MYCORRHIZAL RESPONSE TO SOIL WARMING AND DISTURBANCE IN TEMPERATE FORESTS OF THE EASTERN UNITED STATES

by

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(Under the Direction of Jacqueline Mohan)

ABSTRACT

It is predicted that the planet's global temperature will continue to increase and that more disturbance events will occur. These factors are expected to affect forest productivity as well as soil respiration, decomposition, mineralization, and mycorrhizal activity. The response of ectomycorrhiza (EM) and arbuscular mycorrhiza (AM) to soil warming and disturbance was determined by seedling growth, fungal colonization amounts, foliar nitrogen (N) and $\delta^{15}N$ content, and EM fungal species diversity. It was hypothesized that EM fungal colonization, foliar $\delta^{15}N$, and EM fungal diversity would decrease with increasing soil temperatures and that AM species would be unaffected. The first two hypotheses were not supported but EM fungal diversity was decreased. It was also hypothesized that disturbance would result in greater fungal colonization of seedlings receiving more light but decrease EM fungal diversity. Results demonstrated that support of these hypotheses is tree species dependent.

INDEX WORDS: mycorrhiza, nitrogen (N), soil temperature, global change

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DEDICATION

This work is dedicated to my parents Robin and Gail Bridges and my brother Anthony Bridges for inspiring me and giving me such a strong foundation from which to grow.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

According to the IPCC (2007), global temperatures are increasing primarily due to increasing anthropogenic greenhouse gas emissions. In the Southeastern United States alone, the temperature is expected to increase 3-6°C (Mearns et al 2003). Studies of *plant* response to increasing carbon dioxide (CO_2) and increasing temperatures have become very important. However, most plant species form mycorrhizal relationships with fungal symbionts for their survival and productivity. Therefore, in order to understand how plants will respond to a changing climate it is equally imperative to understand how the mycorrhizal relationship will be affected. A review by Pendall et al (2004) observes that increasing CO_2 and temperature may affect, among others, soil moisture, nutrient mineralization, respiration, decomposition and enzymatic action including mycorrhizal activity.

Mycorrhizal symbiosis is a mutualistic relationship between fungi and plants. Fungi reside on the roots of the plants and receive photosynthetic carbon from the host. In return, the fungi extract biologically unavailable nutrients such as nitrogen and phosphorus from the soil and transfer it to the plant (Smith and Read, 2008). Hobbie (2006) estimated 8-17% plant-derived carbon is allocated to the fungal partner and 61-86% of the plant nitrogen is provided by the fungi. There are several types of mycorrhizal symbiosis, but ectomycorrhiza (EM) and arbuscular mycorrhiza (AM) are the most abundant (Smith and Ready, 2008). Approximately 80-90% of terrestrial plant species are AM and a much smaller proportion is EM (Smith and Read,

2008) but most dominant tree species are EM. Ectomycorrhizal associations involve the fungus residing on the outside of the root cell and are visible to the naked eye. In an arbuscular mycorrhizal relationship, the fungus infiltrates the root cell and nutrient transfer occurs at the interface of plant and fungus cells (Smith and Read, 2008).

As global temperatures increase, it is expected that nitrogen mineralization will increase as well (Rustad et al 2001; Melillo et al 2002, 2011). Nitrogen mineralization is the biological activity of soil microbes converting organic nitrogen to inorganic nitrogen. Inorganic nitrogen is especially important because it is easily taken up by plants. In a mixed temperate forest system typically accepted as nitrogen limited, almost all tree species are host to either EM or AM fungi (Smith and Read, 2008). However, as soil nitrogen becomes more available the mycorrhizal relationship is altered and often reduced as demonstrated in fertilization studies (Baum and Makeschin 2000, Frey et al 2004, Smith and Read 2008, Treseder, 2008). Also, a shift in EM fungal composition has been observed when a change in nitrogen and phosphorus availability occurs (Baum and Makeschin 2000, Frey et al 2004).

EM fungal species are understood to be more efficient in acquiring nitrogen from the soil than are AM fungi (Smith and Read, 2008). Some EM fungi have the ability to access organic sources of nitrogen which gives them an advantage over AM fungi (Smith and Read, 2008). However, AM fungi play an important role in phosphorus uptake.

When transferring nitrogen from soil sources to the tree, the fungus fractionates against ^{15}N so that the tree is depleted in this nitrogen isotope but the fungus is enriched (Hobbie and Hobbie, 2008; Mayor et al 2009). This fractionation is typically more pronounced in EM species, meaning there is a greater depletion of ^{15}N in EM trees, and the ^{15}N content of AM trees is only

slightly depleted or not affected (Hobbie et al 2005). This naturally occurring ratio of $^{15}N/^{14}N$ is often used to study N allocation within mycorrhizal species.

This same technique of natural isotope abundance has also been used for observation of mycorrhizal effects of plant and fungal $^{13}C/^{12}C$ ratios in order to determine carbon allocation. A few studies have found that EM tree species are more enriched in ^{13}C than non-mycorrhizal trees due to increased photosynthesis as a result of greater foliar N content (Handley et al 1993; Hobbie and Colpaert, 2004). This is largely dependent on the fungal species involved in the symbiosis (Hobbie and Colpaert, 2004).

By increasing foliar nitrogen concentrations mycorrhizal symbiosis may increase photosynthesis, and increased light availability surely does. As the globe faces climate change so also does it face human population growth and increased wood product demand. Often, timber management can result in great disturbance of forest structure and composition. There is a great amount of research devoted to observing how tree harvesting practices such as clear cutting (Zhou et al 1997; Zhang et al 2003), thinning (Korb et al 2003), and patch sizes (Luoma et al 2004) are affecting mycorrhizal symbiosis and sporocarp or fruiting body distribution (Luoma et al 2004) .

When timber is harvested and especially clear cut, there is more light available to the regenerating seedlings which can increase plants' photosynthetic rates and carbon balances, thus potentially affecting mycorrhizal associates by making carbon more available. Turner et al (2009) found that *Quercus rubra* seedlings grown in intermediate to high light conditions had greater fungal colonization and species diversity compared to those grown in low light settings. Sugar maple, an AM species, was observed to have decreased fungal colonization in understory or low light conditions (Song et al 2004). In a study of three different EM tree species in a boreal

system, Dehlin et al (2004) found that the effects of light on fungal colonization are species specific. Several studies have observed that mycorrhizal colonization depends on host species shade tolerance (Kyllo et al 2003; Dehlin et al 2004; Druebert et al 2009; Shukla et al 2009).

Soil disturbance due to tree harvesting can also affect mycorrhizal associations. AM fungi are considered generalists (Smith and Read, 2008) and are more likely to recover quickly after a disturbance (Abbott and Robson 1991; McGonigle and Miller 2000; Duan et al 2011. However, EM fungal species diversity and sporocarp production are decreased by disturbance (Jones et al 2003; Luoma et al 2004).

A recent soil warming study at Harvard Forest has revealed an interesting pattern of tree seedling growth where AM species demonstrated more growth in heated soils while EM species were either unaffected or hindered (Mohan et al unpublished). Mycorrhizae were not directly studied in the Harvard Forest warming study, but the disparate tree responses to climate warming may be mediated in part by differential responses of mycorrhizal symbionts.

Similar long-term soil warming studies have been established at Duke Forest, North Carolina and Whitehall Forest, Georgia. Using seedlings from the Harvard Forest and Whitehall Forest sites, it was the goal of this study to determine how mycorrhizal symbiosis may play a role in this pattern. The questions were: 1) Would fungal colonization of seedlings be affected by increased soil temperatures? 2) Would nutrient allocation be affected? 3) Would EM fungal community composition be altered? The hypotheses made were 1) fungal colonization of EM species would be decreased due to increased plant available N, but AM fungal colonization would be unaffected or increased because of their ability to acquire soil phosphorus, 2) EM seedlings would be enriched in ^{15}N and depleted in ^{13}C due to decreased EM fungal colonization,

but AM seedlings ^{15}N would be depleted or unchanged in ^{15}N content due to increased or unaffected fungal colonization, and would be enriched in ^{13}C or unaffected, and 3) there would be a decrease in EM fungal diversity due to increased plant available N.

In the Summer of 2008, an 50x50meter area of Whitehall Forest in Athens, GA was manually cleared to provide space for a soil warming experiment. It is expected that as global population increases so too will urban sprawl and demand for wood products both of which cause great disturbance to forest structure and composition. The goal of this study is to determine the effects that such disturbances might have on mycorrhizal symbiosis and sporocarp distribution in the Southeastern United States. The questions addressed were: 1) How will fungal colonization in a Southeastern temperate forest be affected in a gap vs. understory habitat? 2) How will EM sporocarps be affected? 3) How will EM fungal species diversity of colonized roots be affected? The hypotheses were: 1) Fungal colonization will increase in seedlings grown in the gap due to increased carbon from increased photosynthesis. 2) There will be a decrease in sporocarp production due to removal of host tree species. 3) EM fungal species diversity of colonized roots will be decreased by disturbance.

In summary, understanding mycorrhizal symbiosis will aid in predicting tree response to increasing greenhouse gas emissions and increasing global temperatures. This information is important for understanding a little of how below ground processes will be affected by climate change and the impact it may have on forest productivity and soil nutrient cycling. This will in turn aid in forest management practices to sustain biodiversity, sequester carbon, increase productivity and maintain soil fertility.

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CHAPTER 2

EFFECTS OF INCREASED SOIL TEMPERATURES ON MYCORRHIZAL SYMBIOSIS IN A TEMPERATE FOREST OF THE NORTHEAST

According to the IPCC (2007) global temperatures are increasing due to increasing greenhouse gas emissions (80%) and land use change such as timbering (20%; Canadell et al. 2007). Studies of plant response to increasing CO_2 and increasing temperatures are increasingly important. Most plant species derive the majority of their nutrient from symbiotic mycorrhizal associations (Smith and Read, 2008) making these fungi essential for forest tree growth and survival. In order to understand how plants will respond to a global change it is imperative to understand how the mycorrhizal relationship will be affected. Recent studies provide evidence that increasing global temperatures will strongly affect soil microbial activity including mycorrhizal function (Pendall et al 2004 review).

Mycorrhizal symbiosis is a mutualistic relationship between fungi and plants. Fungi reside on the roots of the plants and receive photosynthetic carbon from the host. Hobbie (2006) estimated 8-17% carbon is allocated to the fungal partner in the mycorrhizal relationship. In return, the fungi extract biologically unavailable nutrients such as nitrogen and phosphorus from the soil and transfer it to the plant (Smith and Read, 2008). There are several types of mycorrhizal symbiosis, but ectomycorrhiza (EM) and arbuscular mycorrhiza (AM) are the most abundant. Approximately 80-90% of terrestrial plant species are AM and a much smaller proportion is EM, although the EM association is important for the majority of eastern temperate tree species (Smith and Read, 2008). Ectomycorrhizal associations characteristically involve the

fungus residing on the outside of the root cell and are visible to the naked eye. In an arbuscular mycorrhizal relationship, the fungus infiltrates the root cell and nutrient transfer occurs at the interface of plant and fungus cells (Smith and Read, 2008).

As global temperatures increase, it is expected that nitrogen mineralization will increase as well (Rustad et al 2001, Melillo et al. 2002, 2011). Nitrogen mineralization is the biological activity of soil microbes converting organic nitrogen to inorganic nitrogen. Inorganic nitrogen is especially important because it is easily taken up by plants or plant available. In a mixed temperate forest system typically accepted as nitrogen limited, almost all tree species are host to either ectomycorrhizal or arbuscular mycorrhizal fungi (Smith and Read, 2008). However, as soil nitrogen becomes more available the mycorrhizal relationship is altered and often reduced as demonstrated in fertilization studies (Baum and Makeschin 2000, Frey et al 2004, Smith and Read 2008, Treseder, 2008 review). Also, a shift in EM fungal composition has been observed when a change in nitrogen and phosphorus availability occurs (Baum and Makeschin 2000, Frey et al 2004).

EM fungal species are understood to be more efficient in acquiring nitrogen from the soil than are AM fungi (Smith and Read, 2008).When transferring nitrogen from soil sources to the tree, the fungus fractionates against ^{15}N so that the tree is depleted in this nitrogen isotope but the fungus is enriched (Emmerton et al 2001; Hobbie and Hobbie, 2008 review; Mayor et al 2009 review). This fractionation is typically more pronounced in EM species (Hobbie et al 2005). This naturally occurring ratio of $^{15}N/^{14}N$ is often used to study N allocation within mycorrhizal species.

This same technique of natural isotope abundance has also been used for observation of mycorrhizal effects of plant and fungal $^{13}C/^{12}C$ ratios in order to determine carbon allocation. A few studies have found that EM tree species are more enriched in ^{13}C than non-mycorrhizal trees due to increased photosynthesis as a result of greater foliar N content (Handley et al 1993; Hobbie and Colpaert, 2004). This is largely dependent on the fungal species involved in the symbiosis (Hobbie and Colpaert, 2004).

A recent soil warming study of tree seedling growth in Harvard Forest has revealed an interesting pattern of AM species demonstrating more growth in heated soils while EM species were either unaffected or hindered (Mohan et al unpublished). Mycorrhiza were not directly studied in the Harvard Forest warming study, but the disparate tree responses to climate warming may be mediated in part by differential responses of mycorrhizal symbionts.

