survival in ectomycorrhizal-associating tree species, and whether CNDD effects on ECM seedling mortality alter seedling community diversity. We then used the survival of naturally occurring saplings to quantify the strength of CNDD across ~~25~~28 co-occurring woody species. Finally, we asked whether mycorrhizal type, shade tolerance, and local abundance predict variation in the strength of CNDD among species.

~~Methods~~

Materials and methods:

Site Description

This study took place on the Prospect Hill tract of the Harvard Forest (HF) located in Petersham, Massachusetts. This forest is in the northern hardwood-hemlock-white-pine transition zone (42.530°'N, 72.190°'W, 300 m elevation above sea level). The mean annual temperature and precipitation are 7.1°C and 1066 mm, respectively. For the observational part of our study, we utilized the 35-hectare HF ForestGEO plot where every woody stem >1 cm diameter at breast height (DBH) has been identified to species, tagged, geolocated and its diameter was measured ~~(Orwig et al. 2015, Anderson-Teixeira et al. 2015).~~(Orwig et al. 2022). The experimental portion of our study took place in plots that are adjacent to the ForestGEO plot (Appendix ~~S1: Fig.~~1: Figure S1).

Experimental Methods

We selected ~~four~~three ectomycorrhizal species: *Pinus resinosa, Quercus rubra*~~,~~ and *Pinus strobus*~~, and *Picea abies*~~ which represent a range in other plant traits, including shade tolerance ~~from highly intolerant (*Pinus resinosa*; shade tolerance 1.89) to highly tolerant (*Picea abies*; shade tolerance 4.45)~~ (Niinemets and Valladares 2006). All ~~four~~three species ~~were~~are common at our study site, although the ~~populations~~population of ~~*Picea abies* and~~ *Pinus resinosa* were planted there in the early 1900s and now consist of both planted and naturally regenerated individuals. In the forest adjacent to the mapped ForestGEO plot, we located 30 experimental plots (Appendix 1: Figure S1). Each circular plot had a diameter of 20 m and was centered on a focal *Pinus resinosa, Quercus rubra*, or *Pinus strobus* with a DBH greater than 28cm such that there were ten plots centered on each of the three species. We chose locations where none of the other study species occurred: e.g., in a plot centered on a *Q. rubra* tree, there were no *Pinus resinosa* or *Pinus strobus*. ~~We purchased bare-root seedlings in May 2019 from~~We used these plots to plant seedlings of each of the three species beneath both conspecific and heterospecific “adult” trees (defined as trees with a DBH greater than 28 cm).

In addition to these three species, we also planted *Picea abies* seedlings. *Picea abies* are present at this site but not native to the area. Like *Pinus resinosa*, *Picea abies* was commonly planted in the early 1900s and now naturally regenerate at this site. We included this species in the seedling planting primarily to increase the diversity of our planted seedling communities. *Picea abies* was chosen as it was available from the same nursery as the three experimental species, was a similar size and age to the other three species, associates with the same type of mycorrhizal fungi, and is a common species at this study site (Table 1). We purchased bare-root seedlings in May 2019 which were grown outdoors at the New Hampshire State Nursery in Boscawen, NH. *Picea abies* and *Quercus rubra* seedlings were two years old at planting while *Pinus strobus* and *Pinus resinosa* were three years old.

~~In the forest adjacent to the mapped ForestGEO plot, we located 30 experimental plots (Appendix S1: Fig S1). Each circular plot had a diameter of 20 m and was centered on a focal *Pinus resinosa, Quercus rubra*, or *Pinus strobus* that was greater than 28 cm DBH, such that there were ten plots centered on each of the three species. We chose locations where none of the other study species occurred: e.g., in a plot centered on a *Q. rubra* tree, there were no *Pinus resinosa* or *Pinus strobus*. We did not center any plots on adult *Picea abies*, which are present at this site but not native to the area, but we included this species in the seedling planting as a common heterospecific to increase the diversity of our planted seedling communities.~~

Experimental planting

Within each plot, we constructed two subplots, 1 m x 1 m, into which the seedlings were planted. Subplots were located approximately 1 meter apart and within 2 meters of the base of the trunk of the focal tree~~.~~, and subplot type ("even" or "uneven") was randomly assigned. In each subplot, we planted 20 seedlings. To test whether CNDD promotes diversity, and whether the effects of CNDD on diversity were dependent on the initial diversity of the community, in one subplot ("Even"), we planted 5 individuals of each species. In the other ("Uneven"), we planted 11 individuals of the same species as the focal tree (conspecifics), and 3 of each of the heterospecific species. Thus, the two subplots had the same total number of seedlings, but the conspecific seedling was either at the same density as each of the heterospecifics, or at a much higher density, as would be more likely under natural regeneration conditions. We used a standard, randomized planting design such that the spatial arrangement of conspecific seedlings relative to heterospecific seedlings was consistent across all subplots of the same type (~~Appendix S1:~~Supplementary Fig ~~S2~~2). All 1200 seedlings were planted between May 31st and June 7th, 2019.

Before planting, each subplot was cleared of aboveground stems greater than 20cm in height with hand clippers, so that competition with herbaceous plants and ferns was minimized across all plots. Resprouts from clipped vegetation, primarily ferns, were rare and were re-clipped when they emerged. Next, leaf litter was removed and set aside. ~~A five-by-five~~A wooden ~~grid~~1m^2 frame with grid lines at 20cm intervals, creating a 5x5 grid, was then placed on the ground to serve as the planting guide~~.~~ (Appendix 1: Figure S2). Using an auger (7.6 cm diameter), we dug holes in 20 locations in each subplot. Removed soil was placed on a tarp and homogenized. The individually tagged seedlings were then planted and covered by the homogenized soil. Throughout the planting process, the roots of the seedlings were kept moist with water in spray bottles~~ and after planting all~~. After the 20 seedlings ~~in each~~were planted, the subplot was ~~completed, the area was ~~flagged, watered, and re-covered by leaf litter.

Within each 10-m radius plot, we also identified and measured the diameter of each tree > 2.5 cm DBH as well as the distance of each stem to the seedling subplots. To account for possible differences in light environment that could influence seedling survival, we took a hemispherical photo using a fisheye lens from the center of each plot to capture the light environment. Photographs were taken between 07:30 and 09:30 am to avoid overexposure. Images were analyzed with WINSCANOPY (Regent Instruments Inc., Quebec, Canada) to calculate the gap fraction (a metric of canopy openness) of each of the 30 plots.

Seedlings were tracked individually throughout the summer. After all seedlings had been in the ground for two weeks, seedlings were censused for survival and their initial heights were measured to account for any differences in survival that were due to variation in initial seedling size. Seedlings were censused again after an additional 10 weeks for their final survival status. Seedlings were presumed dead if their needles had all turned brown (conifers) and if they had no remaining leaves (*Q. rubra*). We continued to monitor all seedlings, regardless of status, for the full 12 weeks of the experiment.

Statistical analysis of experimental seedling mortality

We removed 17 individual seedlings from the analysis (1 *P. strobus*, 13 *Q. rubra* and 3 *P. resinosa*) that died within the first two weeks, presumably due to transplant shock rather than as a result of our experimental treatments. For each seedling species, we calculated the overall odds ratio of survival under conspecific and heterospecific focal adults, such that an odds ratio < 1 indicates a lower chance of survival beneath a conspecific adult. We then fit a binomial mixed model using the R package lme4 (Bates, Maechler, Bolker, & Walker, 2015) to predict seedling survival as a function of the adult species identity of the plot (based on whether the focal tree in the plot was a conspecific or heterospecific), initial seedling height, subplot type (even or uneven) and canopy gap fraction, with the plot as a random effect. For the *~~Picea~~P. abies* seedlings, we fit the same model without the adult identity variable as all seedlings were growing in heterospecific plots.

To account for possible effects of both the focal tree in each plot (our experimental treatment), and also the effects of neighboring trees, we used the full 314-m2 plot to calculate a neighborhood competition index (NCI) following ~~(~~Canham~~ et al. ~~, LePage, and Coates (2004) and modified as in other similar analyses (~~Bai et al. 2012, ~~Zhang et al. 2017~~,~~; Bai et al. 2012; Magee et al. 2020). For each plot, we calculated NCI values to compare the effects of conspecific and heterospecific adults within 10 meters as follows:

$$\mathrm{NCI}_{\mathrm{conspecific}} = \Sigma \frac{DBHconspecific}{distance} \quad \text{eq.1}$$

$$\mathrm{NCI}_{\mathrm{heterospecific}} = \Sigma \frac{DBHheterospecific}{distance} \qquad \text{eq. 2}$$

We then used the results of the first set of models (using the identity of the focal adult tree as our treatment) to inform which parameters to include in the second set (including the NCI as our treatment), including any parameter from the first model set with $p < 0.1$. We ran a second set of binomial mixed models that did not include adult identity, but did include $\mathrm{NCI}_{\mathrm{conspecific}}$, $\mathrm{NCI}_{\mathrm{heterospecific}}$, and any of the parameters identified from model set 1 with plot ID as a random variable.

Finally, we tested whether the observed seedling mortality affected the diversity of the seedling community. To do this, we calculated the overall survival of each seedling subplot. We then simulated random mortality at that level for each subplot, by randomly assigning a survival code to each seedling until the overall observed mortality of the plot was met. We ran this simulation 1000 times for each subplot. As all ~~of~~ our experimental plots were planted with the same species richness (4), we used the Shannon's Equitability Index (J) (Begon, Harper and Townsend 1996):

$$J = \frac{-\sum_{i=1}^{S} p_i * log(p_i)}{log(SR)} \qquad \text{eq. 3}$$

where p_i is the proportion of each species and SR is the species richness. We used this metric to assess whether the diversity we observed at the end of our experiment differed from what the diversity would be if mortality had occurred randomly with respect to species identity of the seedlings.

Observational sapling survival

To determine whether naturally regenerating saplings exhibited a similar pattern to those we experimentally planted, as well as to quantify density dependence in a wider variety of naturally occurring saplings, we used the subset of the Harvard Forest ForestGeo plot that has been re-censused as of 2021. The first census occurred from June 2010 through January 2014. The re-census occurred from May 2018 to September 2019. Individual trees were revisited~~,~~ and their survival status was recorded.

Using the initial census, we calculated the same metrics of neighborhood competition as for our experimental plots (eq.1 and eq. 2) for each individual stem in the forest. We used a maximum distance of 20 m. We only assessed patterns for species with more than 10 individual saplings and more than 20 individual adults. To account for differences in average size of each species as well as maximize the number of species included in our analysis, we defined individuals as saplings if their DBH was less than the median DBH of that species, up to a maximum cutoff of 12.7 cm DBH, and individuals greater than the median as "adults" (Table 1). To confirm that our results were not sensitive to the choice of methods, we re-ran the CNDD estimation using 15m maximum distance to neighbors and 25 m maximum distance to neighbors. ~~Similarly, we~~We also re-ran the CNDD estimation using ~~2~~an alternative ~~methods~~method to distinguish between saplings and adults: using a DBH cutoff of either 3cm, 8cm or 12.7cm for species with a median DBH less than 5cm, greater than 5 but less than 12.7cm, and greater than 12.7cm respectively, as a common concern with CNDD analyses is that the distinction can be arbitrary (Detto et al. 2019). Results of ~~both~~from these alternative analyses can be found in Appendix ~~S1: Fig~~1: Figure S3.

To calculate the overall effect of neighborhood on sapling mortality, we fit a generalized linear mixed-effects model (GLMM) with binomial errors and a complementary log–log link to assess the relative importance of factors determining individual sapling mortality using the R package lme4 (Bates et al. 2015). To account for differences in the length of time between the two censuses for individual trees, we used a log(time) offset of the number of years between the two censuses (range: 5.25 - 9.5 years) for each individual stem as in ~~(~~Johnson et al. (2017). The diameter at breast height (DBH) of the sapling at the first census was included as a covariate(Johnson et al. 2017). Both NCI$_{con}$ and NCI$_{het}$ were also included in the model to account for the overall effects of competition as well as the specific effects of conspecific neighbors. All three variables were scaled by subtracting the overall mean and dividing by two standard deviations (Gelman 2008). Species was included as a random effect, and both NCI$_{con}$ ~~was~~ and NCI$_{het}$ were estimated with random slopes for each species. The model coefficients for each species of NCI$_{con}$ and NCI$_{het}$ were used to estimate ~~CNDD: therefore, a more negative~~CNDD$_{coeff}$, such that CNDD$_{coeff}$ was the difference between the model coefficient ~~indicates a species exhibited stronger CNDD, while a positive coefficient indicates that greater~~

~~conspecific adult density was associated with a higher likelihood of survival.~~for NCI_{con} and NCI_{het}.

