The Impact of Mycorrhizal Networks on *Quercus rubra* Seedling Recruitment

By

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Abstract

Main Objectives – Mycorrhizal fungi are ubiquitous plant mutualists and can be classified into two types: ecto- (EMF) - dominant in temperate forests and arbuscular (AMF) - globally dominant. Both types form mycorrhizal networks (MN), consisting of fungal hyphae that connect plants of the same and different species. The degree to which the MN of adult trees facilitate or inhibit other plants, specifically seedlings, is unclear. This study examines how the MN associated with different species of adult trees affect mycorrhizal colonization, growth, survival, and root fungal community of *Quercus rubra* seedlings, an EMF tree species.

Methods – Seedlings were planted under four adult tree species of varying mycorrhizal types: *Q. rubra* (EMF), *Q. velutina* (EMF), *Carya glabra* (EMF), and *Acer saccharum* (AMF). Before field transplant, two thirds of the seedlings were grown within micromesh bags that allow hyphae to pass while blocking roots. Seedlings were separated into treatment groups: no bag control (C), bagged control (BC), and disturbed (D). C and BC groups were transplanted and allowed to grow undisturbed. Seedlings in the D group had their connection to the MN disrupted every 2-3 days. Seedlings were collected at the end of the growing season and survival and biomass were recorded. A subset of the seedlings was examined for EMF colonization, and all colonized tips were collected. Collected tips had fungal DNA extracted, amplified, and sequenced to determine the EMF community present on the roots of the seedlings.

Results – Seedlings in the D treatment were colonized by a different suite of mycorrhizae than the two control groups, complicating interpretation of the effect of the MN on seedlings performance. *Q. rubra* seedlings benefited from MN connection when grown under con-specific and hetero-specific trees; but the effect of EMF colonization when connected to the MN was negative under *C. glabra*. Furthermore, under *A. saccharum*, seedlings benefited more from the EMF community that colonized the roots when they were disconnected, than from the EMF community when connected to the MN.

Conclusions – The findings in this study underline the importance of MNs in the recruitment of *Q. rubra*, a common canopy tree in the temperate forests of eastern North America. The effects are highly variable, ranging from facilitation to inhibition, and vary on the species and mycorrhizal type associated with neighboring canopy trees. Overall, this study highlights the importance of the MN in structuring temperate forest ecosystems.

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Introduction

Mycorrhizal fungi and plants have one of the most ubiquitous mutualistic relationships, occurring in 85-95% of vascular plant species (van der Heijden & Horton, 2009). In addition to soil exploration for water and nutrients, mycorrhizal fungi form mycorrhizal networks (MNs), webs of interconnected fungal hyphae that link the mycorrhizal colonies of different plants together (Simard & Durall, 2004; Van Der Heijden & Horton, 2009). MNs allow for the transfer of nutrients and photosynthate between the mycorrhizal species and the plants they colonize as well as between the plants themselves (Simard et al., 1997; Teste, Simard, & Durall, 2009). This latest transfer can occur between plants of the same genus and species, but also plants of entirely different genera (Simard et al., 1997); furthermore, it has been speculated this network could be critical for seedlings recruitment under the low light environments characteristic of forest ecosystems (Simard et al., 1997). Specifically, both laboratory and field studies have shown that photosynthate transfer via the MN occurs via a source-sink dynamic, in which plants in low light conditions gain photosynthate from plants with higher light in the overstory (Finlay & Read, 1986; Klein, Siegwolf, & Korner, 2016; Simard et al., 1997). As a result, established plants may play a key role in the success of seedlings in low light conditions. This transfer could be particularly relevant during recruitment because seedlings often experience low-light environments that limit their ability to gain carbon via photosynthesis (Canham et al. 1999, Lee and Ibáñez *in review*) . While this concept has been proposed in previous studies (Klein et al., 2016; Newbery, Alexander, & Rother, 2000) no work has successfully been able to quantify the contribution of MNs to seedling recruitment.

Adult trees may not only affect recruitment via MN carbon transfers, but also by playing an important role in determining what mycorrhizae colonize a plant. This is especially relevant during the seedling stage because different species and communities of mycorrhizae can have varying effects on seedling growth and survival (Bogar et al., 2019). For example, EMF seedlings planted near both con- and hetero-specific EMF trees show increased mycorrhizal colonization, nutrient uptake, and survival when compared to those planted under AMF species (Deniau et al., 2017; Dickie, Koide, & Steiner, 2002; Kennedy, Peay, & Bruns, 2009; Van Der Heijden & Horton, 2009). Thus, the neighborhood surrounding a seedling likely determines the degree of facilitation via the MN.

While existing EMF plants appear to facilitate the growth and survival of other EMF plants, previous work on the effect of conspecific canopies on seedling recruitment has shown that within temperate forests fewer than expected con-specific recruits are found under adult trees (Johnson, Beaulieu, Bever, & Clay, 2012; Hille Ris Lambers, Clark, & Beckage, 2002). This has also been played out on a smaller scale, as planted seedlings of some species tend to perform worse when planted near con-specific adults than they do under hetero-specific conditions (Deniau et al., 2017; Katz & Ibáñez, 2016). This conspecific negative effect is likely the result of an increase in the presence, or virulence, of host specific herbivores and/or soil pathogens (Hersh, Vilgalys, & Clark, 2012; Katz & Ibáñez, 2016; Mccarthy-Neumann & Ibáñez, 2012). The potential trade-off between facilitation, by providing most favorable EMF network, and inhibition, by increasing incidence of natural enemies, when seedlings recruit under con-specific adult trees is currently poorly understood, with some evidence suggesting that mycorrhizal colonization of the roots may help overcome the negative effects of being close to conspecific trees (Booth & Hoeksema, 2010; Brown,

Payne, White, & Peet, 2020; Liang et al., 2016). However, these effects, positive and negative, are likely going to differ among co-occurring species (Katz & Ibáñez, 2016). Therefore, to accurately predict recruitment patterns, and thus population and community dynamics, more work is needed to identify and quantify the benefits and downsides to being connected to the variety of MNs present in a community.

This study examines the role of MNs in the recruitment of *Quercus rubra* L., northern red oak, a dominant tree species in North American eastern forests. More specifically, we aim to quantify the value of being connected to the MN associated with con-specific, con-generic, and hetero-specific (AMF or EMF) adults. We addressed this dynamic by manipulating the connection of seedlings to the MN and measuring their mycorrhizal colonization and associated growth and survival. We addressed two primary questions: 1) what is effect of being connected to the MN on seedling establishment? 2) Do these effects vary based upon the mycorrhizal type and/or the phylogenetic relationship of the adult tree to *Q. rubra* seedlings? We hypothesize that overall, seedlings connected to the MN will display increased performance – as measured by growth and survival – compared to seedlings that are not connected to the MN, facilitated by access to additional resources. We also hypothesized this effect will be strongest under adult trees of the same mycorrhizal type (EMF), and trees that are phylogenetically closer to the seedlings since these conditions will be providing the most beneficial network.

Methods

Study Site

The study was performed in a mixed-oak ecosystem, Radrick Forest, located in southeastern Michigan in Washtenaw County (42.289190 N, 83.660091 W). The site rests

atop a glacial moraine comprised of calcareous, clay loam glacial till. The average temperature is -3.8^{o}C in January and 22.8^{o}C in July with an average annual precipitation of 100.3 cm (National Weather Service Forecast Office, 2018). The overstory is dominated by *Quercus rubra*, *Q. velutina*, *Carya glabra*, *Acer saccharum*, *A. rubrum*, and *Prunus serotina*, while the understory is primarily composed of *Ostraya virginiana* and *P. virginiana*.

Q. rubra is a common canopy tree found throughout the eastern portion of North America. It has a wide range of environments it can tolerate, inhabiting sites ranging from mesic/ dry-mesic to sandy and well drained (Barnes & Wagner, 2004). It is moderately shade tolerant, and can live for more than 300 years, but is relatively fast growing for a long-lived tree (Barnes & Wagner, 2004).

Experimental Design

Field experiments were run during the growing seasons of 2016 and 2018. Acorns collected from three separate populations (see Supplementary Materials Table S1) of *Q. rubra* – 300 in 2016, and 360 in 2018 – were planted in Deepot (313 cm^3 volume) containers in late May of 2016 and early April of 2018 using Metro-Mix ® 830 growing mix (Sungro Horticulture, MA, USA). A subset (n = 240 for both 2016 and 2018) of the acorns were planted in micro-mesh plastic bags with a pore size of 25μm; this mesh size allows mycorrhizal hyphae to grow through and prevents roots from growing out. The remaining acorns (n = 60, n = 120 for 2016 and 2018 respectively) were planted directly into the Deepot containers. Acorns from different populations were randomized between the bagged and non-bagged groups. Acorns germinated and grew in the greenhouse for one month prior to transplantation into field plots. Additionally, prior to field transplantation maternal effects

were estimated by measuring seedlings height (distance from root collar to shoot tip) in 2016 seedlings and by measuring acorns' length and width prior to germination in 2018.