Similar long-term soil warming studies have been established at Duke Forest, North Carolina and Whitehall Forest, Georgia. Using seedlings from the Harvard Forest, it was the goal of this study to determine how mycorrhizal symbiosis may play a role in this pattern. The hypotheses were 1) fungal colonization of EM species would be decreased due to increased plant available N, but AM fungal colonization would be unaffected or increased because of their ability to acquire soil phosphorus. 2) EM seedlings would be enriched in ^{15}N due to decreased EM fungal colonization, but AM seedlings would be depleted or unchanged in ^{15}N content due to increased or unaffected fungal colonization, and 3) there would be a decrease in EM fungal diversity due to increased plant available N.

METHODS

Site Description

The site is a mixed deciduous hardwood forest called 'Barre Woods' in the Harvard Forest Long Term Ecological Research site. It is dominated by *Quercus rubra, Quercus velutina* and *Acer rubrum*. The soils are a Canton series mostly a coarse loamy, mesic Typic Dystrudepts with a pH of approximately 5.5. Mean annual rainfall is 1080mm evenly distributed throughout the year. On an average weekly basis, the air temperature ranges from about 20°C in July to about -6°C in January (Melillo et al, in press).

In 2001, 3.4 miles of conductive cables were buried in a 30x30meter plot in Barre Woods, Massachusetts. The cables were placed 10cm deep and 20cm wide (Melillo et al 2011). These cables have been heating the soil since 2003 to a temperature approximately 6° greater than the ambient temperature. A similar 30x30meter plot was established as a control.

Experimental Design

Naturally recruited seedlings of *Pinus strobus, Quercus rubra, Acer saccharum, Acer pennsylvanicum,* and *Acer rubrum* were harvested from Barre Woods. Eight to ten seedlings of each species were collected, with four to five individuals collected from each of the Control and Heated plots. Seedling heights were approximately uniform by species and ranged from 4.1cm (red maple) to 19cm (white pine). Soil temperature was recorded at every seedling site. The heated soils ranged from 3-6°C greater than the control. Seedlings were carefully extracted by removing the soil and intact root system. Intact seedlings were shipped overnight in cooled packaging to the University of Georgia and stored at 4°C for approximately twenty days. Root systems were separated from shoot systems. Stem and attached leaves were stored at -80°C.

Arbuscular mycorrhizal (AM) root systems of all three of the *Acer* species were immediately stained using the methods of Vierheilig et al (1998). Roots were boiled in KOH, then in black ink (Shaeffer) and vinegar. Percent colonization was determined by sectioning roots into 5mm pieces. These sections were viewed under a compound microscope at 100 and 400x to determine the presence of vesicles, arbuscles or aseptate hyphae. Fifty 5mm sections were taken from each root system unless the system was not large enough; then the entire root system was viewed.

Ectomycorrhizal (EM) root systems of *P. strobus* and *Q. rubra* were viewed under a dissecting microscope at 40x after being rinsed in tap water. All root tips were counted and fungal colonization of root tips was determined by the presence of a mantle. Differing fungal species were determined by morphotyping of fungal characteristics using Agerer (1987-2008) color atlas and the Ectomycorrhizal Description Database.

The foliage of the seedlings which had been stored at -80°C was subjected to C and N isotope analysis. Leaves were freeze dried and lyophilized for 24hours then ground mechanically in Spex 5000D Dual Mixer Mill for homogenization. (Some samples were too small to be ground mechanically so were ground by hand using a glass stirring rod and a vortex. D.I. water was added to decrease static cling. These samples were freeze dried again.) After being ground, a small sample between 1.5-2mg of material was weighed out on a Sartorious Microbalance then deposited in ultrapure tin capsules (5mm x 9mm) for combustion analysis. An Elemental Analyzer-Isotope Ratio Mass Spectrometer (EA-IRMS) was used to determine foliar $\delta^{15}N$ and $\delta^{13}C$ values. Soil ^{13}C and ^{15}N isotopic signatures were determined by almost exactly the same method as foliar isotopes. The only difference is that soil samples were sieved in a 2mm sieve before being homogenized and that 15-20mg of soil sample were analyzed.

Statistical Analysis

Statistics were analyzed using SAS 9.2 (English). A General Linear Model was used. The GLM was followed up with an ANOVA and Tukey's HSD with a p-value of .05. A t-test was used to follow up a barely non-significant ANOVA result. All percentages were arcsine-transformed.

RESULTS

There was no significant difference in soil N concentration between soils in the Heated and Control plots (n=10). The Control plot had a mean %N of 1.0325±0.125 and Heated soils had a mean %N of 0.8859±0.1665 (*p=0.3713*). However, heated soils did have enriched $\delta^{15}N$ values compared to control soils. Whereas control soils had a mean $\delta^{15}N$ of 0.1924±0.2757, heated soil had a mean $\delta^{15}N$ of 1.0436±0.1822 (*p=0.0191*; *Fig. 2.1*). This enriched $\delta^{15}N$ signature of the warmed soils likely reflects in part greater mining of recalcitrant ^{15}N-enriched sources of organic nitrogen by soil microbes. The soil C concentration tended to be decreased in the heated plot (Control: 16.4268±1.3887; Heated: 11.4989±2.6068; *p=0.0799*). The $\delta^{13}C$ value of soils did not differ between plots and was typical of the signature of C3 photosynthetically-derived carbon inputs to the system (Control: -27.3466±0.2045; Heated: -27.0631±0.0458; p=0.2208).

There was no difference in seedling height suggesting that any differences in tissue chemistry or mycorrhizal associations were likely not due to differences in seedling size (*Fig. 2.2*). With the exception of sugar maple stems which were older in the control treatment, stems of a given species did not differ in age between the two temperature treatments (mean age of

sugar maple in control vs. heated was 4.5 and 8 years (*p=0.015*)). Cheng et al (2005) found that AM colonization of sugar maple decreased with age which was not the case in this instance.

Most species from heated plots exhibited decreased percent fungal colonization of roots, with the exception of *Pinus strobus* which showed no difference between treatments (*Fig.2.3*). *Quercus rubra* showed a non-significant trend toward less colonization in the warmed plot (*p=0.45*). The decreased root colonization in the heated plot was particularly notable for maple seedlings which associate with AM fungi. *Acer pennsylvanicum* and *A. rubrum* colonization was slightly decreased in the heated plot (*p=0.0607* and *p=0.1074*, respectively). *A. saccharum* showed significantly decreased root colonization in the heated plot (*p=0.014*).

Only *P. strobus* had significantly increased foliar nitrogen concentrations in the warmed plot compared to the control (*Fig. 2.4*). These heated seedlings also had significantly enriched foliar $\delta^{15}N$ values compared to control seedlings (-4.68 compared to -1.91; *p=0.003; Fig. 2.5*). *A. pennsylvanicum* in the heated plot had lower foliar N concentrations (*p=0.1137*) and $\delta^{15}N$ (*p= 0.0521*; *Fig.2.4* and *2.5*). *A. rubrum, A. saccharum,* and *Q. rubra* showed no difference in foliar N and $\delta^{15}N$ between heated and control conditions.

Foliar % C was not significantly different between the control and heated samples for any tree species, but heated seedlings of *P. strobus* displayed a tendency for decreased %C, likely a 'dilution effect' resulting from the increased foliar % N of this species (Control: 42.95 compared to Heated: 44.26; *Fig. 2.6*). Heated *P. strobus* was significantly enriched in ^{13}C compared to control seedlings as well (-31.15 compared to -32.07; *p=0.0108*; *Fig. 2.7*). All other species demonstrated no difference in foliar carbon concentrations between plots.

Morphotyping of EM fungal species was conducted on the *P. strobus* and *Q. rubra* seedlings. There was greater diversity of fungal types found on control seedlings (*Fig. 2.8*).

DISCUSSION

Percent Colonization

The first hypothesis that EM fungal colonization would decrease and that AM colonization would increase or remain unchanged was not supported by the data. The white pine fungal colonization, a strictly EM tree species, was unaffected by warming and the AM species showed a decrease in root colonization. It appears that greater soil temperature has a negative influence on mycorrhizal colonization of seedling roots except for *P. strobus*. The hardwood species may have been responding to the significantly enhanced rates of nitrogen mineralization in the heated plot (Melillo et al. 2011) by reducing their associations with mycorrhizal fungi. Pines, on the other hand, are obligate mycorrhizal associates (Smith and Read, 2008) and thus are not shifting away from mycorrhizal associations. *Quercus rubra*, on the other hand, has been found to exhibit dual colonization with both EM and AM fungi (Watson et al 1990; Dickie et al 2001).

The greatest decrease in root colonization was found in the AM species: *A. pennsylvanicum, A. rubrum* and *A. saccharum*. This reduction in colonization may reflect reduced importance of the mycorrhizal association when maple trees are growing in warmer soils with higher rates of nitrogen mineralization. This would suggest that AM fungi are not needed by the hosts which are therefore reducing their mycorrhizal associations. A recent study by Hodge and Fitter (2010) demonstrated the ability of AM fungi to acquire organic N and a decrease in root length colonization under N fertilization. Another study in the northern hardwood forests of

Ontario found similar results (van Diepen et al 2010). However, a meta-analysis by Treseder (2004) found significantly varying results in N fertilization studies.

Foliar Nitrogen and Carbon

The second hypothesis that EM tree foliage would be enriched in ^{15}N was not supported for most species. EM-associated plants are generally depleted in ^{15}N whereas AM-associated plants usually have no change or a slight depletion in $\delta^{15}N$ (Hobbie 2005). The heated soils at Barre Woods exhibited a significantly enriched $\delta^{15}N$ signature compared to control soils, with a mean amount of +1‰ enrichment in the heated plot (*Fig. 2.1*). With a decrease in fungal colonization, which occurred for all the maples and the oak, it follows that the plant host would become further enriched in ^{15}N in the heated plot because there are fewer fungi to fractionate against the heavier isotope. However, with decreased fungal colonization in the heated plot, warmer *A. pennsylvanicum* was more depleted in ^{15}N and had less foliar % N (*Fig. 2.3, 2.4 and 2.5*). The more depleted foliar $\delta^{15}N$ may be due to this species gaining a greater proportion of N from soil mineralization, as the biogeochemical process of nitrogen mineralization fractionates against the heavier ^{15}N isotope, enriching the soil (Garten 1993).

The foliar % N was unchanged in heated *A. saccharum* and *A. rubrum* and these species tended to have less fungal colonization. Their $\delta^{15}N$ signatures were not significantly different between plots (*Fig. 2.4* and *2.5*). Given that the heated soils were themselves enriched in ^{15}N and the foliar signatures of the warmer seedlings were not enriched suggests that these hosts are also acquiring their N from a depleted source due nitrogen mineralization or that AM fungal activity cannot be determined with certainty by measuring foliar $\delta^{15}N$ alone.

Quercus rubra exhibited a non-significant tendency for less EM-colonization in the heated plot (*Fig. 2.3*), no change in foliar %N between the plots (*Fig. 2.4*), and an enrichment in $\delta^{15}N$ of about +0.5‰ (*Fig.2.5*). That the foliar enrichment in $\delta^{15}N$ is less than the enrichment in the soil, may result from increased activity in the heated plot of oak fungal associates. (AM fungi were observed in the roots of the Barre Woods oaks but not yet quantified).

Warmed *Pinus strobus* was enriched in $\delta^{15}N$ by about +2.8‰ and had increased foliar % N but showed no difference in fungal colonization (*Fig. 1.3-1.5*). This enrichment in foliar $\delta^{15}N$ above the level observed in the heated soil of +1.0‰ is consistent with enhanced mining of recalcitrant ^{15}N-enriched soil pools in the heated plot, which would also account for the increased foliar %N. These differences also may be due to the shifts in fungal species composition discussed below. Fewer fungal types were found on the roots of *P. strobus* and possibly these fungi are not fractionating as heavily against ^{15}N while taking in more N. The increased foliar $\delta^{13}C$ may also be due fungal species changes (Hobbie and Colpaert, 2004).

Mycorrhizal community

The last hypothesis that the fungal EM community would shift with warming was supported by the results. The abundance of EM fungal colonization was not affected by increased soil temperatures but fungal morphotype/species diversity was decreased in heated conditions for both the red oak and white pine. However, it should be stated that there was not a great abundance of these unshared morphotypes and molecular identification would go a long way in establishing significance in this diversity. Several studies have found that different mycorrhizal species respond differently to increased temperature (Marx and Bryan 1970; Buee et

al 2005; Clemmenson et al 2006) and N availability (Baum and Makeschin 2000; Frey et al 2004; Treseder et al 2004; Alberton and Kuyper, 2009).

CONCLUSION

Increased soil temperatures will likely impact plant-mycorrhizal associations in future temperate forests. This has the potential to affect tree species composition, succession, migration, and carbon sequestration. The data suggests a shift in EM fungal communities with current and future warming and suggest a future climate may also affect fungal species diversity.

These results open several possibilities for future study. To get a more complete idea of what is occurring in this mycorrhizal relationship it would be beneficial to determine the carbon, nitrogen and respective isotope signatures of the mycorrhizal fungi in these associations. Also, determining fungal species by molecular identification would be useful for a more complete understanding of how increased soil temperatures are affecting this symbiosis. Finally, because AM fungi are demonstrating such variability, studying the phosphorus content of the soils in the heated plot may provide more explanation for the results stated here.