To further account for the possibility that ~~associations between NCI_{con} and sapling mortality~~our $CNDD_{coeff}$ estimate could be produced by underlying spatial or other factors not accounted for in this simple statistical model, we additionally performed null model testing (LaManna~~ et al.~~, Mangan, and Myers 2021). Using the same model structure as above, we ~~randomly reassigned~~recalculated values of NCI_{con} ~~among saplings~~and NCI_{het} for each sapling after randomizing the observed proportion of the total NCI that was conspecific: effectively keeping the total neighborhood density the same ~~species, while holding NCI_{het},~~as the observed, but randomizing the density of conspecific neighbors. We also kept the observed values of DBH, and the mortality outcome~~ the same~~. We re-ran this null model 1000 times~~ and used~~, and estimated $CNDD_{null}$ as the difference between the mean ~~null model~~ NCI_{con} and NCI_{het} coefficients ~~for NCI_{con}~~ using the same model structure as described above. We then calculated the final estimate of CNDD for each species ~~to correct~~($CNDD_{est}$) as follows

$$CNDD_{est} = -(CNDD_{coeff} - CNDD_{null}) \qquad \text{eq. 4}$$

where $CNDD_{coeff}$ is the estimate based on the difference between the model coefficients for ~~these patterns. First~~NCI_{con} and NCI_{het} using the observed data, and $CNDD_{null}$ is the estimate based on the difference between the model coefficients for NCI_{con} and NCI_{het} using the randomized NCI_{con}. The negative sign is to make the estimate more interpretable: as all the models were run as hazard functions, with survival coded as zero and mortality coded as one, taking the inverse of the estimate mreans that a negative $CNDD_{est}$ indicates a species exhibited stronger CNDD, while a positive $CNDD_{est}$ indicates that greater conspecific adult density was associated with a higher likelihood of survival. For each species, we took the mean across the 1000 iterations of the null model randomization, and we considered our estimate of CNDD significant if the 95% confidence interval of CNDD_{est} did not cross 0. We also confirmed that the mean null model coefficients were significantly different from the coefficients based on the observed values ~~using a simple linear model with tree species and model type (observed or null) as predictors and estimated CNDD coefficient as the response. We then calculated the final estimate of CNDD for each species (CNDD_{est}) is the initial model coefficient, minus the mean of the null model coefficients (eq. 4).~~ for each species using a paired t-test. Full visualization of the null model distribution for each species relative to CNDD_{obs} can be found in Appendix 1: Figure S4.

~~CNDDest = CNDDcoeff - mean(CNDDnull) eq. 4~~

Finally, we compared this species level estimate of CNDD at this site to species level plant traits. We assigned each species a dominant mycorrhizal association based on values from FungalRoot (Soudzilovskaia et al. 2020) and a shade tolerance value based on ~~(~~Niinemets and Valladares (2006). We then used a linear model to estimate the effects of mycorrhizal association, shade tolerance, and local abundance (log transformed) on CNDD_{est}. Because the species in this study varied dramatically in their typical and mature size, and because AM species were generally smaller (Table 1), we also included the median DBH of the species as a predictor in the model. To ensure that our results were robust to model structure, we also iteratively checked all possible two-way interactions of the four species level predictors (mycorrhizal association, shade tolerance, local abundance and median DBH); none were significant, so we report the results of the additive model. All analyses we performed in R version 4.0.5.

Results

Experimental seedling survival

All seedling species had higher survival rates when planted beneath heterospecific trees than when planted under conspecific trees (Figure 1~~). *Q.*~~, Table 2). *Quercus rubra*, which had the lowest overall mortality, also exhibited the least difference in survival (88.5% under conspecifics, 96.7% under heterospecifics, odds ratio: 0.91). *Pinus resinosa* had the highest overall mortality, with only 3.8% seedling survival beneath conspecifics and 15.4% beneath heterospecifics (odds ratio 0.25). *Pinus strobus* showed the greatest absolute difference in survival between conspecifics (73.9%) and heterospecifics (94.4~~%); though the relative difference in likelihood of survival was intermediate (odds ratio: 0.78)~~%) and the strongest effect of the identity of the neighboring adult tree (Table 2).

Results were qualitatively similar when comparing the density of adult trees in the surrounding community, as measured by NCI, to seedling survival~~ (Table 3). Both *P. strobus* and *P. resinosa*~~. All four species exhibited declining survival with increasing conspecific adult density (NCI_{con}), ~~whereas *P. abies*~~but all but *Q. rubra* also exhibited declining survival ~~declined significantly ~~in response to increasing heterospecific density (NCI_{het}~~). *Q. rubra* survival was not affected by~~) as well (Table 3). When comparing the ~~density~~NCI_{con} coefficient estimates and standard errors with those of ~~either~~NCI_{het}, only *P. strobus* exhibited CNDD: in other words, the coefficient estimate for the negative effect of conspecific ~~or~~neighbors did not overlap with the weaker negative effect of heterospecific ~~adult trees.~~neighbors (Table 3).

None of the seedling species exhibited survival differences between the even and unevenly planted plots (Table 2). In the evenly planted seedling subplots, which began the experiment with perfectly even communities (J=1), observed mortality caused a decline in seedling diversity; however, this decline in diversity was indistinguishable from simulated random mortality (Figure 2a). The seedling diversity in the unevenly planted subplots either stayed the same or, in the case of the unevenly planted subplots beneath *P. resinosa* adults, substantially increased (Figure 3b). In those plots, the seedling community had changed from an equitability index (J) of 0.85 (uneven) to 0.98 at the end of the experiment- almost perfectly even, and far higher than the diversity predicted if the mortality had been random. These locations experienced the highest total mortality (59.7%), and also the highest difference between conspecific seedling mortality (96%) and heterospecific seedling mortality (12.6%). Experimental seedling survival was also affected by factors other than the local neighborhood. For example, *Pinus strobus* and *Pinus resinosa* seedlings that were initially taller had slightly lower survival. *Picea abies* was the only species whose survival increased with increasing light availability as measured by the gap fraction (Table 2).

Observed sapling ~~mortality~~survival

Overall, the probability of sapling ~~mortality~~survival in our observational CNDD analysis ~~increased~~decreased with increasing local density of conspecifics (Table 4). Sapling survival was also positively associated with sapling DBH and negatively associated with local density of heterospecifics; however, the effect of these drivers was considerably lower than the effect of conspecific neighbors (Table 4). The strength of CNDD varied substantially among species. In twenty- three of the twenty-~~five~~ eight species used in our analysis, sapling ~~mortality increased~~survival decreased significantly with increasing conspecific density, whereas in ~~five~~two species sapling survival increased with increasing conspecific density (Figure 3).

Tree species that typically associate with ECM fungi were much more likely to exhibit CNDD than those that typically associate with AM fungi (Figure 3B, Table 5); ~~all~~12 of the 13 ECM-associated species in this analysis exhibited significant CNDD. In contrast, ~~5~~4 of the 15 AM-associated species either did not exhibit significant CNDD ~~and~~or instead were more likely to survive when growing in areas with greater densities of conspecifics~~.~~ (e.g. had significantly positive estimates of CNDD). AM-associated saplings also had a somewhat lower overall survival (61%) than ECM-associated saplings (72%). Although there are a similar number of AM and ECM associating species at this site, ECM species tend to have higher abundance and overall about 66% of saplings are ECM-associating species. In addition, the AM-associating species at this site tend to be smaller (Table 1). However, the median DBH of the species was not associated with stronger CNDD (Table 5). ~~Neither shade tolerance nor local abundance were significant predictors of CNDD at the species level (Figure 4), although the effect of shade tolerance was marginally significant (Table 5).~~

Shade tolerance was also a significant predictor of CNDD at the species level, such that more shade tolerant species generally exhibited stronger, more negative CNDD than shade intolerant speceis (Figure 4). In contrast, local abundance was not a significant predictor of the strength of CNDD (Figure 4).

CNDD estimated in naturally occurring saplings generally aligned with the ~~CNDD estimated by odds ratios~~results from the seedling experiment. *Quercus rubra*, which showed ~~significant but weak CNDD~~the weakest response to the nearby conspecific adults (Table 2) and no response to neighborhood conspecific density (Table 3) in the seedling experiment~~ (odds ratio of 0.91), also ~~, showed ~~relatively weak~~no significant evidence of CNDD at the sapling stage (Figure 3). ~~*Pinus resinosa*, which ~~*Pinus strobus*, which exhibited the strongest response to nearby conspecific adults (Table 2) and neighborhood conspecific densities (Table 3) in the experimental data, showed strong evidence of CNDD in the ~~seedling experiment (odds ratio of 0.25), was among the species exhibiting the strongest CNDD patterns in the ~~analysis of observed sapling survival (Figure 3). *Pinus* ~~*strobus* was~~*resinosa*, which exhibited intermediate CNDD in

~~both~~ the seedling experiment ~~and observational~~, was also intermediate in the observed sapling analysis.

Discussion

CNDD and mycorrhizal associations

The results of both our field experiment and our analysis of sapling survival indicate that ~~young tree~~sapling mortality is higher beneath conspecific adults in this system. In particular, the agreement between these two separate analyses for the three tree species common to the two studies provides strong evidence of CNDD in this temperate forest, particularly in ectomycorrhizal trees. Indeed, the apparent ubiquity of CNDD among ectomycorrhizal species in this forest came as a surprise, as previous work has found weaker or positive patterns of density dependence among ECM associating trees (Bennett et al. 2017~~, Brown et al. 2019,~~; Jiang et al. 2020, 2021; Brown et al. 2019). Notably, however, several of these studies ~~have relied on~~found these patterns primarily in plant growth rates rather than in survival (Bennett et al. 2017~~,~~; Brown et al. 2019). Given that there can be intraspecific tradeoffs between survival and growth rates (Seiwa 2007), it is possible that CNDD estimates based on growth and survival may differ within a species~~.~~ (Brown et al, 2019). In addition, the details of the species included in this study may differ from those in other locations. At this site, while there is a similar number of species that associate with AM and ECM fungi, ECM-associating trees are much more abundant. Thus, the availability of mutualists for AM-associating species is likely lower and/or patchier, and recent evidence shows that AM colonization is greater on plants that grow in stands where other AM-associating plants are more abundant (Grünfeld et al. 2020). This could have the result that regeneration near to conspecifics comes with greater access to appropriate mutualists, which could dampen the negative effects typically associated with proximity to conspecific adults for

AM-associating species (Liang et al. 2021). In contrast, mutualist availability is likely ubiquitous for ECM-associating species, as ECM species are well distributed at this site. Finally, estimates of species level CNDD in one context may not generalize, as there is increasing evidence that the strength of CNDD can change due to variation in the presence of large mammals (Murphy and Comita 2021), climate (Liu and He 2021), and along environmental gradients (Brown, White, and Peet 2021; LaManna et al. 2016~~,~~; Magee et al. 2020; Record et al. 2016~~, Magee et al. 2020, Brown et al. 2021~~).

CNDD and shade tolerance

We also found evidence that shade tolerance predicted the strength of CNDD: shade tolerant species tended to show somewhat greater CNDD, although the effect was strongly driven by a single species (*Picea abies*). This is consistent with some (Jia et al. 2020; García-Guzmán and Heil 2014) but not other (Brown et al. 2019) studies on shade tolerance and CNDD. This discrepancy may be because there are conflicting pressures on trees which result in no clear net effect. Shade tolerant species typically have more conservative life history strategies, which might make them less sensitive to CNDD (McCarthy-Neumann and Kobe 2008; Song et al. 2021). However, shade intolerant species are less likely to be limited by conspecific-associating fungal pathogens, and more likely to be limited by herbivorous insects or pathogens with weaker effects on mortality, perhaps making them less likely to exhibit CNDD (Jia et al. 2020; García-Guzmán and Heil 2014). Taken together with results from this study, it seems that shade tolerance may not have a consistent effect on strength of CNDD in temperate forests.

