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NEGATIVE EFFECTS OF COMMON MYCORRHIZAL NETWORKS AND ROOTS ON
UPLAND OAK SEEDLINGS IN OPEN-CANOPY WOODLANDS AND CLOSED-CANOPY
FORESTS.

A Thesis

Presented for the

Master of Science Degree

Department of Biology

The University of Mississippi

By:

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ABSTRACT

After extensive logging and fire suppression many oak dominated woodlands or forests are in danger of being replaced by a mix of non-pyrogenic and shade tolerant tree species that benefit from fire suppression. Successful advanced regeneration by oaks in forests and woodlands depends both on the persistence of seedlings in the shade and growth within canopy gaps. Through the sharing of carbon and/or water between adults and seedlings, connection to a common mycorrhizal network potentially provides a mechanism by which oak seedlings could persist in shade and/or grow rapidly in dry soils within canopy gaps. A study was conducted to determine the effects of common mycorrhizal networks on seedling growth and survival using four plots with variable canopy density and fire history in north Mississippi. Oak seedlings were grown adjacent to mature oak trees in root exclusion cylinders that allowed seedlings access to fungal networks but isolated them from direct root competition. A subset of seedlings was trenched to disconnect them from the network. Response variables were relative growth rate of height and diameter, above and belowground biomass, root:shoot ratio, lateral root length, total number mycorrhizal tips and mycorrhizal tip density. Contrary to predictions, connection to a common mycorrhizal network did not alleviate either shade stress or water stress, but rather had a negative effect on aboveground biomass. Isolation from roots and common mycorrhizal networks led to an increase in total biomass. Connection to a common mycorrhizal network led to increased mycorrhizal root tips and an increase in the density of mycorrhizal tips per cm

lateral root length. Survival was very high and any treatment effects were negligible. Negative effects of common mycorrhizal connections between adults and seedlings of the same genus could be a previously unappreciated mechanism of negative density-dependent seedling growth. We suggest that research into the effect of CMN interaction with oak seedlings include fire or clipping, and drought as treatments to determine the effects of CMNs on oak seedlings during stressful times to further complete the picture of oak seedling interactions with common mycorrhizal networks.

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INTRODUCTION

One of the most endangered ecosystems in North America is the open oak woodland/savannah, as it occupies only 0.02% of its original area before fire suppression began (Nuzzo 1986). A contributing factor to this decline is the failure of oak species to regenerate at a rate that adequately replaces mortality (Aldrich et al. 2005, Nowacki & Abrams 2008, Rogers et al. 2008). Such oak regeneration failure has been studied extensively (MacDougall et al. 2010, Abrams 2003, Hutchinson et al. 2005, Iverson et al. 2008, McShea et al. 2007, Peterson et al. 2006), but the role of mycorrhizal fungi in this phenomenon has been largely overlooked.

Like most plants, oaks form a symbiotic relationship with mycorrhizal fungi. These relationships are characterized by an exchange of nutrients where the fungi supply soil nutrients to the plant while the plant supplies fixed carbon to the fungi (Johnson et al. 1997). Common mycorrhizal networks (CMNs), formed between the roots of adjacent plants by mycorrhizal fungi connected to both sets of roots (Newman 1988), could play an important role in tree regeneration.

There are two dominant types of mycorrhizal fungi across most terrestrial ecosystems, arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (EMF). AMF are a type of mycorrhizal fungi that have a very broad host range and are often more beneficial to plants in P-poor environments (Johnson et al. 1997). Their hyphae penetrate the cell wall of fine roots and

transport materials directly into root cells (Smith & Read 2008). EMF, on the other hand, associate with mostly trees and other woody plants and are especially beneficial in N poor environments (Johnson et al. 1997). Their hyphae form a sheath (mantle) around the tips of fine roots and penetrate between host root cortical cells, exuding materials into the intercellular space where the root tip absorbs it (Smith & Read 2008). Both AMF and EMF can form CMNs among compatible host plants, and both are known to colonize the roots of oaks (*Quercus* spp.), with AMF predominating earlier in development compared to EMF (Lerat et al. 2002, Egerton-Warburton and Allen 2001, Egerton-Warburton et al. 2007, Querejeta et al. 2009).

Successful regeneration of many oak species of woodlands and forests requires that seedlings endure shade from overstory trees and frequent, low intensity forest fires but then grow rapidly as saplings into the mid- and overstory following the opening of the overstory tree canopy (Abrams 1992). These fires typically damage the above ground parts of the seedling (top kill), but do not kill the roots of oak seedlings (Johnson et al. 2009). Such fires are thought to benefit oak seedlings indirectly by increasing light and reducing competition from taller but more fire-sensitive non-oak saplings (Lorimer et al. 1994), and also by altering the abiotic environment of the forest, which can in turn alter herbaceous plant community composition and density adjacent to oak seedlings in the understory (Arthur et al. 1998, Bowles & Jacobs 2007) and effect competition for water, nutrients, or light (Davis et al. 1998). Topkilled oak seedlings have a large mass of roots to support the regrown sprout and that larger root:shoot ratio can give them a better chance of surviving to eventually recruit into the canopy following canopy gap formation (advanced regeneration; Johnson et al. 2009). CMNs could play a support role in this system where the seedlings must endure shade, water stress and or fires until they can gain enough height and biomass to capture space in the canopy.

CMNs can affect the survival and growth of seedlings facing competition in a forest understory through several possible mechanisms. They could provide access to a pre-existing belowground mycorrhizal network that is much larger than the seedling could support alone (Newman 1988). They could also promote coexistence and species diversity if they permit the transportation of carbon, nitrogen, phosphorus, or water along concentration gradients between individuals of different species (Newman 1988). Such resource transport potentially affects ecosystem productivity by facilitating nutrient cycling (Newman 1988). However, the effect of this symbiosis on seedlings can be anywhere along the gradient from parasitism to mutualism depending on the environmental conditions (Johnson et al. 1997, Jones and Smith 2004).

Some studies suggest that seedlings connected to a CMN can benefit from more consistent access to water, although in most cases, the mechanism has been unclear. For example, Booth and Hoeksema (2010) found that Monterey Pine (*Pinus radiata*) seedlings are more apt to survive the dry summer season when connected to a CMN, likely due to greater access to water that is transferred through the network from mature trees with large tap roots that can access deeper water sources. Egerton-Warburton et al. (2007) used florescent tracer dyes to highlight this potential for CMNs to transfer hydraulically lifted water among plants within a network. Furthermore, Bingham and Simard (2011) experimentally demonstrated that survival and growth of Douglas-fir (*Pseudotsuga menzeisii*) seedlings increased with hydraulic redistribution through mycorrhizal networks (see also Simard 2009, Bingham and Simard 2012).

Most previous studies of the ecological consequences of CMNs have typically used evergreens that exclusively form associations with EMF as focal hosts (Booth and Hoeksema 2010, Bingham and Simard 2011, Warren et al. 2008), but CMNs may behave differently in a

system with oaks because they associate with both EMF and AMF. Associating with either type of fungi can theoretically be beneficial to oaks because the symbiosis may be useful to the plant in a wider range of conditions. McQuattie et al (2004) observed that northern red oak (*Quercus rubra*) seedlings gained a competitive advantage over red maple (*Acer rubrum*) seedlings in burned, open canopy sites. They speculated that this advantage was gained through the oaks ability to associate with either ectomycorrhizal (EMF) or arbuscular mycorrhizal fungi (AMF), compared to red maple that only associates with AMF (see also, Watson et al. 1990).