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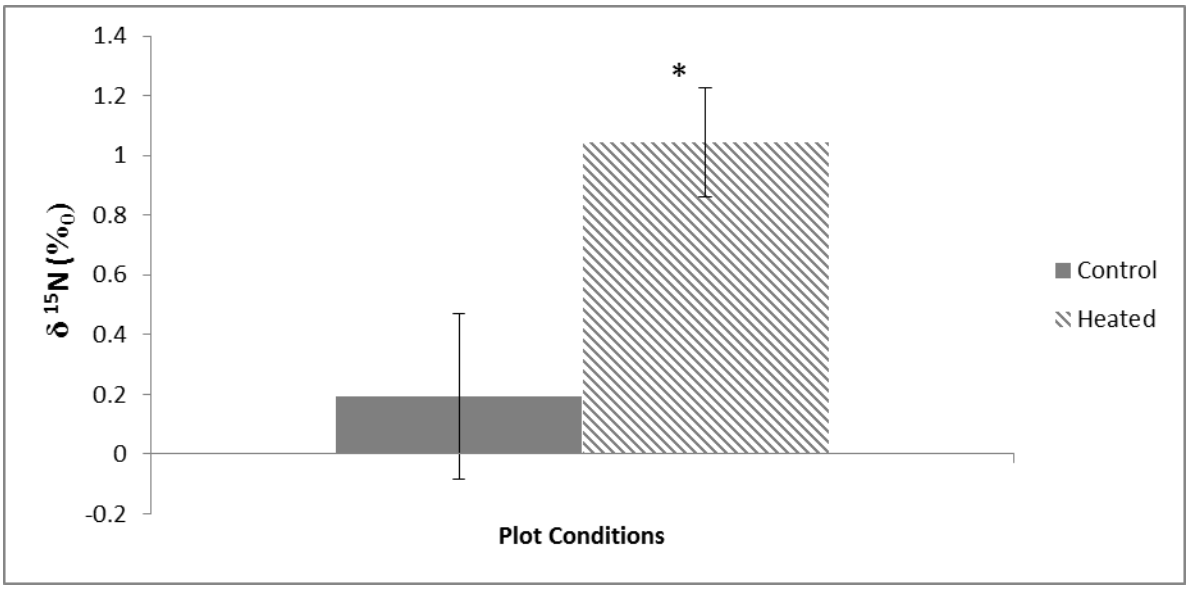

Fig. 2.1 Barre Woods Soil $\delta^{15}N$ Values. $\delta^{15}N$ values of Barre Woods soils in Aug. 2010 in the control (solid) (n=10; P<.05) and the heated (striped) plot (mean and SE bars); Control (solid bar) and Heated (striped bar); (*) denotes statistical significance

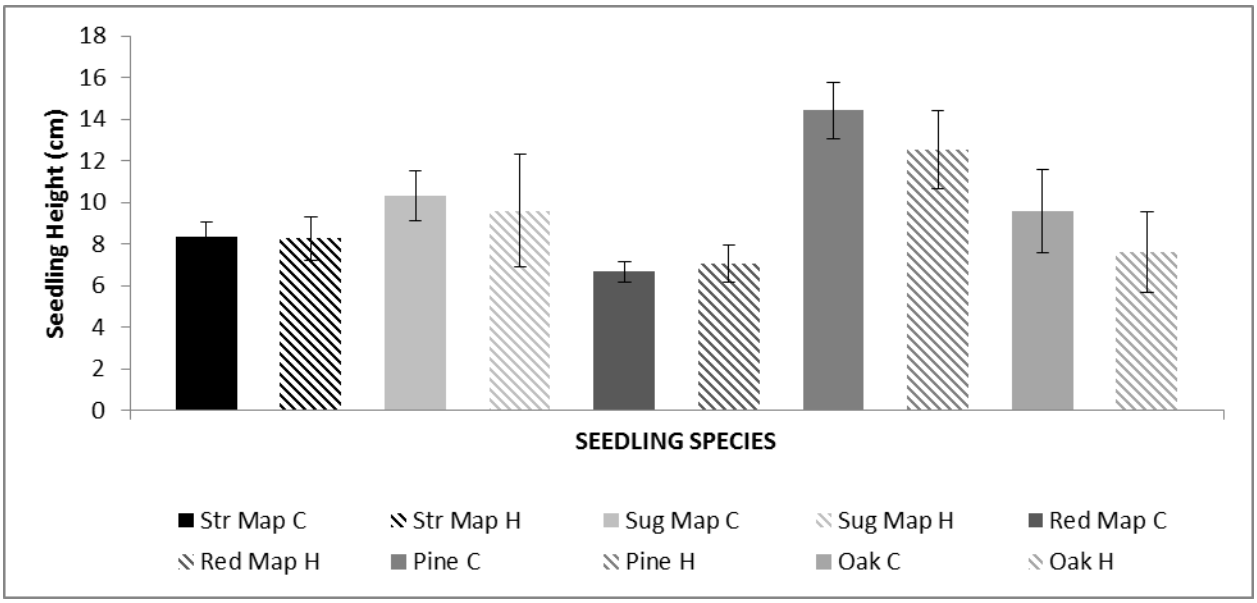

Fig. 2.2 Mean height of seedlings with SE bars; Striped Maple (Str Map), Sugar Maple (Sug Map), Red Map (Red Maple), Pine (White Pine), Oak (Northern Red Oak); C (Control; solid bars) and H (Heated; striped bars).

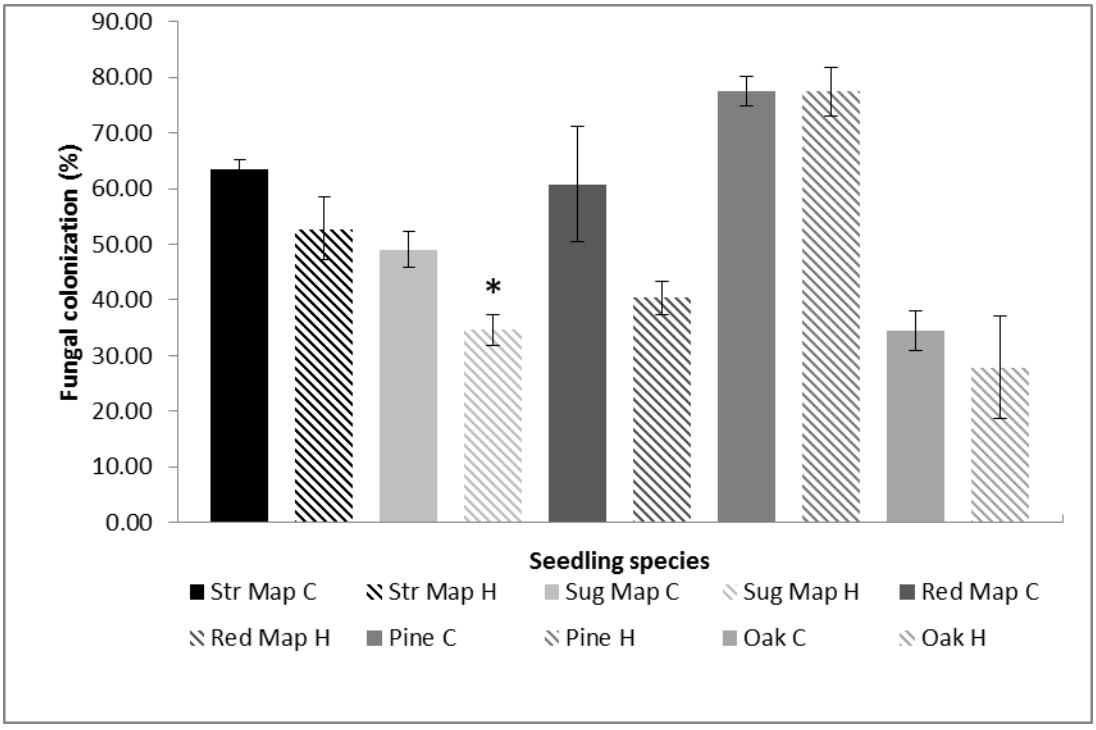

Fig. 2.3 Mean arcsine-transformed percent fungal colonization. Colonization with SE bars of Barre Woods seedlings; Str Map (Striped Maple), Sug Map (Sugar Maple), Red Map (Red

Maple), Pine (White Pine), and Oak (Northern Red Oak); C (Control; solid bars) and H (Heated; striped bars). (*) denotes statistical significance (P<0.05, Tukey's HSD).

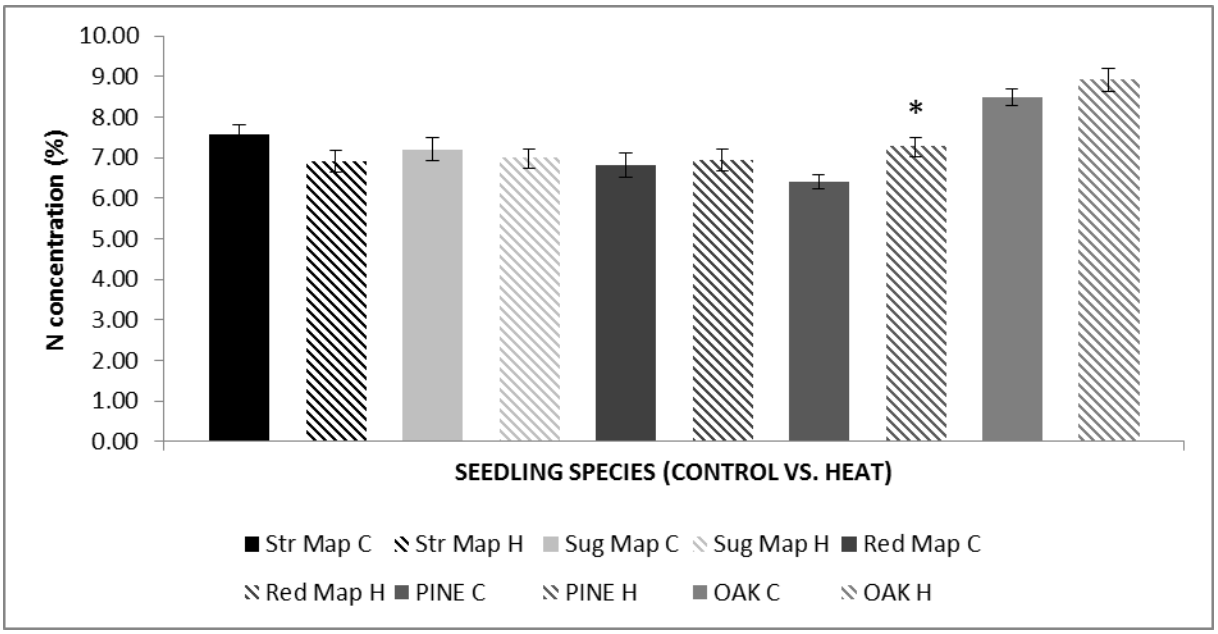

Fig 2.4 Foliar Nitrogen Concentration. Mean arcsine transformed foliar percent N concentration with SE bars; Str Map (Striped Maple), Sug Map (Sugar Maple), Red Map (Red Maple), Pine (White Pine), and Oak (Northern Red Oak); C (Control; solid bars) and H (Heated; striped bars). (*) denotes statistical significance (P<0.05, Tukey's HSD).

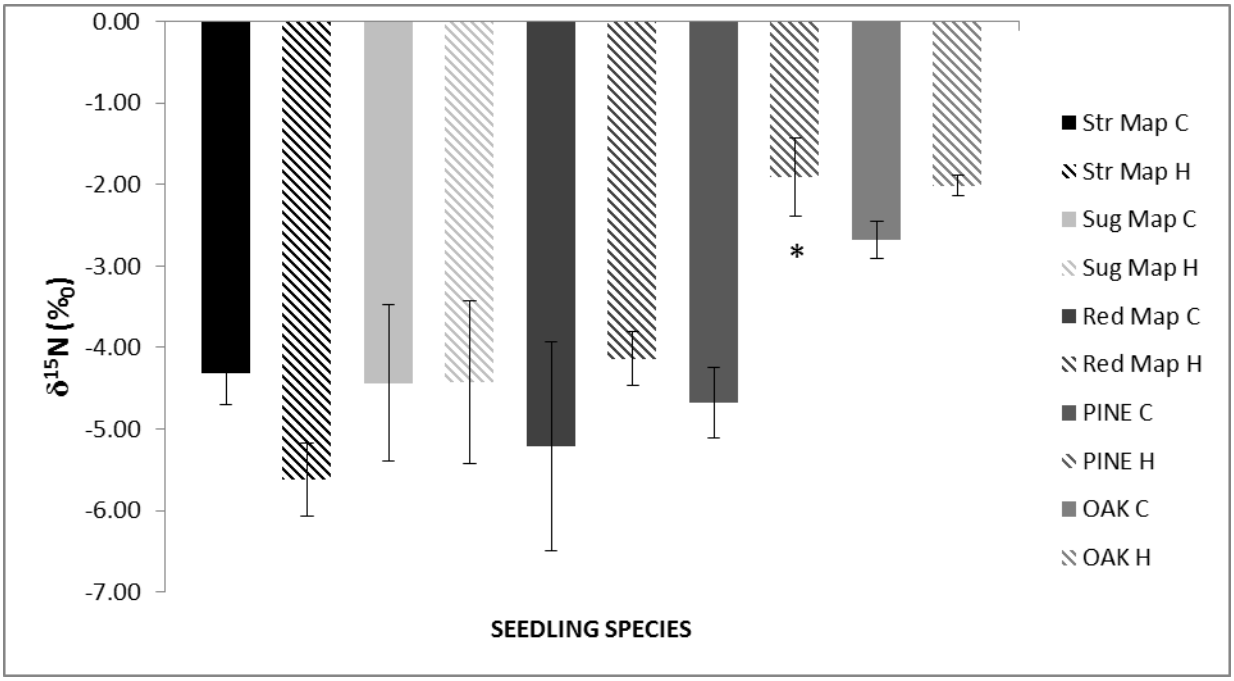

Fig 2.5 Foliar $\delta^{15}N$ Content. Mean foliar ^{15}N content of seedlings with SE bars; Str Map (Striped Maple), Sug Map (Sugar Maple), Red Map (Red Maple), Pine (White Pine), and Oak (Northern Red Oak); C (Control; solid bars) and H (Heated; striped bars). (*) denotes statistical significance ($P<0.05$, Tukey's HSD).

