Girdling: Effects on Carbon-13 Concentrations and Biomass in Mycorrhizal Fungi

Anthony Baldrica, Zac Fortier, Joseph Halick

Abstract

Our study examined the effects of girdling trees on both the total biomass and the stable carbon -13 isotope concentrations of mycorrhizal fungi. In order to conduct this research we used two test sites, the FASET (Forest Accelerated Succession ExperimenT) and the reference plot. During the spring of 2008, in the FASET forest plot all Bigtooth Aspen and Birch trees were girdled, which in turn kills the trees. We used mesh bags to monitor the growth of fungi one year after the girdling process. After the bags were collected we separated the fungi and analyzed them. We found no significant correlation between biomass of the two plots, as well as no variation in carbon-13 levels. In hopes of explaining this inconclusive evidence we looked at tree specific biomass to see if species had any effect on total biomass. The results showed that only Red Maple demonstrated higher levels of biomass between reference and FASET plots. One explanation for these results could be the intermingling of tree roots below ground, which would allow for the mutualistic mycorrhizal fungi to transfer to other tree roots without loss of biomass. Future studies that could be done include monitoring hyphal growth closer to the date of girdling to ensure that population size of fungi are representative of the new growth after girdling. In addition we would like to measure carbon-13 concentrations in the flowering bodies of the fungi precisely after the trees are girdled.

Introduction

Mycorrhizal fungi form mutualisms with many plant species. In this mutualistic relationship Mycorrhizal fungi receive carbon from the plants, while they in turn are responsible for the breakdown of organic materials in the soil to forms of usable nitrogen and phosphorus. There are two different forms of Mycorrhizal fungi that can be found in nature, these are Arbuscular Mycorrhizal fungi and Ectomycorrhizal fungi. Arbuscular Mycorrhizal fungi are found in 85% of all plant species. Their hyphae penetrate through the cell walls of the plants roots in order to exchange nutrients. Ectomycorrhizal fungi on the other hand are much less common and are only found in 10% of all plant species. The signature difference between Ectomycorrhizal and Arbuscular is that in Ectomycorrhizal fungi instead of penetrating through the cell walls of the plant for nutrient exchange like in Arbuscular, they instead surround the cell walls of the plant for their nutrient exchange. In our project we analyzed the total biomass of the Mycorrhizal fungi between experimental and treatment plots. We also analyzed carbon-13 concentrations within the fungi between the experimental and treatment plots. The results received from this analysis should be indicative of the effects of girdling on species mutualisms formed by the Mycorrhizal fungi.

The two test sites that we used for data collection took place in the FASET (Forest Accelerated Succession ExperimenT) research area. The purpose of the FASET forest is to model the shift in tree species composition that is expected to take place over the next 30 years. The FASET forest composition is expected to change from primarily aspen and birch trees, to red maple, which thrives in soils with high nitrogen concentrations. In order to expedite this process 6,500 Aspen and Birth trees were girdled, simulating the composition of the forest's tree species post climate change. A map of the FASET forest (Figure A) shows the scale of the forest

site in which the results were collected. The Girdling process is a disruption in the trees natural Xylem – Phloem interaction. This interaction normally allows the Phloem to transport nutrients between the fungi and the plant, while the Xylem transports H_2O. When girdling occurs two horizontal cuts are made around the circumference of the tree, this is followed by a vertical cut made between the two horizontal cuts. From there a crowbar is used to remove the bark of the tree successfully providing a detachment of the trees phloem and cutting off all nutrient exchange. This causes major stress for nutrients on the tree. When trees are under stress stomata close so that the tree cannot take up anymore CO_2. In order to acquire the carbon needed to maintain functioning life systems the tree can no longer be choose and must resort to taking in C-13. This form of carbon is normally not favored because it is slightly more massive on the atomic level, and as a result is slower in traveling through the tree.