Twelve field plots were established at the field site, distributed under four different neighboring canopy trees: *Q. rubra*, *Q. velutina*, *Acer saccharum* or *Carya glabra*. Canopy trees were selected based upon their relation to *Q. rubra* and/or their mycorrhizal type. Both *Quercus* species and *C. glabra* are EMF, and *A. saccharum* is AMF (Chen, Koide, & Eissenstat, 2018). The selection of these neighboring canopy trees allowed us to establish a variety of MN types – EMF, AMF–, as well as a gradient of phylogenetic relationships, con-specific, con-generic, and hetero-specific. Three replicated trees per species were selected for the experiment, all falling within an area of one hectare.

Seedlings were assigned to one of three treatments: non-bagged or "Control" (C) and bagged were either assigned to a "Bagged Control" treatment (BC) or to "Disturbed" (D) treatment. Seedlings in the C treatment group were removed from their Deepots and planted directly into the soil, but still with the potting soil attached. Seedlings in the BC and D group had their bags transferred to a plastic framework that held the bags and provided direct surface contact with the soil. This contact allowed for potential MN colonization via hyphae. Once planted, BC seedlings were left undisturbed, while D seedlings were slightly shaken three times per week to disrupt the MN (Janos, Scott, Aristizábal, & Bowman, 2013; Jasper, Abbott, & Robson, 1989). Bag effects were assessed by contrasting C and BC treatments; effects of being connected to the MN was assessed by comparing BC and D treatments.

In July of 2016 ten seedlings from both the BC and D groups, and five seedlings from the C group, were planted radially underneath each adult tree at intervals of 30 cm for a total of 25 seedlings planted per tree, and 300 seedlings total across all plots (trees). In May of

2018 in addition to ten BC and D seedlings, ten C seedlings were planted in each plot resulting in a total of 30 seedlings per plot, and a total of 360 seedlings were planted across the 12 plots (trees). Two weeks after planting, seedlings were re-censused to account for deaths due to transplant shock, these were not included in the survival analysis (see below).

Data Collection

Environmental variables - Soil moisture was measured within plots using a FieldScout TDR 300 soil moisture meter (Spectrum Technologies Inc., IL, USA) on a monthly basis. Additionally, at each plot hemispherical photos were taken of the canopy and the global site factor (GSF) was calculated using Hemiview software (Dynamax Inc., TX, USA) (Englund, O'Brien, & Clark, 2000).

Survival, biomass and mycorrhizal colonization - At the end of August in both years, a final re-census to determine survival was conducted. Over a period of three weeks seedlings were collected and stored in a refrigerator at 4^{o}C until they were analyzed for root tip mycorrhizal colonization. Seedlings under either of the bagged treatments were not included in the survival calculations or root tip analysis if their bags were ripped or roots had escaped.

In 2016, root tip analysis was conducted on all collected seedlings by randomly selecting a subset of 10 root tips from each plant, roots were cleared with 10% KOH, and stained with a 5% Schaeffer black ink solution. Stained tips were examined under a microscope at 200x using a magnified intersection method for the presence of arbuscules – indicative of AMF colonization – and a hyphal mantle – indicative of EMF colonization (Ibáñez & McCarthy-Neumann, 2016; Tonn & Ibáñez, 2017).

In 2018, root tip analysis was conducted differently to be able to perform further DNA analyses of the roots (see next section). We implemented this DNA analysis to assess if

there were major differences in the mycorrhizal community associated with each canopy tree or treatment. On a subset of randomly selected seedlings (a minimum of eight per treatment and neighbor combination), seedlings' roots were gently rinsed with tap water to remove all soil. All roots were removed from the seedlings and homogenized. Sections of roots were randomly selected, and tips were visually examined under a microscope at 4.5x for evidence of EMF colonization as shown by the presence of a hyphal mantle. Tips were counted until a total of 200 tips were examined, after which the current root section was finished. Colonized tips were collected and placed in a 2% CTAB buffer solution and stored at -80ºC for DNA extraction and sequencing.

Following collection and root tip analysis seedlings were dried at 70^0C for 72 hours and separated into roots, shoots, and leaves and biomass was measured.

DNA Isolation - To determine if treatment and/or canopy tree species influenced the mycorrhizal community of the seedlings, genomic DNA was extracted from colonized root tips. Root tips were thawed and all excess CTAB was removed. Due to the low number of tips collected in several treatments, fungal root tips were pooled at the treatment level within each canopy tree. Pooled tips were then freeze dried for 12 hours. Freeze dried samples were weighed and, if possible, separated into triplicates of equal weight with a minimum mass of 5 mg. To isolate DNA the DNeasy Plant Mini Kit (Qiagen) was used with a modified manufacturers protocol. Specifically, 200 mg of glass beads and 500 µl of 2% CTAB solution was added to the freeze-dried replicates and samples were then beaten using a benchtop Powerlyzer™ (MO-BIO). Following beating 400µL of Buffer AP1 was added and samples were incubated in a water bath at 60ºC for 30 minutes, before the addition of 175 µl of Buffer P3. The remainder of the protocol matched the manufacturer's provided methods. Following

the completion of the extraction protocol the presence of DNA was confirmed using a NanoDrop 8000 Spectrophotometer (Thermo Scientific) and gel electrophoresis. DNA concentrations were determined using a Quant-iT PicoGreen dsDNA kit (Invitrogen). Replicates of samples were recombined and stored at -80ºC for PCR amplification.

PCR Amplification - In order to isolate and amplify fungal DNA ITS1F and ITS5.8S forward (5' AATGATACGGCGACCACCGAGATCTACAC 3') and reverse (5' CAAGCAGAAGACGGCATACGAGAT 3') primers were utilized. Each primer included a linker sequence and error correcting Golay barcode optimized for use with Illumina MiSeq high throughput sequencing. Triplicate PCR reactions were performed using Phusion High Fidelity Taq Polymerase (New England Laboratories) and a master mix. The concentration of DNA across samples was highly variable and as such the volume of template included in the reactions ranged from 0.75 – 10.0 µL (mean = 5.89 µL). DNA templates were combined with a master mix with a final concentration of 1.5x Phusion High Fidelity buffer, 0.375 µmol forward and reverse primers, 0.42 µmol dNTP, and 0.023 U Phusion High Fidelity Taq. All master mixes were brought to 20 µL with nuclease free water. PCR began with denaturation at 94ºC for 3 min, followed by 27 cycles of 30 seconds at 94 ºC, 45 seconds at 57 ºC, and one minute and 30 seconds at72 ºC, and a final extension step at 72 ºC for 10 minutes. In total, 32 of 34 samples were able to be properly amplified. Following amplification all triplicate reactions were pooled and submitted to the University of Michigan Microbial Systems Molecular Biology Laboratory for a 500V2/Nano Illumina MiSeq run.

Bioinformatic Analysis - All bioinformatic analysis was conducted using QIIME version 2019.7. A total of 2,749,080 raw reads were returned from the Illumina MiSeq Nano run. Because of the poor quality of the reverse reads, only the forward reads were utilized. All

forward reads were demultiplexed, with the first 10 bp trimmed and truncated to 250 bp in length, and assigned to unique samples. Chimeric reads were detected and filtered, while simultaneously operational taxonomic units (OTUs) were assigned to unique reads and taxonomy was determined using a trained UNITE database with the command *feature_classifier* at 97% sequence similarity. Following this step, a total of 737,489 reads remained (mean per sample = 23,046.53, SD = 13901.61). Samples were rarefied at a depth of 9,195 using command *feature_table_rarefy* resulting in the exclusion of eight samples of the original 32, resulting in a final sample count of 24. Finally, sequences found in only a single sample or with five observations or fewer were removed. The resulting data was exported as a BIOM file for statistical analysis.

In order to see if mycorrhizal communities differed by neighboring tree and/or treatment EMF and AMF mycorrhizal genera were separated from the data. EMF genera and lineages were identified using Determination of Ectomycorrhizae (DEEMY) and the EMF genera and lineages identified in Tedersoo et al. (2010). AMF genera were identified using the phylogeny established in Kruger et al. (2012) (Krüger, Krüger, Walker, Stockinger, & Schüßler, 2012; Tedersoo, May, & Smith, 2010).

Data Analysis

Before analyzing, mycorrhizal colonization data were standardized (standard value: (observed-mean)/SD) for each year to account for any effects that the different methodology could have had in the assessment of colonization. To facilitate estimations, we also used standard values for biomass and for the three covariates we included in the analyses, maternal effects, light (GSF) and soil moisture. Always addressing our research questions, we tried several combinations of fixed and random effects, we chose the model with the best

fit (based on Deviance Information Criterion (DIC); Ando, 2007) described below (for other models tried see Supplementary Materials).