CNDD and other species characteristics

Local abundance is a strong driver of CNDD in tropical trees, where rare species typically exhibit the strongest CNDD (Comita et al. 2010). In temperate forests, however, the results have been mixed, with some analyses indicating that rare species exhibit stronger CNDD (Johnson et al. 2012~~,~~; Seiwa et al. 2019) and others finding that more abundant species exhibit stronger CNDD (K. Zhu et al. 2015). Indeed the effect of local abundance may also interact with other characteristics. For example, rare AM species typically exhibit stronger CNDD than common AM species, but the same may not hold true for ECM species (Jiang et al. 2020, 2021). In this study we found that local abundance had no apparent effect on the strength of CNDD, overall or interacting with mycorrhizal type. This may be important for understanding the effects of CNDD on diversity in this forest: theoretical work suggests that if variation among species in CNDD is correlated with abundance, with stronger CNDD for rare species, then diversity may not be maintained. However, if CNDD is unrelated to abundance, as in our study here, or even stronger for common species, then CNDD may help promote the maintenance of diversity (Stump and Comita 2018).

~~*CNDD and other species characteristics*~~

~~We also found little evidence that shade tolerance predicted the strength of CNDD: shade tolerant species tended to show somewhat greater CNDD, but the effect was marginally significant and seemingly driven by a single species (*Picea abies*). This may be because there are conflicting pressures on trees which result in no clear net effect. Shade tolerant species typically have more conservative life history strategies, which might make them less sensitive to CNDD (McCarthy-Neumann and Kobe 2008, Song et al. 2021). However, shade intolerant species are less likely to be limited by conspecific-associating fungal pathogens, and more likely to be limited by herbivorous insects or pathogens with weaker effects on mortality, perhaps making them less likely to exhibit CNDD (García-Guzmán and Heil 2014, Jia et al. 2020). Even among temperate species, there is conflicting evidence for the relationship between shade tolerance and~~

~~CNDD. For example, CNDD effects on survival are greater in shade intolerant species, but CNDD effects on growth are greater in shade tolerant species (Brown et al. 2019). Taken together with results from this study, it seems that shade tolerance does not have a consistent effect on strength of CNDD in temperate trees.~~

Additional plant characteristics may also influence species susceptibility to CNDD. For example, species with larger seeds may be more tolerant to conspecific neighbors as their seeds act as carbohydrate stores, insuring them against biomass losses to herbivores and pathogens (Lebrija-Trejos et al. 2016~~,~~; Seiwa et al. 2019)~~. This may help explain why the large-seeded *Q. rubra* exhibited relatively weak CNDD in both the experiment and the observational study.~~ . This may help explain why the large-seeded *Q. rubra* exhibited either weak or no CNDD in both the experiment and the observational study; however, the effect of cotyledon reserves is likely larger at younger ages. Notably, naturally occurring *Q. rubra* seedlings at this site do exhibit CNDD, but the effect decreases as the size of the seedling increases (Jevon et al. 2020), which is consistent with what we found here (that the evidence of CNDD was stronger in the experiment, which assessed much smaller individuals) The apparent weakening of CNDD in this species as individuals increase in size in also consistent with previous work suggesting that CNDD is often strongest at the seed to seedling transition or at younger age classes (Zhu et al. 2015). We note that as we assessed large seedlings (2 and 3 years old) in the experiment, and saplings (up to 12.7cm DBH) in the observational analysis, the overall results of this study are likely conservative with respect to how many species exhibit CNDD and the apparent strength of the effect relative to the true strength of CNDD that is experienced by trees in this forest.

CNDD and diversity

The change in diversity in one out of ~~the~~ six of our experimental treatments also illustrates how CNDD mortality can affect community diversity (Figure 3). Importantly, it illustrates that alone, differences in mortality in seedlings growing near conspecific and heterospecific adults is not enough to generate greater diversity. The overall mortality must also be relatively high, and the initial diversity low, to result in meaningful changes to seedling community diversity. ~~However,~~This is consistent with conceptual models suggesting that overall differences in mortality rates among life stages, alone or in combination with non-random mortality, can be an important determinant of community diversity (Green, Harms, and Connell 2014). The significant increase in diversity in even one treatment during this short-term experiment provides clear evidence that CNDD can act as a mechanism to support local diversity, particularly in systems or situations with high mortality.

Conclusions

Based on these patterns, we suggest that there is strong evidence for CNDD in temperate tree species. Our results also suggest that tree species associated with ectomycorrhizal fungi exhibit CNDD, which runs counter to previous studies (Bennett et al. 2017~~, Brown et al. 2019,~~; Jiang et al. 2020, 2021; Brown et al. 2019). This suggests caution when generalizing about how plant traits predict CNDD. Instead, integrating information about multiple plant characteristics, as well as the environmental context, will help to better predict species-level patterns in CNDD. We found experimentally that CNDD is capable of increasing seedling community diversity. However, theoretical work suggests that, in some cases, interspecific variation in the strength of CNDD decreases its ability to promote coexistence (Stump and Comita 2018), and results from this study and others show large variation in the strength of CNDD among co-occurring species.

Therefore, although evidence of CNDD in temperate forests is accumulating, the consequences for diversity remain poorly understood.

Acknowledgements

The authors thank our funding sources, including the Smithsonian Institute and CTFS ForestGEO for supporting the mapped plot, the National Science Foundation's LTER program (DEB 06-20443, DEB 12-37491, DEB 18-32210), Harvard University, the Harvard Forest REU program (National Science Foundation DBI-1459519 & DBI-1950364 to Record), the National Science Foundation (award number 1638406 to Matthes and DEB-2024903 to LaManna), the National Aeronautic and Space Administration (award number 20-BIODIV20-0024 to Record), and the New England Botanical Club Graduate Student Research Award. This work would not have been possible without the 22 technicians who collected the adult tree census data in the CTFS-ForestGEO plot. The authors would also like to thank Manisha Patel and everyone involved in the Harvard Forest REU program for supporting this project.

~~Open Research Statement~~

~~All code for the analysis and experimental data is available on Mendeley Data (DOI: 10.17632/ws3cdn28n8.1). Census data is available on the Harvard Forest data archive https://harvardforest1.fas.harvard.edu/exist/apps/datasets/showData.html?id=HF253.~~

References

~~Anderson-Teixeira, K. J., S. J. Davies, A. C. Bennett, E. B. Gonzalez-Akre, H. C. Muller-Landau, S. J. Wright, K. Abu Salim, A. M. Almeyda Zambrano, A. Alonso, J. L. Baltzer, Y. Basset, N. A. Bourg, E. N. Broadbent, W. Y. Brockelman, S. Bunyavejchewin, D. F. R. P.~~

~~Burslem, N. Butt, M. Cao, D. Cardenas, G. B. Chuyong, K. Clay, S. Cordell, H. S. Dattaraja, X. Deng, M. Detto, X. Du, A. Duque, D. L. Erikson, C. E. N. Ewango, G. A. Fischer, C. Fletcher, R. B. Foster, C. P. Giardina, G. S. Gilbert, N. Gunatilleke, S. Gunatilleke, Z. Hao, W. W. Hargrove, T. B. Hart, B. C. H. Hau, F. He, F. M. Hoffman, R. W. Howe, S. P. Hubbell, F. M. Inman-Narahari, P. A. Jansen, M. Jiang, D. J. Johnson, M. Kanzaki, A. R. Kassim, D. Kenfack, S. Kibet, M. F. Kinnaird, L. Korte, K. Kral, J. Kumar, A. J. Larson, Y. Li, X. Li, S. Liu, S. K. Y. Lum, J. A. Lutz, K. Ma, D. M. Maddalena, J.-R. Makana, Y. Malhi, T. Marthews, R. Mat Serudin, S. M. McMahon, W. J. McShea, H. R. Memiaghe, X. Mi, T. Mizuno, M. Morecroft, J. A. Myers, V. Novotny, A. A. de Oliveira, P. S. Ong, D. A. Orwig, R. Ostertag, J. den Ouden, G. G. Parker, R. P. Phillips, L. Sack, M. N. Sainge, W. Sang, K. Sri-Ngernyuang, R. Sukumar, I.-F. Sun, W. Sungpalee, H. S. Suresh, S. Tan, S. C. Thomas, D. W. Thomas, J. Thompson, B. L. Turner, M. Uriarte, R. Valencia, M. I. Vallejo, A. Vicentini, T. Vrška, X. Wang, X. Wang, G. Weiblen, A. Wolf, H. Xu, S. Yap, and J. Zimmerman. 2015. CTFS-ForestGEO: a worldwide network monitoring forests in an era of global change. Global Change Biology 21:528–549.~~

Augspurger, Carol K., and Colleen K. Kelly. 1984. “Pathogen Mortality of Tropical Tree Seedlings: Experimental Studies of the Effects of Dispersal Distance, Seedling Density, and Light Conditions.” ~~Augspurger, C. K., and C. K. Kelly. 1984. Pathogen mortality of tropical tree seedlings: experimental studies of the effects of dispersal distance, seedling density, and light conditions. Oecologia 61:211–217.~~*Oecologia*

~~Bagchi, R., R. E. Gallery, S. Gripenberg, S. J. Gurr, L. Narayan, C. E. Addis, R. P. Freckleton, and O. T. Lewis. 2014. Pathogens and insect herbivores drive rainforest plant diversity and composition. Nature 506:85–88.~~

~~Bai, X., S. A. Queenborough, X. Wang, J. Zhang, B. Li, Z. Yuan, D. Xing, F. Lin, J. Ye, and Z. Hao. 2012. Effects of local biotic neighbors and habitat heterogeneity on tree and shrub seedling survival in an old-growth temperate forest. Oecologia 170:755–765.~~

~~Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67:1–48.~~

~~Bennett, J. A., H. Maherali, K. O. Reinhart, Y. Lekberg, M. M. Hart, and J. Klironomos. 2017. Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. Science 355:181–184.~~

~~Brown, A. J., C. J. Payne, P. S. White, and R. K. Peet. 2019. Shade tolerance and mycorrhizal type may influence sapling susceptibility to conspecific negative density dependence. The Journal of Ecology 4:103.~~

~~Brown, A. J., P. S. White, and R. K. Peet. 2021. Environmental context alters the magnitude of conspecific negative density dependence in a temperate forest. Ecosphere 12.~~

~~Canham, C. D., P. T. LePage, and K. D. Coates. 2004. A neighborhood analysis of canopy tree competition: effects of shading versus crowding. Canadian Journal of Forest Research. Journal Canadien de La Recherche Forestiere 34:778–787.~~

~~Chen, L., N. G. Swenson, N. Ji, X. Mi, H. Ren, L. Guo, and K. Ma. 2019. Differential soil fungus accumulation and density dependence of trees in a subtropical forest. Science 366:124–128.~~

~~Chesson, P. 2000. Mechanisms of Maintenance of Species Diversity.~~

~~Comita, L. S., H. C. Muller-Landau, S. Aguilar, and S. P. Hubbell. 2010. Asymmetric density dependence shapes species abundances in a tropical tree community. Science 329:330–332.~~

~~Comita, L. S., S. A. Queenborough, S. J. Murphy, J. L. Eck, K. Xu, M. Krishnadas, N. Beckman,~~

~~and Y. Zhu. 2014. Testing predictions of the Janzen–Connell hypothesis: a meta-analysis of experimental evidence for distance-and density-dependent seed and seedling survival. The Journal of Ecology 102:845–856.~~

~~Connell, J. H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forests. Pages 298–312 *in* P. J. Den Boer G. R. Gradwell, editor. Dynamics of Populations. Center for Agricultural Publishing and Documentation.~~

~~Corrales, A., S. A. Mangan, B. L. Turner, and J. W. Dalling. 2016. An ectomycorrhizal nitrogen economy facilitates monodominance in a neotropical forest. Ecology Letters 19:383–392.~~