As upland forests and woodlands continue to shift into more mesic, closed-canopy conditions as a result of fire exclusion (a process called mesophication, Nowacki and Abrams 2008), the role of CMNs may change as well. Dickie et al. (2009) found that the fungal community of oak savannah is distinct from the fungal community found on oaks in forests, so the available inoculum to oak seedlings can be different depending on the environment. Furthermore, fungal species that are dominant in shady, closed canopy forests may have a different effect on oak seedlings as the fungal species that are dominant in open canopy forests, since reduced light availability frequently results in decreased growth benefits of mycorrhizal fungi to host plants (Smith and Read 2008).

This research provides insight into the influence of CMNs on oak seedling growth and survival along continuous environmental gradients from open and burned (historic) to closed and unburned (mesophied) conditions. I investigated the following questions:

Question 1. Do mature mycorrhizal networks have an effect on the growth or survival of upland oak seedlings?

Hypothesis 1. Mature trees provide access for oak seedlings to a common mycorrhizal network that can offset the negative effects of deep shade, root competition, and/or water stress.

- If this hypothesis were supported I would expect networked seedlings to grow and survive better than non-networked seedlings.

Question 2. How does canopy openness affect the CMN response?

Hypothesis 2A. A mature CMN can supply carbon to seedlings in the deep shade under closed canopies.

- If the CMN supplies carbon to the seedlings, I would expect the beneficial effect to be greater in the shade than under open canopies.

Hypothesis 2B. A mature CMN can supply water to seedlings in dry conditions under open canopies.

- If the CMN supplies water to the seedlings, I would expect the beneficial effect should be greater in drier soils under open canopies than in moist soils under closed canopies.

Question 3. How does a CMN affect competition between seedlings and herbaceous vegetation?

Hypothesis 3. A mature CMN can increase the survival or growth of oak seedlings under increased herbaceous plant density.

- If the CMN alleviates competition between oak seedlings and herbaceous vegetation, I would expect seedlings within dense herbaceous vegetation to grow or survive better when connected to the CMN.

METHODS

Study Site

The study site was located within the northern hilly coastal plains of Mississippi (Holly Springs National Forest and the Tallahatchie Experimental Forest within the Greater Yazoo River Watershed, U.S.A.; 34.50°N, 89.43°W). These oak-pine upland forests were frequently burned historically and consequently were much more open and primarily composed of the shade intolerant and fire dependent species *Quercus velutina*, *Q. marilandica*, *Q. stellata*, *Q. falcata*, and *Pinus echinata*. Following extensive logging, second growth stands developed and were approximately twice the stand density of the historic forests (Brewer 2001) at the time of the study. The canopy of these second growth forests was primarily composed of the upland oak species mentioned above, except *Q. marilandica*, and tree species such as *Liquidambar styraciflua*, *Acer rubrum*, and *Nyssa sylvatica*, which were historically suppressed by fires or only found in floodplains, now dominate the understory of these forests (Surrette et al. 2008). Soils on the ridges were acidic sandy loams and silt loams, and in bottoms and side slopes they were loamy sands (Surrette et al. 2008).

Study design

Four nested plots (75 x 70 m) were established in mature, closed-canopy upland oak-pine forests in 2000 to examine long-term vegetation changes (see Brewer et al. 2012 for details of

plot arrangement). In February 2008, an F5 tornado struck the Tallahatchie Experimental Forest, creating a ~ 1 km-wide swath of severe canopy damage (50 to 100 % canopy reduction). Two of the four plots were severely damaged by the tornado, wherein the canopy was reduced by 40 - 60% (Brewer et al. 2012, Cannon and Brewer 2013). The other two plots were not visibly damaged by the tornado. Such variation in damage provided an opportunity for ecological restoration of an open, fire-maintained woodland in the damaged area. Hence, in 2010, one of the severely damaged plots and one of the undamaged plots were chosen to receive biennial prescribed fires during the early spring. Prescribed fires were conducted on March 25 and April 1, 2010 and on March 29, 2012 (see Cannon and Brewer 2013 for details regarding the prescribed fires).

In spring 2012 (following the prescribed fire), fifteen mature canopy trees were selected per plot. The trees selected were overstory oaks (*Quercus* spp.) greater than 20cm dbh, located away from the edge of the plot and greater than 5m from the nearest adjacent study tree. In order to isolate the effect of the CMN from the effect of competing tree roots I used root exclusion cylinders (Fig. 1) in combination with trenching to create four treatments. “CMNs only, with cylinder” (common mycorrhizal networks) (n=61 total), that consisted of seedlings grown in a cylinder but with no trenching; “no CMNs/no roots, with cylinder” (n=46 total), consisting of seedlings grown in cylinders and with trenching; “CMNs+roots, no cylinder” (n=18 total), consisting of seedlings grown without cylinders or trenching, and “no CMNs/no roots, no cylinder” (n=23 total), consisting of seedlings grown without cylinders but with trenching. These are the same four treatments created in two previous studies that utilized these same cylinders (Booth 2004, Booth & Hoeksema 2010).

Each PVC cylinder had approximately 50% of the material removed from the sides (perforated) and was wrapped in a 40 micron stainless steel mesh to allow hyphae to grow into the cylinder while excluding roots. These cylinders are 18.5 cm deep, and are installed with the top flush with the soil and have an open bottom to minimize the effect of taproot disturbance because oak seedlings rapidly grow deep taproots.

Four root exclusion cylinders were buried around each mature canopy tree within the ground area defined by the area of the canopy known as the dripline. I installed the cylinders by



slicing a cylindrical slice in the soil with a shovel to sever roots and then inserting the cylinder into the cylindrical slice without removing a soil core. This method installed the cylinder into the soil while still maintaining the soil horizons and proper in situ soil structure. Upon seedling harvest the cylinders were inspected and no live roots were found penetrating the stainless steel mesh or the soil space near the taproots. Two control seedlings per mature

Figure 1. **Root exclusion cylinders.**

tree were grown within individual cylindrical soil volumes that received the same treatment except the PVC cylinder was not inserted into the soil. This approach was meant to control for possible effects of the soil disturbance involved in cylinder installation on the growth and survival of seedlings. A subset of cylinders and controls was conically slit trenched periodically (see Fig. 2.) with a shovel to sever the CMN connection but disturb the water flow as little as possible. The trenching isolated the effect of CMNs from the effect of mycorrhizae alone because it disconnected the subset of seedlings from the network but still allowed them to have un-networked mycorrhizae. The cylinders were installed in June

2012 and no acorns were planted for four months so fungi could have sufficient time to grow through the mesh wall.

