THE EFFECT OF URBANIZATION ON THE MYCORRHIZAL ASSOCIATIONS AND SURVIVAL OF THREE SPECIES OF EASTERN HARDWOODS

by

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Abstract:

Mycorrhizal fungi colonization can be a significant determinant of plant health and establishment success. By protecting roots from pathogens and increasing plant uptake of nutrients and water, mycorrhizal colonization can determine the outcome of competitive interactions between plants, thereby shaping plant community composition. Currently, in remnant forest patches, plants and their fungal symbionts are exposed to varied anthropomorphic effects related to the encroachment of metropolitan areas into rural landscapes. However, little is known about the impact of urbanization on the plant-mycorrhizal fungi association. To assess the effect of urbanization on mycorrhizal fungi root colonization and their role on seedling establishment, we investigated the relationship between mycorrhizal colonization of tree seedlings and seedling survival along an urbanization gradient typical of the mid-western region of the USA. We planted three species of temperate tree seedlings (*Acer rubrum*, *Carya ovata*, and *Quercus rubra*) in each of three landscape types: urban, suburban, and rural forests. We measured the percent of root length of the seedlings colonized by ectomycorrhizal (ECM) and arbuscular mycorrhizal fungi (AMF) and monitored seedling survival during their first growing season. We analyzed the percent root length colonized by mycorrhizae as a function of landscape type (urban-rural) and additional variables known to contribute mycorrhizal colonization (soil phosphorus, soil nitrogen, and initial plant height). We then analyzed seedling survival as a function of the degree of mycorrhizal fungi colonization associated with the landscape gradient and of additional environmental factors (available light and soil moisture).

Within a species, we found no changes in levels of mycorrhizal fungi colonization across the urban landscape gradient. Environmental variables (light, soil moisture, soil nutrients) did not significantly vary along the urban gradient, and differences in these variables did not have a measureable effect upon mycorrhizal colonization or survival. Each seedling species had markedly different levels of colonization and responded differently to increasing levels of mycorrhizal colonization. For *A. rubrum*, survival was independent of mycorrhizal colonization, *Q. rubra* had a statistically non-significant rise in survival as colonization increased, and *C. ovata* had a significant positive survival response to more than 60 % colonization. These findings highlight the resilience of mycorrhizal communities across the rural-urban gradient and the potential sensitivity of some species to lower levels of mycorrhizal colonization.

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I'd like to dedicate this work to the memory of my father, Richard Kelley, and to the loving support of my mother, Patricia Kelley. In the midst of pain, suffering, and loss, they remained present for me. My father wanted so much to live to see me complete this work. I am grateful that I was able to spend time with him during his final months and also continue work on my education from afar. I am also grateful for the unwavering support of many family members and friends, and most notably my husband, David Tonn.

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Introduction:

The plant-mycorrhizal fungi association is one of the most ubiquitous species interactions in terrestrial ecosystems **(**Brundrett 2009**).** For most plants, this association is essential; not only do plants grow less without their mycorrhizal partners, but during seedling stages, survival is much less likely in the absence of mycorrhizal fungal colonization (Menkis et al. 2007). The degree and effect of mycorrhizal fungi colonization can be greatly affected by the environment (Tinker & Gildon 1983; Treseder 2004). Thus, any changes in environmental conditions, like those taking place in urbanized landscapes, could affect this symbiosis.

Seedling survival has decreased in some urban areas (Broshot 2007; Lehvavirta et al. 2014). This may be due, in part, to the effects of pollution and drought, which have been well studied in urban environments (Guerrero et al. 2013; Gillner et al. 2013; McDonald & Urban 2004). Although the below-ground dynamics of the urban forest are less well known, there is a growing body of evidence suggesting that what happens below-ground is just as crucial to ecosystems, and maybe even more so, than many of the changes observed on the surface (O'Brien et al. 2011; Horton et al. 1999; Horton et al. 2005). Potentially, mycorrhizal fungi species and the degree to which they colonize plant roots could be affected by the micro-climate, soil nutrients or heavy metal accumulation, and limited dispersal characteristic of urban environments (Treseder 2013; Fitter et al. 2004; Bainard et al. 2011). Because mycorrhizal fungi are critical drivers of forest population dynamics (Hetrick et al. 1989), a better understanding of the interaction between plants and mycorrhizal fungi within the urban environment will assist in better assessment and management of urban forests.

Mycorrhizal fungal symbionts provide many benefits to their host plant in exchange for carbohydrates produced via photosynthesis. Their large hyphal networks increase the absorptive surface area of the roots and provide greater access to water and nutrients (Hohenheim 1994; Allen et al. 2003; Augé 2001). Thus, mycorrhizae can often increase the drought tolerance of a plant (Augé 2001) by giving the host plant a cue to close the stomata sooner and/or increase the absorptive surface area of the roots allowing the plant to use what little water may be available (Augé 2001). Mycorrhizal associations increase the amount of phosphorus and biomass in multiple genera of plants (Treseder 2013). As a result of providing carbohydrates to the fungus and receiving important limiting nutrients in return, plants are able to grow more above-ground biomass than they would without the fungus (Allen et al. 2003). This increase in biomass can be key in determining the outcome of competitive interactions between plant species (Bray et al. 2003

There are two major groups of mycorrhizal fungi that colonize tree species. Arbuscular mycorrhizal fungi (AMF) colonize plant roots intra-cellularly and are the oldest group of mycorrhizae (400 mya), and it is believed that at one time all land plants formed relationships with AMF (Cairney 2000). The second mycorrhizal group, ectomycorrhizal fungi (EMF), colonize plant roots extracellularly (Cairney 2000). Although approximately 1.9% of vascular plants form relationships with EMF, the majority of EMF hosts are trees and shrubs (Brundrett 2009). Trees are colonized by a variety of different mycorrhizal species, but in most cases, mature trees tend to form relationships predominantly with one of the groups, either EMF or AMF (Cairney 2000; Wang & Qiu 2006). There are many notable exceptions to this phenomenon, but young tree seedlings in particular, especially *Quercus* and *Carya*, have been known to form relationships with both EMF and AMF

species (Dickie et al. 2002).

The plant-soil-fungus association has been found to play a large role in seedling success for many different species of trees (O'Brien et al. 2011; Horton et al. 1999; Nara & Hogestsu 2004). For example, the lack of suitable mycorrhizal species explains why exotic Douglas-fir has not dispersed far from plantations on Isla Victoria, Argentina yet the trees easily grow off the plantations when mycorrhizal inoculum from con-specifics is added at the time of planting (Nunez 2009). When mycorrhizae are removed by fungicide applications, non-mycorrhizal species that were previously sub-dominant experience competitive release resulting in more species diverse plots in the absence of the dominant mycorrhizal-dependent competitor (Hartnett & Wilson 1999). Therefore, by limiting the survival of some species and/or facilitating the growth and survival of others, the aggregate mycorrhizal community present in the soil can shape the structure of the above-ground plant community (Hartnett & Wilson 1999; Van Der Heijden et al. 1998; Teste & Simard 2008).

The level and nature of the mycorrhizal fungi association can vary according to local conditions. The biotic underground environment associated with adult trees can cause substantial changes in seedling success dependent upon whether they are con-specific or heterospecific trees (Van Der Heijden & Horton 2009). For example, *Quercus rubra* seedlings had higher growth rates and mycorrhizal colonization when grown near other *Quercus* species than when grown near *Acer rubrum* trees (Dickie et al. 2002). Environmental conditions such as light, soil moisture, and soil nutrients, also play a role in the plant-fungal relationship. Low light conditions may cause the plant-fungal relationship to be parasitic, weakening the plant for the benefit of the fungus (Ibáñez & McCarthy-Neumann 2016). In general, mycorrhizal colonization increases with increasing soil

moisture, but plants may benefit the most from mycorrhizal fungi under low water environments (Martinez-Garcia 2015; Hartnett & Wilson 1999). Nutrients also change the nature of the symbiosis. Plants in general, do not have as much mycorrhizal fungal colonization when N and P levels are high (Treseder 2004). Phosphorus especially controls the mycorrhizal relationship; effects of mycorrhizal fungi on plants are positive when phosphorus is limited and negative when phosphorus is high (Propster & Johnson 2015; Johnson et al. 2015). The mycorrhizal relationship may also shift from mutualism to commensalism or parasitism dependent on the N:P ratio, tending towards mutualism in P limited systems and parasitism in N limited systems (Johnson et al. 2015).