~~Detto, M., M. D. Visser, S. J. Wright, and S. W. Pacala. 2019. Bias in the detection of negative density dependence in plant communities. Ecology Letters 22:1923–1939.~~

~~García-Guzmán, G., and M. Heil. 2014. Life histories of hosts and pathogens predict patterns in tropical fungal plant diseases. The New Phytologist 201:1106–1120.~~

~~Gelman, A. 2008. Scaling regression inputs by dividing by two standard deviations. Statistics in Medicine 27:2865–2873.~~

~~Grünfeld, L., M. Wulf, M. C. Rillig, A. Manntschke, and S. D. Veresoglou. 2020. Neighbours of arbuscular-mycorrhiza associating trees are colonized more extensively by arbuscular mycorrhizal fungi than their conspecifics in ectomycorrhiza dominated stands. The New Phytologist 227:10–13.~~

~~Janzen, D. H. 1970. Herbivores and the Number of Tree Species in Tropical Forests. The American Naturalist 104:501–528.~~

~~Jiang, F., J. A. Lutz, Q. Guo, Z. Hao, X. Wang, G. S. Gilbert, Z. Mao, D. A. Orwig, G. G. Parker, W. Sang, Y. Liu, S. Tian, M. W. Cadotte, and G. Jin. 2021. Mycorrhizal type influences plant density dependence and species richness across 15 temperate forests.~~

~~Ecology 102:e03259.~~

~~Jiang, F., K. Zhu, M. W. Cadotte, and G. Jin. 2020. Tree mycorrhizal type mediates the strength of negative density dependence in temperate forests. The Journal of Ecology.~~

~~Jia, S., X. Wang, Z. Yuan, F. Lin, J. Ye, G. Lin, Z. Hao, and R. Bagchi. 2020. Tree species traits affect which natural enemies drive the Janzen-Connell effect in a temperate forest. Nature Communications 11:286.~~

~~Johnson, D. J., W. T. Beaulieu, J. D. Bever, and K. Clay. 2012. Conspecific negative density dependence and forest diversity. Science 336:904–907.~~

~~Johnson, D. J., N. A. Bourg, R. Howe, W. J. McShea, A. Wolf, and K. Clay. 2014. Conspecific negative density-dependent mortality and the structure of temperate forests. Ecology 95:2493–2503.~~

~~Johnson, D. J., R. Condit, S. P. Hubbell, and L. S. Comita. 2017. Abiotic niche partitioning and negative density dependence drive tree seedling survival in a tropical forest. Proceedings of the Royal Society B: Biological Sciences 284:20172210.~~

~~LaManna, J. A., S. A. Mangan, A. Alonso, N. A. Bourg, W. Y. Brockelman, S. Bunyavejchewin, L.-W. Chang, J.-M. Chiang, G. B. Chuyong, K. Clay, R. Condit, S. Cordell, S. J. Davies, T. J. Furniss, C. P. Giardina, I. A. U. N. Gunatilleke, C. V. S. Gunatilleke, F. He, R. W. Howe, S. P. Hubbell, C.-F. Hsieh, F. M. Inman-Narahari, D. Janík, D. J. Johnson, D. Kenfack, L. Korte, K. Král, A. J. Larson, J. A. Lutz, S. M. McMahon, W. J. McShea, H. R. Memiaghe, A. Nathalang, V. Novotny, P. S. Ong, D. A. Orwig, R. Ostertag, G. G. Parker, R. P. Phillips, L. Sack, I.-F. Sun, J. S. Tello, D. W. Thomas, B. L. Turner, D. M. Vela Díaz, T. Vrška, G. D. Weiblen, A. Wolf, S. Yap, and J. A. Myers. 2017. Plant diversity increases with the strength of negative density dependence at the global scale. Science 356:1389–~~

~~1392.~~

~~LaManna, J. A., S. A. Mangan, and J. A. Myers. 2021. Conspecific negative density dependence and why its study should not be abandoned. Ecosphere 12.~~

~~LaManna, J. A., M. L. Walton, B. L. Turner, and J. A. Myers. 2016. Negative density dependence is stronger in resource-rich environments and diversifies communities when stronger for common but not rare species. Ecology Letters 19:657–667.~~

~~Lebrija-Trejos, E., P. B. Reich, A. Hernández, and S. J. Wright. 2016. Species with greater seed mass are more tolerant of conspecific neighbours: a key driver of early survival and future abundances in a tropical forest. Ecology Letters 19:1071–1080.~~

~~Levine, J. M., and J. HilleRisLambers. 2009. The importance of niches for the maintenance of species diversity. Nature 461:254–257.~~

~~Liang, M., L. Shi, D. F. R. P. Burslem, D. Johnson, M. Fang, X. Zhang, and S. Yu. 2021. Soil fungal networks moderate density-dependent survival and growth of seedlings. The New Phytologist.~~

~~Liu, Y., and F. He. 2021. Warming intensifies soil pathogen negative feedback on a temperate tree. The New Phytologist.~~

~~Magee, L., A. Wolf, R. Howe, J. Schubbe, K. Hagenow, and B. Turner. 2020. Density dependence and habitat heterogeneity regulate seedling survival in a North American temperate forest. Forest Ecology and Management:118722.~~

~~Mangan, S. A., S. A. Schnitzer, E. A. Herre, K. M. L. Mack, M. C. Valencia, E. I. Sanchez, and J. D. Bever. 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. Nature 466:752–755.~~

~~McCarthy-Neumann, S., and R. K. Kobe. 2008. Tolerance of soil pathogens co-varies with shade~~

~~tolerance across species of tropical tree seedlings. Ecology 89:1883–1892.~~

~~McCarthy-Neumann, S., and R. K. Kobe. 2010. Conspecific and heterospecific plant-soil feedbacks influence survivorship and growth of temperate tree seedlings. The Journal of Ecology 98:408–418.~~

~~Murphy, S. J., and L. S. Comita. 2021. Large mammalian herbivores contribute to conspecific negative density dependence in a temperate forest. The Journal of Ecology 109:1194–1209.~~

~~Niinemets, Ü., and F. Valladares. 2006. Tolerance to shade, drought, and waterlogging of temperate northern hemisphere trees and shrubs. Ecological Monographs 76:521–547.~~

~~Orwig, D., D. Foster, and A. Ellison. 2015. Harvard Forest CTFS-ForestGEO Mapped Forest Plot since 2014. Harvard Forest Data Archive: HF253.~~

~~Qin, J., Y. Geng, X. Li, C. Zhang, X. Zhao, and K. von Gadow. 2021. Mycorrhizal type and soil pathogenic fungi mediate tree survival and density dependence in a temperate forest. Forest Ecology and Management 496:119459.~~

~~Ramage, B. S., D. J. Johnson, E. Gonzalez-Akre, W. J. McShea, K. J. Anderson-Teixeira, N. A. Bourg, and K. Clay. 2017. Sapling growth rates reveal conspecific negative density dependence in a temperate forest. Ecology and Evolution 7:7661–7671.~~

~~Record, S., R. K. Kobe, C. F. Vriesendorp, and A. O. Finley. 2016. Seedling survival responses to conspecific density, soil nutrients, and irradiance vary with age in a tropical forest. Ecology 97:2406–2415.~~

~~Seiwa, K. 2007. Trade-offs between seedling growth and survival in deciduous broadleaved trees in a temperate forest. Annals of Botany 99:537–544.~~

~~Seiwa, K., K. Masaka, M. Konno, and S. Iwamoto. 2019. Role of seed size and relative abundance in conspecific negative distance-dependent seedling mortality for eight tree~~

~~species in a temperate forest 453.~~

~~Smith, L. M., and H. L. Reynolds. 2015. Plant–soil feedbacks shift from negative to positive with decreasing light in forest understory species. Ecology 96:2523–2532.~~

~~Song, X., J. Yang, M. Cao, L. Lin, Z. Sun, H. Wen, and N. G. Swenson. 2021. Traits mediate a trade-off in seedling growth response to light and conspecific density in a diverse subtropical forest. The Journal of Ecology 109:703–713.~~

~~Soudzilovskaia, N. A., S. Vaessen, M. Barcelo, J. He, S. Rahimlou, K. Abarenkov, M. C. Brundrett, S. I. F. Gomes, V. Merckx, and L. Tedersoo. 2020. FungalRoot: global online database of plant mycorrhizal associations. The New Phytologist 227:955–966.~~

~~Stump, S. M., and L. S. Comita. 2018. Interspecific variation in conspecific negative density dependence can make species less likely to coexist. Ecology Letters 21:1541–1551.~~

~~Terborgh, J. 2012. Enemies maintain hyperdiverse tropical forests. The American Naturalist 179:303–314.~~

~~Xu, M., Y. Wang, and S. Yu. 2015, December. Conspecific negative density dependence decreases with increasing species abundance.~~

~~Zhang, Z., M. J. Papaik, X. Wang, Z. Hao, J. Ye, F. Lin, and Z. Yuan. 2017. The effect of tree size, neighborhood competition and environment on tree growth in an old-growth temperate forest. Journal of Plant Ecology 10:970–980.~~

~~Zhu, K., C. W. Woodall, J. V. D. Monteiro, and J. S. Clark. 2015. Prevalence and strength of density-dependent tree recruitment. Ecology 96:2319–2327.~~

61 (2): 211–17.

Bagchi, Robert, Rachel E. Gallery, Sofia Gripenberg, Sarah J. Gurr, Lakshmi Narayan, Claire E. Addis, Robert P. Freckleton, and Owen T. Lewis. 2014. "Pathogens and Insect Herbivores Drive Rainforest Plant Diversity and Composition." *Nature* 506 (7486): 85–88.

Bai, Xuejiao, Simon A. Queenborough, Xugao Wang, Jian Zhang, Buhang Li, Zuoqiang Yuan, Dingliang Xing, Fei Lin, Ji Ye, and Zhanqing Hao. 2012. "Effects of Local Biotic Neighbors and Habitat Heterogeneity on Tree and Shrub Seedling Survival in an Old-Growth Temperate Forest." *Oecologia* 170 (3): 755–65.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. "Fitting Linear Mixed-Effects Models Using lme4." *Journal of Statistical Software* 67 (1): 1–48.

Bennett, Jonathan A., Hafiz Maherali, Kurt O. Reinhart, Ylva Lekberg, Miranda M. Hart, and John Klironomos. 2017. "Plant-Soil Feedbacks and Mycorrhizal Type Influence Temperate Forest Population Dynamics." *Science* 355 (6321): 181–84.

Brown, Alissa J., Christopher J. Payne, Peter S. White, and Robert K. Peet. 2019. "Shade Tolerance and Mycorrhizal Type May Influence Sapling Susceptibility to Conspecific Negative Density Dependence." Edited by Pierre Mariotte. *The Journal of Ecology* 4 (July): 103.

Brown, Alissa J., Peter S. White, and Robert K. Peet. 2021. "Environmental Context Alters the Magnitude of Conspecific Negative Density Dependence in a Temperate Forest." *Ecosphere* 12 (3). https://doi.org/10.1002/ecs2.3406.

Canham, Charles D., Philip T. LePage, and K. Dave Coates. 2004. "A Neighborhood Analysis of Canopy Tree Competition: Effects of Shading versus Crowding." *Canadian Journal of Forest Research. Journal Canadien de La Recherche Forestiere* 34 (4): 778–87.

Chen, Lei, Nathan G. Swenson, Niuniu Ji, Xiangcheng Mi, Haibao Ren, Liangdong Guo, and Keping Ma. 2019. "Differential Soil Fungus Accumulation and Density Dependence of Trees in a Subtropical Forest." *Science* 366 (6461): 124–28.

Chesson, Peter. 2000. "Mechanisms of Maintenance of Species Diversity." *Annual Review of Ecology and Systematics*. https://doi.org/10.1146/annurev.ecolsys.31.1.343.

Comita, Liza S., Helene C. Muller-Landau, Salomón Aguilar, and Stephen P. Hubbell. 2010. "Asymmetric Density Dependence Shapes Species Abundances in a Tropical Tree Community." *Science* 329 (5989): 330–32.