Mycorrhizal colonization - Seedling mycorrhizal colonization (standardized values) was modeled as a function of neighboring canopy tree species, treatment (Control, Bagged Control, Disturbed), soil moisture, light availability (GSF), and maternal effects (standardized height at planting or standardized acorn size). We analyzed the proportion of seedling (*i*)'s roots that were colonized by EMF with likelihood:

$$EMF\ Colonization_i \sim Normal\ (ectom_i, \sigma^2)$$

and process model:

$$ectom_i = \alpha_{neighbor(i),treatment(i)} + \delta_1 \cdot Maternal\ Effect_i + \delta_2 \cdot Light\ Availability_{plot(i)} + \delta_3 \cdot Soil\ Moisture_{plot(i)} + PYRE_{plot(i),year(i)}$$

Included in the process model was a plot and year random effect (PYRE) for each plot (replicated tree) and year (2016 and 2018) to account for additional variability arising from differences across plots and years. Because only a subset of seedlings was examined for EMF colonization, we used parameters of this model to predict EMF colonization (EMFpred) for each seedling. We then used these estimates in the biomass and survival analyses.

Biomass – Standardized seedlings biomass (*BM*) was analyzed as a function of the same covariates (maternal effects, light and soil moisture), neighboring canopy tree and treatment combinations, and of EMF colonization (estimated for each seedling). This last effect was estimated for each canopy tree and treatment combination; our rationale here is that if the mycorrhizal community varies across neighboring canopy trees its effects may also be different, i.e., beneficial in some (con-specific), detrimental or neutral in others (hetero-specific). Also included were plot and year random effects. Biomass was estimated using likelihood:

$$BM_i \sim Normal\ (B_i,\ \sigma^2)$$

And process Model:

$$BM_i = \beta 1_{neighbor(i),treatment(i)} + \beta 2_{neighbor(i),treatment(i)} \cdot EMF\ Colonization_{(i)} + \delta_1 \cdot Maternal\ Effect_i + \delta_2 \cdot Light\ Availability_{plot(i)} + \delta_3 \cdot Soil\ Moisture_{plot(i)} + PYRE_{plot(i),year(i)}$$

Survival - Seedling survival (*Survival*) was analyzed as a function of the same variables, but with likelihood:

$$Survival_i \sim Bernoulli\ (P_i)$$

and process model:

$$Logit\ (P_i) = \mu 1_{neighbor(i),treatment(i)} + \mu 2_{neighbor(i),treatment(i)} \cdot EMF\ Colonization_{(i)} + \delta_1 \cdot Maternal\ Effect_i + \delta_2 \cdot Light\ Availability_{plot(i)} + \delta_3 \cdot Soil\ Moisture_{plot(i)} + PYRE_{plot(i),year(i)}$$

All parameters were estimated using a Bayesian framework, and were given non-informative priors, $\alpha_*, \beta_*, \mu_*, \delta_* \sim Normal(0,1000)$. Random effects were estimated as $PYRE*_i \sim Normal(0, \sigma_*^2)$, and $1/\sigma_*^2 \sim Gamma(0.001,0.001)$. Analyses were performed in OpenBUGS (Lunn, Spiegelhalter, Thomas, & Best, 2009). Two MCMC chains were run simultaneously until convergence was reached (~25,000 iterations) (see Supplementary Materials for code). Posterior means and 95% CIs were calculated from ~50,000 iterations following convergence.

DNA analysis - Data rarefied at 9,195 sequences from the bioinformatic analysis was visualized using Principle Coordinate Analysis (PCoA) and Nonmetric Multidimensional Scaling (NMDS). Shannon and Simpson diversity metrics were calculated for all samples and were compared across treatments (n = 3) and neighboring canopy tree species (n = 4) using a two-way ANOVA. A Bray-Curtis distance matrix was calculated for use in PERMANOVAs to determine if there were community differences by plot, neighbor, and/or

treatment. Prior to the calculation of Bray-Curtis distances the data was transformed by taking the square root of each value. First, a PERMANOVA was performed on data that included all sequences – not just mycorrhizal. Following this, a second PERMANOVA was conducted including only the EMF and AMF lineages identified. Lastly, because *Q. rubra* is an EMF dominated species, a final PERMANOVA was run including only those sequences that were identified as EMF lineages. For all PERMANOVAs beta-dispersion tests were run to ensure that all estimations were not resulting from the overdispersion of the data. All calculations were performed using R Statistical Software (version 3.6.2; R Core Team, 2019). ANOVAs were run using R base software. PERMANOVAs were run using the adonis command in the package vegan (Oksanen et al., 2019). Specific differences between groups in PERMANOVAs were calculated using the function pairwise-adonis in the package pairwiseAdonis (Martinez Arbizu, 2017). Diversity and distance matrices were also calculated using the vegan package.

Results

Overall, 375 out of 548 (68.4%) *Q. rubra* seedlings survived the duration of the experiment, 220 of which were examined for EMF colonization. Detailed descriptions of mycorrhizal colonization, biomass and survival, is provided in the Supplementary Materials. Results from the field experiments were highly variable with no clear trends emerging prior to analysis. Across plots and years the mean proportion of *Q. rubra* seedling root tips colonized by EMF ranged from 0.37 ± 0.92 % to 0.07 ± 0.39 % (mean ± SD; Supplementary Materials Table S2), seedling biomass varied from 1.2 ± 0.53 g to 2.0 ± 1.2 g (Supplementary Materials Table S3), and proportion of surviving seedlings ranged from 0.87 ± 0.16 to 0.39 ± 0.35 (Supplementary Materials Table S4). All mean posterior values, standard deviations, and

95% confidence intervals for all parameters estimated in the analysis are reported in the supplementary materials (Supplementary Materials Tables S6 – S8). Soil moisture and light values recorded can be found in the Supplementary Materials Fig. S1.

Mycorrhizal colonization – Model fit (predicted *vs* observed) for the mycorrhizal colonization model for EMF was $R^2 = 0.24$ (Supplementary Materials Fig. S2). The bag treatment (non-bagged Control *vs* Bag Control) resulted in reduced mycorrhizal colonization of the roots under *C. glabra* and *A. saccharum* canopies (Fig. 1A). Being disconnected from the MN (Bag Control *vs* Disturbed) significantly decreased root mycorrhizal colonization under *A. saccahrum* (Fig. 1A). There was no significant effect of maternal effects or either environmental variable on mycorrhizal colonization (Fig. 1B).

Biomass - Seedling biomass model fit was R^2 of 0.42 (Supplementary Materials Fig.S2). Bag effect on biomass was significant under *Q. velutina*, with seedlings having higher biomass if bagged (Fig. 2A). Being disconnected from the MN was associated with higher biomass under *A. saccharum*. Also, among disconnected seedlings, those under *A. saccharum* had higher biomass than those under *C. glabra* (Fig. 2A). There was no effect of mycorrhizal colonization on biomass across all neighbor tree canopy and treatment combinations (Fig. 2B). Seedling biomass was positively impacted by maternal effects, and unaffected by light and soil moisture (Fig. 2C).

Survival - The survival model had a model fit of AUC = 0.99 (Supplementary Materials Fig. S2). Survival was similar across canopy tree/treatment combinations (Fig. 3A). Overall EMF colonization had a positive effect on survival (Fig. 3B). Bags substantially affected the effect of EMF colonization (Control *vs* Bag Control). EMF had a positive effect on survival among bagged seedlings under *C. glabra* and *Q. velutina*, but it had a negative effect under *A.*

saccharum (Fig. 3B). Disruption of the MN (Bagged Control *vs* Disturbed) was positively associated with survival under *A. saccharum* (Fig. 3B). Among control treatments the positive effect of EMF on survival was considerably lower under *Q. velutina* (Fig. 3B). Among bagged control treatments the EMF effect ranged from negative under *A. saccharum* to positive in the other three neighboring species. When disconnected from the MN only seedlings under *A. saccharum* and *Q. velutina* benefited from EMF colonization (Fig. 3B).

Bioinformatic Analysis – Alpha rarefaction curves calculated at a sampling depth of 9,195 were primarily asymptotic, indicating that an adequate sampling depth was reached to represent the species richness present in the root tip samples (Supplementary Materials Fig. S 3). In total across all samples 114 fungal OTUs were identified when clustered at 97% similarity. Within those 114 OTUs 31 fungal genera were identified.

Simpson and Shannon diversity were lowest in the Disturbed treatment; however, ANOVA revealed no statistically significant difference by treatment (ANOVA: $F = 2.345$, $p = 0.135$), neighbor (ANOVA: $F = 0.487$, $p = 0.697$), or the interaction of neighbor and treatment (ANOVA: $F = 0.497$, $p = 0.773$) (Supplementary Materials Table S9).

Across all fungal taxa identified from our samples and grouped at the genus level, treatment had a significant effect on the community composition (PERMANOVA: $F = 2.08$, $p = 0.028$, $R^2 = 0.163$), while neighboring canopy tree (PERMANOVA: $F = 0.817$, $p = 0.641$, $R^2 = 0.096$) and the interaction canopy/treatment (PERMANOVA: $F = 1.17$, $p = 0.25$, $R^2 = 0.229$) did not (Fig. 5A).