As forests along urbanization gradients experience varying conditions, it is likely that the plant-mycorrhizal fungi association is altered. Urban forests usually are small, isolated forest fragments (Ruddiman 2013). Compared to their rural counterparts, urban forests experience increased temperature due to heat island effects (Oke 1973), an increase in N deposition (Hosseini Bai et al. 2015; O'Brien et al. 2012), and heavy metal accumulation (Sun et al. 2009). Although plant-mycorrhizal associations seem to be very resilient to disturbances, they can persist in the soil despite clear-cutting and weathering (Haug et al. 2013), mycorrhizal fungi can be affected detrimentally by certain environmental conditions present in most urban environments. Karpati et al. (2011) found less mycorrhizal diversity in urban and highly disturbed soils and explained that pollution and increased anthropogenic deposition of nitrogen might have been a cause of this shift. Some AMF fungi have shown failure to sporulate under high nitrogen conditions (Egerton-Warburton & Allen 2000) and hyphal growth is often decreased under N deposition (Treseder & Allen 2000). In addition, heavy metals such as zinc and lead can inhibit or reduce mycorrhizal colonization (Yang et

al. 2015). Soil aggregation is also a positive mycorrhizal indicator; in soils with more recent physical disturbance and smaller soil aggregates, mycorrhizal communities are less diverse (Duchicela 2013). In an extensive study of Canadian urban forests, Bainard et al. (2011) documented that fewer or potentially different mycorrhizal fungi are present at urban sites than rural sites of the same region. However, Rillig et al. (2002) found that mycorrhizal colonization increases with artificially increased temperature, so we can likely expect multiple opposing forces acting on mycorrhizal communities within urban forests.

Despite the potential consequences of plants lacking or altering their mycorrhizal symbionts, we know little about how changes in the mycorrhizal community along urban gradients might affect plant recruitment. Thus, if we want to assess urban forest resilience and forecast future forest dynamics in urban systems, we need to know more about environmental effects of urbanization on the plant-mycorrhizal fungi relationship.

This study investigates the mycorrhizal fungi associations in establishing seedlings of three dominant tree species in the northeastern USA, *Quercus rubra*, *Acer rubrum*, and *Carya ovata*, across an urbanization gradient and quantifies the effects of the fungal associations on seedling survival. We hypothesized that mycorrhizal colonization of tree seedlings may shift along a rural-to-urban gradient due to the environmental conditions associated with urbanization, i.e., increase N and light and decreased soil moisture. We additionally expected that survival would shift along a gradient of mycorrhizal fungal colonization, i.e., from parasitism to mutualism mediated by availability of resources. We address the following questions: 1) Are plant-mycorrhizae symbiosis maintained across an urban gradient? And, 2) what is the role of mycorrhizal colonization on seedling survival across an urban gradient? Answers to these questions will assist in better assessments of

how urban forests may be affected by potential changes in mycorrhizal fungi colonization of tree seedlings.

Methods:

Study Sites and Experimental Design

Study sites were located in and near Ann Arbor, Michigan in the Great Lakes Region of the United States (Table 1 and Figure 1**)**. Ann Arbor has a population of 118,000 (US Census 2010) in an area of 27.8 square miles (US Census 2010). Approximately 33% of the area within city boundaries is covered by tree canopy and 24% of the urban forests are on public land and managed green spaces (City of Ann Arbor 2014). The city of Ann Arbor is representative of many small/medium-size cities in the eastern part of the USA, which make up the majority of North American cities (US Census 2014). Nine oak-hickory forest sites were selected on an urban-rural gradient with 3 rural, 3 suburban, and 3 urban forest sites. Rural sites had 0-2% impervious surface in the area within a 1 km radius, suburban sites were located closer to the city and had 3-14% impervious surface, urban sites were located within city limits and had 14-30% impervious surface within 1 km.

We planted seedlings of three tree species dominant in the local forests, *Quercus rubra*, *Acer rubrum*, and *Carya ovata* under the canopy of trees of the same species. *Quercus rubra* is a relatively fast-growing tree with intermediate shade tolerance (Barnes & Wagner 2004). *Q. rubra* acorns average 7.51 g (Miao 1995). *Carya ovata* is a slow growing tree with shade tolerant seedlings (Barnes & Wagner 2004). *C. ovata* nuts average 4.54 g (Burns & Honkala 1990). As adults, both *Q. rubra* and *C. ovata* are generally colonized by ectomycorrhizal fungi but as seedlings can also be colonized by arbuscular mycorrhizal

fungi (Dickie et al. 2002; Comas & Eissenstat 2009). *Acer rubrum* traditionally has occupied swamps and lowland sites, but has expanded its range into the understory of many different types of forests due to fire exclusion (Abrams & Ruffner 1995). The seedlings are shade tolerant, moderately fast-growing, and present in most forests types of the region. Average seed weight is 0.015 g per samara (Burns & Honkala 1990). *A. rubrum* is an arbuscular mycorrhizal species (Phillips & Fahey 2006).

Under each canopy tree, one per species and site, we established 1.25-m x 1.25-m plots in which we planted 10 seedlings of each species (see planting methods below). Each plot was located 50 cm from the base of the canopy tree. We planted a total of 810 seedlings (3 landscape types x 3 replicates x 3 canopy types x 3 seedling species x 10 seedlings).

Seedling Plantings and Harvests

We used wild seed sources from locations nearby our study region (Table A1 of Appendix). The seeds were stratified and then germinated in containers filled with potting soil (Metro Mix 380). To account for differences in maternal effects due to differential allocation of resources to the seeds, initial seedling height was measured before transplant into the field when the seedlings were ~4 weeks old in mid-May (Ibáñez & McCarthy-Neumann 2014). A few seedlings were chosen from each potting container for initial colonization analysis and little to no mycorrhizal fungi were found colonizing the seedlings at that time. We recorded seedling survival 2 weeks after the field transplants and again at the end of the summer (mid-September). To exclude any mortality due to transplant shock survival rates per plot were calculated as the ratio between the number of seedlings surviving at the end of the summer and the number of seedlings alive two weeks after the transplant.

All surviving seedlings were harvested at the end of the growing season. Roots were gently rinsed with deionized water and allowed to air dry for 30 minutes. Seedling height was measured, above- and belowground sections were weighed, and approximately 10 fine lateral roots were removed for microscope analysis (roots were selected at random from the various heights along the primary root). Harvesting occurred in late September during leaf fall with some seedlings still retaining leaves and others just recently abscised. For this reason, seedling biomass was not analyzed.

Mycorrhizal Fungi Colonization Assessment

The selected root tips were cleared with a 10% KOH solution and then stained with a 5% Schaeffer black ink and acetic acid solution. Microscopic analysis was performed at 200X magnification using the magnified intersections method (McGonigle et al. 1990). Microscope slides of roots were scanned using a microscope cross hair eye-piece. Percent AMF colonization and percent EMF colonization was assessed by counting the number of root-fungus intersections bisecting the microscope cross-hair. Similar to Bainard et al. (2011) AMF were identified by vesicles, arbuscles, and fungal hyphae growing within and penetrating the cell walls of plant roots; EMF were identified by mantle of hyphae growing predominantly on the exterior of the root and between the cells (Hartig nets) towards the edges of roots.

Environmental Sampling

Nutrient information for each plot was collected using Ag Manager Resin Capsules (Unibest International, WA, USA) that simulate plant root uptake using ion-exchange and store plant available nutrients inside the capsule (Woodward et al. 2013, Skogley 1992). Capsules were buried 5 cm deep at the top of each plot (~40 cm from the base of the canopy

tree). The capsules were unearthed at the end of the season and sent to UniBest International for analysis of plant available nutrients (Unibest International, WA, USA). A full list of nutrients is available in Table A2 in the appendix.

Volumetric water content (%VWC), was measured six times throughout the growing season with a Fieldscout 300 soil moisture meter (Spectrum Technologies, IL, USA) at approximately 7 cm depth. Measurements were taken from the 4 corners and the middle of each plot at each of the six times water content was measured. We used the average of the growing season's measurements to characterize the water availability at each plot.