Comita, Liza S., Simon A. Queenborough, Stephen J. Murphy, Jenalle L. Eck, Kaiyang Xu, Meghna Krishnadas, Noelle Beckman, and Yan Zhu. 2014. "Testing Predictions of the Janzen--Connell Hypothesis: A Meta-Analysis of Experimental Evidence for Distance-and Density-Dependent Seed and Seedling Survival." *The Journal of Ecology* 102 (4): 845–56.

Connell, J. H. 1971. "On the Role of Natural Enemies in Preventing Competitive Exclusion in Some Marine Animals and in Rain Forests." In *Dynamics of Populations*, edited by P. J. Den Boer G. R. Gradwell, 298–312. Center for Agricultural Publishing and Documentation.

Corrales, Adriana, Scott A. Mangan, Benjamin L. Turner, and James W. Dalling. 2016. "An Ectomycorrhizal Nitrogen Economy Facilitates Monodominance in a Neotropical Forest." *Ecology Letters* 19 (4): 383–92.

Detto, Matteo, Marco D. Visser, S. Joseph Wright, and Stephen W. Pacala. 2019. "Bias in the Detection of Negative Density Dependence in Plant Communities." *Ecology Letters* 22

(11): 1923–39.

García-Guzmán, Graciela, and Martin Heil. 2014. “Life Histories of Hosts and Pathogens Predict Patterns in Tropical Fungal Plant Diseases.” *The New Phytologist* 201 (4): 1106–20.

Gelman, Andrew. 2008. “Scaling Regression Inputs by Dividing by Two Standard Deviations.” *Statistics in Medicine* 27 (15): 2865–73.

Green, Peter T., Kyle E. Harms, and Joseph H. Connell. 2014. “Nonrandom, Diversifying Processes Are Disproportionately Strong in the Smallest Size Classes of a Tropical Forest.” *Proceedings of the National Academy of Sciences of the United States of America* 111 (52): 18649–54.

Grünfeld, Leonie, Monika Wulf, Matthias C. Rillig, Annette Manntschke, and Stavros D. Veresoglou. 2020. “Neighbours of Arbuscular-Mycorrhiza Associating Trees Are Colonized More Extensively by Arbuscular Mycorrhizal Fungi than Their Conspecifics in Ectomycorrhiza Dominated Stands.” *The New Phytologist* 227 (1): 10–13.

Janzen, Daniel H. 1970. “Herbivores and the Number of Tree Species in Tropical Forests.” *The American Naturalist* 104 (940): 501–28.

Jevon, Fiona V., Sydne Record, John Grady, Ashley K. Lang, David A. Orwig, Matthew P. Ayres, and Jaclyn Hatala Matthes. 2020. “Seedling Survival Declines with Increasing Conspecific Density in a Common Temperate Tree.” *Ecosphere* 11 (11): e03292.

Jiang, Feng, James A. Lutz, Qingxi Guo, Zhanqing Hao, Xugao Wang, Gregory S. Gilbert, Zikun Mao, et al. 2021. “Mycorrhizal Type Influences Plant Density Dependence and Species Richness across 15 Temperate Forests.” *Ecology* 102 (3): e03259.

Jiang, Feng, Kai Zhu, Marc W. Cadotte, and Guangze Jin. 2020. “Tree Mycorrhizal Type Mediates the Strength of Negative Density Dependence in Temperate Forests.” *The Journal* *of Ecology*, May. https://doi.org/10.1111/1365-2745.13413.

Jia, Shihong, Xugao Wang, Zuoqiang Yuan, Fei Lin, Ji Ye, Guigang Lin, Zhanqing Hao, and Robert Bagchi. 2020. "Tree Species Traits Affect Which Natural Enemies Drive the Janzen-Connell Effect in a Temperate Forest." *Nature Communications* 11 (1): 286.

Johnson, Daniel J., Wesley T. Beaulieu, James D. Bever, and Keith Clay. 2012. "Conspecific Negative Density Dependence and Forest Diversity." *Science* 336 (6083): 904–7.

Johnson, Daniel J., Norman A. Bourg, Robert Howe, William J. McShea, Amy Wolf, and Keith Clay. 2014. "Conspecific Negative Density-Dependent Mortality and the Structure of Temperate Forests." *Ecology* 95 (9): 2493–2503.

Johnson, Daniel J., Richard Condit, Stephen P. Hubbell, and Liza S. Comita. 2017. "Abiotic Niche Partitioning and Negative Density Dependence Drive Tree Seedling Survival in a Tropical Forest." *Proceedings of the Royal Society B: Biological Sciences* 284 (1869): 20172210.

LaManna, Joseph A., Scott A. Mangan, Alfonso Alonso, Norman A. Bourg, Warren Y. Brockelman, Sarayudh Bunyavejchewin, Li-Wan Chang, et al. 2017. "Plant Diversity Increases with the Strength of Negative Density Dependence at the Global Scale." *Science* 356 (6345): 1389–92.

LaManna, Joseph A., Scott A. Mangan, and Jonathan A. Myers. 2021. "Conspecific Negative Density Dependence and Why Its Study Should Not Be Abandoned." *Ecosphere* 12 (1). https://doi.org/10.1002/ecs2.3322.

LaManna, Joseph A., Maranda L. Walton, Benjamin L. Turner, and Jonathan A. Myers. 2016. "Negative Density Dependence Is Stronger in Resource-Rich Environments and Diversifies Communities When Stronger for Common but Not Rare Species." Edited by Marcel

Rejmanek. *Ecology Letters* 19 (6): 657–67.

Lebrija-Trejos, Edwin, Peter B. Reich, Andres Hernández, and S. Joseph Wright. 2016. “Species with Greater Seed Mass Are More Tolerant of Conspecific Neighbours: A Key Driver of Early Survival and Future Abundances in a Tropical Forest.” *Ecology Letters* 19 (9): 1071–80.

Levine, Jonathan M., and Janneke HilleRisLambers. 2009. “The Importance of Niches for the Maintenance of Species Diversity.” *Nature* 461 (7261): 254–57.

Liang, Minxia, Liuqing Shi, David F. R. P. Burslem, David Johnson, Miao Fang, Xinyi Zhang, and Shixiao Yu. 2021. “Soil Fungal Networks Moderate Density-Dependent Survival and Growth of Seedlings.” *The New Phytologist*, no. nph.17237 (January). https://doi.org/10.1111/nph.17237.

Liu, Yu, and Fangliang He. 2021. “Warming Intensifies Soil Pathogen Negative Feedback on a Temperate Tree.” *The New Phytologist*, April. https://doi.org/10.1111/nph.17409.

Magee, Lukas, Amy Wolf, Robert Howe, Jonathan Schubbe, Kari Hagenow, and Benjamin Turner. 2020. “Density Dependence and Habitat Heterogeneity Regulate Seedling Survival in a North American Temperate Forest.” *Forest Ecology and Management*, November, 118722.

Mangan, Scott A., Stefan A. Schnitzer, Edward A. Herre, Keenan M. L. Mack, Mariana C. Valencia, Evelyn I. Sanchez, and James D. Bever. 2010. “Negative Plant-Soil Feedback Predicts Tree-Species Relative Abundance in a Tropical Forest.” *Nature* 466 (7307): 752–55.

McCarthy-Neumann, Sarah, and Richard K. Kobe. 2008. “Tolerance of Soil Pathogens Co-Varies with Shade Tolerance across Species of Tropical Tree Seedlings.” *Ecology* 89 (7):

1883–92.

———. 2010. "Conspecific and Heterospecific Plant-Soil Feedbacks Influence Survivorship and Growth of Temperate Tree Seedlings." *The Journal of Ecology* 98 (2): 408–18.

Murphy, Stephen J., and Liza S. Comita. 2021. "Large Mammalian Herbivores Contribute to Conspecific Negative Density Dependence in a Temperate Forest." *The Journal of Ecology* 109 (3): 1194–1209.

Niinemets, Ülo, and Fernando Valladares. 2006. "Tolerance to Shade, Drought, and Waterlogging of Temperate Northern Hemisphere Trees and Shrubs." *Ecological Monographs* 76 (4): 521–47.

Orwig, David A., Jason A. Aylward, Hannah L. Buckley, Bradley S. Case, and Aaron M. Ellison. 2022. "Land-Use History Impacts Spatial Patterns and Composition of Woody Plant Species across a 35-Hectare Temperate Forest Plot." *PeerJ* 10 (January): e12693.

Qin, Jianghuan, Yan Geng, Xiaoyu Li, Chunyu Zhang, Xiuhai Zhao, and Klaus von Gadow. 2021. "Mycorrhizal Type and Soil Pathogenic Fungi Mediate Tree Survival and Density Dependence in a Temperate Forest." *Forest Ecology and Management* 496 (September): 119459.

Ramage, Benjamin S., Daniel J. Johnson, Erika Gonzalez-Akre, William J. McShea, Kristina J. Anderson-Teixeira, Norman A. Bourg, and Keith Clay. 2017. "Sapling Growth Rates Reveal Conspecific Negative Density Dependence in a Temperate Forest." *Ecology and Evolution* 7 (19): 7661–71.

Record, Sydne, Richard K. Kobe, Corine F. Vriesendorp, and Andrew O. Finley. 2016. "Seedling Survival Responses to Conspecific Density, Soil Nutrients, and Irradiance Vary with Age in a Tropical Forest." *Ecology* 97 (9): 2406–15.

Seiwa, Kenji. 2007. “Trade-Offs between Seedling Growth and Survival in Deciduous Broadleaved Trees in a Temperate Forest.” *Annals of Botany* 99 (3): 537–44.

Seiwa, Kenji, Kazuhiko Masaka, Miki Konno, and Susumu Iwamoto. 2019. “Role of Seed Size and Relative Abundance in Conspecific Negative Distance-Dependent Seedling Mortality for Eight Tree Species in a Temperate Forest” 453 (117537). https://doi.org/10.1016/j.foreco.2019.117537.

Smith, Lauren M., and Heather L. Reynolds. 2015. “Plant–soil Feedbacks Shift from Negative to Positive with Decreasing Light in Forest Understory Species.” *Ecology* 96 (9): 2523–32.

Song, Xiaoyang, Jie Yang, Min Cao, Luxiang Lin, Zhenhua Sun, Handong Wen, and Nathan G. Swenson. 2021. “Traits Mediate a Trade-off in Seedling Growth Response to Light and Conspecific Density in a Diverse Subtropical Forest.” *The Journal of Ecology* 109 (2): 703–13.

Soudzilovskaia, Nadejda A., Stijn Vaessen, Milagros Barcelo, Jinhong He, Saleh Rahimlou, Kessy Abarenkov, Mark C. Brundrett, Sofia I. F. Gomes, Vincent Merckx, and Leho Tedersoo. 2020. “FungalRoot: Global Online Database of Plant Mycorrhizal Associations.” *The New Phytologist* 227 (3): 955–66.

Stump, Simon Maccracken, and Liza S. Comita. 2018. “Interspecific Variation in Conspecific Negative Density Dependence Can Make Species Less Likely to Coexist.” *Ecology Letters* 21 (10): 1541–51.

Terborgh, John. 2012. “Enemies Maintain Hyperdiverse Tropical Forests.” *The American Naturalist* 179 (3): 303–14.

Xu, Meng, Yongfan Wang, and Shixiao Yu. 2015. “Conspecific Negative Density Dependence Decreases with Increasing Species Abundance.” https://doi.org/10.1890/ES15-00144.1.

Zhang, Zhaochen, Michael J. Papaik, Xugao Wang, Zhanqing Hao, Ji Ye, Fei Lin, and Zuoqiang Yuan. 2017. “The Effect of Tree Size, Neighborhood Competition and Environment on Tree Growth in an Old-Growth Temperate Forest.” *Journal of Plant Ecology* 10 (6): 970–80.

Zhu, Kai, Christopher W. Woodall, Joao V. D. Monteiro, and James S. Clark. 2015. “Prevalence and Strength of Density-Dependent Tree Recruitment.” *Ecology* 96 (9): 2319–27.

Zhu, Yan, Liza S. Comita, Stephen P. Hubbell, and Keping Ma. 2015. “Conspecific and Phylogenetic Density-Dependent Survival Differs across Life Stages in a Tropical Forest.” Edited by Richard Shefferson. *The Journal of Ecology* 103 (4): 957–66.