Within just mycorrhizal genera identified in our samples, treatment again had a significant effect on community composition (PERMANOVA: $F = 3.609$, $p = 0.002$, $R^2 = 0.245$). Neither neighboring canopy tree (PERMANOVA: $F = 0.978$, $p = 0.45$, $R^2 = 0.10$) nor

the interaction canopy/treatment (PERMANOVA: $F = 1.264$, $p = 0.202$, $R^2 = 0.214$) had a significant effect (Fig. 5B). Specifically, pairwise differences indicated that seedlings in the Disturbed treatment differed significantly from seedlings in both the Control and Bag Control groups ($F = 3.10$, $p = 0.03$, $R^2 = 0.205$ and $F = 5.62$, $p = 0.012$, $R^2 = 0.273$ respectively), while the two control groups did not differ significantly from one another ($F = 1.458$, $p = 0.492$, $R^2 = 0.089$). Additionally, genera that were key drivers of these differences were *Inocybe*, *Dactylella*, *Glomus*, *Pachyphloeus*, *Piloderma*, *Acephala*, and *Serendipita* (Fig. 6).

The same pattern was found for the analysis on only the EMF genera identified (Fig. 5C). Overall treatment was a significant factor in determining the composition of the EMF community present on the seedlings' root tips (PERMANOVA: $F = 3.65$, $p = 0.002$, $R^2 = 0.247$), with neither neighboring canopy tree (PERMANOVA: $F = 0.986$, $p = 0.451$, $R^2 = 0.10$) nor canopy/treatment (PERMANOVA: $F = 1.27$, $p = 0.20$, $R^2 = 0.215$) having an impact. Again, the EMF community of seedlings in the Disturbed treatment differed significantly from seedlings in the Control ($F = 3.13$, $p = 0.039$, $R^2 = 0.207$) and the Bag Control ($F = 5.63$, $p = 0.006$, $R^2 = 0.273$), while they did not differ from each other ($F = 1.48$, $p = 0.48$, $R^2 = 0.090$).

Discussion

The seedling stage represents an important bottleneck in the recruitment of plant species (e.g., Haper 1977, Grubb 1977), yet recruitment patterns determine the spatial structure of tree communities (Green, Harms, & Connell, 2014). Key to understanding the forest assembly process is determining the mechanisms that drive seedlings performance and survival. The sharing of resources via MNs has the potential to be a vital mechanism during recruitment by playing a large role in facilitating seedling establishment (Simard et al., 1997;

Teste et al., 2009). Despite its potential impact, we still have little knowledge of the impact of MNs on seedling recruitment. This study aimed to quantify the contribution of the MNs associated with con-specific, con-generic, and hetero-specific adult trees of the same and different mycorrhizal type. We hypothesized that seedlings connected to the network would show improved performance – measured as higher mycorrhizal colonization, biomass, and survival – when compared to seedlings that were disconnected from the network. Furthermore, we hypothesized that this effect would be greatest for seedlings planted under species of the same mycorrhizal type (EMF), and that were more closely related phylogenetically to the seedlings. Our results corroborate these hypotheses but also shed new light on the intricacies of these interactions. DNA analysis of the roots showed that when seedlings are not connected to the MN they are being colonized by a different community of EMF. This shift in EMF colonizing species impacted seedling survival as a function of the neighboring tree species they were growing under. Seedlings benefited from connection to the MN when growing under con-specific and hetero-specific trees; however, the effect of EMF colonization when connected to the MN was negative when growing under a hetero-specific tree of the same mycorrhizal type. Furthermore, under an AMF tree, seedlings benefited more from the EMF community that colonized the roots when they were disconnected, than from the EMF community when connected to the MN. These results illustrate one more dimension involved in recruitment dynamics taking place in forest ecosystems.

We found no evidence to support that neighboring canopy trees influenced the mycorrhizal community associated with seedlings roots; however, we discovered that treatment did (Figs. 5 and 6). When MN connections were disturbed, the mycorrhizal

community associated with the plant was different than those developed when connected. Previous work has shown the importance of priority effects on EMF colonization, with EMF species that are able to colonize first often dominating the mycorrhizal community of the root tips during the early stages of seedling development (Kennedy & Bruns, 2005; Kennedy et al., 2009). Consistent disruption of the seedlings in the Disturbed treatment beginning soon after the transplantation in our experiment may have inhibited the ability of mycorrhizal symbionts to maintain the association, allowing other inoculum the opportunity to establish.

Alternatively, colonization may be governed by seedling preference for mycorrhizal symbionts. Prior research has shown that the mycorrhizal symbiotic relationship is a complex balance of mycorrhizae competing for the root trips that will provide them the most benefit, and plants opting for the more beneficial symbionts over others (Bogar et al., 2019; Werner & Kiers, 2015). One of the primary benefits of the MN is the addition of carbon, nutrients and water via fungal mycelium transfer (Egerton-Warburton, Querejeta, & Allen, 2007; Simard et al., 1997; Teste et al., 2009). The possibility of augmenting resources available to the seedling via this transfer potentially makes colonization of root tips by mycorrhizae connected to the MN more beneficial and therefore preferred by plants. However, once this connection is severed the mycorrhizal community composing the MN may now be a less beneficial symbiont than other mycorrhizae present in the surrounding soil. These differences between control and Disturbed treatments complicated our analysis, making it difficult to separate the effects of connection to the MN from the effect of a different mycorrhizal community, yet, they also indicate an important role of the MN in promoting colonization.

The effect of the mycorrhizal community on seedling performance varied depending on the canopy tree they were growing under. Under con-specific *Q. rubra* adults, EMF

colonization and biomass were unaffected by any treatment. The impact of EMF colonization of seedlings under con-specific *Q. rubra* on survival was positive for both control treatments, indicating that the mycorrhizal community that colonizes the roots when connected to the MN can facilitate seedling survival. When growing under con-generic *Q. velutina*, seedlings experienced an increase in biomass within the bag treatments; still, the amount of mycorrhizal colonization did not affect biomass although it did increase survival (Fig. 2 and 3); and unlike under *Q. rubra*, in this case the mycorrhizal community in the disrupted seedlings also had a positive effect in survival. Here, we can only speculate that this lack of effect under disrupted conditions might be not due to a lack of benefit but rather to stronger negative plant-soil-feedbacks commonly associated with con-specific adults (Liang et al., 2016; McCarthy-Neumann & Ibanez, 2013), indicating that the mycorrhizal community associated with the MN has a larger effect in ameliorating negative plant-soil-feedbacks.

Under the hetero-specific and EMF associated *C. glabra* EMF colonization was much higher than in the bagged seedlings, thus the negative effect on survival under this treatment (Fig. 3B) could be attributed to heavy colonization of a suboptimal EMF community. We observed a similar negative effect of the EMF community associated with the MN, this time in the Bagged Control treatment (which experienced highest colonization), when growing under hetero-specific, AMF associated *A. saccharum*. Mycorrhizal associations are not always mutually beneficial, and can end up being more parasitic than symbiotic, with the fungi gaining substantially more than the plant (Ibáñez & McCarthy-Neumann, 2014). We also observed that under *A. saccharum* disturbed seedlings had the highest biomass and the effect of EMF on survival was positive (unlike the bagged treatment). Previous work has shown that hetero-specific EMF seedlings typically perform well in soil collected from near

A. saccharum (McCarthy-Neumann & Kobe, 2010; McCarthy-Neumann and Ibáñez 2012); this effect had been attribute to the presence of differing microbial pathogens under *A. saccharum* that have less effect on EMF hetero-specifics; but, these experiments were done in a greenhouse setting, and here we show that this positive effect may only take place if disconnected from the MN. Other work has shown the negative effect that growing near AMF neighbors can have on other plants (e.g., Bennett et al., 2017); in our study we got one step closer to the mechanisms that may be driving that pattern, suboptimal symbionts included in the MN mycelium.

The lack of differences in *Q. rubra* seedling survival based upon species of neighboring tree runs counter to previous studies that examined the effects of con-specific negative density dependence. Irrespective of connection to the MN past work indicates that seedlings should experience lower biomass and survival in con-specific environments (Bennett et al., 2017). However, we found no evidence of this. This could owe to the fact that seedlings in our experiment were planted with acorns attached, offering seedlings a large store of additional resources from which to draw on during the single growing season they were left in the field (Ofcarcik & Burns, 1971). It is possible that if left in the field for longer differential effects of neighboring canopy trees would be more apparent. The positive effect of maternal effects on seedling biomass is consistent with previous studies, as initial resource stores has a strong positive impact on first year seedling growth (Ibáñez & McCarthy-Neumann, 2016). The lack of effect of other covariates is initially surprising, especially given that light is such a limiting factor for seedlings on the forest floor. However, a lack of variability between measurements in both light and soil moisture likely explains this discrepancy (see Sup. Fig. S1).