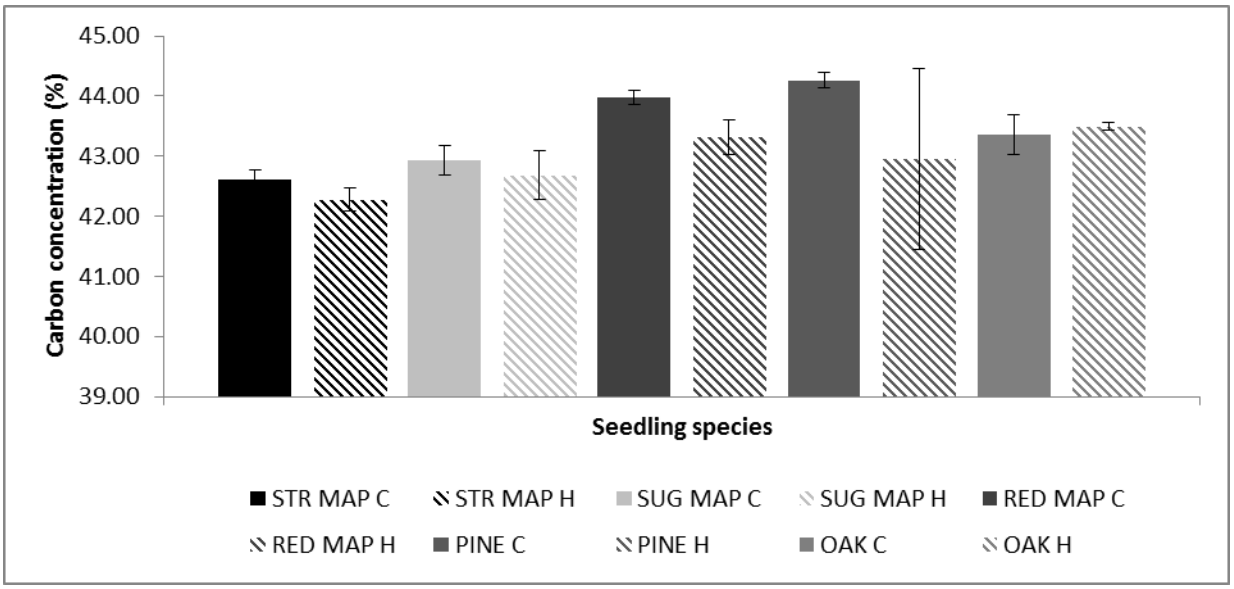

Fig 2.6 Foliar Carbon Concentration. Mean arcsine transformed percent foliar C concentration with SE bars; Str Map (Striped Maple), Sug Map (Sugar Maple), Red Map (Red Maple), Pine (White Pine), and Oak (Northern Red Oak); C (Control; solid bars) and H (Heated; striped bars).

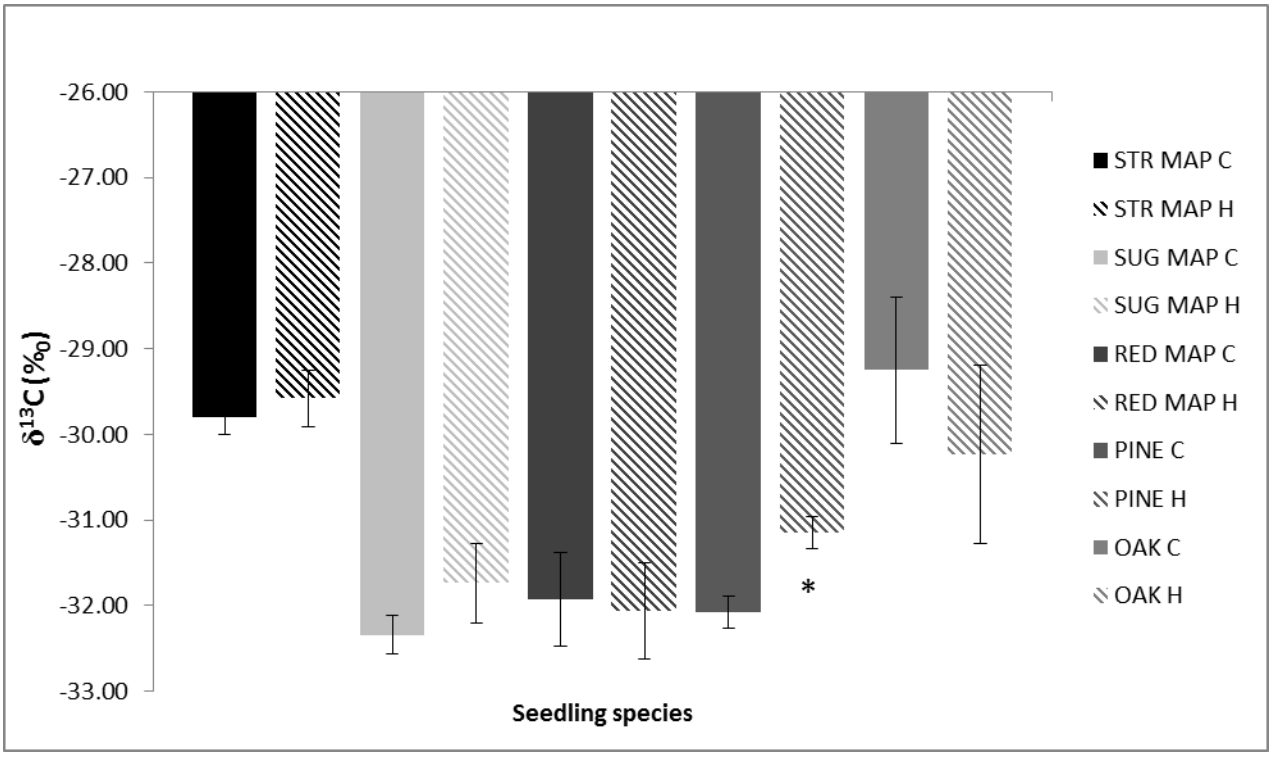

Fig 2.7 Foliar ^{13}C Concentration. Mean foliar ^{13}C content with SE bars; Str Map (Striped Maple), Sug Map (Sugar Maple), Red Map (Red Maple), Pine (White Pine), and Oak (Northern Red Oak); C (Control; solid bars) and H (Heated; striped bars). (*) denotes statistical significance ($P<0.05$, Tukey's HSD).

A. B.

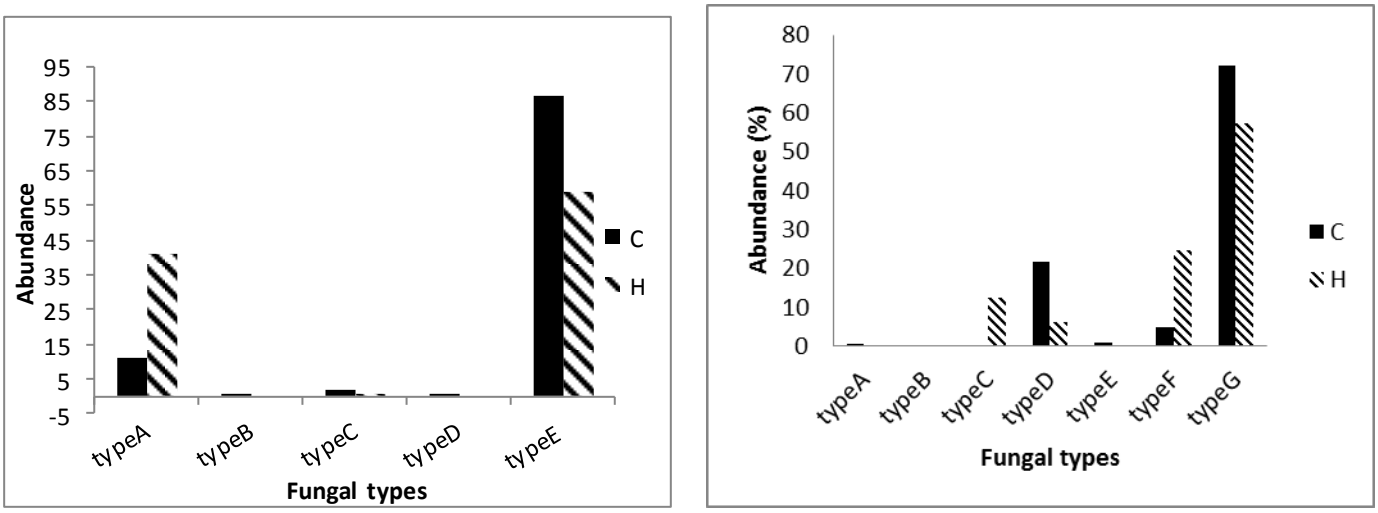

Fig. 2.8 Fungal diversity. Abundance of each morphotype on roots of A) *P. strobus* and B) *Q. rubra.*

CHAPTER 3

MYCORRHIZAL RESPONSE OF SWEET GUM (*LIQUIDAMBAR STYRACIFLUA*) TO SOIL WARMING AND DISTURBANCE IN A TEMPERATE FOREST OF THE SOUTHEAST

According to the IPCC (2007), global temperatures are increasing due to increasing emissions of greenhouse gases (80%) and tropical deforestation (20%; Canadell et al. 2007). Studies of plant responses to increasing CO_2 and increasing temperatures are clearly important but most plant species form mycorrhizal relationships with fungal symbionts for their survival and productivity. Yet few climate change studies examine this plant-mycorrizal association in relevant settings such as actual forest ecosystems. Recent studies do suggest that increasing global temperatures will strongly affect soil microbial activity which includes mycorrhizal functioning (Pendall et al 2004 review).

Mycorrhizal symbiosis is a mutualistic relationship between fungi and plants. Fungi reside on or in the roots of the plants and receive photosynthetic carbon from the host. Hobbie (2006) estimated 8-17% of plant-derived carbon is allocated to the fungal partner in the mycorrhizal relationship. In return, the fungi extract biologically unavailable nutrients such as nitrogen and phosphorus from the soil and transfer it to the plant (Smith and Read, 2008). There are several types of mycorrhizal symbiosis, but ectomycorrhiza (EM) and arbuscular mycorrhiza (AM) are the most abundant. Approximately 80-90% of terrestrial plant species are AM (Smith and Read, 2008). Most studies of AM response to warming have been conducted on crop and

grassland systems (Staddon et al 2002, Gavito et al 2005, Heinemeyer et al 2006). Little research has been focused on forest systems.

As global temperatures increase, it is expected that nitrogen mineralization will increase as well (Rustad et al 2001, Melillo et al. 2002, 2011). Nitrogen mineralization is the biological activity of soil microbes converting organic nitrogen to inorganic nitrogen. Inorganic nitrogen is especially important because it is easily taken up by plants. In a mixed temperate forest system typically accepted as nitrogen limited, almost all tree species are host to either ectomycorrhizal or arbuscular mycorrhizal fungi (Smith and Read, 2008). However, as soil nitrogen becomes more available the mycorrhizal relationship is altered and in the case of EM species often reduced as demonstrated in fertilization studies (Baum and Makeschin 2000, Frey et al 2004, Smith and Read 2008, Treseder, 2008 review).

EM fungal species are understood to be more efficient in acquiring nitrogen from the soil than are AM fungi (Smith and Read, 2008).When transferring nitrogen from soil sources to the tree, the fungus fractionates against ^{15}N so that the tree is depleted in this nitrogen isotope but the fungus is enriched (Hobbie and Hobbie, 2008 review; Mayor et al 2009 review). This fractionation is typically more pronounced in EM than AM species (Hobbie et al 2005). However, Handley et al (1993) saw a $2‰$ enrichment in foliar ^{15}N content in AM compared to non-mycorrhizal plants. Ames et al (1983) found similar results.

A recent soil warming study of tree seedling growth in Harvard Forest has revealed an interesting pattern of AM species demonstrating more growth in heated soils while EM species were either unaffected or hindered (Mohan et al, unpublished data). Mycorrhizae were not

directly studied in the Harvard Forest warming study, but the disparate tree responses to climate warming may be mediated in part by differential responses of mycorrhizal symbionts.