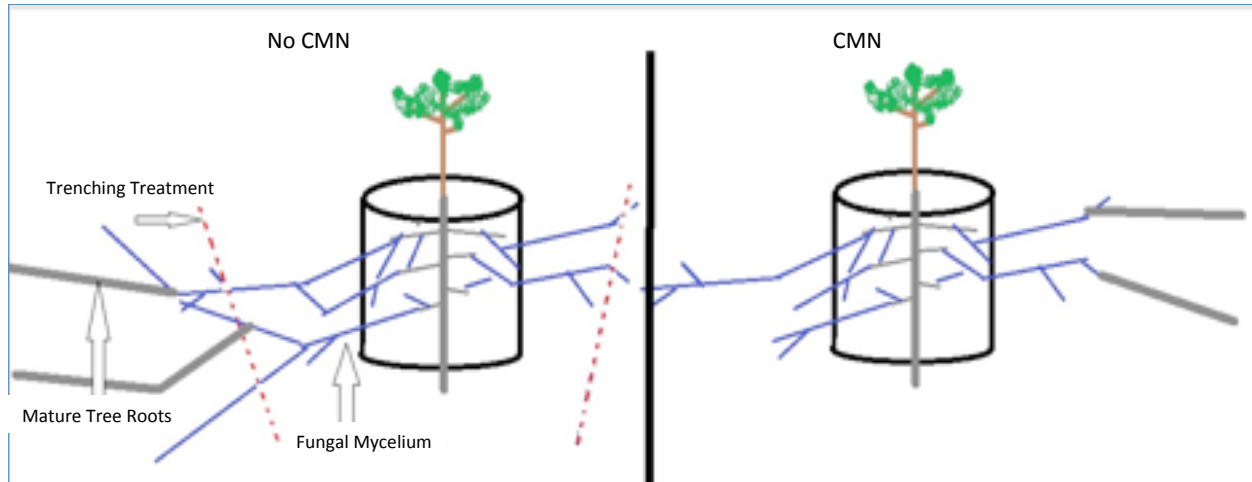


Figure 2. Trenching treatments. A subset of seedlings received the conical slit trenching treatment that disconnected the seedlings from the CMN but still allowed them to have un-networked mycorrhizal fungi.

Southern red oak (*Quercus falcata*, n=720) and white oak (*Quercus alba*, n=720) acorns were collected locally and two of each were planted into the cylinders and non-cylinder control soil volumes in November of 2012. This was done because white oak acorns germinate in the fall and then remain dormant through the winter. In addition, acorns were planted instead of seedlings to allow mycorrhizal networks to be in contact with all study plants as soon as they germinated.

The cylinders were left uncovered during the fall to allow accurate leaf litter depths to accumulate until January 2013. During that time there was significant squirrel predation on the planted acorns resulting in an unbalanced number of observations per plot. In January 2013, wire mesh cages were installed to prevent any additional acorn predation. When the acorns began to grow or germinate, they were thinned to a density of one per cylinder in May 2013. The final relative abundance of each species depended on the relative germination and predation rates of each species (Table 1).

Table 1. Acorns planted. The number of acorns planted and the number that survived and germinated in each canopy openness and burning category. There were 15 canopy trees per plot with four experimental cylinders and two non-cylinder control planting sites per study tree.

Canopy / Fire	Planted		Final	
	Experimental	Control	Experimental	Control
Open/Burned	60	30	33	12
Open/Unburned	60	30	16	9
Closed/Burned	60	30	50	19
Closed/Unburned	60	30	8	1

Seedling Measurements and Mycorrhizal Colonization of Root Tips

The seedlings were monitored for survival until late October 2013. Initial and final seedling height and basal diameter were measured. The seedlings were then destructively harvested and stored at ~3°C until they could be processed. The shoot of each seedling was severed at the root collar. The roots of each seedling were washed with water over a sieve to remove the soil. The lateral roots were removed from the taproot, cut into approximately 1 cm pieces and spread out in a 9mm glass petri dish and suspended in approximately 0.5 cm of water. The shoots and taproots were collected in a paper envelope and then dried in an oven at 60°C for 72 hrs. A dissecting microscope was then used to count the number of times any lateral root intersected a line on a 1cm grid and that value was then used to calculate the lateral root length with the equation:

$$R = \pi NA / 2H$$

Where R = root length, N = number of root intercepts, A = area of the petri dish, H = total length of the grid lines.

Using the dissecting microscope, the root tips colonized by ectomycorrhizal fungi were directly counted using the 1cm grid as a guide to inspect all root pieces. The lateral roots were stored in 50% ethanol for a possible future analysis of arbuscular fungal colonization. After the shoots and roots were dried, the above and below ground biomass was obtained to calculate the total biomass and the root:shoot biomass ratio.

Environmental measurements

To quantify natural variation in light, moisture, and groundcover competition, slope angle, slope position, aspect, soil texture, groundcover vegetation leaf area index, and canopy openness were measured for each cylinder. A Sonin Combo Pro laser distance meter was used to measure the distance from the nearest ridge top to determine slope position, and a clinometer was used to determine the elevation relative to the nearest ridge top. Slope angle was mathematically derived from the relative elevation and distance to the nearest ridge top. Aspect was determined by using a compass to find the direction perpendicular to the plane of the slope; forty-five degrees was then subtracted and that was then converted to radians and cosine transformed to create an aspect index where northeast was positive and southwest was negative. Soil texture was obtained from soil samples taken adjacent to each cylinder; the texture was determined through sedimentation tests using the laMOTTE soil texture test kit. An abiotic covariate moisture index (MI) associated with each seedling was derived from an additive combination of slope aspect, slope position, slope angle and % sand, wherein all variables were first standardized using z-transformation, as follows:

MI = aspect index + distance below nearest ridge - slope angle - log-odds proportion sand

Canopy openness was determined by taking spherical (fish eye lens) canopy photos directly above each cylinder and then using the *gap light analyzer II* program (Cary Institute of Ecosystem Studies, Millbrook New York) to obtain canopy openness values. To examine the impact of adjacent herbaceous plants on the growth and survival of the seedlings, I used a Licor Plant Canopy Analyzer to obtain the neighborhood scale leaf area index (LAI) of groundcover vegetation adjacent to each seedling.

Analysis

Survival was so high that only growth was analyzed. To address whether CMNs had an effect on the growth or ectomycorrhizal development of oak seedlings under open or closed canopy or in response to the gradient of herbaceous groundcover density, the following continuous response variables were analyzed: total biomass, above ground biomass, below ground biomass, root:shoot ratio, relative growth rate for height, relative growth rate for basal diameter, lateral root length, number of colonized tips, and the density of colonized tips. Each of these variables was analyzed using linear models. The predictor variables were Trt (CMN/root treatment), MI (moisture index), LAI_grnd (neighborhood scale groundcover), Canopy (% canopy openness), Damage (canopy damage) and Fire (fire history). Hypothesis 1 was tested by the main effect of Trt, hypothesis 2 was tested by the interaction of Trt and Canopy or MI and hypothesis 3 was tested by the interaction of Trt and LAI_grnd; the interaction of damage and fire was used as a categorical blocking factor. I did not include oak species as a predictor variable in the final model because it was not significant for any response variable. Type III

sums of squares were used first to check for interactions; if they were not present, Type II sums of squares were used to estimate main effects because they provide unbiased and more powerful estimates of effects when analyzing unbalanced sample sizes.

If a main effect was significant I then used three orthogonal *a priori* contrasts to test the following specific hypotheses. First, to test for a cylinder effect in the absence of CMNs or root competition, I compared the trenched seedlings within a cylinder (i.e. “No CMNs/no roots, with cylinder”) with trenched seedlings that were not within a cylinder (i.e. “No CMNs/no roots, no cylinder”). Second, I checked for the effect of eliminating both root competition and connection to CMNs simultaneously (i.e., classic trenching) by comparing the only group of seedlings that experienced both root competition and CMNs (i.e., the “CMNs + roots, no cylinder” group) with the group of seedlings that were not grown within a cylinder but were trenched (i.e., the “no CMNs/no roots, no cylinder” group). Third, to test for the effect of eliminating connection to a CMN in the absence of roots, I compared the seedlings grown in a cylinder and connected to the CMN (i.e. the “CMNs only, with cylinder” group) with seedlings that were grown in a cylinder and disconnected from the CMN by trenching (i.e. the “no CMN/no roots, with cylinder” group). The intensity of root competition (which could not be reduced without also severing connection to a CMN) was inferred indirectly by comparing the results of the second and third contrasts, with the assumption that the maintenance of a CMN does not interact with root competition to influence the seedling’s performance.