Conclusions

The findings in this study underline the importance of the MN in the recruitment of a common canopy tree in the temperate forests of Northeast North America. However, these effects are highly variable, ranging from beneficial to sub-optima, and based upon the species and mycorrhizal type associated with neighboring canopy trees. Additionally, this study finds the importance of connection to the MN in establishing the most beneficial mycorrhizal community colonizing a seedling's roots, highlighting the potentially important role additional resources that are supplied via the MN in making mycorrhizal associations mutually beneficial for host and symbiont. Future work on temperate forest recruitment should factor in the degree to which MNs are present or absent, as roles of mycorrhizae on seedling performance can shift accordingly.

Figures

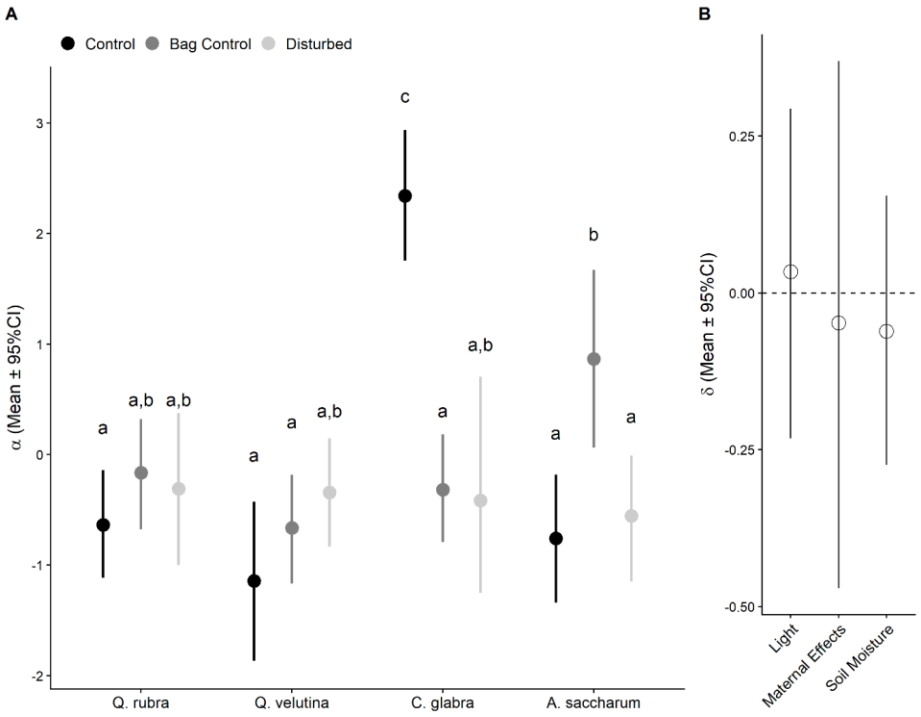

Figure 1 – Results from the mycorrhizal colonization analysis, mean parameter posterior values and 95% credible intervals (CI). (A) Parameter α for each treatment/neighbor canopy tree combinations organized by phylogenetic relatedness to *Q. rubra* seedlings, *A. saccharum* is AMF and all other canopy trees are EMF. Overlapping 95% credible intervals (CI) indicate combinations do not differ from each other, indicated by similar letters. (B) Parameter δ values associated to the covariates included in the analysis; coefficients which 95%CIs overlap with zero are not statistically significant.

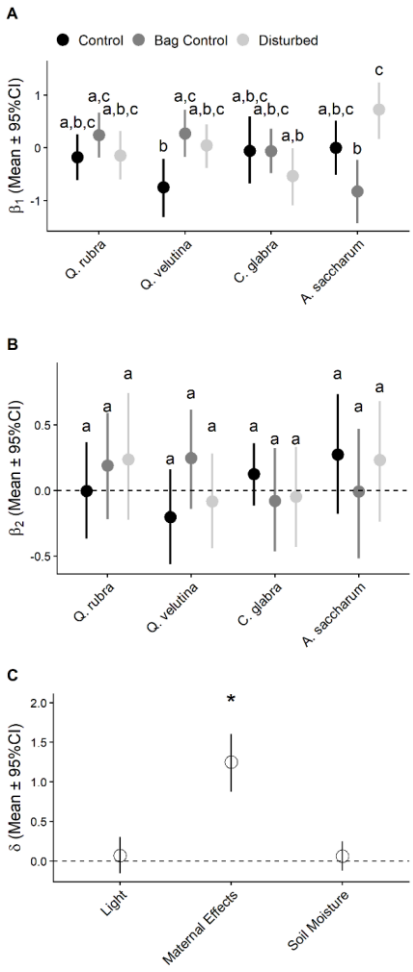

Figure 2 - Mean Posterior values and 95% credible intervals (CI) for the model parameters of biomass. Canopy trees in (A) and (B) are ordered by phylogenetic relatedness to *Q. rubra* seedlings, *A. saccharum* is AMF and all other canopy trees are EMF. (A) represents effect of canopy tree species and treatment on biomass; (B) display the effects of EMF colonization across neighboring canopy tree species and treatment; (D) represents the effects of covariates on seedling biomass. Asterisks represent values that differ significantly from zero (covariates), and different letters display significant differences between parameter values.

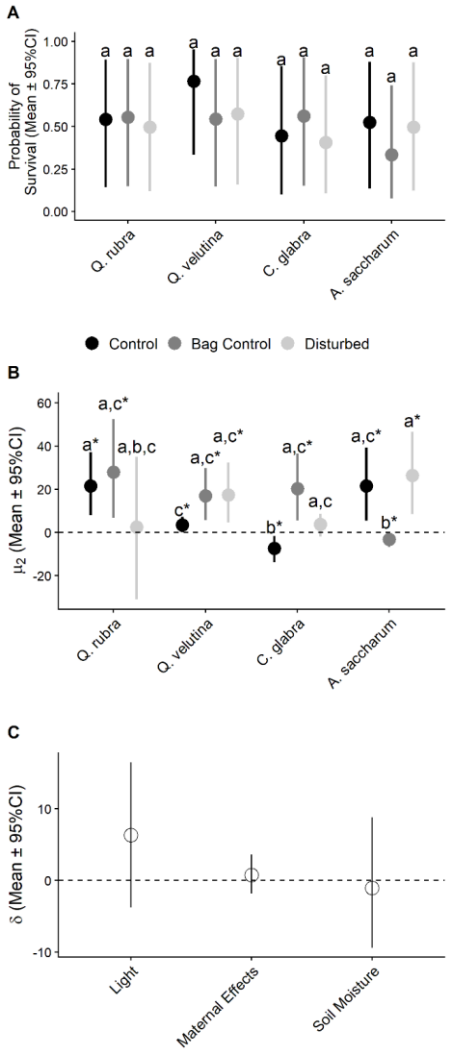

Figure 3 - Mean Posterior values and 95% credible intervals of the parameters associated to the seedling survival model. Canopy trees in (A) and (B) are ordered by phylogenetic relatedness to *Q. rubra* seedlings, *A. saccharum* is AMF and all other canopy trees are EMF. (A) Survival (back-transformed parameter μ_1) across neighboring canopy tree and treatments under average mycorrhizal colonization and average covariate values. (B) Display of the effects of EMF colonization on survival. (D) Effects of covariates on seedling survival. Asterisks represent values that differ significantly from zero, and different letters display significant differences between parameter values.

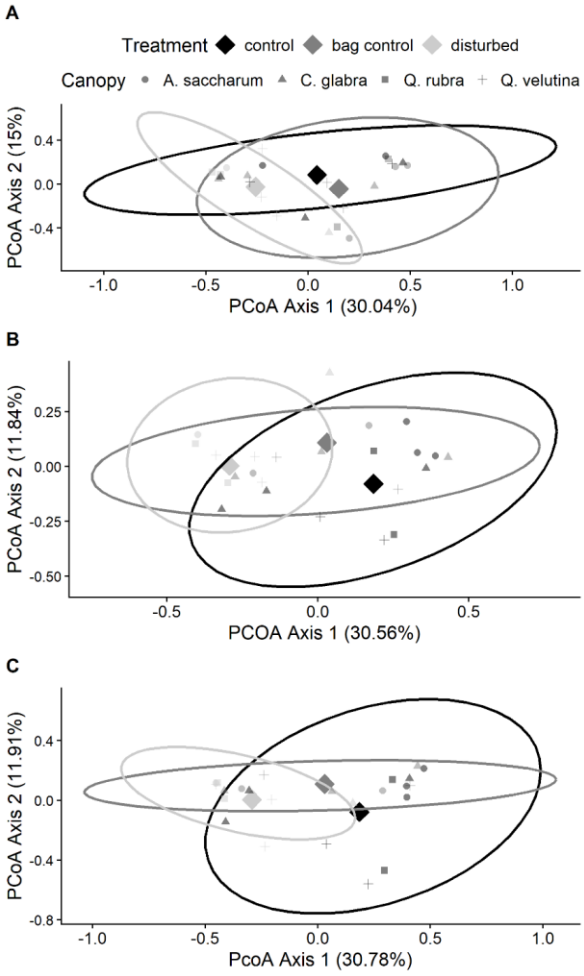

Figure 5 – Principle Coordinate Analysis (PCoA) for all fungal genera identified in the samples (A), only mycorrhizal genera (B), and only ectomycorrhizal genera (C). Ellipses represent 95% confidence interval around the points and large diamonds indicate centroid of the points. Across all three groupings treatment had a significant effect on community.