The amount of light reaching the forest floor was estimated using canopy photos. We used a Sigma 4.2 m 180° fish eye lens to take photos 1.15 m above the ground for each plot (Ronkokoma, NY, USA). Photos were taken in early August well after the canopy had fully developed. The amount of light reaching the canopy floor (% of full sun) was calculated using Hemiview software (Delta-T Devices, Cambridge, UK).

Data Analysis

We carried out extensive exploratory data analysis to identify patterns within and between all the variables we measured. We then developed two models to quantify 1) mycorrhizal colonization for each landscape-canopy tree combination and 2) seedling survival, the two response variables were analyzed as a function of the environmental variables we measured and in the case of seedling survival, also as a function of, the percent of mycorrhizal fungi colonization estimated. Each seedling species was analyzed independently.

Mycorrhizal Colonization Model - We developed identical models for AMF and EMF colonization for each of the tree species planted. Colonization counts for each *seedling i* (C_i),

were analyzed as a function of the combination of landscape $landscape_{(i)}$ (rural, suburban, urban) and canopy tree $canopy_{(i)}$ (*A. rubrum, C. ovata, Q. rubra*), where they were planted, of the environmental variables in the plot (standardized soil moisture [$Soilm_{plot(i)}$], standardized nitrogen [$N_{plot\,(i)}$], and standardized phosphorous [$P_{plot\,(i)}$]), and of the standardized initial size of the seedling at the time of planting ($InitHeight_i$). To account for the large variation observed on the observed mycorrhizal colonization data, we also included individual random effects $IRE_{(i)}$, then the amount of mycorrhizal colonization was estimated using a Gaussian likelihood limited to be positive (colonization can only be positive):

$$C_i \sim Normal(Cm_i, \sigma^2)$$

and process model:

$$Cm_i = \alpha_{landscape(i),canopy(i)} + \beta_1 InitHeight_i + \beta_2 Soilm_{plot(i)} + \beta_3 N_{plot(i)} + \beta_4 P_{plot(i)} + IRE_i$$

Parameters $\alpha_{landscape,canopy}$ were estimated following a hierarchical approach from species level parameters, $\alpha_{landscape,canopy} \sim \text{Normal}(\alpha_{species}, \sigma_\alpha^2)$. We chose to run our final model using only one type of mycorrhizal colonization per species. *A. rubrum* was run with only AMF data as we did not find EMF present in our samples, and *C. ovata* and *Q. rubra* were run with only EMF data. Though AMF mycorrhizal species were present in *C. ovata* and *Q. rubra*, they made up less than 2% of the total colonization values and were therefore not present in sufficient numbers to warrant an analysis. Mycorrhizal colonization by plot (*MP*, a latent variable) was estimated using parameter values from the mycorrhizal colonization model. They reflect the predicted percent of mycorrhizal colonization for each species given the landscape-canopy plot combination and the environmental conditions associated

with those plots (average soil moisture, P and N); these predicted values were used in the analysis of the survival model (see next section).

Survival Model - We analyzed the survival rates at each plot, *p*, using a Binomial likelihood distribution where the number of surviving seedlings at the end of the summer ($Survival_p$) was estimated as a function of the probability of survival (S_p) and initial number of seedlings, i.e., seedlings still alive two weeks after the transplant (N_p):

$$Survival_p \sim Binomial(S_p, N_p)$$

And process model:

$$logit(S_p) = \alpha + \beta_1 Light_p + \beta_2 Soilm_p + \beta_3 MP_p$$

Seedling survival at the plot level is estimated as a function of standardized light, standardized soil moisture (*Soilm*), and the predicted amount of mycorrhizal colonization by plot (*MP*).

We used a Bayesian framework to estimate our parameter values. To let the observed data lead the analysis, all parameter values in the process models were estimated from non-informative prior distributions, α_* , β_* ~ Normal (0, 1000), IRE ~ Normal(0, σ_{IRE}^2) and $1/\sigma_*^2$ ~ Uniform(0,1000). We ran three Markov Monte Carlo chains using OpenBugs 3.2.3 (Thomas 2006). Parameter values were estimated from 200,000 to 300,000 iterations after the chains converged. We estimated the posterior mean, 95% credible interval, and standard deviation for each parameter value. Model fit was evaluated by plotting predicted values for percent mycorrhizal colonization with observed data. Alpha parameter values were considered significantly different from each other if their 95% credible intervals did not overlap. Beta parameter values whose 95% credible intervals did not cross zero were considered significant. Model code is available in the appendix section A8.

Results:

Although there were differences between plots, the environmental variables measured did not vary consistently along the urban-rural gradient (Figure 2). Light, soil moisture, and phosphorus values were evenly spread across all landscape types. Total nitrogen values did not vary much between sites except for three plots in two suburban forests whose values were notably higher than the rest (Figure 2). A two-way ANOVA was run in R (version 3.2.0, R core team, 2015). No significant differences were found in nitrogen levels between landscape or canopy types.

Mycorrhizal Colonization Model - Goodness of fit, predicted *vs.* observed, had R^2 values of 0.95 for *Q. rubra* and 0.99 for *C. ovata* and *A. rubrum.* Effects on mycorrhizal colonization varied by seedling species but within a species they were similar along landscape-canopy combinations (Figure 3a). *Q. rubra* had the highest mycorrhizal colonization values, *A. rubrum* had the lowest. The urban-rural gradient had little to no effect on the amount of mycorrhizal colonization present, nor did the canopy tree (Figure 3a). Initial seedling height and total nitrogen and phosphorus in soil also had no significant effect on the amount of mycorrhizal colonization present on seedling roots (See Figure 3b). All parameter values are reported in the Appendix Tables A3-A8.

Survival Model - Goodness of fit, predicted *vs.* observed, had R^2 values of 0.94 for *Q. rubra*, 0.93 for *C. ovata* and 0.45 for *A. rubrum.* Survival across all plots was not significantly different by seedling species (Figure 4a). *A. rubrum* and *Q. rubra* had similar survival levels (mean ± SD) 36 ± 6% and 31 ± 8% respectively. *C. ovata* had a much higher average survival level of 74 ± 15%.

Light and soil moisture by plot did not have significant effects on seedling survival

(Figure 4b). There was a significant positive effect on survival of increased mycorrhizal colonization in the survival rates of *C. ovata*, and also a positive, but not significant, effect on *Q. rubra* (Figure 4b).

Survival probability as a function of mycorrhizal fungi colonization varied by species as mycorrhizal colonization increased (Figure 5). *Q. rubra* and *A. rubrum* had much higher natural variability in their survival response than *C. ovata*. *A. rubrum* had even survival probabilities, around 35-46%, across the range of mycorrhizal colonization values. Both *C. ovata* and *Q. rubra* had lower survival probability until mycorrhizal colonization reached 60%. *C. ovata* seedlings had the strongest survival response to increasing fungal colonization and reached much higher survival levels at higher colonization.

Discussion:

Benefits and costs of mycorrhizal fungi to plants are well documented, but landscape-level changes in mycorrhizal abundance and diversity are less well understood (Cousins et al. 2003; Fitter et al. 2004; Karliniski et al. 2014). Our study focused on these landscape-level changes. In particular, we investigated differences in mycorrhizal colonization of seedlings across an urban gradient typical of many North American landscapes. We also asked if differing levels of mycorrhizal colonization would cause a differential effect on seeding survival. We found that mycorrhizal colonization did not change across our urban gradient, but that survival did change across a gradient of mycorrhizal colonization for two of our three species. These observations imply that, if the levels of mycorrhizal fungi abundance were to change, potentially due to urbanization, we should expect shifts in some species recruitment success.

Studies focused on the effects of urbanization are often located in large metropolitan regions (O'Brien et al. 2012; Setala et al. 2013; Vailshery et al. 2013) or study street trees (Guerrero et al. 2013; Youngsteadt et al. 2015). Neither of these types of studies are representative of the dynamics taking place in vegetation patches frequently found in small and mid-size cities. Many healthy forest fragments commonly found in small and medium-size cities do not have consistent conservation plans or funding available, and cities rely on their self-sustainable dynamics for their preservation (Kielbaso 1990). Therefore, to be able to maintain current dynamics in these remnant forests it is important to address the driving factors affecting recruitment, e.g., such as the mycorrhizal fungi and plant relationship.