Table 1. Characteristics of species included in the analysis of CNDD for saplings from two censuses. Species used in the planting experiment are in bold. AM: species that typically associate with arbuscular mycorrhizae; ECM: species that typically associate with ectomycorrhizae*. The operational cutoff between saplings and adults was the median DBH for unshaded species and 12.7 cm for shaded species.

Species	Total abundance	Mycorrhizal association	Median DBH	Species ~~abbreviation~~code
Viburnum dentatum	53	AM	1.1	Vibude
Viburnum lantanoides	96	AM	1.2	Vibual
Viburnum cassinoides	1846	AM	1.3	Vibuca
Lindera benzoin	83	AM	1.4	Lindbe
Ilex verticillata	1266	AM	1.5	Ilexve
Amelanchier laevis	354	AM	1.4	Amella
Crataegus spp.	259	AM	1.5	Cratsp
Acer pennsylvanicum	425	AM	1.8	Acerpe
Nemopanthus mucronatus	377	AM	1.8	Nemomu
Castanea dentata	1020	ECM	2.2	Castde
Hamamelis virginiana	3578	AM	2.45	Hamavi
Fagus grandifolia	4362	ECM	3.0	Fagugr
Sorbus americana	74	AM	3.25	Sorbam
Tsuga canadensis	24,222	ECM	5.4	Tsugca
Betula alleghaniensis	5015	ECM	5.6	Betula
Betula lenta	1545	ECM	8.6	Betule
Betula populifolia	123	ECM	9.5	Betupo
Fraxinus americana	197	AM	10.1	Fraxam
Nyssa sylvatica	193	AM	10.6	Nysssy
Acer rubrum	12,967	AM	11.9	Acerru
Prunus serotina	266	AM	13.3	Prunse
Betula papyrifera	590	ECM	15.35	Betupa
Picea abies	911	ECM	16.5	Piceab
Picea rubens	106	ECM	18.15	Piceru
Pinus strobus	2149	ECM	22.1	Pinust

Quercus rubra	4407	ECM	28.6	Querru
Quercus velutina	227	ECM	30.4	Querve
Pinus resinosa	789	ECM	32.8	Pinure

*Mycorrhizal associations determined according to the FungalRoot database (Soudzilovskaia et al. 2020)

Table 2. Coefficient estimates (standard error in parentheses) from binomial mixed effects model of seedling survival for each of the four seedling species. In all models, plot was included as a random effect. * indicates $p< 0.05$, †indicates $p < 0.1$.

Seedling species	Conspecific adult	Seedling height	Gap Fraction	~~Uneven subplot~~Subplot type (uneven)
Pinus resinosa	-1.96 (0.86)*	-0.10 (0.04)*	0.18 (0.12)	0.25 (0.46)
Quercus rubra	-1.38 (0.57)*	-0.01 (0.03)	0.00 (0.09)	0.07 (0.47)
Pinus strobus	-2.76 (1.09)*	-0.06 (0.03)*	-0.11 (0.17)	-0.25 (0.41)
Picea abies	na	-0.05 (0.04)	0.22 (0.13)†	-0.43 (0.38)

Table 3. Coefficient estimates (standard error in parentheses) from binomial mixed effects model of survival for each of the four experimental seedling species using the quantitative variables NCI_{con} and NCI_{het}. In all models, plot was included as a random effect. * indicates p<

0.05, †indicates $p < 0.1$~~, na indicates that parameter was not included in the model for that species.~~ .

Seedling species	NCI_{con}	NCI_{het}	Seedling height	Gap Fraction	Subplot type (uneven)
Pinus resinosa	-0.~~029~~032 (0.013)*	-0.~~020~~016 (0.015)	-0.~~093~~097 (0.~~041~~042)*	~~na~~0.145 (0.120)	0.282 (0.463)
Quercus rubra	-0.~~012~~015 (0.~~014~~015)	0.005 (0.010)	-0.~~007~~009 (0.~~009~~031)	~~na~~ -0.045 (0.098)	~~na~~0.029 (0.469)
Pinus strobus	-0.~~057~~061 (0.~~019~~021)*	-0.~~021~~022 (0.016)	-0.~~064~~061 (0.030)*	~~na~~ -0.105 (0.152)	-0.283 (0.407)
Picea abies	-0.021 (0.~~028~~029)	-0.~~022~~023 (0.~~011)*~~012)†	~~na~~-0.042 (0.040)	0.~~17~~170 (0.~~11~~118)	-0.426 (0.379)

Table 4. Overall standardized coefficient estimates~~ (standard error in parentheses)~~ from GLMM predicting sapling mortality as a function of initial size and local tree community. $\text{NCI}_{\text{con}}$and NCI_{het} represent local densities of conspecific trees, and heterospecific trees, respectively.

Species was included as a random effect with NCI_{con} and NCI_{het} both estimated with a random slope for each species. Individual random effects coefficients for each species can be found in Appendix 1: Table S1.

Predictor	Estimate (SE)	P
(intercept)	-1.~~55~~867 (0.~~480~~249)	≤0.001
DBH	-0.~~78~~805 (0.~~035~~036)	<0.001
NCI_{Het}	-0.~~21~~106 (0.~~036~~147)	~~<~~0.~~001~~468
NCI_{Con}	~~-1.53 (~~0.~~570~~948 (0.271)	≤0.~~007~~001
Random Effects		
σ^2	1.64	
τ_{00} species	~~4.71~~1.41	
τ_{11} species * NCIcon	~~6.33~~1.20	
~~ρ_{01}~~τ_{11} species * NCIhet	0.~~92~~40	
ICC	0.~~68~~46	
N species	28	
Observations	~~26443~~24250	
Marginal R^2 / Conditional R^2	0.~~091~~121 / 0.~~707~~527	

Table 5. Coefficient estimates ~~(standard error in parentheses)~~ from linear model predicting the strength of the species level estimate of CNDD as a function of four species level characteristics:

mycorrhizal association, shade tolerance, local abundance and median DBH of the species at this site.

Predictor	Estimate (SE)	P
(Intercept)	-0.~~48 (2.08~~250 (1.00)	0.~~82~~805
Mycorrhizal type (ECM)	-~~2.27~~1.111 (0.~~95~~482)	0.~~028~~033
Shade tolerance	-0.~~84~~540 (0.240)	0.~~09~~036
Median DBH	~~-~~0.~~003~~001 (0.~~048~~025)	0.~~95~~965
Log(abundance)	0.~~41~~228 (0.~~26~~130)	0.~~13~~095

Overall model R^2_{adjusted} = 0.~~21~~257

Figure captions

Figure 1. Plot level survival of each of the four seedling species when planted beneath a conspecific (~~blue~~pale green) or a heterospecific (dark green) adult tree. Seedlings from both subplots in each 20m diameter plot are included in each point, such that each point represents survival of 40 planted seedlings. N = 30 plots for each species.

Figure 2. Mean Shannon’s equitability index (J), a metric of community evenness, at the end of the experiment (green) relative to 1000 model simulations of equivalent level of mortality occurring randomly (black). Values for seedling subplots that were planted with equal numbers of each seedling species (A) and subplots that were planted with the conspecific seedling dominating (B), averaged across the 10 replicated adult trees. Error bars represent 95% confidence intervals. Dashed lines represent Shannon's equitability index of the community as it was initially planted.

Figure 3. Estimates of conspecific negative density dependence (CNDD) for saplings of 28 tree species at the Harvard Forest (A). Error bars represent the total range of estimates using 1000 iterations of the null model (see statistical methods section for details). Blue points represent species that typically associate with arbuscular mycorrhiza (AM), green points represent species that typically associate with ectomycorrhizae (ECM). B) Boxplot of all estimates by mycorrhizal type, showing the significant difference between AM tree species and ECM tree species. Species used in the seedling experiment are in bold. Species codes as in Table 1.

Figure 4. Estimates of conspecific negative density dependence (CNDD) for saplings of 28 tree species at the Harvard Forest as a function of shade tolerance (A) and local abundance (B). Blue points represent species that typically associate with arbuscular mycorrhiza (AM), green points represent species that typically associate with ectomycorrhizae (ECM). Gray line in A) illustrates significant negative relationship between shade tolerance and CNDD (see Table 5). Vertical error bars represent 95% confidence intervals (see statistical methods section for details). Horizontal error bars in A) are standard error from Niinemets and Valladares (2006). Note that *Crataegus sp., Nemopanthus mucronatuso*, *Viburnum lantanoides* and *Viburnum cassinoides* are missing from a), as there was no estimate of shade tolerance available.

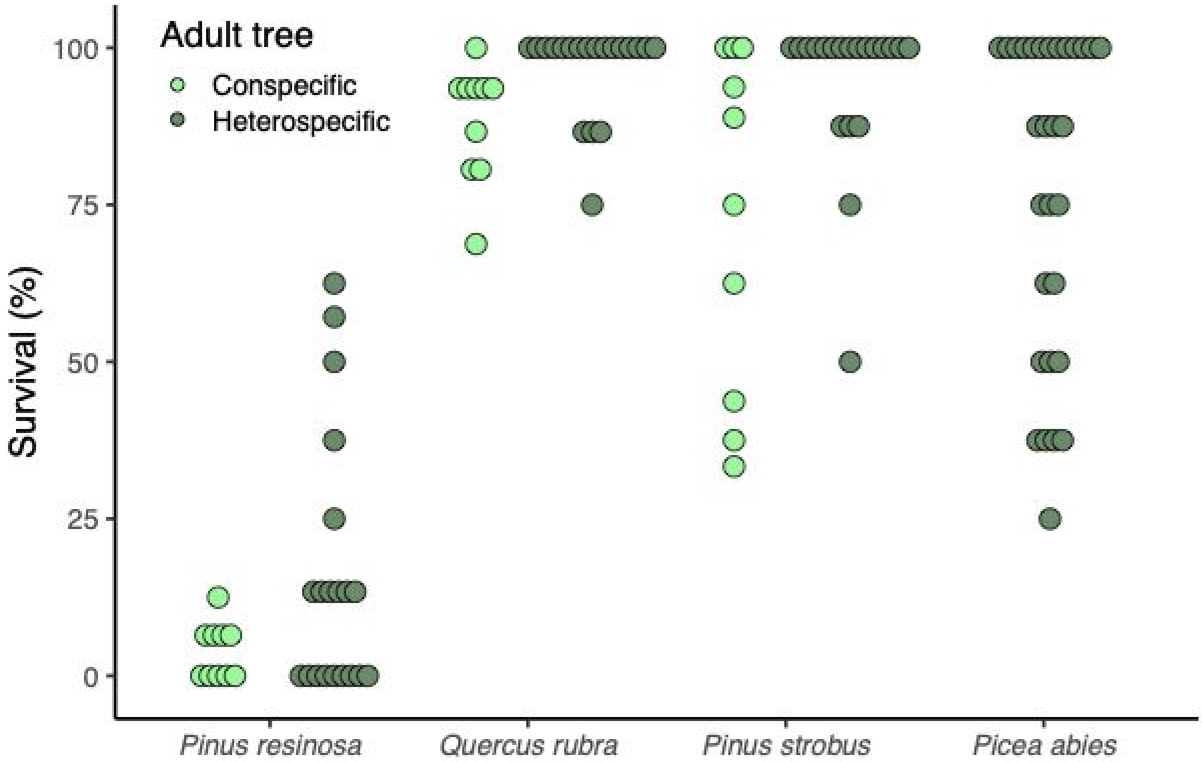


Figure 1. Plot level survival of each of the four seedling species when planted beneath a conspecific (pale green) or a heterospecific (dark green) adult tree. Seedlings from both subplots in each 20m diameter plot are included in each point, such that each point represents survival of 40 planted seedlings. N = 30 plots for each species.