RESULTS

This experiment investigated the growth and survival of oak seedlings grown from acorns through a data collection period of one growing season. There were very few seedlings ($n=4$) that did not survive through the single season and therefore seedling survival was not analyzed.

Several aspects of seedling growth were affected by the cylinder and/or trenching treatments in ways that indicated negative effects of root competition and/or connection to the CMN. There was a significant effect of treatment on total biomass of the seedlings ($F_{3, 114}=5.325$, $p=0.002$). Contrasts indicated that the total biomass of seedlings was decreased by the combination of both root competition and connection to CMNs ($T=3.075$, $df=129$, $p=0.007$), but was not significantly affected by CMNs alone ($T=1.491$, $df=129$, $p=0.335$). Seedlings without a cylinder that were cut off from both roots and CMNs by trenching had 70% more biomass than ones that had not been trenched, whereas within cylinders, seedlings that were cut off from CMNs by trenching had only 16% more biomass than the seedlings still connected to the CMN. There was also a significant effect of having a cylinder ($T=-2.621$, $df=129$, $p=0.028$) where seedlings without a cylinder had 38% more biomass.

Trenching increased aboveground biomass of oak seedlings in part by eliminating the connection between seedlings and a common mycorrhizal network. There was a significant main effect of treatment on shoot mass ($F_{3, 125}=4.894$, $p=0.003$) and results of the contrasts indicated a significant effect of isolation from both roots and CMNs by trenching on above

ground biomass ($T= 2.431$, $df= 140$, $p= 0.046$), and an effect of isolation from CMNs approaching significance ($T=2.118$, $df=140$, $p=0.097$). Where the seedlings without cylinders that did not experience CMNs or root competition had on average 74% more aboveground biomass than seedlings with both roots and CMNs (Fig. 3A, 3 vs. 4), and within cylinders the seedlings without CMNs had 29% more aboveground biomass than seedlings with CMNs (Fig. 3A, 1 vs. 2). There was no significant cylinder effect for aboveground biomass ($T=-0.966$, $df=140$, $p=0.672$) (Fig. 3A, 2 vs. 4).

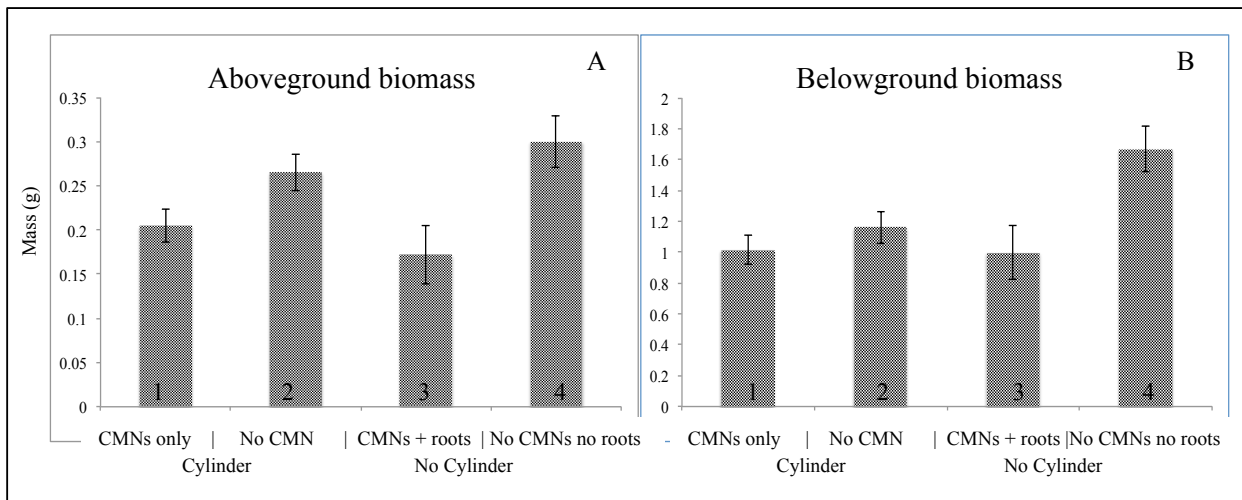


Figure 3. Leverage residual bar graphs of variation in average biomass of oak seedlings among CMN/root manipulation treatments. The contrast between columns 1 and 2 tests the effect of CMNs; between columns 3 and 4 tests the effect of CMNs and roots, and between columns 2 and 4 tests the effect of the cylinder.

For taproot mass I found that elimination of both roots and CMNs increased below ground biomass for seedlings, but eliminating connection to CMNs alone did not. The main effect of treatment was ($F_{3, 114}= 5.167$, $p= 0.002$). Contrasts showed that seedlings that were isolated from root competition and CMN connection had 67% more belowground biomass (Fig. 3B, 3 vs. 4), which was statistically significant ($T= 2.919$, $df=129$, $p= 0.012$) whereas the Seedlings disconnected from CMNs alone had only 14% more belowground biomass (Fig. 3B, 1

vs. 2). Hence, the effect of eliminating connection to CMNs alone was not statistically significant ($T=1.344$, $df=129$, $p=0.421$). Seedlings grown without a cylinder had 44% more belowground biomass (see Fig. 3B, 2 vs. 4) than seedlings grown with a cylinder, resulting in a statistically significant cylinder effect ($T=-2.784$, $df=129$, $p=0.018$).

Above and below ground biomass responses to the treatments were accompanied by a significant change in the root:shoot ratio due to the interaction of the treatments and the density of groundcover vegetation ($F_{3,114} = 5.647$, $p=0.001$). Contrasts revealed that the differences in root:shoot ratio were due to an interaction between the cylinder effect and groundcover vegetation ($T= -3.386$, $df= 62$, $p= 0.001$). Seedlings grown within a cylinder maintained a large taproot relative to shoot mass as the groundcover became denser, whereas seedlings grown without a cylinder had a reduced taproot mass relative to shoot mass as the groundcover became denser. There was no significant effect for the CMN contrast ($T= 0.547$, $df=91$, $p= 0.586$) and a near-significant effect of the root contrast ($T= 1.704$, $df=34$, $p= 0.097$).

I found that the number of ectomycorrhizal root tips per seedling increased as the neighboring groundcover became denser, as indicated by a positive effect of leaf area index (LAI) of neighboring groundcover on the total number of colonized tips per seedling that approached statistical significance ($F_{1,112}= 3.583$, $p=0.061$). I also found an almost significant interaction of treatment and groundcover density on the number of colonized tips per unit lateral root length ($F_{3,111}= 2.636$, $p=0.053$). The difference in colonized root tip density between seedlings connected to the CMN and those not connected to the CMN increased as neighboring groundcover vegetation increased or decreased from the mean ($T= -2.262$, $df=88$, $p= 0.026$). In dense vegetation, seedlings connected to the CMN had much greater root tip colonization than

did those not connected to the CMN (Fig. 4A). The presence of roots coincided with a near significant decrease ($T = -1.842$, $df = 34$, $p = 0.074$) in colonized root tip density as neighboring groundcover increased (not shown). In addition, the difference in root tip colonization between seedlings not grown within a cylinder and those grown within a cylinder was large at low groundcover density and decreased as neighboring groundcover vegetation increased ($T = 2.308$, $df = 62$, $p = 0.024$). In sparse vegetation, seedlings grown within a cylinder had greater root tip colonization than did those not grown within a cylinder (Fig. 4B).