While mycorrhizal symbiosis may increase photosynthesis, increased light availability surely does. As the globe faces climate change so also does it face human population growth and increased wood product demand. Often, timber management can result in great disturbance of forest structure and composition. There has been a great amount of research devoted to observing how tree harvesting practices such as clear cutting (Zhang et al 2003), thinning (Korb et al 2003), and patch sizes (Luoma et al 2004) affect mycorrhizal symbiosis.

When timber is harvested and especially clear cut, there is more light available to the regenerating seedlings. Turner et al (2009) found that *Quercus rubra* seedlings grown in intermediate to high light conditions had greater fungal colonization and species diversity compared to those grown in low light settings. Sugar maple, an AM species, was observed to have decreased fungal colonization in understory or low light conditions (Song et al 2004).

Soil disturbance due to tree harvesting can also affect mycorrhizal associations. Most AM fungal colonization tends to recover quickly or is unaffected by soil disturbance (Abbott and Robson 1991; McGonigle and Miller 2000; Duan et al 2011). AM fungi are considered generalists (Smith and Read, 2008) and likely to recover their populations quickly.

A long-term soil warming study similar to the study in Harvard Forest was established at Whitehall Forest, Georgia. A gap condition was established by clear cutting a 50x50m area. Using seedlings from the Whitehall Forest site, it was the goal of this study to determine how mycorrhizal symbiosis may play a role in tree response to increasing soil temperatures and light availability. The hypotheses were 1) fungal colonization would increase in the heated plots due

to more available N and the need for P acquisition by the fungus, 2) fungal colonization would increase in the gap due to more C availability from the host and 3) seedlings with greater fungal colonization would be depleted in ^{15}N due to increased fractionation.

METHODS

Site description

The 280 ha Whitehall Forest is a warm temperate forest managed by UGA's Warnell School of Forestry and Natural Resources, located ~3 miles from campus in the Piedmont of Georgia ($33^{o}54'N$, $83^{o}22'W$). This site includes 3600 m^2 of even-aged, mixed deciduous forest dominated by *Quercus alba, Q. rubra,* and *A. rubrum,* and is representative of deciduous forests of the Georgia Piedmont (Forkner and Hunter 2000). Soils are of the Cecil/Pacolet association (fine, kaolinitic, thermic Typic Kanhapludults) with low organic matter content, low fertility, and medium to slow permeability. Mean January temperature is $9.1^{o}C$ and mean July, $28^{o}C$. Precipitation is evenly distributed throughout the year and growing season droughts are relatively common due to high evapotranspiration. The long-term precipitation mean is 125 cm yr^{-1} (1945-2003); recent mean is 113 cm yr^{-1} (2003-2007). The total precipitation for the time that these sweet gum seedlings were growing (September 2010-January 2011) was 40.39cm and average air temperature was $14.6^{o}C$. It was a naturally-recruited, unmanaged stand originating in the first half of the 20th Century following agricultural land abandonment.

Experimental Design

Twenty-four chambers were erected in Whitehall. The chambers are halved with twelve under forest canopy (understory) and twelve under a gap condition (gap). Within both sets of twelve, three chambers were controls, fenced in with chicken wire, to determine chamber effects.

The other nine chambers were constructed with non-treated 2x4's and greenhouse plastic. All chambers were 18.7m^2 and had open tops. Conductive cables used to warm the soil were buried 10cm deep and 20 cm apart in all nine chambers, but only six were heated while the other three were used as controls. The temperature of heated soils were 3°C above ambient. The heat was initiated October 2010. The 50x50m area used as the gap treatment was cleared July 2008.

All *L. styraciflua* seedlings were germinated in a sterile medium of sand and peat moss. Once they were mature enough, they were transplanted to the field chambers September 2010. Ten centimeter soil cores were extracted from the chamber soils by a tulip bulb planter. These cores were placed undisturbed (unhomogenized) into mesh bags and reset into the holes from which they were taken. The seedlings were then planted in the bagged cores after height and root length was measured. Seedlings were transplanted to the field in September 2010 and grown under field conditions for 16 weeks at which time they were harvested and measured for final height and root length.

To determine amount of fungal colonization, root systems of the seedlings were immediately stained using the methods of Vierheilig et al (1998). Roots were boiled in KOH, then in Shaeffer black ink and vinegar. Percent colonization was determined by sectioning roots into 5mm pieces. These sections were viewed under a compound microscope at 100X to determine the presence of vesicles, arbuscles or aseptate hyphae. Fifty 5mm sections were taken from each root system unless the system was not large enough; then the entire root system was viewed.

The foliage of the seedlings which had been stored at -80°C was subjected to C and N isotope analysis. Leaves were freeze dried and lyophilized for 24hours then ground manually.

The samples were too small to be ground mechanically so were ground by hand using a glass stirring rod and a vortex. D.I. water was added to decrease static cling. These samples were freeze dried again. After being ground, a small sample between 1.5-2mg of material was weighed out on a Sartorious Microbalance then deposited in ultrapure tin capsules (5mm x 9mm) for combustion analysis. An Elemental Analyzer-Isotope Ratio Mass Spectrometer (EA-IRMS) was used to determine foliar ^{15}N and ^{13}C content.

Soil $\delta^{13}C$ and $\delta^{15}N$ isotopes were determined by almost exactly the same method as foliar isotopes. The only difference is that soil samples were sieved in a 2mm sieve before being homogenized and that 10-25mg of soil sample were used for analysis.

Statistical Analysis

Data was analyzed using SAS 9.2 (English). A General Linear Model was used. The GLM was followed up with an ANOVA and Tukey's HSD with a p-value of 0.05. A T-Test was used to follow up a barely non-significant ANOVA result. All percentages were arcsine-transformed.

RESULTS

Greater survivorship occurred in the gap habitat as opposed to the understory (78% and 58% respectively). Final height of seedlings was not affected by warming treatments in the gap but was significantly affected by warming treatment in the understory (*p=0.6782* and *p=0.0319* respectively; *Fig 3.1*). Warmed seedlings in the understory had greater growth than the control (Control: 4.1cm±0.329 and Heated: 5.04cm±0.167).

There was no effect of habitat (gap vs. understory) on percent soil carbon (*p=0.6804*) or $\delta^{13}C$ content (*p=0.7982*). Also, there was no difference in carbon concentration or soil ^{13}C content between warming treatments in either the gap or the understory (%C *p=0.4413*; $\delta^{13}C$ *p=0.6404* and %C *p=0.8213*; $\delta^{13}C$ *p=0.5951* respectively). Finally, there was no interaction between habitat and warming treatment with respect to soil C or $\delta^{13}C$ (*p=0.4333*).

Soil nitrogen concentration was unaffected by habitat (*p=0.2896*) or warming treatments (*p=0.9628*). There was no significant interaction effect between habitat and warming treatment (*p=0.3309*). The soil $\delta^{15}N$ content was also unaffected by habitat, treatments or an interaction (*p=0.6721, p=0.9722,* and *p=0.4331* respectively).

There was no difference in percent fungal colonization of sweet gum roots between warming treatments in the gap (*p=0.3373*). The treatment had a significant effect on percent fungal colonization in the understory *(p=0.0286*) *(Fig. 3.2*). The control seedlings had a mean colonization of 35.18±2.13 and warmed seedlings had a mean colonization of 79.94±7.27 (*p=0.0286*). Seedlings from the non-chambered control were not significantly different in fungal colonization from either the heated or control seedlings but had a very strong tendency to be greater than the control (*p=0.0575*).

Foliar nitrogen concentrations were significantly elevated in seedlings in the gap condition compared to those in the understory (7.28±0.23 and 6.23±0.16 respectively; *p=0.0011; Fig 3.3*). The non-chambered control in the gap had significantly greater nitrogen concentrations than the same treatment in the understory (*p=0.0011)* (*Fig. 3.4*). Nitrogen concentrations were unaffected by warming treatments within the gap or understory (*p=0.0742* and *p=0.8701* respectively).

The $\delta^{15}N$ values of sweet gum foliage were not affected by warming treatments in the gap or understory (*p=0.9543* and *p=0.2597* respectively). However, in the understory, seedlings of the control treatment were significantly enriched compared to seedlings of the same treatment in the gap (Gap Control mean: -1.7497±0.2223 and Understory Control mean: 0.6655±1.1233, *p=0.02; Fig. 3.5*). The same was also true of seedlings in the warmed treatment with the understory seedlings being more enriched in ^{15}N (Gap +3^{o}C mean: -1.9178±0.3490 and Understory +3^{o}C mean: -0.00092±0.5013, *p=0.0052*). Non-chambered control seedlings were unaffected by habitat (*p=0.7252*; *Fig. 3.5*).

Foliar carbon concentrations were unaffected by warming treatments, habitat, or interactions (*p=0.6598, p=0.2879*, and *p=0.5842* respectively; *Fig 3.6*). The $\delta^{13}C$ values of sweet gum foliage was not different between warming treatments in the gap condition (*p=0.1078*). Foliar $\delta^{13}C$ content was not affected by warming treatment in the understory, but the non-chambered control seedlings were slightly more depleted in $\delta^{13}C$ (*p=0.0638*). However, habitat did affect $\delta^{13}C$ content of sweet gum foliage. All seedlings were more enriched in $\delta^{13}C$ in the gap condition compared to the understory (Non-chambered control *p=0.0035*, Control *p<0.0001*, and Heated *p=0.0168; Fig. 3.7*). There was no interaction effect.

DISCUSSION

The first hypothesis that there would be increased colonization in the heated plots was not supported. There was an interesting result in the control which demonstrated a significant decrease from the heated and non-chambered control plots. It is unclear what may have brought about this result as the soil moisture and temperature were consistent with that of the non-

chambered control plots. . As these chambers were only heated for a short four month period, it may be that an extended time period will see different and more definite results

The second hypothesis that there would be greater colonization of seedlings in the gap was also not supported. There was no significant difference in colonization between light treatments. This was unexpected but may be explained by the time of year. Even though several seedlings still retained green leaves, most were senescing and entering a dormant state for the colder season. Therefore, less photosynthetic carbon was available for the fungal symbionts in all the treatments.

Lastly, the third hypothesis that the seedlings with greater colonization would be more depleted in ^{15}N was not supported. However, the seedlings with the least amount of colonization, those of the understory control plot, were the most enriched in ^{15}N. It may be that such a drastic decrease in colonization did affect foliar ^{15}N content. Hodge and Fitter (2010) found that AM fungi can play an important role in N acquisition. It may be that AM fungi do not fractionate against ^{15}N very heavily and would be difficult to use this ratio to determine how colonization is affecting N uptake.

CONCLUSIONS

From this data it would appear that forest practices which result in greater light availability and disturbance have a greater impact on AM fungal colonization than does soil temperature. However, as mentioned earlier, an extended period of soil heating may see different results especially during the growing season. This has the potential to affect tree species composition, succession, migration, and carbon sequestration.

These results reveal that much more information regarding AM fungal activity in N acquisition is needed. Since the importance of AM fungi in Phosphorus uptake is well known, it would be very beneficial to know soil and foliar Phosphorus content and how that may be affected by clear cutting as well as soil heating.

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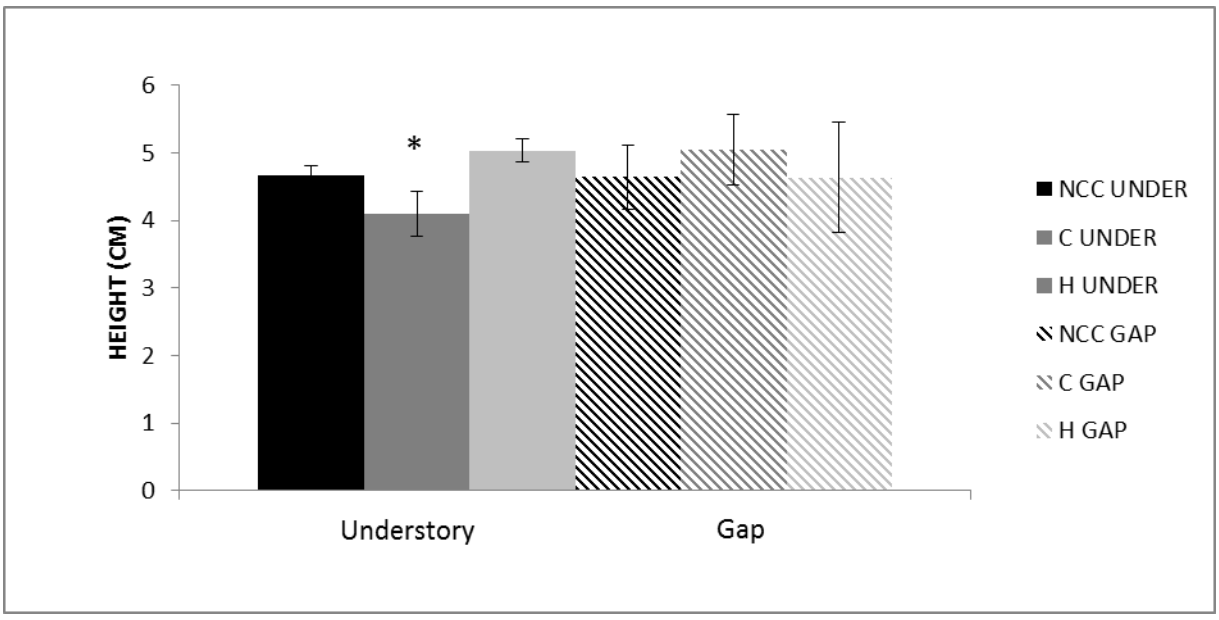

Fig. 3.1 *Liquidambar styraciflua* mean height at harvest per treatment with SE bars. NCC (non-chambered control), C (control), and H (heated). Asterisks (*) denote statistical significance at the $p \leq 0.05$ level for the difference between Understory and Gap habitats.