Although urban environments have the potential to detrimentally effect mycorrhizal colonization by altering soil nutrients, hampering spore dispersal, disturbing the soil, and depositing heavy metals (Egerton-Warburton & Allen 2000; Yang et al. 2015; Duchicela 2013), some cities do not have a decrease in mycorrhizal colonization (Karpati et al. 2011; Karlinski et al. 2014, although they found a decrease in mycorrhizae diversity). Consistent with Karpati et al. (2011), we did not find a negative effect of urbanization on colonization in our forest patches. It is well supported in the literature that nitrogen, phosphorus, and soil moisture affect mycorrhizal colonization (Egerton-Warburton & Allen 2000; Treseder 2004; Propster & Johnson 2015), however in our study they had little to no effect on mycorrhizal colonization. This pattern could be explained by the low variation in soil nutrients, soil moisture, and light across the urban-rural gradient for our study sites (Figure 2). Thus, it is not surprising that these variables were not associated with significant changes in mycorrhizal colonization. Moreover, this pattern may be representative of many healthy forest patches in small and medium size cities in which urbanization may not have a strong

effect (Karlinski et al. 2014). Nevertheless, we did not survey mycorrhizal diversity, an important component for long-term forest health and tree species diversity (Amaranthus 1998; Jeffries et al. 2003). Subsequent studies will be needed to investigate any potential changes in fungal diversity in these type of urban landscapes.

It is likely that the diversity, and possibly even the functionality, of the mycorrhizal community changes dependent on the canopy tree species (Van Der Heijden & Horton 2009; O'Brien et al. 2011), However, unlike what it has been reported in others studies (Dickie et al. 2002; Lewis et al. 2008; Teste & Simard 2008), our study revealed little effect of canopy tree identity on the overall amount of mycorrhizal colonization each seedling species experienced (Figure 3a). Thus further studies are necessary to shed light on the importance of the identity of the canopy trees on the mycorrhizal community affecting seedling recruitment.

Different levels of light and soil moisture have positive and negative effects on seedling survival (e.g. Propster & Johnson 2015; Ibáñez & McCarthy Neumann 2014). Too little light may cause carbon starvation (Maguire & Kobe 2015), too much is usually associated to higher competition with other ground vegetation and an increased risk in desiccation (Parker et al. 2009). Likewise with soil moisture, higher levels of soil moisture are associated with increased effects of soil pathogens (Mordecai 2012), whereas dry conditions are a major cause of seedling mortality during establishment (Maguire & Kobe 2015). The relationship between light levels and soil moisture is also important as a seedling's water needs increase with increasing levels of photosynthesis (Rodriguez-Calcerrada et al. 2010). In our analysis we did not observe any effects of light and soil moisture on seedling survival (Figure 4b), this again may be due to the small range of variability recorded among our study's plots

(Figure 2).

The most relevant finding in our study is the differential effect of mycorrhizal fungi colonization on the seedling survival of our three species. We found that two of our three species, *Q. rubra* and *C. ovata*, had improved survival levels with increasing amounts of mycorrhizal colonization. *A. rubrum*, a species which is increasing in northeastern forest systems (Thomas-Van Gundy et al. 2014; Katz et al. 2010) and nearby local forests as well (Hartmann et al. 2005), shows no changes in survival with increased mycorrhizal colonization. In contrast, *C. ovata* showed a strong positive survival response to increasing mycorrhizal colonization, and *Q. rubra* survival also had a positive (but non-significant) relationship with mycorrhizal fungi colonization. During the seedling establishment phase, water and nutrients are often difficult to access, but when seedlings link into mycorrhizal networks, they not only benefit from an increased supply of nutrients, but also from being connected to neighboring trees from whose carbon assimilates they may benefit (Bingham & Simard 2012; File et al. 2012). In this way, mycorrhizae from neighboring, often conspecific, trees can offset harsh conditions like drought or very low light levels, which may be lethal to seedlings (Rodriguez-Calcerrada et al. 2010).

In past studies, small seeded species seemed to benefit the most from mycorrhizal fungi (Jin et al. 2009), thus we were surprised to find that *A. rubrum* (our smallest seeded species) had far less dependence on the mycorrhizal network than larger seeded species like *C. ovata* (Figure 5). This finding requires more investigation, but may explain part of the shift in forest community structure towards more *A. rubrum* dominated understories (Abrams & Ruffner 1995). Researchers have cited many different factors like climate change (Reinmann & Templer 2016) and decreased forest fire (Abrams & Ruffner 1995; Thomas-

Van Gundy et al. 2014) that may account for these population increases, but a lack of sensitivity to changes in mycorrhizal colonization could also contribute to *A. rubrum's* success. Moreover, given our results, we should expect seedling recruitment for this species to remain unchanged should mycorrhizal abundance decrease in our study system. While we may expect *C. ovata* to be quite sensitive to any decreases in mycorrhizal abundance and subsequently have a substantial decrease in recruitment. It is important to note that many other researchers have used biomass instead of survival as a metric of the mycorrhizal benefit to the host plant (Martinez-Garcia et al. 2015; Roger et al. 2013; Millar & Ballhorn 2013). Due to the early loss of leaves by some seedlings, we were not able to use biomass to assess the potential effects of mycorrhizal colonization on growth along the urbanization gradient. We therefore cannot assure there was a total lack of mycorrhizal effect on *A. rubrum* seedling performance.

Much attention is given to studies showing drastic changes in mycorrhizal colonization (Bainard et al. 2011), but of the ecological studies done in North America, most of them are located in heavily populated areas (Martin et al. 2012) that do not represent the majority of urban forests located in small- to mid-size cities such as ours. We suspect that different cities may show different mycorrhizal colonization responses dependent on size, land-use history, soil disturbance, and industrial activity.

Our study revealed that, in our urban gradient, representative of small to mid-size cities in the Northeastern part of the USA, there was no shortage of available mycorrhizae for inoculation of seedlings in urban forest fragments. Our seedlings had abundant levels of mycorrhizal colonization and there were no differences in these colonization levels when planted under conspecifics vs. heterospecifics. Survival and mycorrhizal colonization were

not influenced by the range of recorded environmental conditions. Seedling survival however, improved greatly for *C. ovata* with increased mycorrhizal colonization and remained unchanged for *A. rubrum*. These results suggest that forest recruitment patterns may change should mycorrhizae become less abundant in the urban matrix.

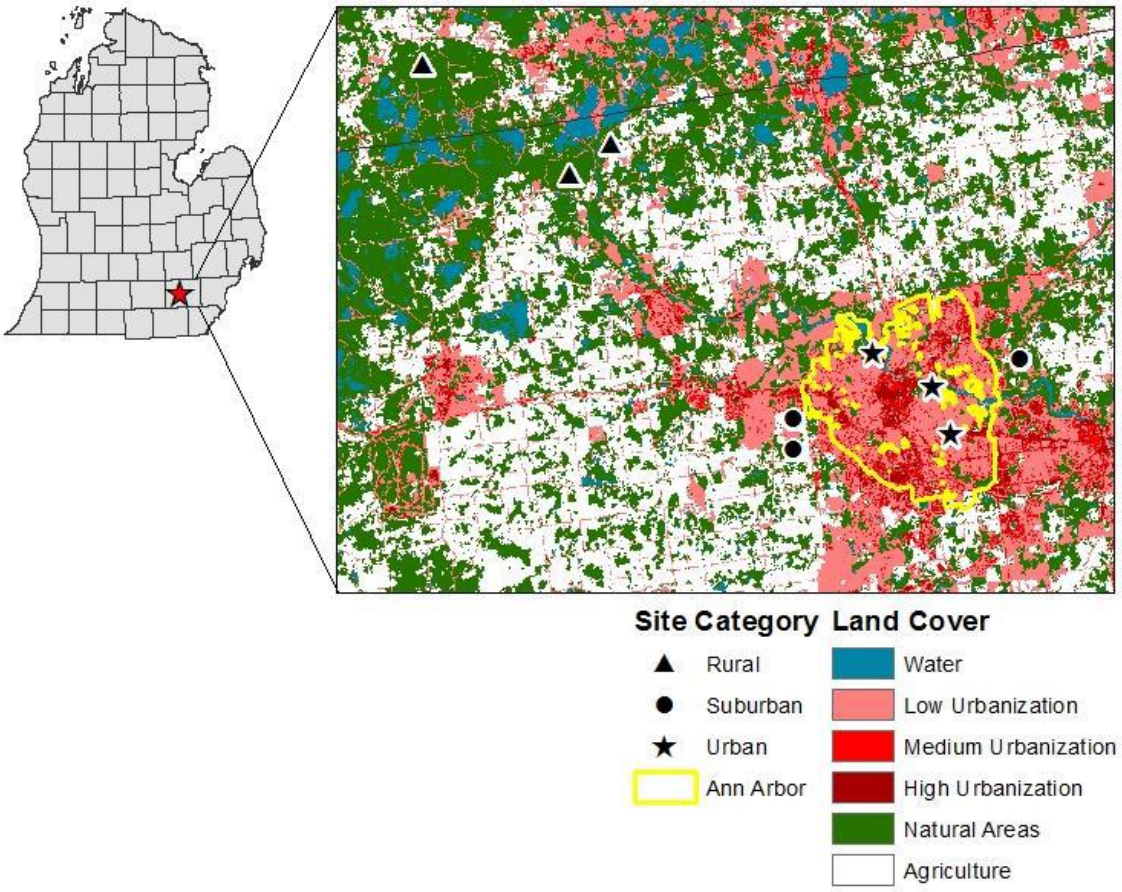
Table 1. Landscape information for each of our 9 study sites. Average Volumetric Water content is a measure of the entire season's water content measurements. Vegetation and Soil types were classified by visual survey and on site soil texture tests.