In the first hypothesis of our experiment we expect find that the total bio-mass of the Mycorrhizal fungi will differ significantly between the experimental and control plots. Our prediction is that there will be less mass of Mycorrhizal fungi in the experimental plant because there are less living trees to transfer nutrients to the fungus. Our experiment will also look to test the hypothesis that concentrations of C-13 contained in Mycorrhizal fungi of the experimental plot will differ from the control plot. Our prediction for this hypothesis is that we expect higher levels of C-13 concentrations within the Mycorrhizal fungi of the experimental plot because of the stress caused by the girdling process.

Methods

The methods section of our experiment can be divided into two clear and concise sections. There are the pre-experimental methods and the post-experimental methods. Pre-

experimental processes were conducted by FASET research specialists beginning with the girdling of 6,500 Birch and Aspen trees in the spring of 2008 with the help of a forestry company. All girdled trees started to die, which was intended to be a sped up representative simulation of the succession event occurring in the forests of Northern Michigan. Shortly after Mesh bags were buried in each plot (experimental and control) with openings only large enough for hyphae of fungi to pass through. Some of the bags were placed as close as 2.1 meters of specific species of trees with the hopes of giving an accurate representation of that trees Mycorrhizal fungi mutualisms, other bags were planted in the absence of any tree connection. The sample bags contained sterilized sand to ensure that only the Mycorrhizal fungi would be living in them after removal from their one year stay at respective FASET and Reference plots. These bags were then left in the freezer at lakeside lab where we were able to collect them for our personal experimental methods.

The first step in our process was accurately categorizing bags by their Plot, Tree Number, and Bag number. From there we began to clean the bags of any humus or other debris that could construe our results. The next step in our methods was to open the bags and empty them into one liter beakers. After the soil and fungi were emptied into the beakers, we filled them with approximately half a liter of water and swirled the solution in order to separate the semi buoyant hyphae from the sand. While the swirling action is going on the hyphae glom together and are poured into a sieve. From there the fungi were moved into petri dishes where we used spray bottles to clean out remaining grains of sand. Once the vast majority of sand was removed from the samples we used tweezers and a microscope to remove any threads from the bag that got into the fungus. After this preparatory process we freeze dried the samples and took the biomass of them before grinding the samples into a fine powder using the grinder in the boat well. Finally,

we had the samples analyzed for their C-13 concentrations by having an isotope analysis doen by lakeside lab. With all the preparation of the samples now complete we were able to input the data into Microsoft Excel. Microsoft Excel allowed us to analyze the differences between tree species biomass between the FASET and Reference and successfully make histograms to demonstrate these differences. We also used the SPSS software and imported the data from Excel. Once in SPSS we used a T-Test which documented the difference in comparative means in biomass and C-13 concentrations from the FASET and Reference plots.

Results

Figures 1.1-1.5 display the varying levels of hyphal mass contained by each bag of tree species in both the experimental FASET plot and the control reference plot. Red Maple is the only species that supports our hypothesis ($p=0.007$) which states that control plot should have more hyphal mass than the FASET plot. White Pine ($p=0.781$), Red Oak ($p=0.603$), Paper Birch ($p=0.717$), Bigtooth Aspen ($p=0.822$) all showed inclusive results that infer from our hypothesis that there is no difference in biomass between the two plots.

The FASET plot had an average of 242.5 kg/ha of hyphae with a standard deviation of 145.8 kg/ha, and the control plot had an average of 295.3 kg/ha of hyphae with a standard deviation of 183.7 kg/ha. The p-value was 0.364 which is no less than 0.05 so our null hypothesis cannot be confidently rejected.

The range of d13C in all of the trees is from -25 to -28, so there is not much range for a trend to be formed between the experimental and control plot. FASET plot showed an average of -26.68 with a standard deviation of 0.54, and control plot showed an average of -26.88 with a standard deviation of 0.53. The p-value of this t-test proved to be inconclusive at 0.637.