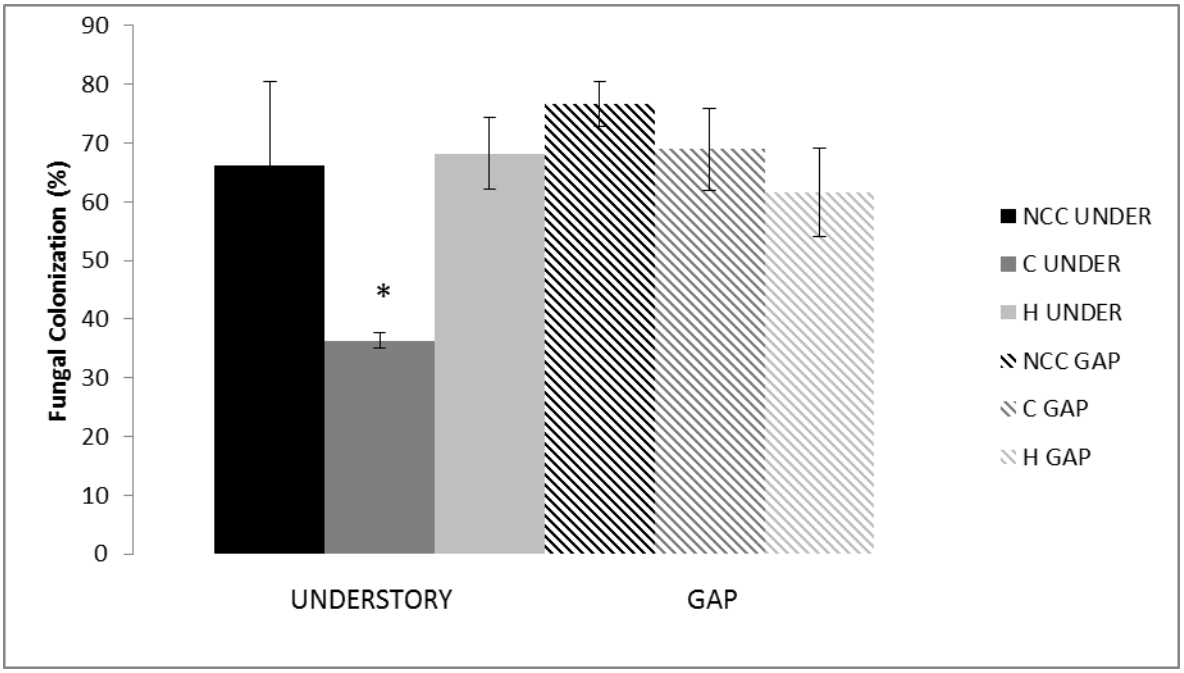

Fig. 3.2 Mean fungal arcsine-transformed percent colonization with SE bars. NCC (non-chambered control), C (control), and H (heated). (*) denote statistical significance ($P<0.05$, Tukey's HSD) between treatments within light conditions.

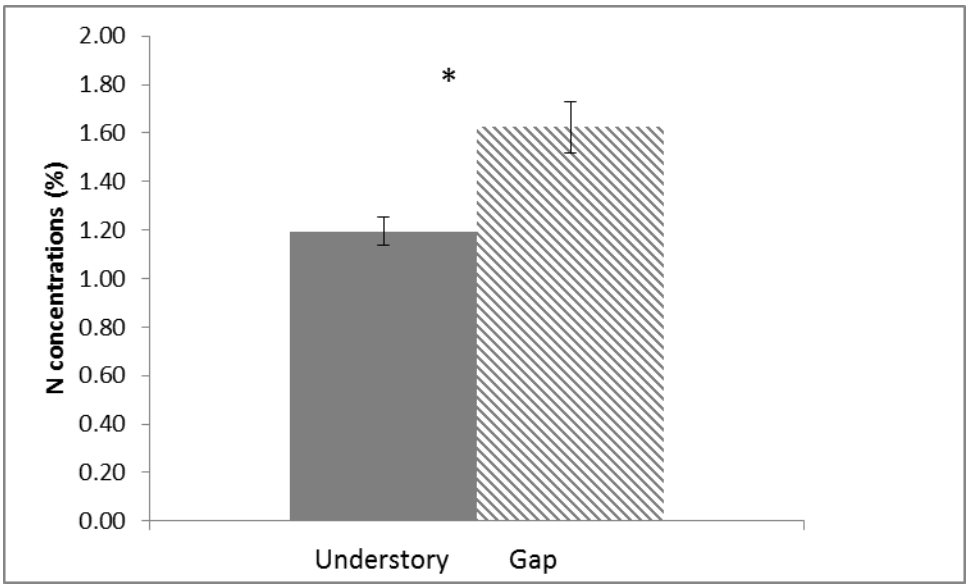

Fig 3.3 Mean arcsine-transformed percent foliar N concentration. All seedlings within both light conditions with SE bars

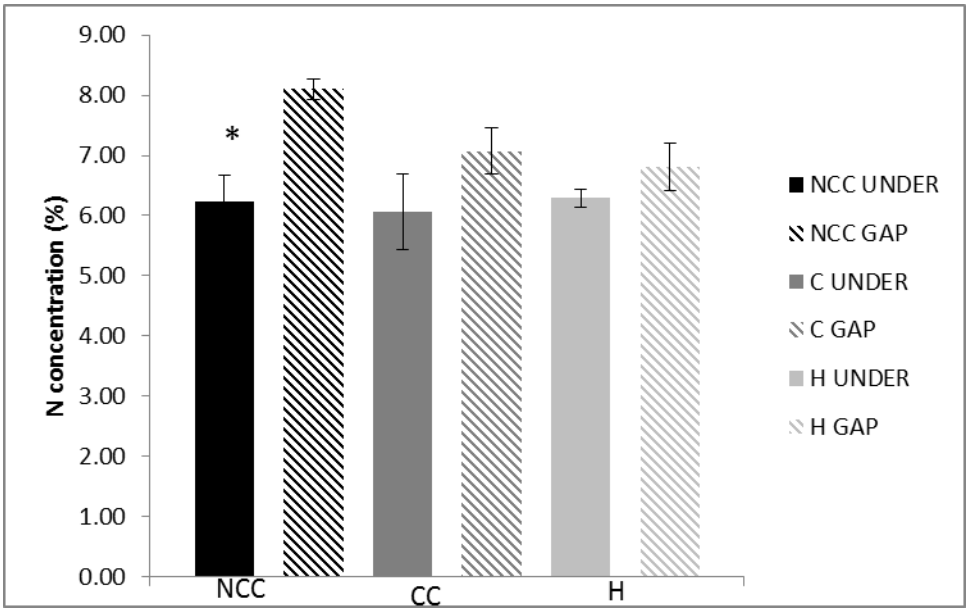

Fig 3.4 Mean arcsine-transformed foliar N concentration of seedlings between light conditions; NCC (non-chambered control), C (control), and H (heated).Under (understory) and Gap (Gap); (*) denote statistical significance ($P<0.05$, Tukey's HSD)

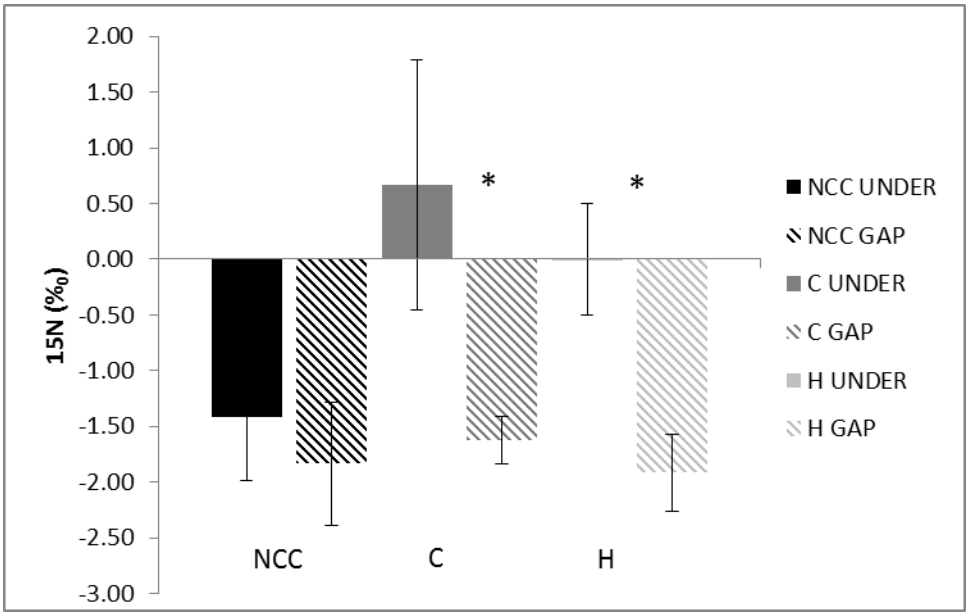

Fig 3.5 Mean foliar ^{15}N content of seedlings between light conditions with SE bars; NCC (non-chambered control), C (control), and H (heated); Under (understory) and Gap (Gap); (*) denote statistical significance ($P<0.05$, Tukey's HSD)

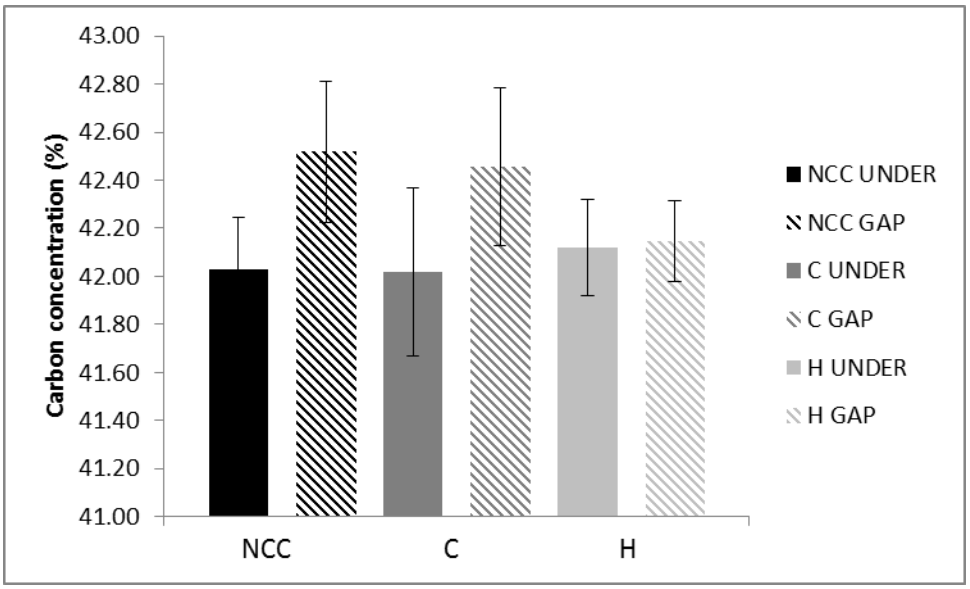

Fig 3.6 Mean arcsine-transformed foliar C concentration of seedlings between light conditions; NCC (non-chambered control), C (control), and H (heated).Under (understory) and Gap (Gap)

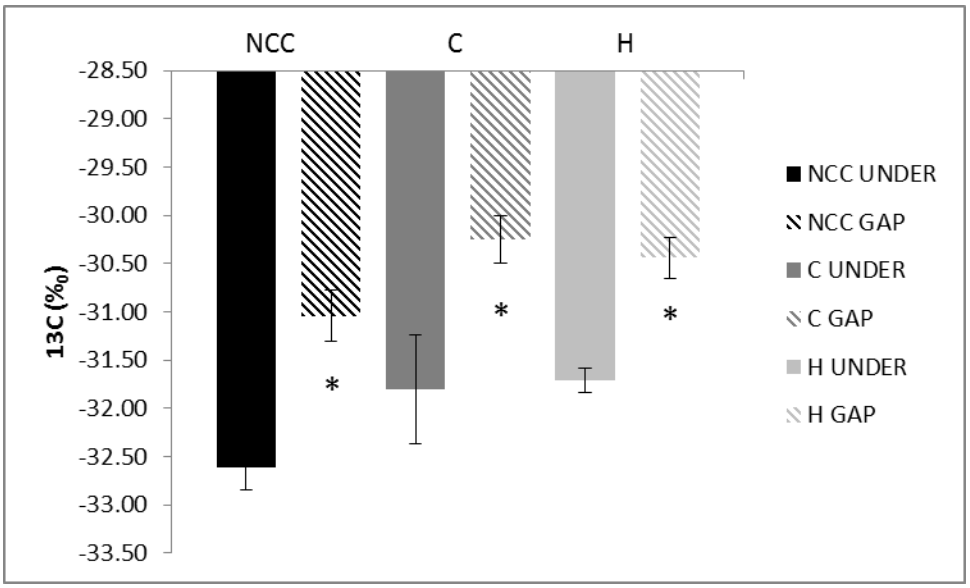

Fig. 3.7 Mean foliar ^{13}C content of seedlings between light conditions with SE bars; NCC (non-chambered control), C (control), and H (heated); Under (understory) and Gap (Gap); Asterisks (*) denote statistical significance ($P<0.05$, Tukey's HSD)

CHAPTER 4

RESPONSE OF MYCORRHIZA AND FUNGAL SPOROCARPS TO GAP VS. UNDERSTORY HABITATS IN A TEMPERATE FOREST OF THE SOUTHEAST

As the globe faces climate change so also does it face population growth and increased wood product demand. Often, timber management can result in great disturbance of forest structure and composition and contribute to increased greenhouse gas emissions (Canadell et al 2007). There is a great amount of research devoted to observing how tree harvesting practices such as clear cutting (Zhou et al 1997; Zhang et al 2003), thinning (Korb et al 2003), and patch sizes (Luoma et al 2004) are affecting mycorrhizal symbiosis and sporocarp (fruiting body) distribution (Luoma et al 2004; Luoma et al 2006) .