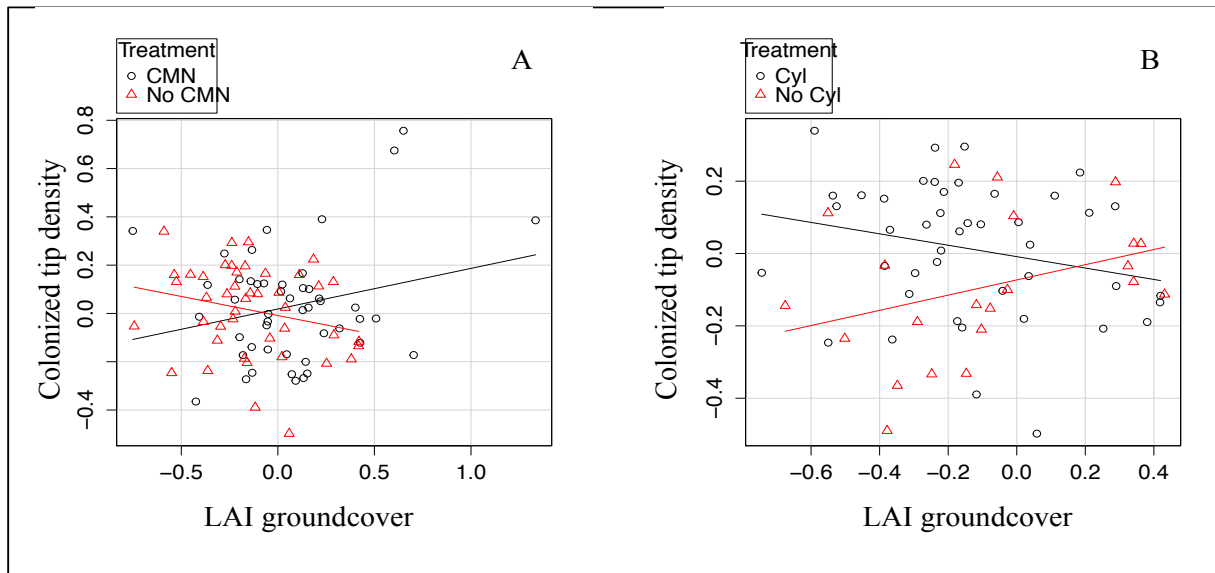


Figure 4. Leverage residual scatterplots for colonized tip density. 4A shows the effect of CMNs on density as groundcover vegetation changes; 4B shows the effect of cylinders on density as groundcover vegetation changes.

For the relative growth rates of height and basal diameter and for lateral root length I found no significant effects of any predictor variable or interaction. See the appendix for R code, complete anova tables, and contrasts.

DISCUSSION

Successful advanced regeneration by oaks in forests and woodlands depends both on the persistence of seedlings in the shade and growth within canopy gaps. Through the mast reproduction strategy of oaks, some acorns are distributed widely but most germinate in a shady understory near other mature oaks. Depending on mass, acorns can grow a substantial seedling from energy stored in the cotyledon that will then persist under the canopy and occasionally die back to the roots until they receive enough light through a canopy gap. When a large enough gap opens in the canopy, the oak seedlings respond to the increased light by increasing their height and leaf area and recruiting to the sapling or tree size class if they have access to enough water (Johnson et al. 2009).

Through the sharing of carbon and/or water between adults and seedlings, connection to a common mycorrhizal network potentially provides a mechanism by which oak seedlings could persist in shade and/or grow rapidly in dry soils within canopy gaps. As oak seedlings germinate under the canopy of a mature tree they are colonized by mycorrhizal fungi present in the soil. These same fungi can be connected to adjacent mature tree roots and can serve as a potential network through which a mature tree could facilitate the growth or survival of seedlings through a more even distribution of water that the seedling could not reach alone, or through the transportation of carbon that the seedling could not capture without more light. This experiment investigated what effect a CMN has on the growth or survival of oak seedlings during the first

season of growth, and variation in effects of a CMN under closed canopy forest conditions versus open canopy woodland conditions in conjunction with a history of burning.

Contrary to predictions, connection to a CMN did not alleviate either shade stress or water stress, but rather had a negative effect on growth and the accumulation of aboveground biomass in all environmental conditions encountered. Several studies involving evergreen conifers, which rely exclusively on EMF, have shown a positive CMN effect of increased survival or growth (Booth 2004, Booth and Hoeksema 2010, Bingham and Simard 2012). The authors concluded or suggested that the benefit was due to hydraulic redistribution of water that was shown to occur with *Pinus ponderosa* by Warren et al. (2008). At nearly the same time, hydraulic redistribution was also shown to occur through CMNs with oaks by Egerton-Warburton et al (2007). However, in our study we found that CMN connection reduced the aboveground biomass of seedlings, and increased the density of colonized tips on the lateral roots of seedlings. This effect was present regardless of canopy openness or water availability, as there were no interactions of treatment with canopy density or the moisture index. I should note that the seedlings were all grown within the drip line of a mature tree and, although I refer to the damaged canopy plots as open canopy, the lowest canopy cover value for any individual seedling was 57.87%.

Negative effects of common mycorrhizal connections between adults and seedlings of the same genus could be a previously unappreciated mechanism of negative density-dependent seedling growth. Exactly why such connections are maintained despite detrimental effects on seedlings is not clear, but their presence indicates a parasitic relationship between mycorrhizal fungi and oak seedlings under the range of conditions examined here. Perhaps benefits of EMF

colonization later in oak ontogeny outweigh these negative effects during the first year. A popular hypothesis to address the regeneration failure of upland oaks in forests excluded from fire is increased shade from a denser canopy (Abrams 1992, Lorimer et al. 1994) along with increased competition from taller or faster growing non-oaks in canopy gaps (Brose et al. 1999, Iverson et al. 2008). In light of the results of this experiment, we suggest that, along with canopy density and fire exclusion, density-dependent growth reduction mediated through CMNs may reduce natural oak regeneration.

This experiment showed that the aboveground biomass of an oak seedling growing within the dripline of an oak tree in a woodland or forest during the first growing season is suppressed by the connection via a CMN to a mature tree. It seems that with higher tree density there is not only a suppression of growth through light limitation but also belowground competition from mature roots mediated through the CMN. So in regards to our first research question: yes there is an effect of a CMN on the growth of oak seedlings and, in this case, it is a small negative effect on aboveground biomass relative to the effect of root competition. The transportation of carbon or water, in regards to my second question, is not supported by my results, as there was no interaction between treatment and canopy openness or moisture potential. But I did find a partial answer for the third question; while the interaction of groundcover density and treatments did not affect the seedlings survival or biomass directly it did have an effect on the density of ectomycorrhizal root tips on the lateral roots. As the groundcover becomes denser, so do the colonized tips on the lateral roots of seedlings, and with a higher density of colonized tips it is likely that any effect the seedling experiences through the CMN would be stronger as the seedling is more connected to the network. Also, it is important to note the strong negative effect of root competition on the oak seedlings in this experiment. The seedlings that were

exposed to root competition and then isolated from that competition by trenching had on average 70% more total biomass than seedlings that remained exposed to root competition throughout the experiment. This result clearly indicates belowground competitive suppression by mature oaks on oak seedlings growing in their root zones; this has largely been unstudied for oaks and it is assumed that shade, rather than belowground competition, is the dominant factor suppressing oak seedling growth in forests. These results support the practice of tree thinning to reduce density and prescribed burning to promote oak regeneration.