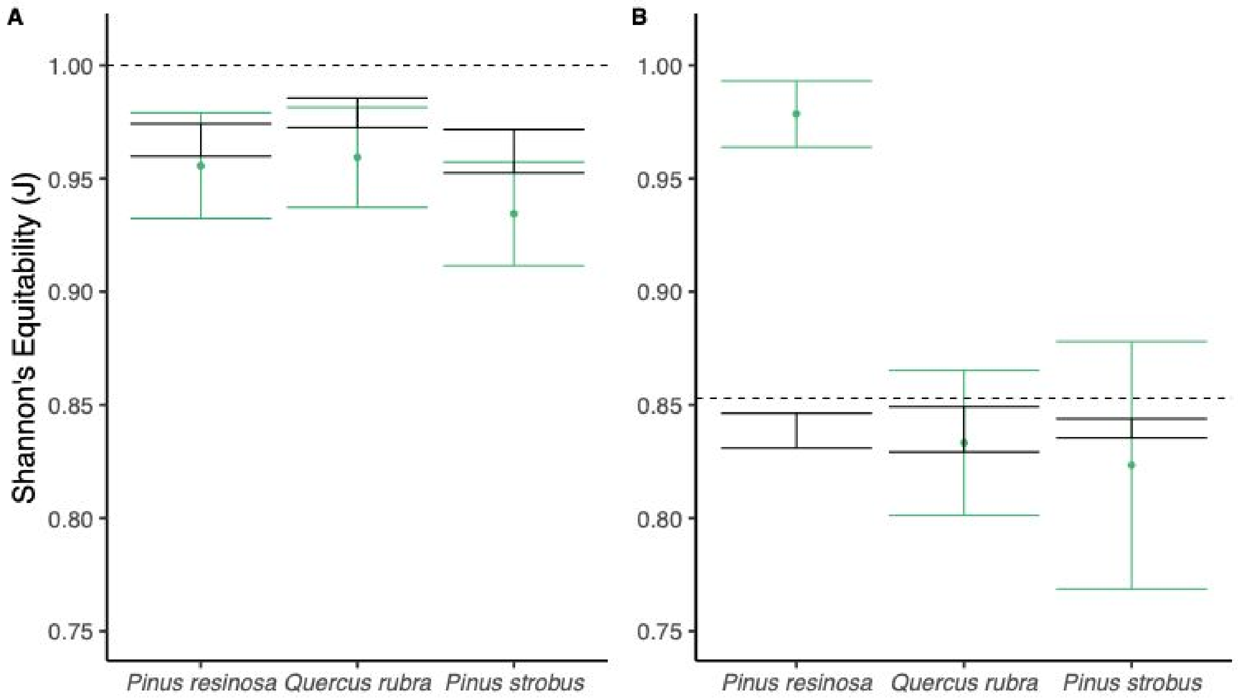


Figure 2. Mean Shannon's equitability index (J), a metric of community evenness, at the end of the experiment (green) relative to 1000 model simulations of equivalent level of mortality occurring randomly (black). Values for seedling subplots that were planted with equal numbers of each seedling species (A) and subplots that were planted with the conspecific seedling dominating (B), averaged across the 10 replicated adult trees. Error bars represent 95% confidence intervals. Dashed lines represent Shannon's equitability index of the community as it was initially planted.

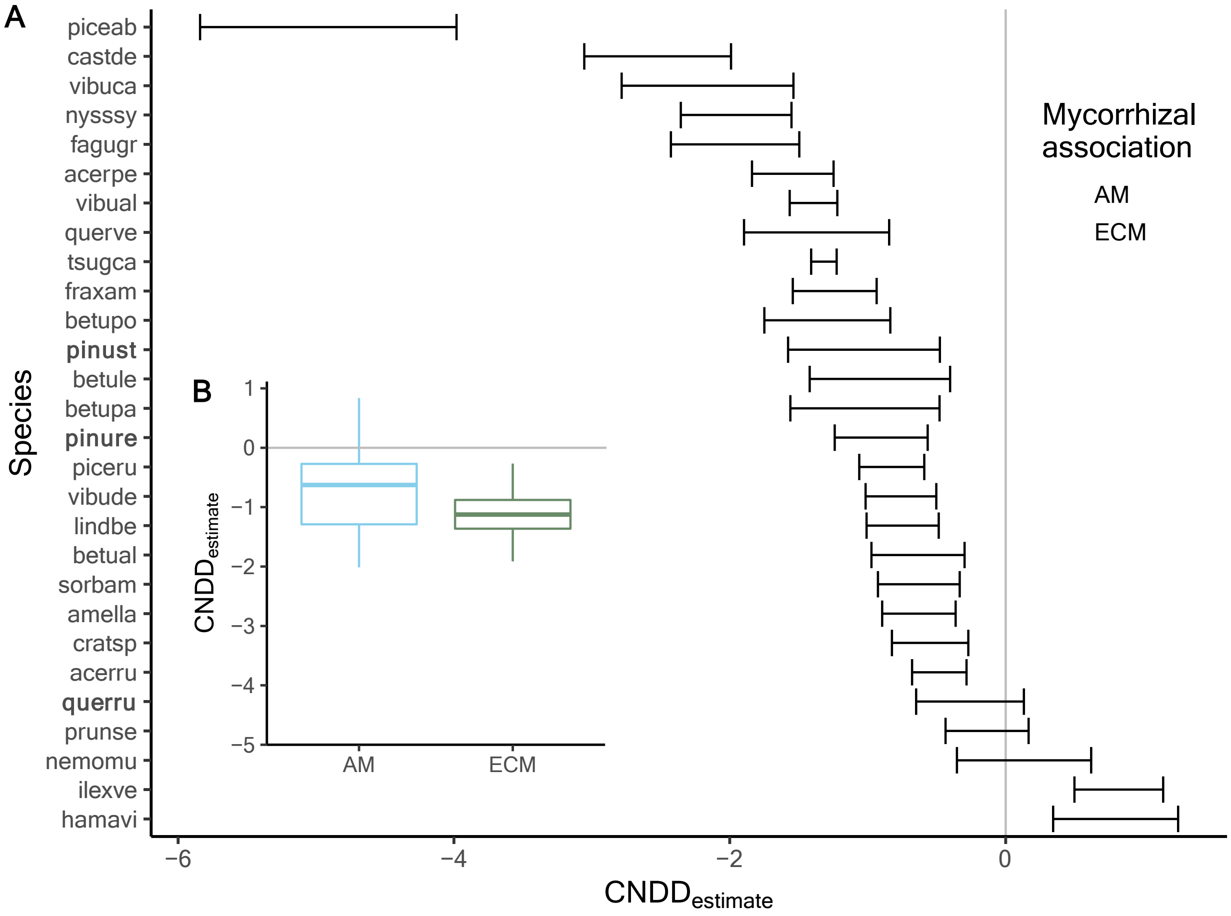
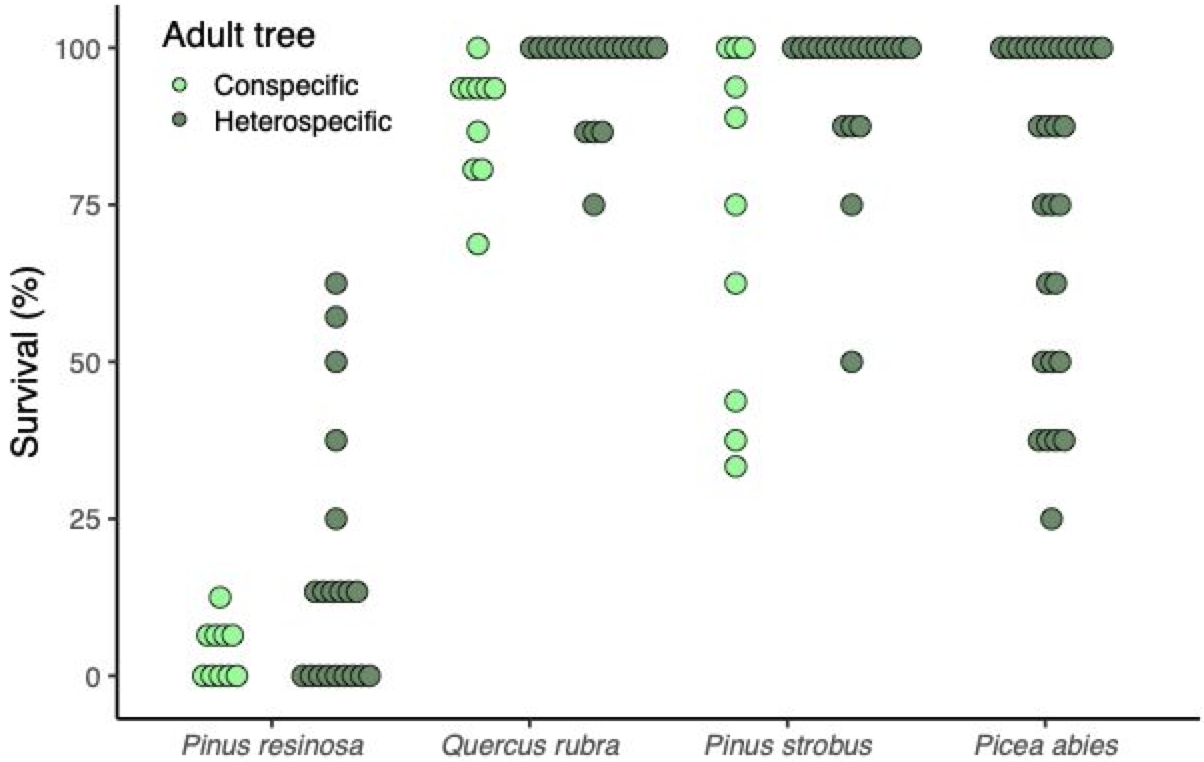


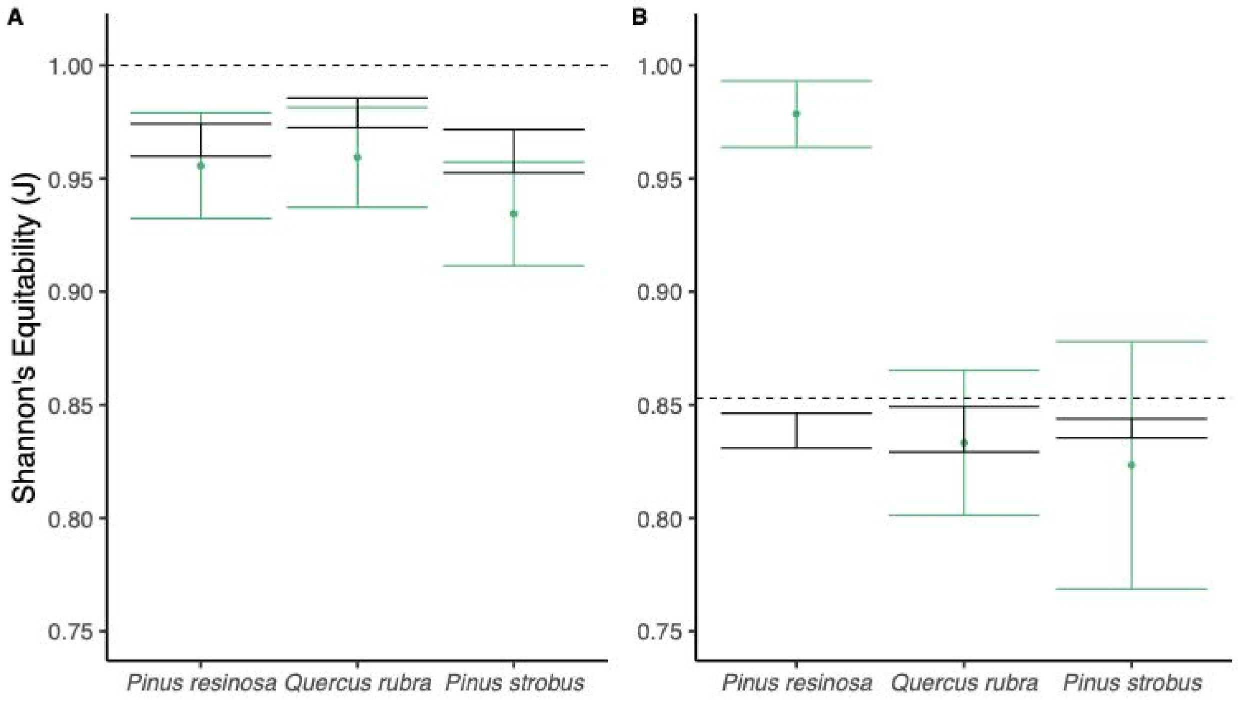
Figure 3. Estimates of conspecific negative density dependence (CNDD) for saplings of 28 tree species at the Harvard Forest (A). ~~Error bars represent the total range of estimates using 100 iterations of the null model (see statistical methods section for details). Blue points represent species that typically associate with arbuscular mycorrhiza (AM), green points represent species that typically associate with ectomycorrhizae (ECM). B) Boxplot of all estimates by mycorrhizal type, showing the significant difference between AM tree species and ECM tree species. Species used in the seedling experiment are in bold. Species codes as in Table 1.~~