When timber is harvested and especially clear cut, there is more light available to the regenerating seedlings. Turner et al (2009) found that *Quercus rubra* seedlings grown in intermediate to high light conditions had greater fungal colonization and species diversity compared to those grown in low light settings. Sugar maple, an AM species, was observed to have decreased fungal colonization in understory or low light conditions (Song et al 2004). In a study of three different EM tree species in a boreal system, Dehlin et al (2004) found that the effects of light on fungal colonization are species specific. Several studies have observed that mycorrhizal colonization depends on host species shade tolerance (Kyllo et al 2003; Dehlin et al 2004; Druebert et al 2009; Shukla et al 2009).

Soil disturbance due to tree harvesting can also affect mycorrhizal associations. Most AM fungal colonization tends to recover quickly or is unaffected by soil disturbance (Abbott and Robson 1991; McGonigle and Miller 2000; Duan et al 2011). AM fungi are considered generalists (Smith and Read, 2008) and likely to recover their populations quickly. However, EM fungal species diversity and sporocarp production are decreased by disturbance (Jones et al 2003; Luoma et al 2004). Only the recovery of tree hosts on a severely disturbed site will restore EM fungal diversity (Perry et al 1990, review).

In the Summer of 2008, an 50x50meter area of Whitehall Forest in Athens, GA was cleared to provide space for a soil warming experiment. In conjunction with the warming study, it was decided that a disturbance experiment should also be conducted. It is expected that as global human population increases so too will urban sprawl and demand for wood products both of which cause great disturbance to forest structure and composition. The goal of this study is to determine the effects that such disturbances might have on mycorrhizal symbiosis and mushroom distribution in the Southeastern United States, a region currently undergoing human population increases at rates exceeding the national average with concomitant 'ex-urbanization' and suburbanization. The hypotheses addressed were: 1) Fungal colonization will increase in seedlings grown in the gap due to increased carbon from increased photosynthesis. 2) There will be a decrease in sporocarp production due to removal of host tree species. 3) Species or morphotypic diversity of colonized seedling roots will be greater in the understory than the gap.

METHODS

Site description

The 280 ha Whitehall Forest is a warm temperate forest managed by UGA's Warnell School of Forestry and Natural Resources, located ~3 miles from campus in the Piedmont of Georgia (33^{o}54'N, 83^{o}22'W). This site includes 3600 m^2 of even-aged, mixed deciduous forest dominated by *Quercus alba, Q. rubra,* and *A. rubrum,* and is representative of deciduous forests of the Georgia Piedmont (Forkner and Hunter 2000). Soils are of the Cecil/Pacolet association (fine, kaolinitic, thermic Typic Kanhapludults) with low organic matter content, low fertility, and medium to slow permeability. Mean January temperature is 9.1^{o}C and mean July, 28^{o}C. Precipitation is evenly distributed throughout the year and growing season droughts are relatively common due to high evapotranspiration. The long-term precipitation mean is 125 cm yr^{-1} (1945-2003); recent mean is 113 cm yr^{-1} (2003-2007). It was a naturally-recruited, unmanaged stand originating in the first half of the 20th Century following agricultural land abandonment.

Experimental design

Twenty-four 18.7-m^2 plots were erected in Whitehall Forest. Twelve plots were located under forest canopy (understory) and twelve in a 50x50m gap (gap) that had been manually-cleared of timber in July 2008. Light availability of the understory and gap was approximately 10 and 90%, respectively.

All seedlings (*Pinus taeda, Quercus alba,* and *Acer rubrum)* were germinated in a soil-free, low-nutrient medium of sand and peat moss. At one month old, they were transplanted to field chambers. Soil cores were extracted from the chamber soils by a tulip bulb planter. Cores were 10cm deep and 5cm in diameter. These cores were placed undisturbed (unhomogenized)

into mesh bags and reset into the holes from which they were taken. The seedlings were then planted in the bagged cores after height and root length were measured. Seedlings were transplanted to the field in Summer 2010 and grown under field conditions for 16 weeks at which time they were harvested and measured for final height, root length, and fungal colonization of roots. Twenty-seven red maples, 9 white oaks, and 20 loblolly pines were collected from the understory. Twelve red maples, 8 white oaks, and 29 pines were harvested from the gap.

Arbuscular mycorrhizal (AM) root systems of the *Acer rubrum* seedlings were immediately stained using the methods of Vierheilig et al (1998). Roots were boiled in KOH, then in black ink (Shaeffer) and vinegar. Percent colonization was determined by sectioning roots into 5mm pieces. These sections were viewed under a compound microscope at 100x to determine the presence of vesicles, arbuscles or aseptate hyphae. Fifty 5mm sections were taken from each root system unless the system was not large enough; then the entire root system was viewed. *Quercus* sp. have been found to exhibit both EM and AM relationships (Dickie et al 2001; Smith and Read 2008); therefore, randomly sampled *Q. alba* roots from the gap and understory conditions were stained and examined for evidence of AM fungi.

Ectomycorrhizal (EM) root systems of *P. taeda* and *Q. alba* were viewed under a dissecting microscope at 40x after being rinsed in tap water. All root tips were counted and fungal colonization of root tips was determined by the presence of a mantle. Differing fungal species were determined by morphotyping of fungal characteristics using Agerer (1987-2008) color atlas and the Ectomycorrhizal Description Database.

Once a week for one growing season from October 2009-January 2011, above ground fruiting bodies were counted in each 18.7m^2 growth chamber. Fruiting bodies were identified

using the field guides *Mushrooms Demystified* by David Arora and the *National Audobon Society's Field Guide to North American Mushrooms*.

Statistical Analysis

Statistics were analyzed using SAS 9.2 (English). A General Linear Model was used as were ANOVA, Tukey's significant difference with a p-value of 0.05, and 2-tailed t-tests All percentages were arcsine-transformed and the square root of seedling growth was used. The Shannon-Wiener diversity index was used for fungal morphotypes.

RESULTS

The average final height of all seedlings was greater in the gap habitat ($p<0.0001$; *Fig. 4.1*). All seedlings displayed positive above ground growth in the gap while maples and oaks demonstrated a negative average growth in the understory ($p<0.0001$; *Fig 4.2*). There was greater survivorship of maples in the understory and the opposite for pines. There was slightly greater survivorship of oaks in the understory (*Fig. 4.3*). Average root length of seedlings was not affected by plot condition ($p=0.906$).

Fungal colonization of seedlings was significantly affected by plot habitat. Maple seedlings had greater average colonization in the gap than the understory (71.487±3.81 and 41.702±5.34 respectively; $p=0.0008$). Oak seedling fungal colonization had a tendency to be decreased in gap conditions (32.998±2.37 and 25.4±5.45 respectively; $p=0.2029$). Fungal colonization of pine seedlings was greater in the gap compared to the understory (57.68±1.93 and 41.7±3.33 respectively; $p<0.0001$) (*Fig.4.4*).

There was a strong effect on seedling growth by fungal colonization, seedling species, plot habitat, and the interaction of colonization, species and plot habitat (*p<0.0001, p<0.0001, p<0.0001,* and *p=0.0159* respectively; R^2=0.552). On a per species basis, growth of maples was positively correlated with percent colonization (R^2=0.474; *p=0.0267; Fig. 4.5*) but not with plot habitat. The growth of oak seedlings was not correlated with colonization or plot habitat (*p=0.3898; Fig. 4.6*). Pine seedling growth was positively correlated with colonization and exhibited significant differences between habitat with higher colonization of gap seedlings (*p<0.0001* and *p<0.0001*; R^2=0.593; *Fig. 4.7*).

Observation of fungal colonization of oak and pine roots revealed increased fungal species richness of oak seedlings grown in understory conditions. Seven fungal types were found in the understory while only two types were observed in the gap (*Fig. 4.8* and *4.9; Table 4.1*). However, the pine seedlings were observed to have greater fungal diversity on roots of seedlings grown in gap conditions. Three types were found in the understory, but seven types were found in the gap (*Fig. 4.10* and *4.11; Table 4.1*).). Greater diversity resulted in more evenness of fungal abundance for both of the tree species. In the roots of understory oaks, no arbuscles or vesicles of AM fungi were found, but hyphae were present. No sign of AM colonization was observed on the oaks of the gap habitat.

There were more total fruiting bodies in 2010 in both the understory and gap conditions. The understory had more total fruiting bodies than the gap in both years (*Fig. 4.12*). Most of the fruiting bodies in the gap were small and were identified to be saprotrophic and not mycorrhizal. There was also a greater variety of species in the understory treatment (*Fig. 4.13*).

DISCUSSION

The first hypothesis that there would be greater fungal colonization in the gap was supported by the results of maple and pine data but not oak. The greater amount of light likely provided the fungal symbionts with more carbon allowing for increased colonization which in turn resulted in greater growth of the seedlings.

The second hypothesis that sporocarp (fruiting body) production would be decreased in the gap was also supported. The clear cutting of the gap treatment removed the host species from that area resulting in the removal of EM fungal sporocarps. The vegetative remains of that process such as wood chips and tree stumps provided a carbon source for saprotrophic fungi. In the understory treatment which had almost all of its original trees continued to have an abundance of sporocarps. The tree hosts provided sufficient carbon to the mycorrhizal fungi to allow for sporocarp production. The lower diversity and number of fungal sporocarps in 2009 compared to 2010 may be attributed to the disturbance of the soil from chamber and soil cable establishment. It is expected that as time after disturbance increases and no more disturbance occurs, sporocarp diversity and abundance will continue to increase.

The third hypothesis that fungal species (morphotype) diversity would be greater in the understory was supported by oak data but not by pine. As with aboveground sporocarps, it was expected that below ground diversity would also be greater in the understory or non-disturbed condition. This was true for the white oak seedlings, but the pine seedlings from the gap had greater diversity of morphotypes on their roots. This result was unexpected because there is not a great population of conifers on this site, therefore, few sources of inoculation for mycorrhizal fungal species that are specific to conifers. The fungal species colonizing the pines may have

been generalists. It may also be due to wind transfer of fungal spores from pine plantations located within a kilometer of this site.

CONCLUSION

From this data it may be concluded that for maple and pine seedlings greater light availability greatly increases mycorrhizal fungal colonization and that colonization, in turn, is correlated with higher seedling growth. Also, fungal morphotypic diversity is decreased by disturbance for oak seedlings but not loblolly pine seedlings. Lastly, mushroom (sporocarp) abundance and diversity are greater in undisturbed sites than in clear cut sites. It may also be concluded that mycorrhizal symbiosis plays a very important role in plant succession, fungal biodiversity, forest structure and composition, and how these ecological aspects will be affected by climate change and land use changes.

There is much room for future studies following the results of this project. It would be interesting to know why the pine seedlings in the gap exhibited greater fungal diversity and why the fungal colonization of oak seedlings was not more affected by light availability. It is strongly suggested that molecular identification of fungal species colonizing plant roots be used along with morphotyping. DNA analysis will likely result in the identification of more species than morphotyping alone. It is also suggested that analysis of foliar and soil nitrogen and carbon content be conducted to determine the ecological effects that nutrient content and mycorrhizal symbiosis may have on each other in this system.

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Table 4.1 Shannon-Wiener Diversity Index. Diversity index of fungal morphotypes found in oaks and pines in both the understory and gap conditions.