Although my results highlight a negative effect on seedlings from a CMN, it's important to keep the big picture in mind. Lifelong fitness of long-lived perennials may be better gauged through the ability to survive stressful disturbances such as fire or drought, and not solely through rapid growth during relatively stable, moderate conditions. When contemplating our results we should note that the oaks in our experiment showed a very similar growth response as the pine seedlings did in the Booth and Hoeksema (2010) study. The pine seedlings connected to the CMN had reduced biomass during the first year, but by the second year those same seedlings had significantly better survival than disconnected seedlings. That experiment was carried out in coastal California, which has a Mediterranean climate with a cool wet winter season and a warm dry summer season. The connected seedlings had better survival through the second warm dry season, which was drier than average when that study was conducted. Furthermore, Bingham and Simard (2011) showed that growth of Douglas fir seedlings was increased by CMNs, and demonstrated that drought stress intensified this facilitation in a growth chamber experiment. Our experiment only tested the CMN effect through one growing season that had no drought-like dry spells. It is possible that the seedling-mycorrhizal fungi-mature tree system is somewhat analogous to an insurance policy. Where the seedlings are constantly paying “premiums” that

result in reduced biomass during moderate conditions but their survival is facilitated through stressful periods such as fire or drought stress

This paper provides a glimpse into the dynamics of oak seedling interactions with a CMN in a forest or woodland under moderate conditions, but we would also like to know the effect of a CMN on seedlings during times of stress. I suggest that research into the effect of CMN interaction with oak seedlings include fire, or clipping, and drought as treatments to determine the effects of CMNs on oak seedlings during stressful times to further complete the picture of oak seedling interactions with common mycorrhizal networks.

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LIST OF APPENDICES

APPENDIX A: TOTAL BIOMASS

Appendix A total biomass

```
biomass<- lm(data=dat4,Biomass ~ Trt*Covin + Trt*canopy + Trt*LAI_grnd +  
damage*Fire, contrasts=list(Trt=contr.sum,damage=contr.sum,Fire=contr.sum))
```

```
Anova(biomass,type="3")
```

Anova Table (Type III tests)

Response: Biomass

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	2.361	1	3.6287	0.05931
Trt	1.204	3	0.617	0.60538
Covin	0.001	1	0.0008	0.97774
canopy	1.644	1	2.5276	0.11464
LAI_grnd	0.929	1	1.4274	0.23467
damage	0.983	1	1.5111	0.22151
Fire	0	1	0	0.99822
Trt:Covin	1.904	3	0.9753	0.40704
Trt:canopy	2.398	3	1.2285	0.30268
Trt:LAI_grnd	3.555	3	1.8215	0.1472
damage:Fire	0.807	1	1.2407	0.26768
Residuals	74.164	114		

Anova(biomass)

Anova Table (Type II tests)

Response: Biomass

	Sum Sq	Df	F value	Pr(>F)	
Trt	10.393	3	5.3253	0.001803	**
Covin	0.172	1	0.2651	0.607625	
canopy	1.997	1	3.0691	0.082482	.
LAI_grnd	1.208	1	1.8568	0.17568	
damage	1.102	1	1.6933	0.195796	
Fire	0.001	1	0.0011	0.973764	
Trt:Covin	1.904	3	0.9753	0.407045	
Trt:canopy	2.398	3	1.2285	0.302681	
Trt:LAI_grnd	3.555	3	1.8215	0.147202	
damage:Fire	0.807	1	1.2407	0.267678	
Residuals	74.164	114			

```
contr.trt <- rbind("CMN vs no CMN"=c(-1,1,0,0),"Roots+CMN vs. Neither"=c(0,0,-1,1),"Cylinder effect"=c(0,1,0,-1))
```

```
contr.bio <- lm(data=biodata, Biomass ~ damage*Fire + canopy + LAI_grnd + Covin)
```

```
con <- lm(data=biodata, contr.bio$residuals ~ Trt)
```

```
contr.bio <- summary(glht(con, linfct=contr.trt))
```

```
contr.bio
```

Simultaneous Tests for General
Linear Hypotheses

Fit: lm(formula = contr.bio\$residuals ~ Trt, data = biodata)

Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(> t)	
CMN vs no CMN == 0	0.3789	0.2541	1.491	0.33475	
Roots+CMN vs. Neither == 0	0.8073	0.2625	3.075	0.00742	**
Cylinder effect == 0	-0.5467	0.2086	-2.621	0.02799	*

APPENDIX B: BIOMASS RATIO

Appendix B biomass ratio

```
bratio<- lm(data=dat4,bioratio ~ Trt*Covin + Trt*canopy + Trt*LAI_grnd +  
damage*Fire, contrasts=list(Trt=contr.sum,damage=contr.sum,Fire=contr.sum))
```

```
Anova(bratio,type="3")
```

Anova Table (Type III tests)

Response: sqrat

	Sum Sq	Df	F value	Pr(>F)	
(Intercept)	25.936	1	83.3327	2.97E-15	***
Trt	1.781	3	1.9077	0.1323	
Covin	0.047	1	0.1494	0.69979	
canopy	0.018	1	0.0581	0.8099	
LAI_grnd	0.442	1	1.4198	0.2359	
damage	0.074	1	0.2366	0.62763	
Fire	0.042	1	0.1347	0.71433	
Trt:Covin	0.565	3	0.6055	0.61277	
Trt:canopy	0.917	3	0.9816	0.40409	
Trt:LAI_grnd	5.272	3	5.6466	0.00121	**
damage:Fire	0.315	1	1.0129	0.31633	
Residuals	35.481	114			

Anova(bratio)

Anova Table (Type II tests)

Response: sqrat

	Sum Sq	Df	F value	Pr(>F)	
Trt	2.005	3	2.1476	0.09813	.
Covin	0.002	1	0.0049	0.94408	
canopy	0	1	0.0002	0.98821	
LAI_grnd	0.028	1	0.0901	0.76455	
damage	0.056	1	0.1802	0.67204	
Fire	0.05	1	0.1591	0.69069	
Trt:Covin	0.565	3	0.6055	0.61277	
Trt:canopy	0.917	3	0.9816	0.40409	
Trt:LAI_grnd	5.272	3	5.6466	0.00121	**
damage:Fire	0.315	1	1.0129	0.31633	
Residuals	35.481	114			

APPENDIX C: ABOVEGROUND BIOMASS

Appendix C aboveground biomass

```
shootmass<- lm(data=dat4,shootmass_g ~ Trt*Covin + Trt*canopy + Trt*LAI_grnd +  
damage*Fire, contrasts=list(Trt=contr.sum,damage=contr.sum,Fire=contr.sum))
```

```
Anova(shootmass,type="3")
```

Anova Table (Type III tests)