~~**Figure 4.** Estimates of conspecific negative density dependence (CNDD)for saplings of 28 tree species at the Harvard Forest are not predicted by shade tolerance (A) or local abundance (B).~~

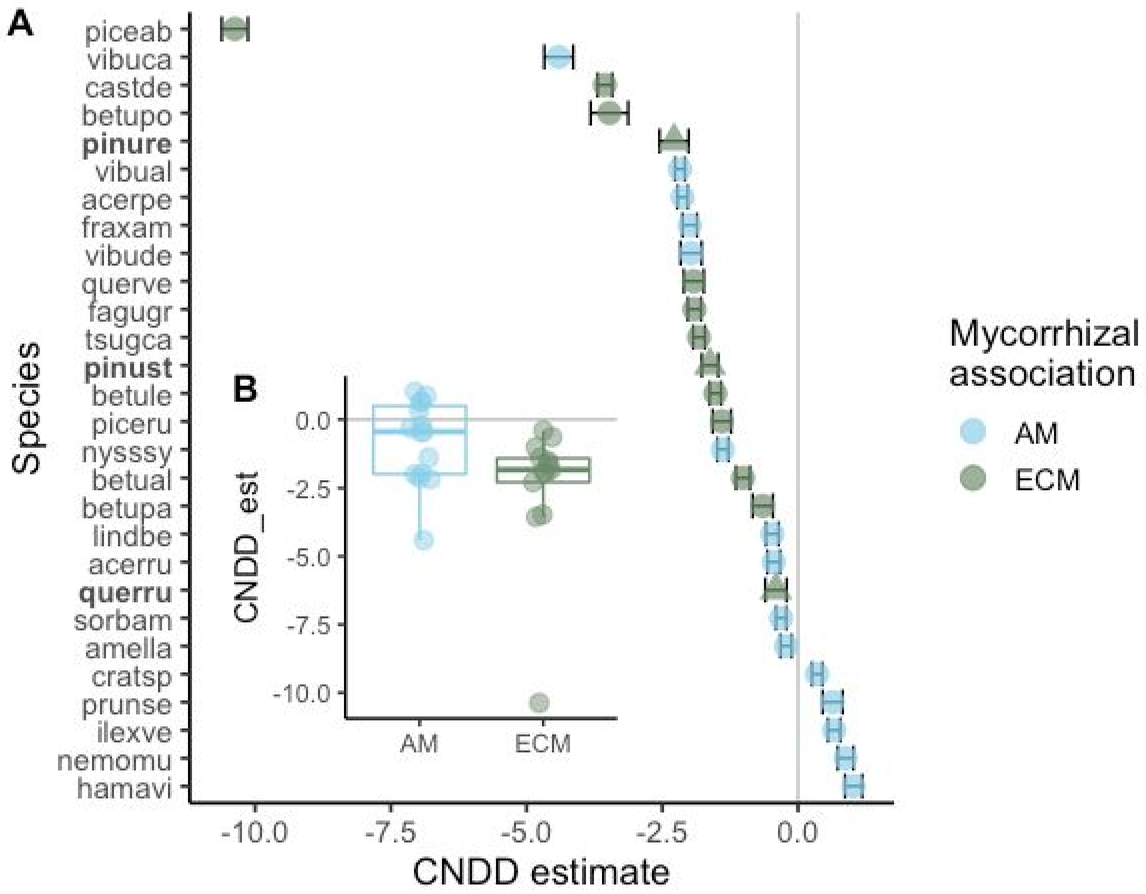
~~Blue points represent species that typically associate with arbuscular mycorrhiza (AM), green points represent species that typically associate with ectomycorrhizae (ECM). Vertical error bars represent the total range of CNDD estimates using 100 iterations of the null model (see statistical methods section for details). Horizontal error bars in A) are standard error from (Niinemets and Valladares 2006). Note that *Crataegus sp.*, *Nemopanthus mucronatuso*, *Viburnum lantanoides* and *Viburnum cassinoides* are missing from a), as there was no estimate of shade tolerance available.~~



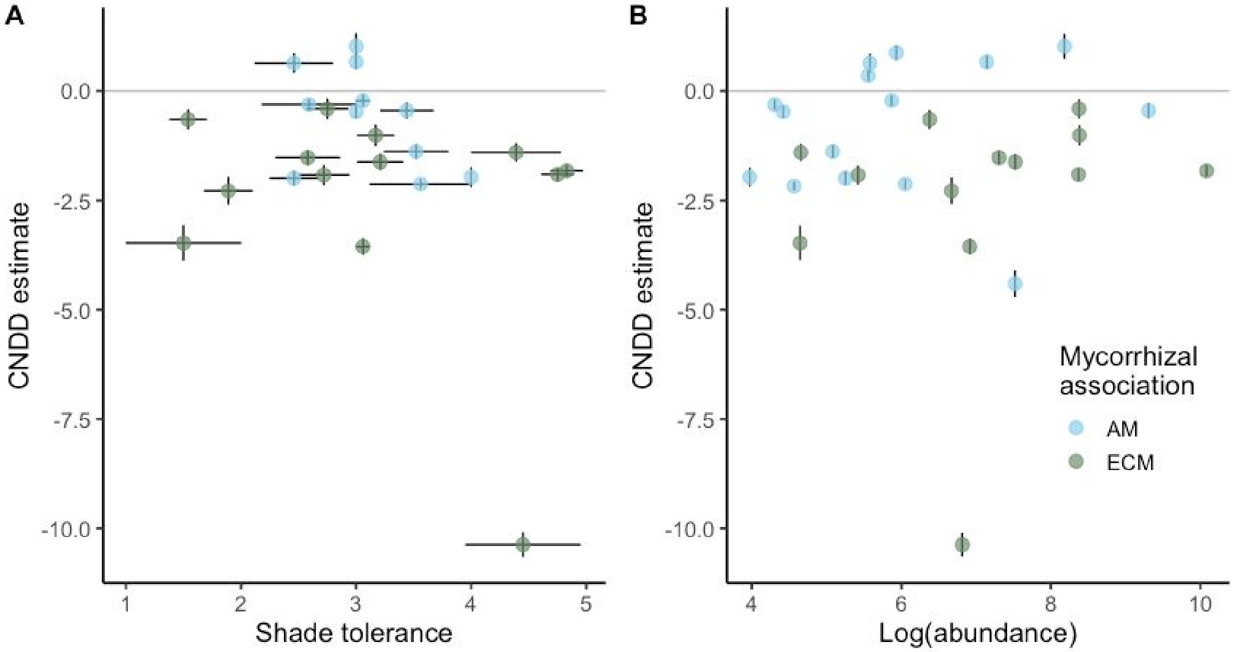
~~**Figure 1.** Plot level survival of each of the four seedling species when planted beneath a conspecific (blue) or a heterospecific (green) adult tree. Seedlings from both subplots in each 20m diameter plot are included in each point, such that each point represents survival of 40 planted seedlings. N = 30 plots for each species.~~



~~**Figure 2.** Mean Shannon's equitability index (J), a metric of community evenness, at the end of the experiment (green) relative to 1000 model simulations of equivalent level of mortality occurring randomly (black). Values for seedling subplots that were planted with equal numbers of each seedling species (A) and subplots that were planted with the conspecific seedling dominating (B), averaged across the 10 replicated adult trees. Error bars represent 95% confidence intervals. Dashed lines represent Shannon's equitability index of the community as it was initially planted.~~



~~**Figure 3.** Estimates of conspecific negative density dependence (CNDD) for saplings of 28 tree species at the Harvard Forest (A).~~ Error bars represent the total range of estimates using ~~100~~1000 iterations of the null model (see statistical methods section for details). Blue points represent species that typically associate with arbuscular mycorrhiza (AM), green points represent species that typically associate with ectomycorrhizae (ECM). B) Boxplot of all estimates by mycorrhizal type, showing the significant difference between AM tree species and ECM tree species. Species used in the seedling experiment are in bold. Species codes as in Table 1.



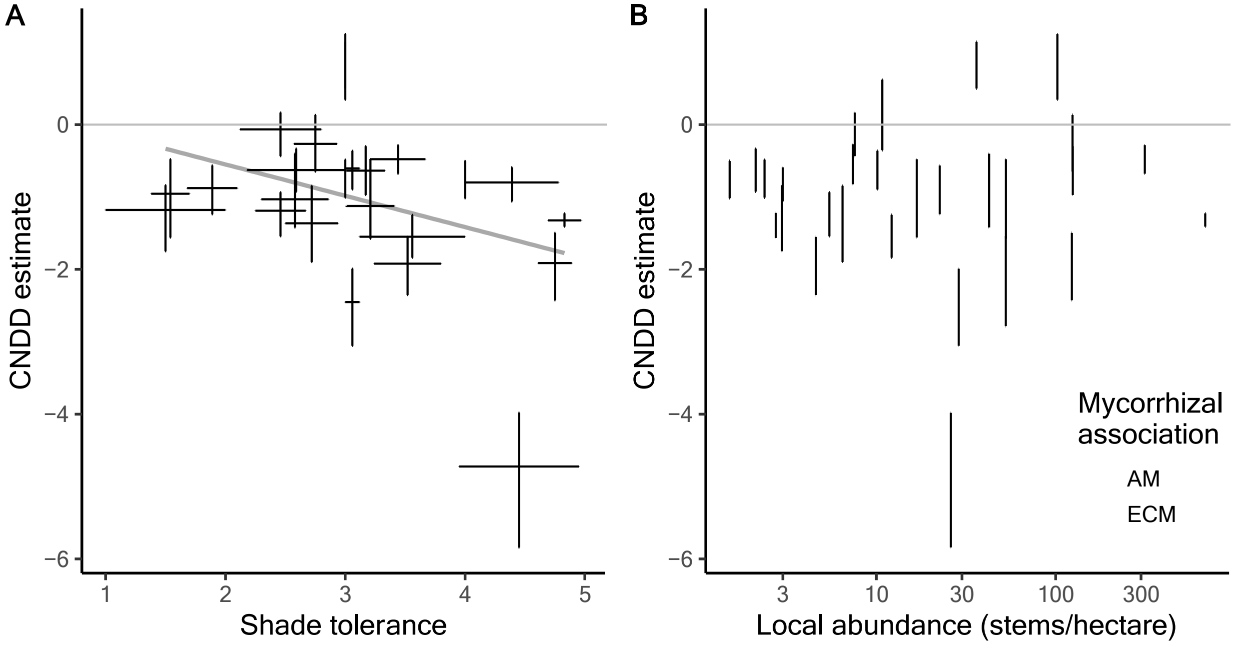


Figure 4. Estimates of conspecific negative density dependence (CNDD) for saplings of 28 tree species at the Harvard Forest ~~are not predicted by~~ as a function of shade tolerance (A) ~~or~~and

local abundance (B). Blue points represent species that typically associate with arbuscular mycorrhiza (AM), green points represent species that typically associate with ectomycorrhizae (ECM). ~~Blue points represent species that typically associate with arbuscular mycorrhiza (AM), green points represent species that typically associate with ectomycorrhizae (ECM).~~ Gray line in A) illustrates significant negative relationship between shade tolerance and CNDD (see Table 5). Vertical error bars represent ~~the total range of CNDD estimates using 100 iterations of the null model~~95% confidence intervals (see statistical methods section for details). Horizontal error bars in A) are standard error from ~~(~~Niinemets and Valladares (2006). Note that *Crataegus sp., Nemopanthus mucronatuso*, *Viburnum lantanoides* and *Viburnum cassinoides* are missing from a), as there was no estimate of shade tolerance available.