Forest Site	Landscape	Owner	Latitude	Longitude	Average % Volumetric Water Content ± SD	% Impervious surface (1km radius)	Vegetation	Type of Soil
Edwin S. George Reserve	**Rural**	University of Michigan	42.45899	-84.011392	20.3 ± 5.6	0.1	Oak-Hickory	sandy-loam
Stinchfield Woods	**Rural**	University of Michigan	42.39971	-83.928818	23.0 ± 5.7	0.1	Oak-Hickory	sandy-clay-loam
Newcomb Tract	**Rural**	University of Michigan	42.41094	-83.901458	15.9 ± 7.1	1.3	Oak-Hickory	sandy-loam
Scio Woods Preserve	**Suburban**	Washtenaw County Natural Areas Preservation	42.27844	-83.69857	23.2 ± 8.2	3.6	Oak-Hickory-Sugar Maple-Beech	clay
Saginaw Forest	**Suburban**	University of Michigan	42.27444	-83.803887	26.3 ± 5.4	13.9	Oak-Hickory	sandy-clay-loam
Radrick Forest	**Suburban**	University of Michigan	42.28755	-83.659765	17.9 ± 8.7	3.7	Oak-Hickory	loamy-sand
Nichols Arboretum	**Urban**	University of Michigan	42.28053	-83.71744	24.1 ± 7.7	17.6	Oak-Hickory-Sugar Maple	clay-loam
Kuebler Langford Nature Area	**Urban**	City of Ann Arbor	42.29989	-83.75203	15.3 ± 9.3	14.1	Oak-Hickory	sandy-loam
County Farm Park	**Urban**	Washtenaw County Parks	42.25711	-83.710125	29.5 ± 10.7	27.1	Oak-Hickory	clay-loam

Table 2. Nutrient Information by Plot. Soil nutrient amounts in parts per million of extracted solution. Soil Nutrient data collected in Unibest capsules and analyzed by Unibest International.

Forest	Landscape	Canopy	Total N	NO_3	NH_4	Al	B	Ca	Cu	Fe	K	Mg	Mn	Na	P	S	Zn
Edwin S. George Reserve	Rural	*A. rubrum*	4.9	0.0	4.9	1.1	0.0	72.7	0.0	0.5	3.2	16.2	0.9	0.9	0.1	3.8	0.0
	Rural	*C. ovata*	6.0	0.9	5.1	1.2	0.0	83.4	0.0	0.4	21.1	17.1	1.4	1.9	0.3	7.1	0.1
	Rural	*Q. rubra*	6.5	1.2	5.3	1.6	0.0	69.1	0.0	0.3	13.1	13.7	1.4	1.3	0.2	4.4	0.0
Newcomb Tract	Rural	*A. rubrum*	6.1	1.3	4.8	1.9	0.0	45.7	0.0	0.6	28.2	10.7	1.7	1.6	0.4	5.8	0.2
	Rural	*C. ovata*	7.4	1.8	5.6	1.0	0.0	33.0	0.0	0.4	29.5	7.5	0.9	1.7	0.2	6.3	0.5
	Rural	*Q. rubra*	7.2	1.1	6.0	1.0	0.0	51.4	0.0	0.4	42.1	10.2	1.0	1.9	1.3	6.5	0.3
Stinchfield Woods	Rural	*A. rubrum*	6.4	2.2	4.3	2.3	0.0	102.6	0.0	0.9	24.8	18.7	3.9	2.0	1.9	6.1	0.0
	Rural	*C. ovata*	18.2	9.3	8.8	4.5	0.0	96.4	0.0	2.5	92.8	20.4	2.9	4.7	2.1	16.0	0.1
	Rural	*Q. rubra*	5.5	1.0	4.5	1.3	0.1	68.8	0.0	0.6	75.3	13.2	0.7	3.3	1.8	9.0	0.1
Radrick Forest	Suburban	*A. rubrum*	34.3	29.8	4.5	2.8	0.0	91.2	0.0	0.7	35.3	14.1	1.8	1.9	1.6	7.2	0.1
	Suburban	*C. ovata*	10.1	4.8	5.3	0.8	0.0	139.7	0.0	0.4	37.7	17.0	0.6	4.1	0.3	10.8	0.0
	Suburban	*Q. rubra*	12.3	7.5	4.8	1.7	0.0	43.3	0.0	0.6	35.6	8.1	1.3	2.9	1.5	6.1	0.1
Saginaw Forest	Suburban	*A. rubrum*	5.1	0.7	4.4	1.5	0.0	42.9	0.0	0.9	19.6	13.0	1.5	2.1	0.5	5.0	0.0
	Suburban	*C. ovata*	31.2	26.2	5.0	3.6	0.0	194.1	0.0	1.8	73.8	35.3	2.8	3.1	1.2	12.1	0.1
	Suburban	*Q. rubra*	4.4	0.3	4.1	3.5	0.0	95.1	0.0	1.4	14.5	25.3	2.4	2.5	0.7	5.2	0.1
Scio Woods Preserve	Suburban	*A. rubrum*	26.8	6.8	20.0	1.2	0.0	60.2	0.0	0.5	28.0	15.7	0.6	2.8	2.1	8.7	0.1
	Suburban	*C. ovata*	6.9	1.2	5.7	2.7	0.0	31.4	0.0	0.8	22.4	10.2	0.4	2.5	0.4	9.4	0.0
	Suburban	*Q. rubra*	7.0	2.5	4.5	1.7	0.0	29.6	0.1	1.0	32.0	8.3	0.3	3.3	0.4	8.2	0.1
Nichols Arboretum	Urban	*A. rubrum*	10.6	3.6	7.0	0.7	0.0	33.3	0.0	0.5	26.9	9.7	0.5	2.4	1.7	6.8	0.0
	Urban	*C. ovata*	8.7	4.7	4.0	0.9	0.0	52.4	0.0	0.3	23.4	11.4	0.9	1.3	0.9	9.4	0.0
	Urban	*Q. rubra*	9.3	4.6	4.7	1.2	0.0	23.8	0.0	0.7	20.2	5.9	0.7	1.6	1.1	6.9	0.0
County Farm Park	Urban	*A. rubrum*	15.5	9.3	6.3	1.5	0.0	65.9	0.0	0.7	11.9	14.3	0.8	2.8	0.4	8.8	0.0
	Urban	*C. ovata*	11.5	6.9	4.5	1.1	0.0	36.7	0.0	0.5	46.7	14.8	0.4	6.9	0.4	15.0	0.1
	Urban	*Q. rubra*	4.8	0.4	4.5	0.6	0.0	7.2	0.0	0.4	2.9	2.6	0.2	1.6	0.1	3.5	0.0
Kuebler Langford Nature Area	Urban	*A. rubrum*	2.6	0.2	2.4	1.2	0.0	18.6	0.0	0.4	5.8	4.2	0.7	0.7	0.1	0.9	0.0
	Urban	*C. ovata*	10.2	2.5	7.7	1.8	0.1	75.0	0.0	0.6	37.1	25.0	2.6	3.4	1.3	11.5	0.1
	Urban	*Q. rubra*	4.5	0.2	4.4	1.0	0.0	9.7	0.0	0.4	6.6	3.5	0.2	1.6	0.1	4.8	0.0

Figure 1. Location of study sites in SE Michigan, USA. Data from the National Land Cover dataset (Xian et al. 2011). Pixels averaged in a 250m radius.