Response: shootmass_g

	Sum Sq	Df	F value	Pr(>F)	
(Intercept)	0.07197	1	3.4984	0.06377	.
Trt	0.00679	3	0.1101	0.95409	
Covin	0.00559	1	0.2718	0.60305	
canopy	0.02976	1	1.4465	0.23136	
LAI_grnd	0.09193	1	4.4685	0.03651	*
damage	0.01292	1	0.6281	0.42956	
Fire	0.00014	1	0.0067	0.93479	
Trt:Covin	0.05094	3	0.8254	0.48223	
Trt:canopy	0.02815	3	0.4561	0.71346	
Trt:LAI_grnd	0.0551	3	0.8929	0.44693	
damage:Fire	0.06666	1	3.2402	0.07426	.
Residuals	2.57154	125			

Anova(shootmass)

Anova Table (Type II tests)

Response: shootmass_g

	Sum Sq	Df	F value	Pr(>F)	
Trt	0.30207	3	4.8944	0.002991	**
Covin	0.01429	1	0.6945	0.406225	
canopy	0.04339	1	2.1092	0.148919	
LAI_grnd	0.05512	1	2.6795	0.10416	
damage	0.01254	1	0.6094	0.436475	
Fire	0.00088	1	0.0426	0.836846	
Trt:Covin	0.05094	3	0.8254	0.482226	
Trt:canopy	0.02815	3	0.4561	0.713462	
Trt:LAI_grnd	0.0551	3	0.8929	0.446927	
damage:Fire	0.06666	1	3.2402	0.074262	.
Residuals	2.57154	125			

```
contr.sh<-lm(data=shootdata,shootmass_g~ damage*Fire + canopy + LAI_grnd + Covin)
```

```
cons<-lm(data=shootdata,contr.sh$residuals ~ Trt)
```

```
contr.sho <- summary(glht(cons,linfct=contr.trt))
```

```
contr.sho
```

Simultaneous Tests for General
Linear Hypotheses

Fit: lm(formula = contr.sh\$residuals ~ Trt, data = shootdata)

Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(> t)	
CMN vs no CMN == 0	0.09089	0.04291	2.118	0.0969	.
Roots+CMN vs. Neither == 0	0.12799	0.04454	2.874	0.0135	*
Cylinder effect == 0	-0.03493	0.03614	-0.966	0.6719	

APPENDIX D: BELOWGROUND BIOMASS

Appendix D belowground biomass

```
rootmass<- lm(data=dat4,Root_mass ~ Trt*Covin + Trt*canopy + Trt*LAI_grnd +  
damage*Fire, contrasts=list(Trt=contr.sum,damage=contr.sum,Fire=contr.sum))
```

```
Anova(rootmass,type="3")
```

Anova Table (Type III tests)

Response: Root_mass

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	1.737	1	3.5473	0.06219
Trt	1.268	3	0.8626	0.46276
Covin	0.004	1	0.0086	0.92633
canopy	1.186	1	2.4222	0.1224
LAI_grnd	0.425	1	0.8682	0.35343
damage	0.748	1	1.5265	0.21917
Fire	0	1	0	0.99973
Trt:Covin	1.39	3	0.9459	0.42101
Trt:canopy	2.026	3	1.3787	0.25289
Trt:LAI_grnd	3.543	3	2.4111	0.07052
damage:Fire	0.421	1	0.8605	0.35555
Residuals	55.837	114		

Anova(rootmass)

Anova Table (Type II tests)

Response: Root_mass

	Sum Sq	Df	F value	Pr(>F)	
Trt	7.592	3	5.1665	0.002197	**
Covin	0.077	1	0.1568	0.692858	
canopy	1.409	1	2.8759	0.092643	.
LAI_grnd	0.732	1	1.495	0.223961	
damage	0.823	1	1.6794	0.197628	
Fire	0	1	0.0009	0.976402	
Trt:Covin	1.39	3	0.9459	0.421006	
Trt:canopy	2.026	3	1.3787	0.25289	
Trt:LAI_grnd	3.543	3	2.4111	0.070522	.
damage:Fire	0.421	1	0.8605	0.355549	
Residuals	55.837	114			

```
contr.rt<-lm(data=rootdata,Root_mass~ damage*Fire + canopy + LAI_grnd + Covin)
```

```
> conr<-lm(data=rootdata,contr.rt$residuals ~ Trt)
```

```
> contr.sho <- summary(glht(conr,linfct=contr.trt))
```

```
> contr.sho
```

Simultaneous Tests for General Linear Hypotheses

Fit: lm(formula = contr.rt\$residuals ~ Trt, data = rootdata)

Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(> t)	
CMN vs no CMN == 0	0.2977	0.2215	1.344	0.4207	
Roots+CMN vs. Neither == 0	0.6678	0.2288	2.919	0.012	*
Cylinder effect == 0	-0.5062	0.1818	-2.784	0.0178	*

APPENDIX E HEIGHT RGR

Appendix E height RGR

```
height<- lm(data=dat4,ht_rgr ~ Trt*Covin + Trt*canopy + Trt*LAI_grnd + damage*Fire,  
contrasts=list(Trt=contr.sum,damage=contr.sum,Fire=contr.sum))
```

```
Anova(height,type="3")
```

Anova Table (Type III tests)

Response: ht_rgr

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	2.65E-06	1	2.8536	0.09366
Trt	3.45E-06	3	1.2356	0.29968
Covin	1.10E-06	1	1.1824	0.27897
canopy	7.00E-08	1	0.0758	0.78359
LAI_grnd	1.37E-06	1	1.4703	0.22758
damage	1.29E-06	1	1.384	0.24166
Fire	2.03E-06	1	2.1787	0.14244
Trt:Covin	3.77E-06	3	1.3514	0.26084
Trt:canopy	2.28E-06	3	0.8157	0.48749
Trt:LAI_grnd	1.16E-06	3	0.417	0.74112
damage:Fire	1.44E-06	1	1.5441	0.21634
Residuals	1.16E-04	125		

Anova(height)

Anova Table(Type II tests)

Response: ht_rgr

	Sum Sq	Df	F value	Pr(>F)
Trt	5.45E-06	3	1.951	0.1248
Covin	1.76E-07	1	0.1895	0.6641
canopy	6.15E-07	1	0.6615	0.4176
LAI_grnd	1.68E-06	1	1.8078	0.1812
damage	1.14E-06	1	1.2231	0.2709
Fire	2.02E-06	1	2.1686	0.1434
Trt:Covin	3.77E-06	3	1.3514	0.2608
Trt:canopy	2.28E-06	3	0.8157	0.4875
Trt:LAI_grnd	1.16E-06	3	0.417	0.7411
damage:Fire	1.44E-06	1	1.5441	0.2163
Residuals	1.16E-04	125		

APPENDIX F DIAMETER RGR

Appendix F diameter RGR

```
diameter<- lm(data=dat4,dia_rgr ~ Trt*Covin + Trt*canopy + Trt*LAI_grnd +  
damage*Fire, contrasts=list(Trt=contr.sum,damage=contr.sum,Fire=contr.sum))
```

```
Anova(diameter,type="3")
```

Anova Table (Type III tests)