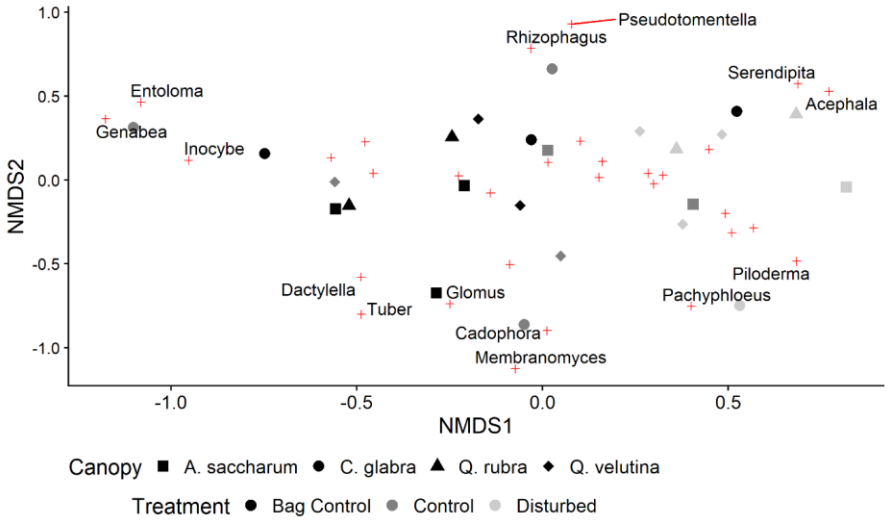

Figure 6 – NMDS for mycorrhizal genera (k = 3, stress = 0.155). Shape and color combinations represent the ordination of the mycorrhizal communities of associated with the twelve plots, red crosses indicate particular fungal genera. For ease of viewing only those genera furthest from one another are labeled. Distance between points represents rank dissimilarity calculated using the Bray-Curtis distance. Treatment was the only factor that had a significant effect on mycorrhizal community, with communities in the Disturbed treatment (Blue) differing from the two control treatments.

Supplementary Materials

Tables

Supplementary Table S1 – Sources of acorns utilized in this study. Seedlings were randomly selected during planting from the three populations.

Source No.	Source
1	Michigan Wild Types
2	Sheffield, PA
3	Sheffield, IL

Supplementary Table S2 – Summary of measured ectomycorrhizal (EMF) colonization in 2016 and 2018 and arbuscular Mycorrhizal (AMF) colonization in 2016. Values represent mean and standard deviation across all plots within a neighboring canopy tree.

Neighboring Canopy Tree	Treatment	Mean EMF ± SD	Mean AMF ± SD
A. saccharum	C	0.11 ± 0.15	0.02 ± 0.05
A. saccharum	BC	0.15 ± 0.14	0.02 ± 0.04
A. saccharum	D	0.08 ± 0.08	0.02 ± 0.04
C. glabra	C	0.37 ± 0.21	0.04 ± 0.03
C. glabra	BC	0.17 ± 0.14	0.01 ± 0.02
C. glabra	D	0.11 ± 0.11	0.02 ± 0.02
Q. rubra	C	0.16 ± 0.17	0.01 ± 0.02
Q. rubra	BC	0.18 ± 0.14	0.01 ± 0.01
Q. rubra	D	0.07 ± 0.13	0.00 ± 0.01
Q. velutina	C	0.15 ± 0.11	0.04 ± 0.05
Q. velutina	BC	0.11 ± 0.10	0.02 ± 0.02
Q. velutina	D	0.10 ± 0.10	0.02 ± 0.03

Supplementary Table S3 – Summary of measured biomass. Values represent mean and standard deviation, as well as the range of biomass of living seedlings across all plots within a neighboring canopy tree and across years.

Neighboring Canopy Tree	Treatment	Mean Biomass ± SD	Range
A. saccharum	C	1.35 ± 0.58	(0.72 , 2.84)
A. saccharum	BC	1.56 ± 0.96	(0.4 , 3.84)
A. saccharum	D	1.22 ± 0.52	(0.18 , 2.42)
C. glabra	C	1.45 ± 0.81	(0.44 , 2.94)
C. glabra	BC	1.99 ± 1.15	(0.27 , 4.32)
C. glabra	D	1.69 ± 1.12	(0.18 , 4.66)
Q. rubra	C	1.23 ± 0.69	(0.38 , 2.67)
Q. rubra	BC	1.35 ± 0.88	(0.48 , 3.42)
Q. rubra	D	1.39 ± 1.01	(0.25 , 3.2)
Q. velutina	C	1.62 ± 0.64	(0.64 , 3.49)
Q. velutina	BC	1.39 ± 0.54	(0.73 , 2.85)
Q. velutina	D	1.49 ± 0.65	(0.46 , 3.34)

Supplementary Table S4 - Summary of survival. Values represent total seedlings across all plots under the different canopy tree neighbors alive following transplant (N) and total surviving at the end of the experiment (Total Alive), and the proportion that survived.

Neighboring Canopy Tree	Treatment	N	Total Alive	Prop Surviving
A. saccharum	C	26	17	0.65
A. saccharum	BC	49	39	0.80
A. saccharum	D	46	39	0.85
C. glabra	C	30	19	0.63
C. glabra	BC	57	45	0.79
C. glabra	D	56	49	0.88
Q. rubra	C	33	13	0.39
Q. rubra	BC	57	26	0.46
Q. rubra	D	53	24	0.45
Q. velutina	C	31	19	0.61
Q. velutina	BC	55	41	0.75
Q. velutina	D	55	44	0.80

-

Supplementary Table S5 – Deviance Information Criterion (DIC) for all models tried. Lowest DIC value represents the best model.

Model	DIC
No PRE	2446
No PRE, Plot RE in Habitat	2429
No PRE, plot RE in Habitat, YearRE	2415
EMF in Survival	2131
Final Model	2073

Supplementary Table S6 - Mean posterior values (±SD) and 95% credible intervals for Ectomycorrhizal Colonization Model. Parameters with same letters indicate they do not significantly differ from each other within that parameter. Parameters with an asterisk indicate statistically significant covariates.

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Parameter	Neighboring Canopy Tree	Treatment	Mean Posterior Value ± SD	95% CI	sig
alpha	A. saccharum	Control	-0.7596 ± 0.2941	(-1.3387 , -0.1813)	a
alpha	A. saccharum	Bag Control	0.8653 ± 0.4139	(0.0648 , 1.6714)	b
alpha	A. saccharum	Disturbed	-0.5558 ± 0.2916	(-1.1493 , -0.0069)	a
alpha	C. glabra	Control	2.3422 ± 0.3016	(1.7564 , 2.9394)	c
alpha	C. glabra	Bag Control	-0.3171 ± 0.2452	(-0.7926 , 0.1802)	a
alpha	C. glabra	Disturbed	-0.4145 ± 0.4675	(-1.2529 , 0.7042)	a,b
alpha	Q. rubra	Control	-0.6358 ± 0.2473	(-1.1158 , -0.1419)	a
alpha	Q. rubra	Bag Control	-0.1634 ± 0.2517	(-0.677 , 0.32)	a,b
alpha	Q. rubra	Disturbed	-0.3108 ± 0.3793	(-1.0023 , 0.3763)	a,b
alpha	Q. velutina	Control	-1.1452 ± 0.3766	(-1.8678 , -0.4253)	a
alpha	Q. velutina	Bag Control	-0.6644 ± 0.2476	(-1.1648 , -0.1848)	a
alpha	Q. velutina	Disturbed	-0.3429 ± 0.2490	(-0.8341 , 0.1486)	a,b
delta1(Maternal Effects)			-0.0477 ± 0.2165	(-0.4706 , 0.3691)	
delta2(Liight)			0.0335 ± 0.1326	(-0.2322 , 0.2934)	
delta3(Soil Moisture)			-0.0614 ± 0.1079	(-0.2742 , 0.1552)	
variance			1.1797 ± 0.1176	(0.9606 , 1.4203)	
variance(PYRE)			0.1847 ± 0.1053	(0.0282 , 0.3977)	

Supplementary Table S7 - Mean posterior values (±SD) and 95% credible intervals for Biomass Model. Within each parameter, treatments with same letters indicate they do not significantly differ from each other. Parameters with an asterisk indicate statistically significant covariates.