As percent carbon increases the percent of other minerals from the sand decreases as well as the total hyphal mass because hyphae weigh less than sand. Figure 2 shows a negative slope which correlates with our assumptions that total hyphal mass will decrease with increasing percent carbon. Since not ll of our bags are equally as pure it is difficult to distinguish meaningful results in hyphal mass and d13C levels.

Another way to test our methods and whether or not the total number of hyphal mass is affected by the number of bags in the plot is by graphing number of bags versus per bag hyphal mass. We should hope for a slope of 0 to show no correlation between the two. Instead a slightly increasing slope is exhibited in Figure 3, but this could be caused due to the single outlier point of 8 bags. Therefore it can still be assumed that the number of bags is not influencing how much hyphael mass is collected per plot area.

Figure 1.1

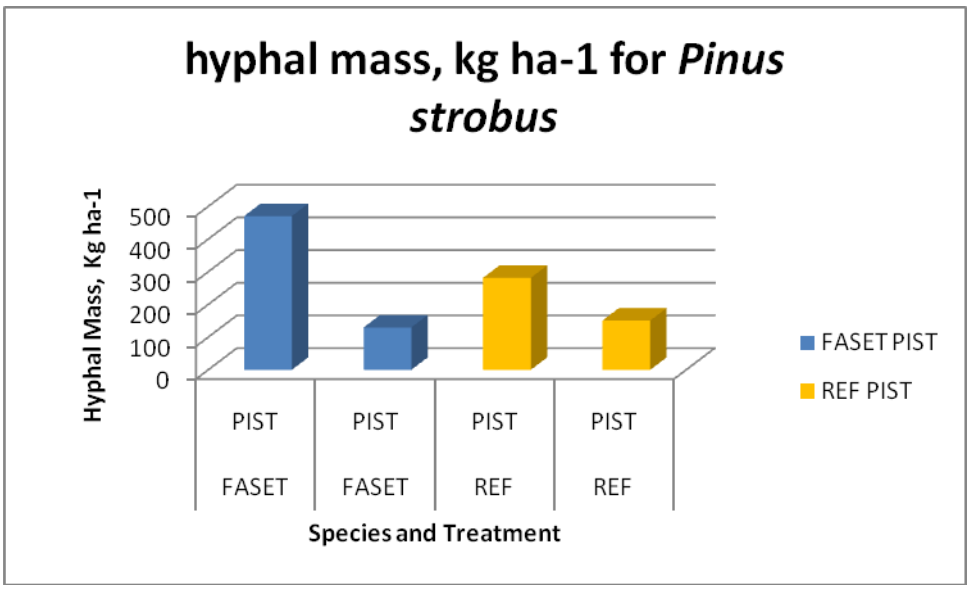


Figure 1.1 shows the difference between plots of hyphal mass in White Pine