Response: dia_rgr

	Sum Sq	Df	F value	Pr(>F)	
(Intercept)	0.00007108	1	14.4032	0.0002288	***
Trt	0.00001814	3	1.2252	0.303441	
Covin	0.0000027	1	0.5478	0.4606108	
canopy	0.00000464	1	0.9394	0.3343067	
LAI_grnd	0.00000045	1	0.092	0.7621291	
damage	0.0000019	1	0.3855	0.5358304	
Fire	0.00000208	1	0.4211	0.5176002	
Trt:Covin	0.00001178	3	0.7956	0.4985313	
Trt:canopy	0.00001176	3	0.7941	0.4993714	
Trt:LAI_grnd	0.00000622	3	0.4203	0.738784	
damage:Fire	0.00000347	1	0.7034	0.403233	
Residuals	0.00061686	125			

Anova(diameter)

Anova Table (Type II tests)

Response: dia_rgr

	Sum Sq	Df	F value	Pr(>F)
Trt	0.00001235	3	0.8345	0.4774
Covin	0.00000027	1	0.0544	0.816
canopy	0.00000294	1	0.596	0.4416
LAI_grnd	0.00000112	1	0.2276	0.6341
damage	0.00000162	1	0.3283	0.5677
Fire	0.00000206	1	0.418	0.5191
Trt:Covin	0.00001178	3	0.7956	0.4985
Trt:canopy	0.00001176	3	0.7941	0.4994
Trt:LAI_grnd	0.00000622	3	0.4203	0.7388
damage:Fire	0.00000347	1	0.7034	0.4032
Residuals	0.00061686	125		

APPENDIX G: TOTAL COLONIZED TIPS

Appendix G total colonized tips

```
tips<- lm(data=dat4,Intips ~ Trt*Covin + Trt*canopy + Trt*Inlai + damage*Fire,  
contrasts=list(Trt=contr.sum,damage=contr.sum,Fire=contr.sum))
```

```
Anova(tips,type="3")
```

Anova Table (Type III tests)

Response: Intips

	Sum Sq	Df	F value	Pr(>F)	
(Intercept)	96.417	1	81.2429	6.22E-15	***
Trt	2.992	3	0.8405	0.47447	
Covin	0.035	1	0.0293	0.86432	
canopy	0.313	1	0.2639	0.60843	
Inlai	4.253	1	3.5834	0.06094	.
damage	0.123	1	0.1035	0.7483	
Fire	0.086	1	0.0726	0.78814	
Trt:Covin	2.169	3	0.6093	0.61033	
Trt:canopy	2.399	3	0.6738	0.56985	
Trt:Inlai	1.306	3	0.3669	0.77703	
damage:Fire	0.054	1	0.0452	0.83208	
Residuals	132.918	112			

Anova(tips)

Anova Table (Type II tests)

Response: Intips

	Sum Sq	Df	F value	Pr(>F)
Trt	6.346	3	1.7824	0.1546
Covin	0.005	1	0.0042	0.9484
canopy	0.017	1	0.0145	0.90432
Inlai	4.185	1	3.5262	0.06301
damage	0.103	1	0.0866	0.76903
Fire	0.072	1	0.0604	0.8063
Trt:Covin	2.169	3	0.6093	0.61033
Trt:canopy	2.399	3	0.6738	0.56985
Trt:Inlai	1.306	3	0.3669	0.77703
damage:Fire	0.054	1	0.0452	0.83208
Residuals	132.918	112		

APPENDIX H: COLONIZED TIP DENSITY

Appendix H colonized tip density

```
tipden<- lm(data=dat4,coldensity ~ Trt*Covin + Trt*canopy + Trt*LAI_grnd +  
damage*Fire, contrasts=list(Trt=contr.sum,damage=contr.sum,Fire=contr.sum))
```

```
Anova(tipden,type="3")
```

Anova Table (Type III tests)

Response: coldensity

	Sum Sq	Df	F value	Pr(>F)	
(Intercept)	4.7228	1	110.5853	< 2e-16	***
Trt	0.1459	3	1.1389	0.33662	
Covin	0.0434	1	1.016	0.31566	
canopy	0	1	0.0002	0.98765	
LAI_grnd	0.0354	1	0.8282	0.36476	
damage	0.0292	1	0.6847	0.40976	
Fire	0.0044	1	0.1039	0.74782	
Trt:Covin	0.0355	3	0.2771	0.84184	
Trt:canopy	0.1932	3	1.5079	0.21645	
Trt:LAI_grnd	0.3378	3	2.6362	0.05326	.
damage:Fire	0.0297	1	0.6964	0.40579	
Residuals	4.7405	111			

Anova(tipden)

Anova Table (Type II tests)

Response: coldensity

	Sum Sq	Df	F value	Pr(>F)
Trt	0.2331	3	1.8194	0.14777
Covin	0.0321	1	0.7505	0.38817
canopy	0.0131	1	0.3056	0.5815
LAI_grnd	0.036	1	0.8439	0.36027
damage	0.0229	1	0.5354	0.46591
Fire	0.0072	1	0.169	0.68182
Trt:Covin	0.0355	3	0.2771	0.84184
Trt:canopy	0.1932	3	1.5079	0.21645
Trt:LAI_grnd	0.3378	3	2.6362	0.05326
damage:Fire	0.0297	1	0.6964	0.40579
Residuals	4.7405	111		

APPENDIX I: LATERAL ROOT LENGTH

Appendix I lateral root length

```
latlen<- lm(data=dat4,root_length ~ Trt*Covin + Trt*canopy + Trt*LAI_grnd +  
damage*Fire, contrasts=list(Trt=contr.sum,damage=contr.sum,Fire=contr.sum))
```

```
Anova(latlen,type="3")
```

Anova Table (Type III tests)

Response: root_length

	Sum Sq	Df	F value	Pr(>F)	
(Intercept)	188094	1	13.9247	0.0003006	***
Trt	29656	3	0.7318	0.5351319	
Covin	476	1	0.0352	0.8515022	
canopy	10301	1	0.7626	0.384378	
LAI_grnd	23153	1	1.714	0.1931453	
damage	3793	1	0.2808	0.5972039	
Fire	10631	1	0.787	0.3769078	
Trt:Covin	19148	3	0.4725	0.7020498	
Trt:canopy	34948	3	0.8624	0.4629302	
Trt:LAI_grnd	77845	3	1.921	0.1302508	
damage:Fire	1	1	0.0001	0.9943036	
Residuals	512886	112			

Anova(latlen)

Anova Table (Type II tests)

Response: root_length

	Sum Sq	Df	F value	Pr(>F)
Trt	9072	3	0.2239	0.8796
Covin	6817	1	0.5046	0.4789
canopy	7340	1	0.5434	0.4626
LAI_grnd	19550	1	1.4473	0.2315
damage	3838	1	0.2841	0.5951
Fire	10763	1	0.7968	0.374
Trt:Covin	19148	3	0.4725	0.702
Trt:canopy	34948	3	0.8624	0.4629
Trt:LAI_grnd	77845	3	1.921	0.1303
damage:Fire	1	1	0.0001	0.9943
Residuals	512886	112		

VITA

William Chase Bailey was born in Grenada Ms. in early January 1983. He grew up playing in the forests and working in the fields of a small family cattle farm just outside of Grenada Ms. He attended kindergarten through high school at Kirk Academy in Grenada, where he discovered his love for biology and ecology. He then received his BA in Biology from the University of Mississippi with minors in Chemistry and Anthropology in 2007. After his Bachelors degree he worked at a ski resort as a photographer and on whitewater rivers as a raft guide for four years before beginning his graduate studies in August 2011 at the University of Mississippi. He received his Masters of Science in Biology in May 2015 after four years of research, work and writing and began searching for a job to begin his career.