Parameter	Neighboring Canopy Tree	Treatment	Mean Posterior Value ± SD	95% CI	sig
beta1	A. saccharum	Control	-0.0018 ± 0.2619	(-0.5168 , 0.5142)	a,b
beta1	A. saccharum	Bag Control	-0.8283 ± 0.3067	(-1.4343 , -0.2338)	a
beta1	A. saccharum	Disturbed	0.7249 ± 0.2733	(0.167 , 1.2424)	b
beta1	C. glabra	Control	-0.0564 ± 0.3264	(-0.6836 , 0.5914)	a,b
beta1	C. glabra	Bag Control	-0.0641 ± 0.2130	(-0.4837 , 0.3585)	a,b
beta1	C. glabra	Disturbed	-0.5378 ± 0.2759	(-1.0976 , -0.0097)	a,c
beta1	Q. rubra	Control	-0.1776 ± 0.2177	(-0.6136 , 0.2489)	a,b
beta1	Q. rubra	Bag Control	0.2415 ± 0.2178	(-0.192 , 0.6654)	b,c
beta1	Q. rubra	Disturbed	-0.1474 ± 0.2338	(-0.6038 , 0.3158)	a,b
beta1	Q. velutina	Control	-0.7490 ± 0.2790	(-1.3167 , -0.2172)	a
beta1	Q. velutina	Bag Control	0.2715 ± 0.2283	(-0.1711 , 0.7237)	b,c
beta1	Q. velutina	Disturbed	0.0472 ± 0.2085	(-0.381 , 0.4436)	a,b
beta2	A. saccharum	Control	0.2759 ± 0.2334	(-0.1784 , 0.7365)	a
beta2	A. saccharum	Bag Control	-0.0069 ± 0.2531	(-0.5181 , 0.4716)	a
beta2	A. saccharum	Disturbed	0.2325 ± 0.2345	(-0.2371 , 0.6829)	a
beta2	C. glabra	Control	0.1253 ± 0.1219	(-0.1149 , 0.3619)	a
beta2	C. glabra	Bag Control	-0.0779 ± 0.2024	(-0.4639 , 0.3237)	a
beta2	C. glabra	Disturbed	-0.0475 ± 0.1953	(-0.4313 , 0.3332)	a
beta2	Q. rubra	Control	-0.0033 ± 0.1880	(-0.3675 , 0.3688)	a
beta2	Q. rubra	Bag Control	0.1916 ± 0.2085	(-0.2201 , 0.5921)	a
beta2	Q. rubra	Disturbed	0.2375 ± 0.2469	(-0.2246 , 0.7445)	a
beta2	Q. velutina	Control	-0.2038 ± 0.1858	(-0.5631 , 0.1616)	a
beta2	Q. velutina	Bag Control	0.2473 ± 0.1940	(-0.1391 , 0.619)	a
beta2	Q. velutina	Disturbed	-0.0818 ± 0.1851	(-0.4413 , 0.2812)	a
delta1(Maternal Effects)			1.2488 ± 0.1854	(0.8742 , 1.6029)	*
delta2(Liight)			0.0691 ± 0.1158	(-0.1533 , 0.3027)	
delta3(Soil Moisture)			0.0626 ± 0.0942	(-0.122 , 0.2505)	
variance			0.6729 ± 0.0553	(0.5659 , 0.7806)	
variance(PYRE)			0.1467 ± 0.0759	(0.0303 , 0.2958)	

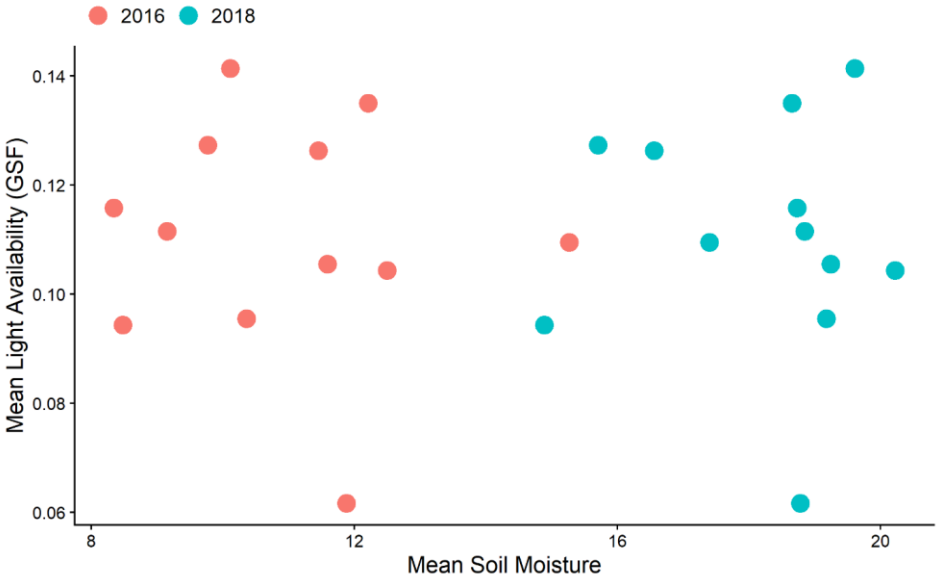
Supplementary Table S8 - Mean posterior values (±SD) and 95% credible intervals for Survival Model. Parameters with same letters indicate they do not significantly differ from each other within that parameter. Parameters with an asterisk indicate statistically significant covariates.

Parameter	Neighboring Canopy Tree	Treatment	Mean Posterior Value ± SD	95% CI	sig
mu1	A. saccharum	Control	0.0975 ± 0.9931	(-1.8646 , 2.0041)	a
mu1	A. saccharum	Bag Control	-0.6939 ± 0.9083	(-2.4844 , 1.0562)	a
mu1	A. saccharum	Disturbed	-0.0146 ± 0.9979	(-1.9673 , 1.9579)	a
mu1	C. glabra	Control	-0.2194 ± 1.0104	(-2.1807 , 1.7646)	a
mu1	C. glabra	Bag Control	0.2504 ± 1.0092	(-1.714 , 2.2485)	a
mu1	C. glabra	Disturbed	-0.3780 ± 0.8958	(-2.1323 , 1.3836)	a
mu1	Q. rubra	Control	0.1665 ± 0.9984	(-1.7861 , 2.1262)	a
mu1	Q. rubra	Bag Control	0.2159 ± 0.9946	(-1.7436 , 2.1615)	a
mu1	Q. rubra	Disturbed	-0.0136 ± 1.0103	(-2.0081 , 1.9387)	a
mu1	Q. velutina	Control	1.1822 ± 0.9416	(-0.6923 , 2.9956)	a
mu1	Q. velutina	Bag Control	0.1784 ± 0.9980	(-1.7524 , 2.1593)	a
mu1	Q. velutina	Disturbed	0.2986 ± 1.0013	(-1.6743 , 2.2448)	a
mu2	A. saccharum	Control	21.5651 ± 8.8957	(5.4905 , 39.3532)	a,c*
mu2	A. saccharum	Bag Control	-3.2114 ± 1.5964	(-6.5716 , -0.4637)	b*
mu2	A. saccharum	Disturbed	26.4080 ± 10.0607	(8.5434 , 46.4557)	a*
mu2	C. glabra	Control	-7.3540 ± 3.1365	(-13.771 , -1.648)	b*
mu2	C. glabra	Bag Control	20.3075 ± 8.3815	(5.5189 , 36.4812)	a,c*
mu2	C. glabra	Disturbed	3.7880 ± 2.5659	(-1.865 , 8.6415)	a,c
mu2	Q. rubra	Control	21.4654 ± 7.6375	(7.9596 , 37.1634)	a*
mu2	Q. rubra	Bag Control	27.9426 ± 12.0745	(6.8654 , 52.471)	a,c*
mu2	Q. rubra	Disturbed	2.4544 ± 20.0496	(-30.8916 , 34.9578)	a,b,c
mu2	Q. velutina	Control	3.5045 ± 1.6489	(0.2151 , 7.1785)	c*
mu2	Q. velutina	Bag Control	17.0181 ± 6.4318	(5.7683 , 29.8281)	a,c*
mu2	Q. velutina	Disturbed	17.3795 ± 7.3602	(4.5597 , 32.3381)	a,c*
delta1(Maternal Effects)			0.7775 ± 1.3853	(-1.8097 , 3.5975)	
delta2(Liight)			6.3271 ± 5.8994	(-3.7548 , 16.4839)	
delta3(Soil Moisture)			-1.0342 ± 4.5168	(-9.3468 , 8.7969)	
variance			563.1959 ± 375.8329	(26.4904 , 1308.7916)	