Map credit: Teegan McClung

Figure 2. Site environmental characteristics. The landscape of the site is indicated by the shading of the points and the nearest adult individual is indicated by the shape of the points.

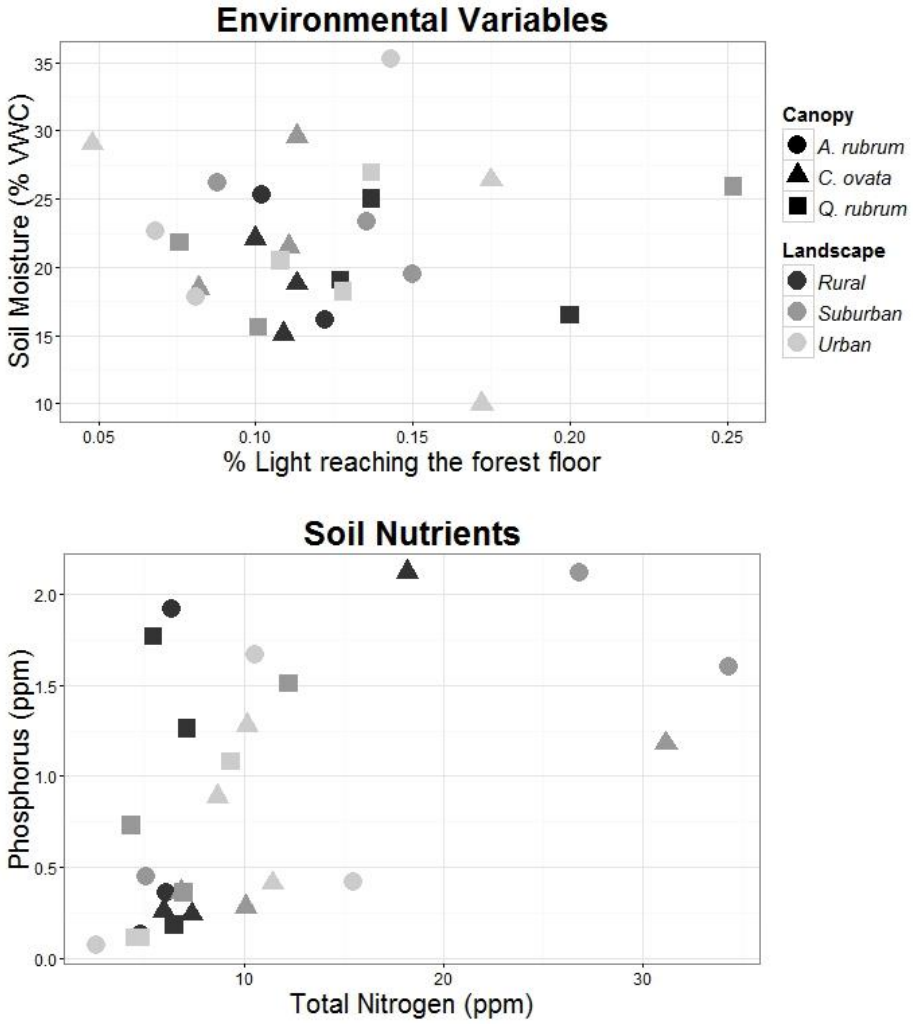
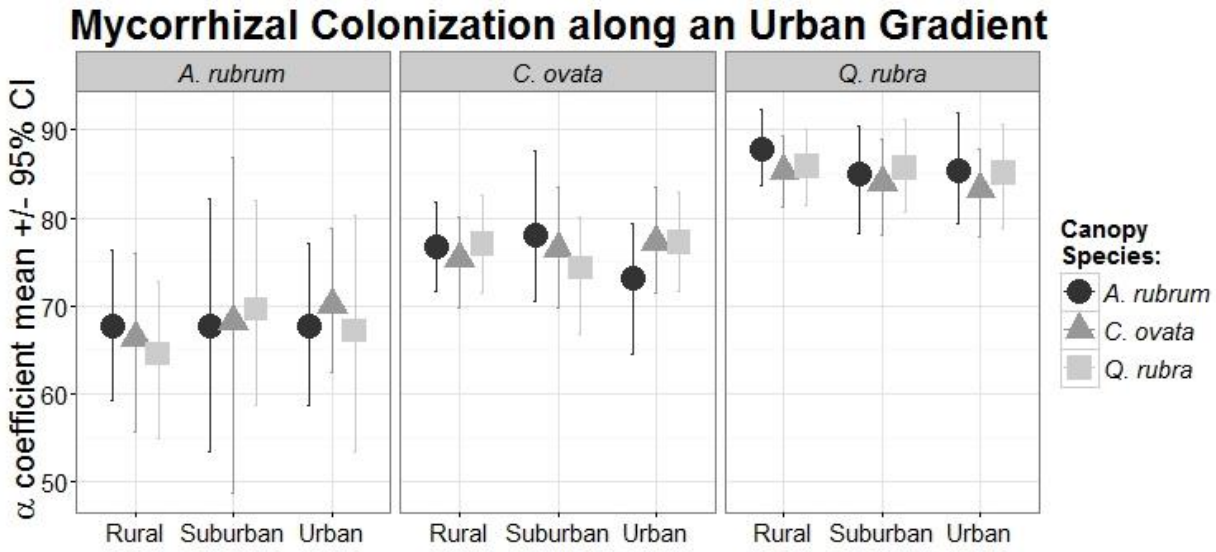


Figure 3. a) The amount of mycorrhizal colonization (α parameter) in each species along the urban gradient, and among canopy species, while P, N, and initial height are at their average levels. Parameters which 95%CI overlap are not statistically different. b) Effect of seedling initial height (H), Nitrogen (N), and Phosphorus (P) on mycorrhizal colonization (mean+95%CI), 95% CI that do not cross the zero line are not statistically significant.

a)



b)

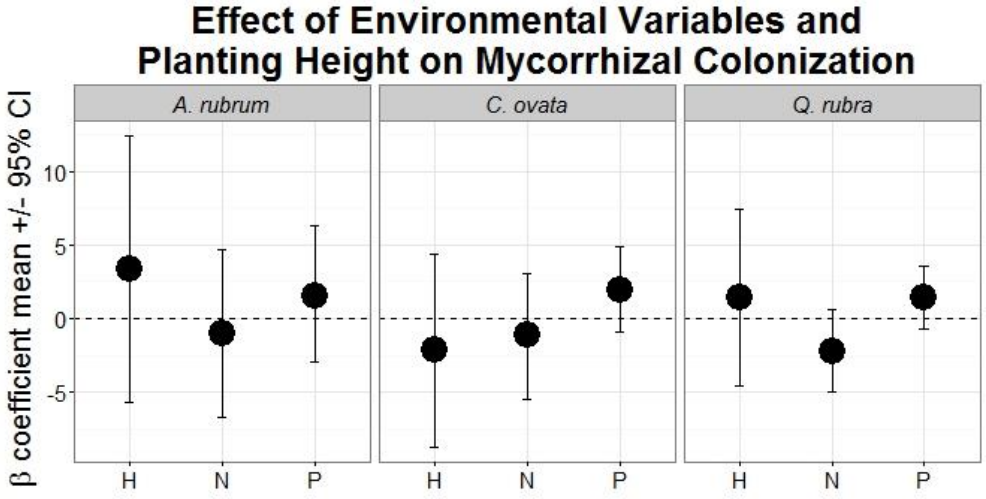
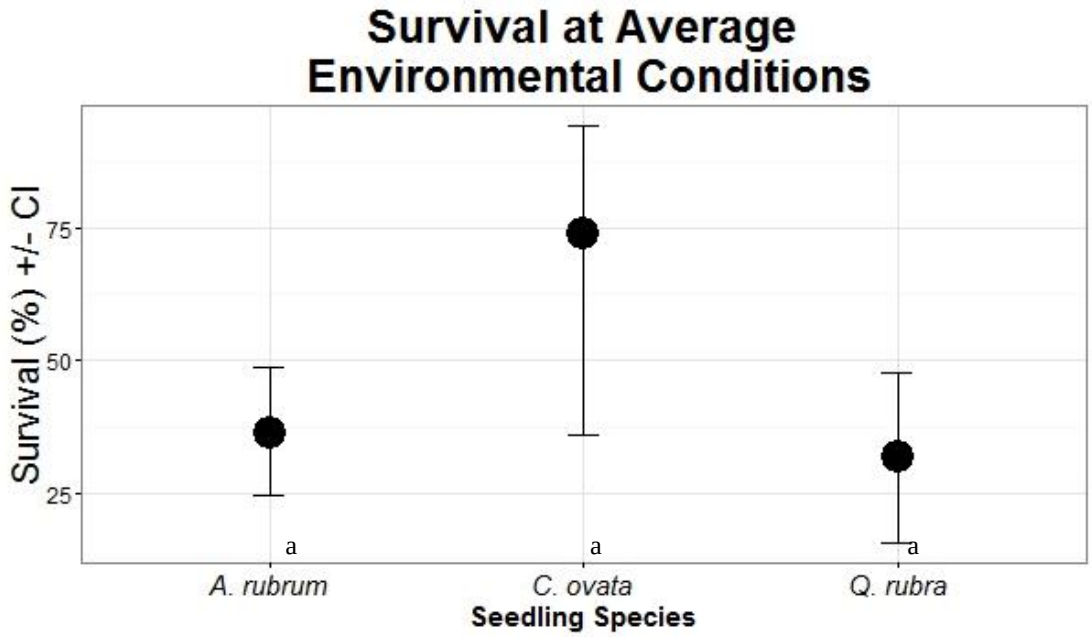