Figure 1.2

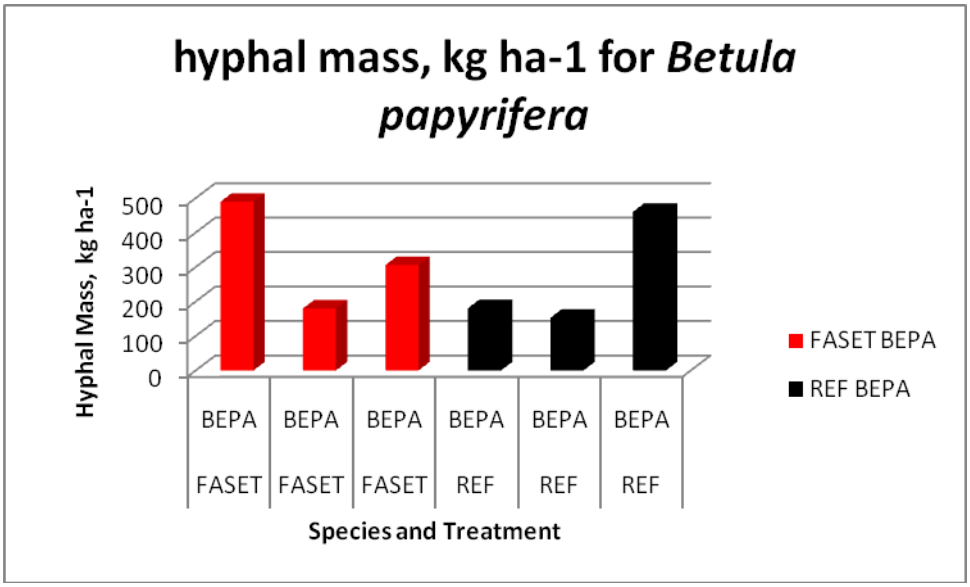


Figure 1.2 shows the difference between plots of hyphal mass in Paper Birch

Figure 1.3

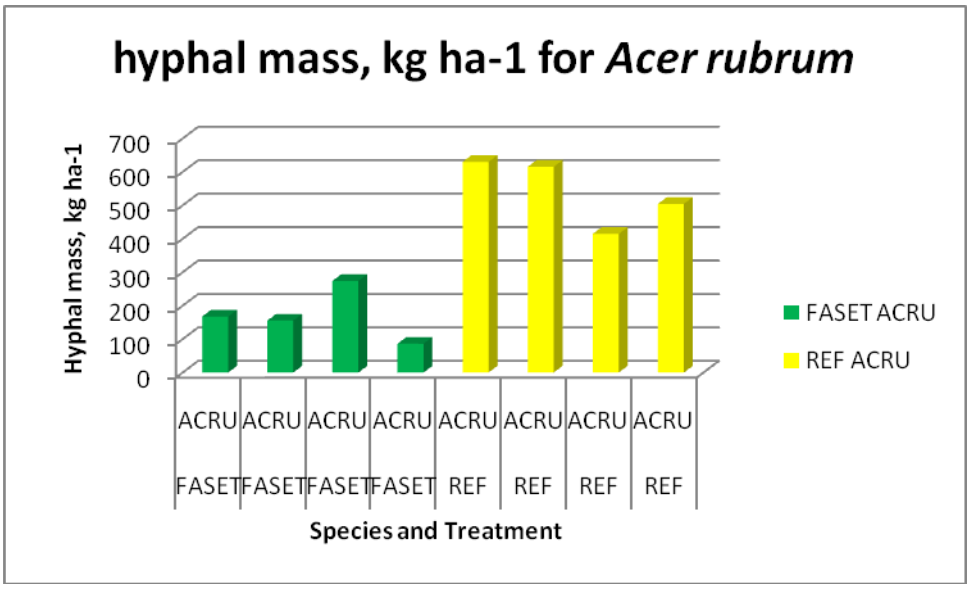


Figure 1.3 shows the difference between plots of hyphal mass in Red Maple

Figure 1.4

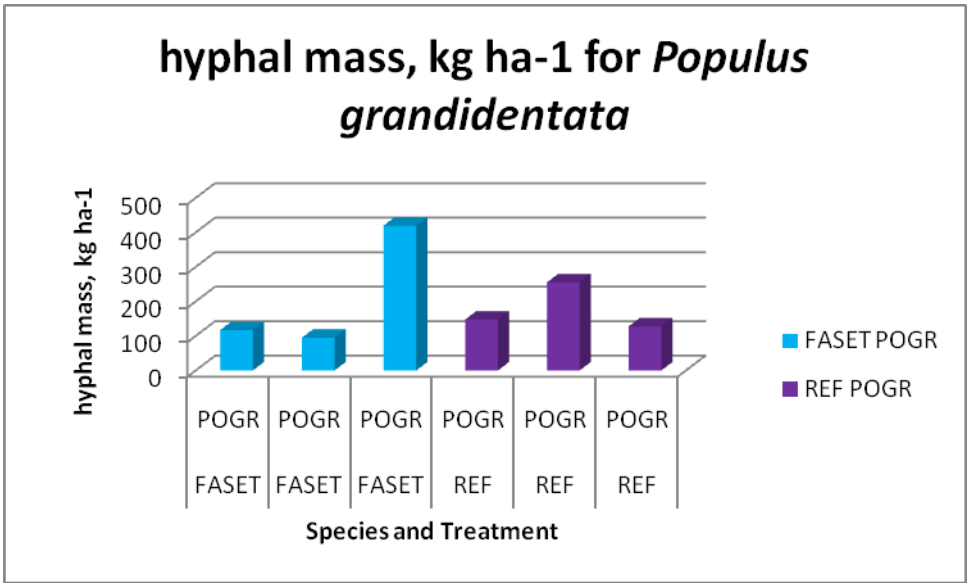


Figure 1.4 shows the difference between plots of hyphal mass in Bigtooth Aspen

Figure 1.5

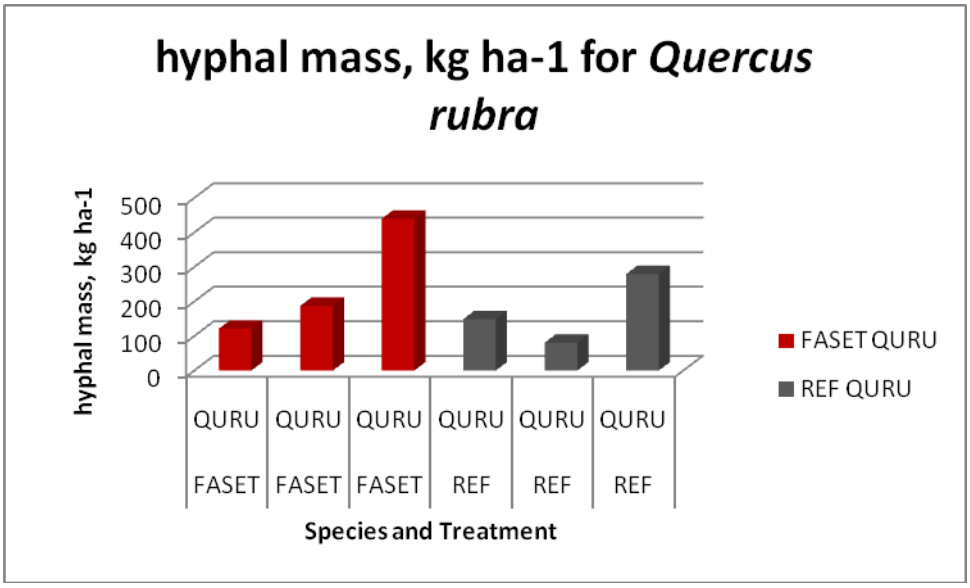
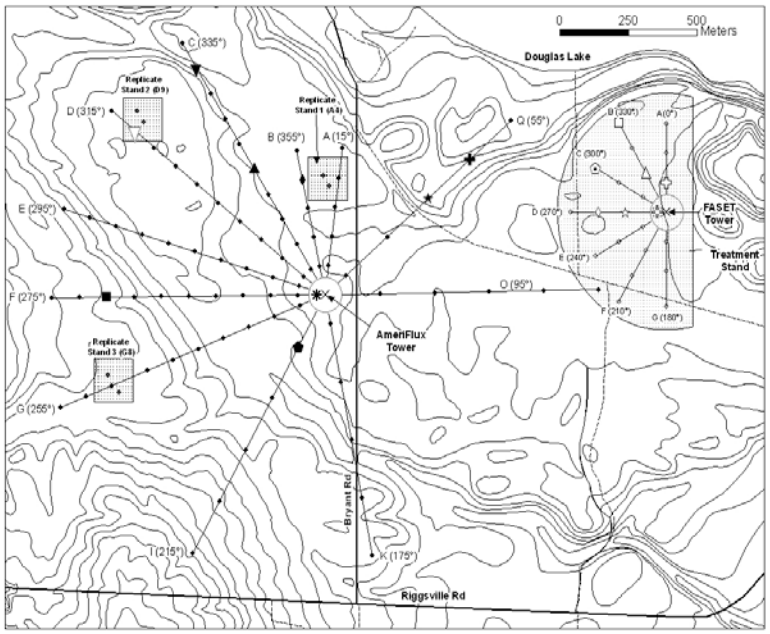