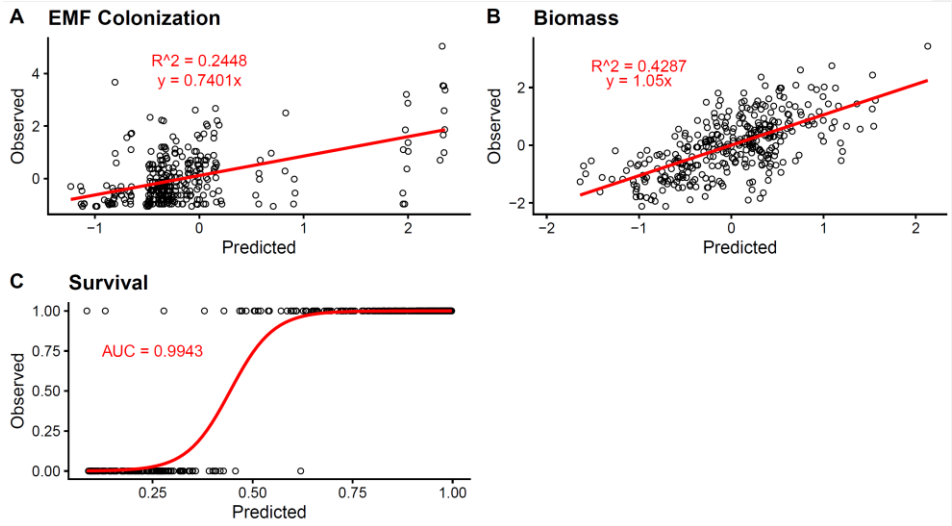
Supplementary Table S9- Mean and standard deviation of Shannon and Simpson diversity metrics, calculated for neighboring canopy trees and Treatments. No significant differences were found.

Habitat	Treatment	Shannon	SD1	Simpson	SD2
A. saccharum	BC	15.000	5.568	16.333	5.033
A. saccharum	C	14.000	11.314	11.500	9.192
A. saccharum	D	2.000	NaN	2.000	NaN
C. glabra	BC	11.667	4.509	11.000	4.583
C. glabra	C	17.667	6.506	16.667	6.429
C. glabra	D	4.000	NaN	3.000	NaN
Q. rubra	BC	11.000	11.314	13.000	12.728
Q. rubra	D	5.000	5.657	5.000	5.657
Q. velutina	BC	12.500	6.364	14.000	8.485
Q. velutina	C	17.500	3.536	16.000	4.243
Q. velutina	D	13.667	9.018	14.667	8.505

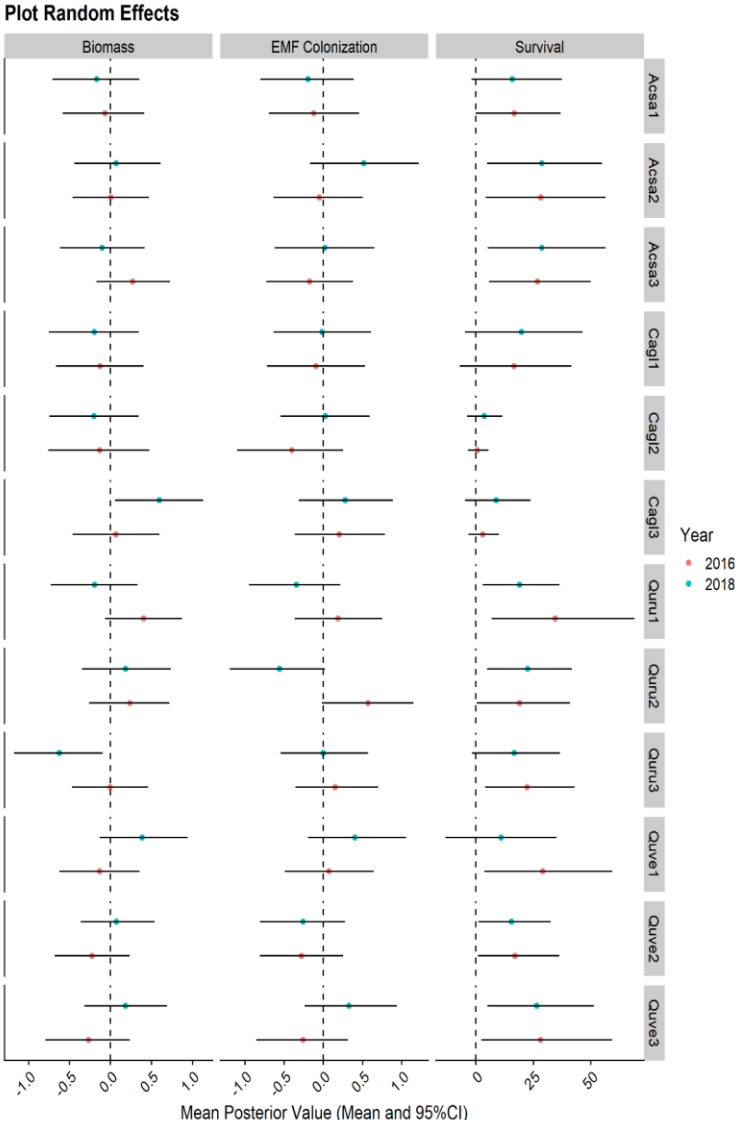
Figures



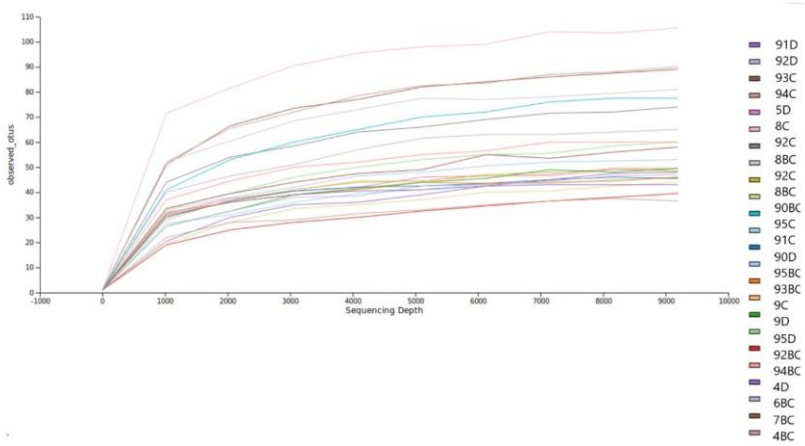
Supplementary Fig. S1 – Relationship between soil moisture and light availability measured as the global site factor (GSF), showing that range of both light and soil moisture were relatively narrow.



Supplementary Fig. S2 – Observed vs. Predicted values for (A) ectomycorrhizal colonization, (B) arbuscular mycorrhizal colonization (C) biomass and (D) Survival.



Supplementary Fig. S3 – Mean posterior values and the 95% credible interval (CI) for plot and year random effects for all plots and both 2016 (red) and 2018 (blue) for the three models included in this study. Dashed line represents zero line. No clear pattern is evident.



Supplementary Fig. S4 – Alpha rarefaction curves calculated using QIIME 2 command alpha-rarefy at a depth of 9,195. Most samples are close to asymptotic indicating desired sampling depth was reached.

OpenBUGS Code

```
model{

for( i in 1:548){

emfS[i]~dnorm(ECTOm[i], tau[1])
emf.p[i]~dnorm(ECTOm[i], tau[1])
ECTOm[i]<-alpha1[hab[i],treat[i]]+delta[1,1]*stan_mat[i]+delta[1,2]*gsfS[i]+delta[1,3]+
PYRE1[plot[i],year[i]]

biomS[i]~dnorm(B[i], tau[2])
biom.p[i]~dnorm(B[i], tau[2])
B[i]<-beta1[hab[i],treat[i]]+beta2[hab[i],treat[i]]*emfS[i]+delta[2,1]*stan_mat[i]+
delta[2,2]*gsfS[i]+delta[2,3]*smS[i]+PYRE2[plot[i],year[i]]

status_end[i]~dbern(pp[i])
surv.p[i]~dbern(pp[i])
pp[i]<-max(0,p0[i])
logit(p0[i])<-mu1[hab[i],treat[i]]+mu2[hab[i],treat[i]]*emfS[i]+
delta[3,1]*stan_mat[i]+delta[3,2]*gsfS[i]+delta[3,3]*smS[i]+PYRE3[plot[i],year[i]]

}

#priors

for(h in 1:4){
  for(t in 1:3){
  alpha1[h,t]~dnorm(0,0.0001)
  beta1[h,t]~dnorm(0,0.0001)
  mu1[h,t]~dnorm(0,00001)
  beta2[h,t]~dnorm(0,0.001)
  mu2[h,t]~dnorm(0,0.001)
  }
}

for(a in 1:3){
for (i in 1:3){
  delta[a,i]~dnorm(0,0.001)
```

```
}
}

for(p in 1:12){
  for(y in 1:2){
  PYRE1[p,y]~dnorm(0,tau[3])
  PYRE2[p,y]~dnorm(0,tau[4])
  PYRE3[p,y]~dnorm(0,tau[5])
}}
for(i in 1:5){
  tau[i]<- 1/variance[i]
  variance[i]~dgamma(0.001,0.001)
}

} #end model
```

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