Figure 4. a) Survival (mean+95%CI) across all landscapes at average light, soil moisture, and mycorrhizal colonization (similar letters indicate no significant differences between species). b) Effect of light (Light), mycorrhizal colonization (Myco), and soil moisture (SM) on survival (mean+/-95%CI). Credible intervals (CI) that cross the zero line are not statistically significant.

a)



b)

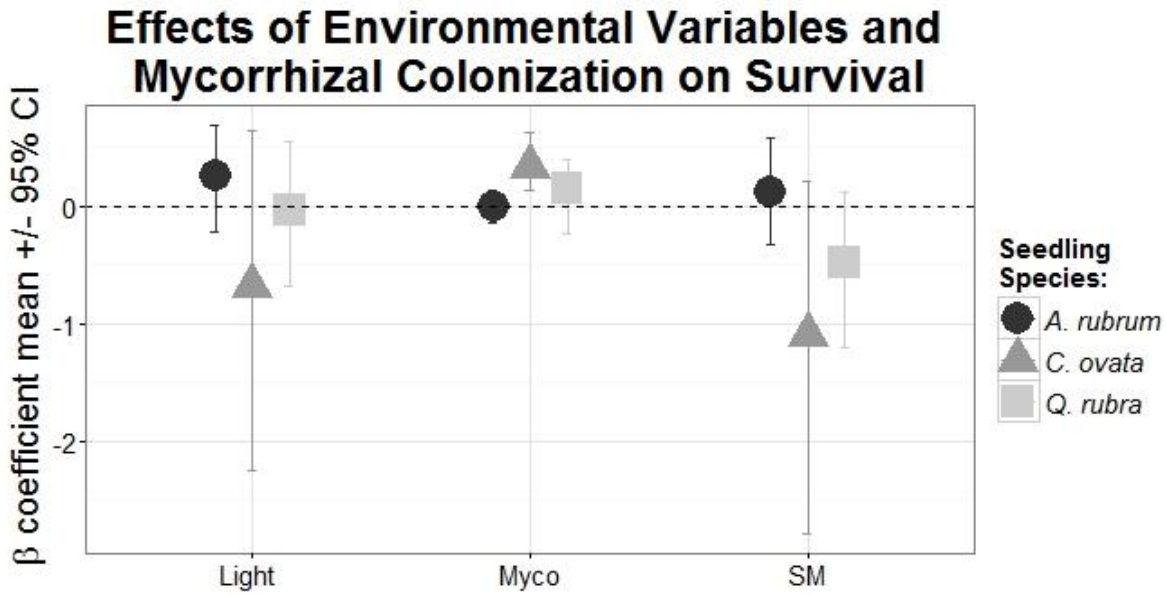
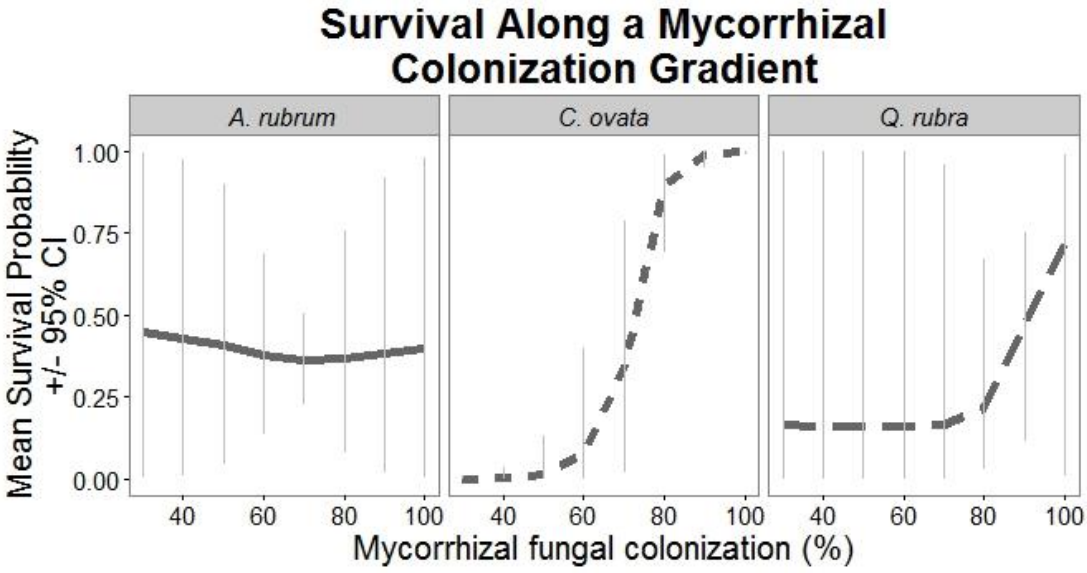


Figure 5. Predicted survival (mean and 95% CI) at several levels of mycorrhizal colonization (ranges based on observed data). Light gray vertical lines are 95% confidence intervals.



Appendix:

Table A1. Seed Sources

Species	Collector	Location
Carya ovata	Sheffield Seed Co.	Illinois
Quercus rubra	Sheffield Seed Co.	Illinois
Acer rubrum	New Forests	Michigan

Table A2. Alpha E.

Intercept: Mycorrhizal colonization at average nitrogen, phosphorus, and average initial height.

Species	Landscape	Canopy	Mean	St. Dev.	95% Confidence Interval	
A. rubrum	Urban	*A. rubrum*	67.76	4.575	58.65	77.05
A. rubrum	Urban	*C. ovata*	70.16	4.13	62.37	78.73
A. rubrum	Urban	*Q. rubra*	67.12	6.544	53.38	80.27
A. rubrum	Suburban	*A. rubrum*	67.79	6.797	53.49	82.17
A. rubrum	Suburban	*C. ovata*	68.14	8.984	48.65	86.8
A. rubrum	Suburban	*Q. rubra*	69.57	5.765	58.71	81.98
A. rubrum	Rural	*A. rubrum*	67.73	4.245	59.16	76.3
A. rubrum	Rural	*C. ovata*	66.37	5.065	55.63	76.07
A. rubrum	Rural	*Q. rubra*	64.52	4.492	54.95	72.77
C. ovata	Urban	*A. rubrum*	73.17	3.756	64.56	79.28
C. ovata	Urban	*C. ovata*	77.31	3.032	71.44	83.56
C. ovata	Urban	*Q. rubra*	77.2	2.848	71.58	82.94
C. ovata	Suburban	*A. rubrum*	78.01	4.217	70.45	87.7
C. ovata	Suburban	*C. ovata*	76.45	3.399	69.74	83.58
C. ovata	Suburban	*Q. rubra*	74.28	3.391	66.74	80.13
C. ovata	Rural	*A. rubrum*	76.81	2.573	71.65	81.84
C. ovata	Rural	*C. ovata*	75.31	2.608	69.87	80.18
C. ovata	Rural	*Q. rubra*	77.03	2.81	71.42	82.6
Q. rubra	Urban	*A. rubrum*	85.33	3.044	79.38	91.98
Q. rubra	Urban	*C. ovata*	83.28	2.582	77.78	87.88
Q. rubra	Urban	*Q. rubra*	85.11	2.938	78.83	90.71
Q. rubra	Suburban	*A. rubrum*	84.95	2.976	78.33	90.44
Q. rubra	Suburban	*C. ovata*	84.04	2.731	78.14	89
Q. rubra	Suburban	*Q. rubra*	85.71	2.574	80.77	91.2
Q. rubra	Rural	*A. rubrum*	87.81	2.203	83.69	92.3
Q. rubra	Rural	*C. ovata*	85.38	2.011	81.31	89.3
Q. rubra	Rural	*Q. rubra*	85.8	2.156	81.51	90.09

Table A3. Mycorrhizal Model Parameters (Beta).
Effect of initial height (IH), and soil nutrients phosphorus (P) and nitrogen (N) on mycorrhizal colonization.