Figure 1.5 shows the difference between plots of hyphal mass in Red Oak

Figure A



This is a map of the FASET forest that shows the location compared to Douglas Lake and its surroundings.

Discussion

The process of girdling trees has an impact on what would normally be expected of C-13 levels in dying trees, which is an elevated concentration of C-13 (Hogberg et al.). Although the girdled trees were stressed and dying, C-13 concentrations did not differ in the experimental and control plots. If carbon transfer to the fungi was still occurring post girdling, there would be an increased concentration of C-13 within the fungi. Without an intact phloem due to girdling, the transfer of carbon to the tree's roots and mycorrhizal mutualists ceases. This explains why we didn't see higher concentrations of C-13 in the experimental plot than in the control plot.

While the process of girdling trees results in killing trees, it doesn't necessarily affect the population size of mycorrhizal fungi associated with them, so long as many living trees are still

surrounding the living ones. Although all of the birch and aspen trees were girdled in the FASET forest, biomass of the mycorrhizal fungi in the FASET forest did not significantly vary from the control site in white pine, red oak, big tooth aspen, and paper birch. However, biomass of mycorrhizal fungi did differ significantly in red maples between plots. A potential reason to explain why biomass doesn't differ between treatments in white pine, red oak, big tooth aspen, and paper birch is how tree roots are very inter-mingled (Perry). We propose that because tree roots are so close together, that when the roots of a tree are dying, mycorrhizal populations in the forest won't diminish. Instead of dying with the girdled tree, the fungi only need to grow a very small distance to the next nearest tree root, and carryout life processes with that new partner. The intermingledness of tree roots also makes our assumption that sample bags placed next to a certain species only contain hyphae associating with that species false. Tree roots of one tree species can grow very close to the base of another tree species. If the hyphae from sample bags placed next to an aspen or birch tree were truly associated only with the aspen or birch tree's roots, that sample bag wouldn't contain any fungi. Any fungi would be dead because they would have no food source. We have shown that carbon transfer is cut off from fungi post girdling due to C-13 concentrations not changing. This explains how biomass of mycorrhizal fungi doesn't differ between white pine, red oak, big tooth aspen, and paper birch between treatments.

The significantly higher biomass of fungi near red maples in the reference plot is probably due to a sampling flaw. With only four samples taken from each treatment, we didn't really get a very good representation of all red maples in the area. There was also variability within the cleanliness of our samples taken. Mycorrhizal fungi all contain about 45% carbon, so if any sample was found to have less than 40% carbon, we can be sure it was contaminated, primarily from left over grains of sand from the sample bags. We can know that our data is not flawed, because of the negative slope of the linear regression line (figure 2).

Our data ended up to support each of our null hypotheses, which is interesting. Total biomass of mycorrhizal fungi between plots did not differ, nor did C-13 concentrations of mycorrhizal fungi. We can find some significance in the experiment due to the insignificance of our data. Girdling trees turns out to be an easy way to kill trees while keeping the mycorrhizal population levels stable. Mycorrhizae are not harmed by the girdling of trees so long as there are living trees nearby. Tree girdling is done in order to control population levels of unwanted and or invasive tree species, as well as to increase the taste of fruit grown (Goldschmidt et al.). These practices should not have an effect on the total biomass of mycorrhizal fungi in an area with some girdled trees.

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