Parameter	Species	Mean	St. Dev	95%CI	
Effect of P on myco. colonization	*A. rubrum*	1.56	2.34	-2.931	6.252
Effect of N on myco. colonization	*A. rubrum*	-1.022	2.844	-6.742	4.622
Effect of IH on myco. colonization	*A. rubrum*	3.362	4.585	-5.702	12.4
Effect of P on myco. colonization	*C. ovata*	1.908	1.449	-0.9123	4.843
Effect of N on myco. colonization	*C. ovata*	-1.149	2.135	-5.496	3.038
Effect of IH on myco. colonization	*C. ovata*	-2.159	3.303	-8.711	4.319
Effect of P on myco. colonization	*Q. rubra*	1.389	1.095	-0.7187	3.57
Effect of N on myco. colonization	*Q. rubra*	-2.24	1.419	-4.99	0.6296
Effect of IH on myco. colonization	*Q. rubra*	1.378	3.043	-4.59	7.368

Table A4. Survival Model Parameters (Beta)
Effect of light (L), soil moisture (SM), and mycorrhizal colonization (MC) on seedling survival.

Parameter	Species	Mean	St. Dev	95%CI	
Effect of L on survival	*A. rubrum*	0.2527	0.2268	-0.219	0.6846
Effect of SM on survival	*A. rubrum*	0.123	0.2325	-0.3368	0.5789
Effect of MC on survival	*A. rubrum*	-0.00824	0.07161	-0.1493	0.1283
Effect of L on survival	*C. ovata*	-0.6817	0.7282	-2.252	0.6393
Effect of SM on survival	*C. ovata*	-1.1	0.7523	-2.782	0.2081
Effect of MC on survival	*C. ovata*	0.3336	0.1365	0.1352	0.627
Effect of L on survival	*Q. rubra*	-0.04054	0.3055	-0.6758	0.5453
Effect of SM on survival	*Q. rubra*	-0.4786	0.3276	-1.199	0.1087
Effect of MC on survival	*Q. rubra*	0.1451	0.1567	-0.2408	0.39

Table A5. Survival Model (SP0)
Species level survival at average soil moisture, light, and average predicted mycorrhizal colonization.

Species	Mean	St.Dev	95% CI	
A. rubrum	0.3628	0.06027	0.2465	0.4877
C. ovata	0.7398	0.1521	0.3588	0.9432
Q. rubra	0.3179	0.08013	0.1572	0.4766

Table A6. Predicted Survival
Predicted Survival at various mycorrhizal colonization (MC) levels.

Species	MC	Mean	St. Dev	95%CI	
A. rubrum	30	0.4486	0.3467	0.003273	0.9935
A. rubrum	40	0.4306	0.3023	0.01207	0.9733
A. rubrum	50	0.4065	0.2352	0.04332	0.897
A. rubrum	60	0.3784	0.135	0.1378	0.6873
A. rubrum	70	0.3605	0.06878	0.2267	0.5045
A. rubrum	80	0.3663	0.1744	0.08149	0.7555

A. rubrum	90	0.3819	0.2592	0.0217	0.9182
A. rubrum	100	0.3973	0.3157	0.005207	0.9764
C. ovata	30	0.001098	0.006758	5.62E-13	0.009765
C. ovata	40	0.003989	0.01573	2.79E-10	0.03637
C. ovata	50	0.01629	0.04075	1.34E-07	0.1331
C. ovata	60	0.07367	0.1109	5.75E-05	0.3976
C. ovata	70	0.3405	0.2264	0.01885	0.7848
C. ovata	80	0.8903	0.07858	0.6924	0.9876
C. ovata	90	0.9896	0.01452	0.948	0.9999
C. ovata	100	0.9984	0.003718	0.9877	1
Q. rubra	30	0.1641	0.3568	2.51E-10	1
Q. rubra	40	0.1633	0.353	1.11E-08	1
Q. rubra	50	0.1625	0.3463	4.81E-07	0.9996
Q. rubra	60	0.1622	0.3311	2.04E-05	0.9959
Q. rubra	70	0.1671	0.2885	0.000882	0.9558
Q. rubra	80	0.2154	0.1637	0.03241	0.6683
Q. rubra	90	0.4717	0.1598	0.115	0.7531
Q. rubra	100	0.7185	0.3101	0.01206	0.99

Table A7. Alpha Sm

Survival model intercept. Species level mycorrhizal colonization at average N and P.

Species	Mean	St.Dev	95% CI	
A. rubra	-0.01216	4.877	-9.333	9.639
C. ovata	-24.31	10.67	-47.24	-8.853
Q. rubra	-13.18	13.46	-34.41	19.76

A8.

Model code for OpenBugs 3.2.3

```
model{

for(i in 1:207){

#mising values
LS[i]~dunif(-1.85,3.35)  # data's range

#mycorrhizal model - predicting colonization based on Species, Urban, Canopy and environmental variables

   EMF[i]~dnorm(E[i],tau[Species[i]])C(0,100)    #likelihood
```

```
   EMF.h[i]~dnorm(E[i],tau[Species[i]])C(0,100)  # predictions

        E[i] <-  alphaE[Species[i],Urban[i],Canopy[i]]+
                 betaE[Species [i],1 ]*PS[i]+
                 betaE[Species [i],2 ]*NS[i]+
                 betaE[Species[i],3]*HS[i]+
                 IRE[i]

        IRE[i]~dnorm(0,tauEre[Species[i]])

}


for(i in 1:71){

# predicting EMF in each plot for each species- 10 plots missing data for one species

        LSs[i]~dunif(-1.85,3.35)  # data's range

   EMF.p[i]~dnorm(E.p[i],tau[Speciess[i]])C(0,100)  # predictions based on the parameters
                                                                  # calculated from the data
                                                                 #at an average seedling height


        E.p[i]<- alphaE[Speciess[i],Urbans[i],Canopys[i]]+
                 betaE[Speciess[i],1]*PSs[i]+

                 betaE[Speciess [i],2]*NSs[i]



#Survival model

        S[i] ~ dbin(p[i],N[i])              #likelihood  for each plot


        logit(p[i]) <- alphaSm[Speciess[i]]+
                 betaS[Speciess[i],1]*LSs[i]+
                 betaS[Speciess[i],2]*SMs[i]+
                  betaS[Speciess[i],3]*EMF.p[i]
                  }


#priors


for(sp in 1:3){   #number of species
tauE[sp]<-1/varE[sp]
varE[sp]~dunif(0,1000)
tau[sp]<-1/var[sp]
```

```
var[sp]~dunif(0,1000)
tauEre[sp]<-1/varEre[sp]
varEre[sp]~dunif(0,1000)

alphaEm[sp]~dnorm(50,0.001)C(0,100) #associated with alphaE,
alphaSm[sp]~dnorm(0,0.001)C(-50,50)
  for(la in 1:3) {  #number of landscapes

  for(ca in 1:3) {  #number of canopies
    alphaE[sp,la,ca] ~ dnorm(alphaEm[sp],tauE[sp])C(0,100)

}
}
}


for(sp in 1:3) {   #number of species

for(i in 1:3) { # of betas
betaS[sp,i] ~ dnorm(0,0.001)
betaE[sp,i]~dnorm(0,0.001)
}
}

}
```

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