Fungal communities	**Shannon-Wiener**	**Species Richness**	**Simpson**	**Evenness**
			D	
Oak Understory	1.679	7	0.224	0.863
Oak Gap	0.573	2	0.615	0.827
Pine Understory	0.603	4	0.691	0.435
Pine Gap	1.309	7	0.329	0.673

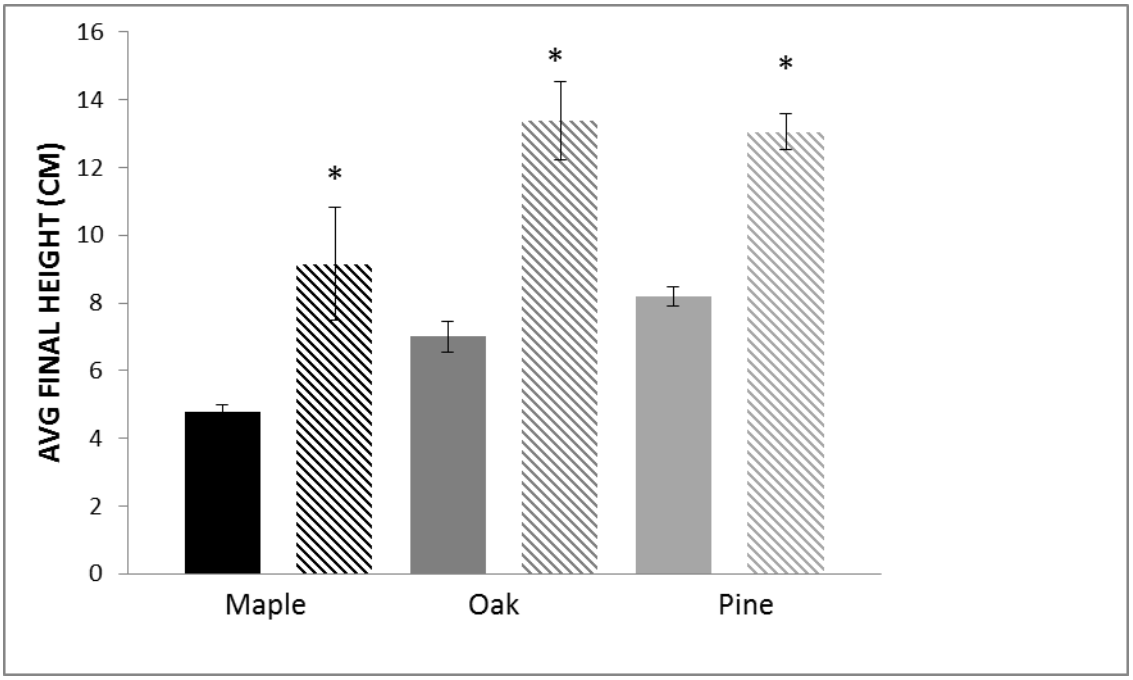

Fig 4.1 Mean final height of seedlings. Seedlings in the understory (solid) and gap (striped) conditions with SE bars. An asterisk (*) denotes significance.

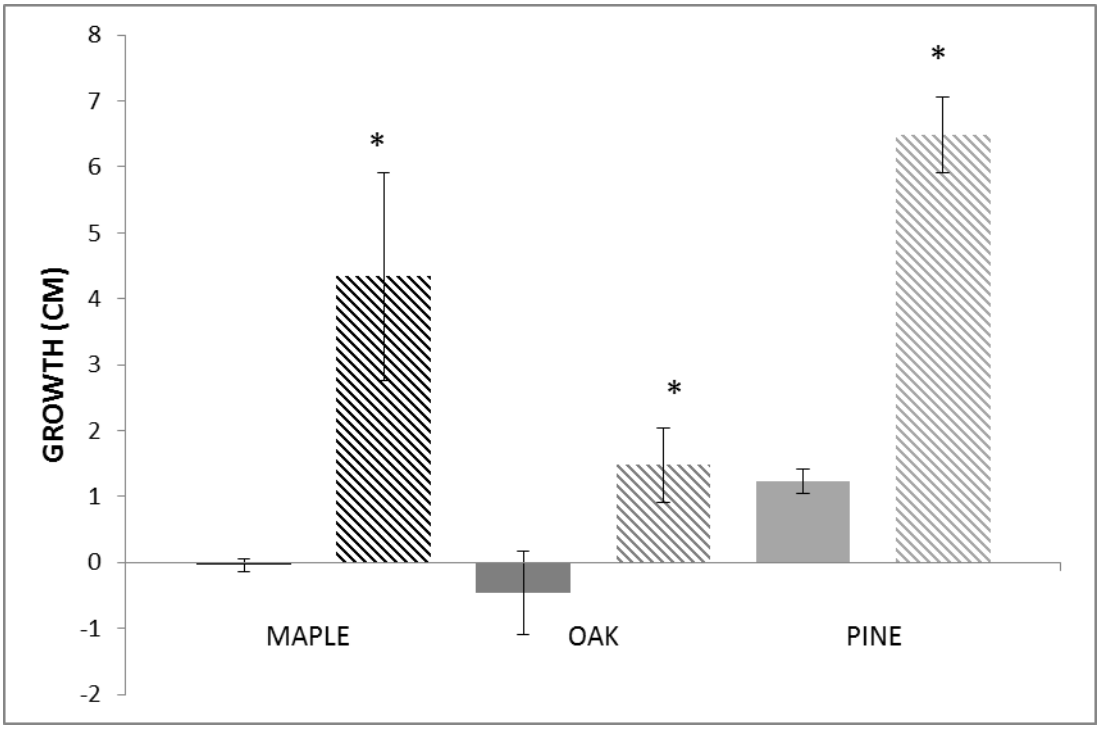

Fig 4.2 Mean growth (Final height-Initial height) of seedlings. Growth in the understory (solid) and gap (striped) with SE bars. An asterisk (*) denotes significant difference between understory and gap habitats.

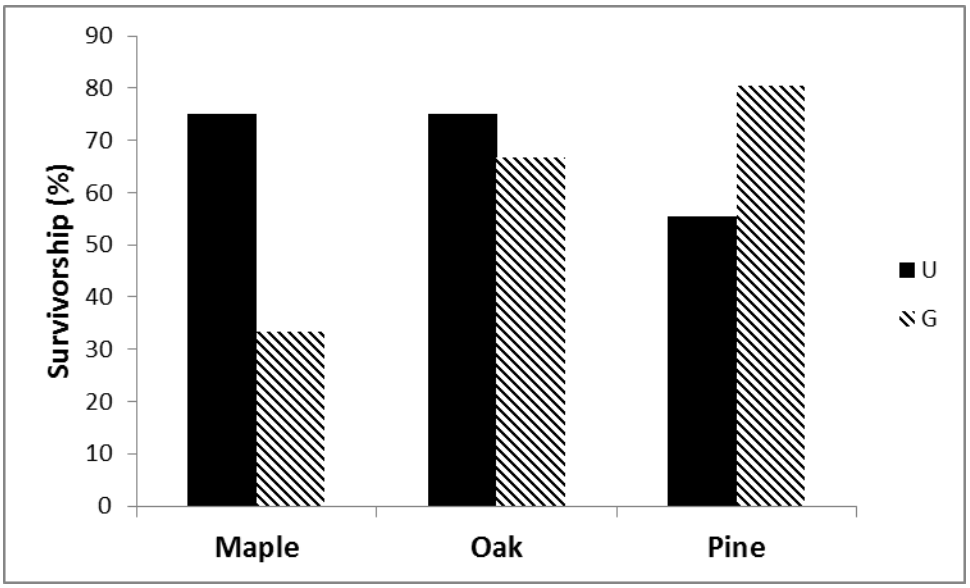

Fig 4.3 Percent survivorship**.** Percent survivorship of seedlings in the understory (solid) and gap (striped).

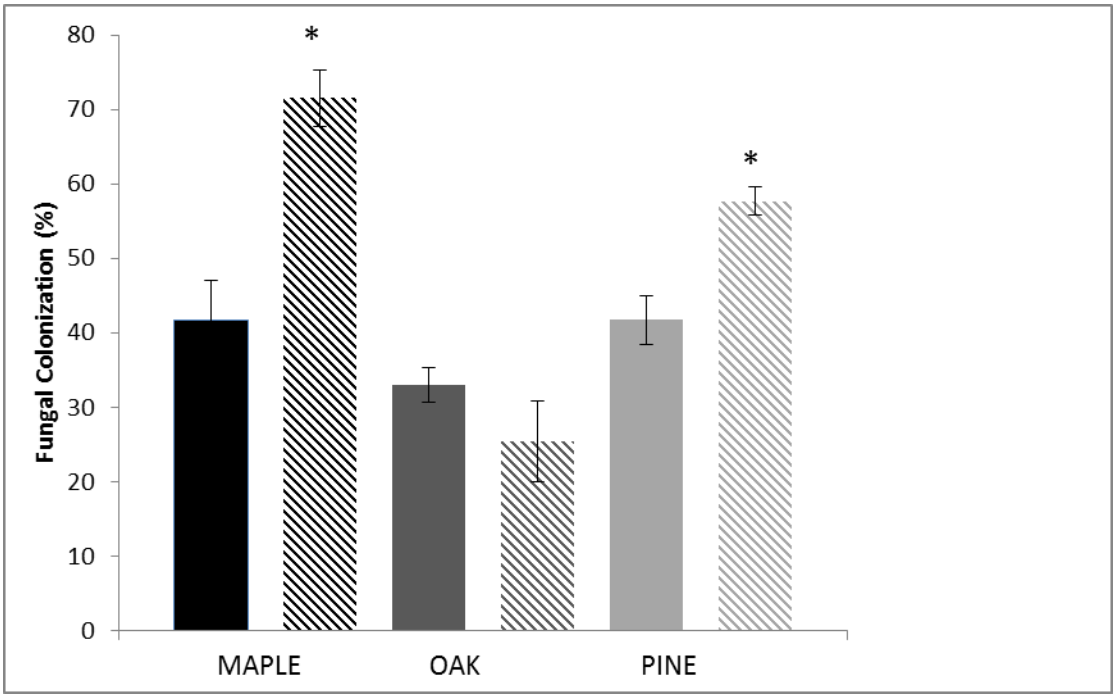

Fig. 4.4 Mean arcsine- transformed percent fungal colonization of seedlings. Colonization in the understory (solid) and gap (striped) with SE bars. An asterisk (*) denotes significant difference between understory and gap habitats.

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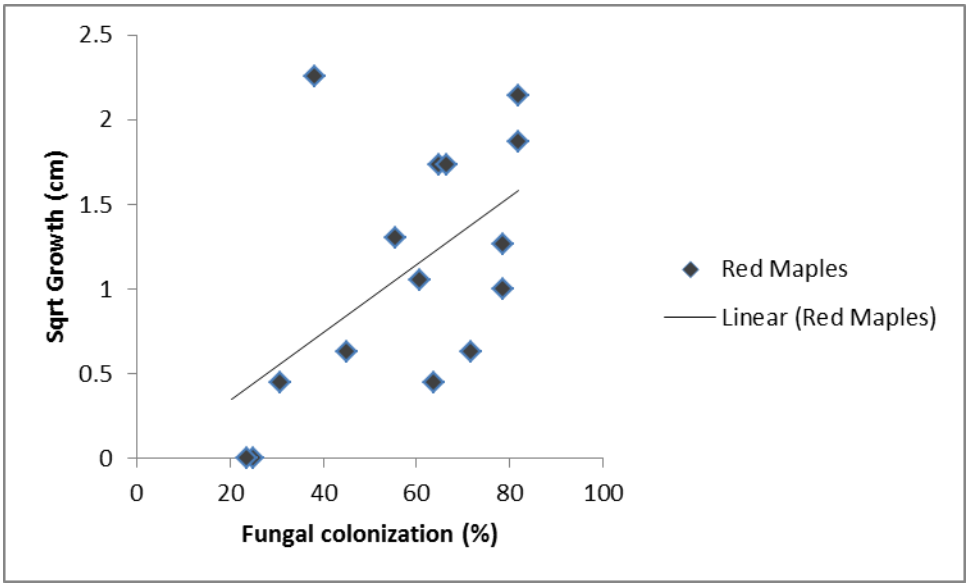


Fig. 4.5 Maple growth vs. colonization**.** Growth of red maple seedlings influenced by arcsine-transformed percent fungal colonization with regression line. R^2=0.474. n=15

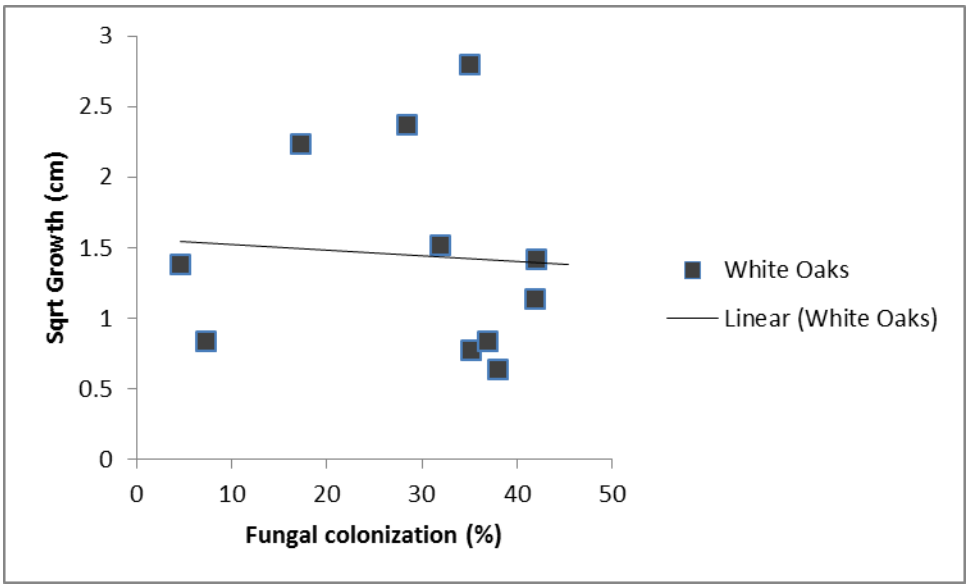


Fig 4.6 Oak growth vs. colonization. Growth of white oak seedlings influenced by arcsine-transformed percent fungal colonization with regression line. R2=0.0098. n=11

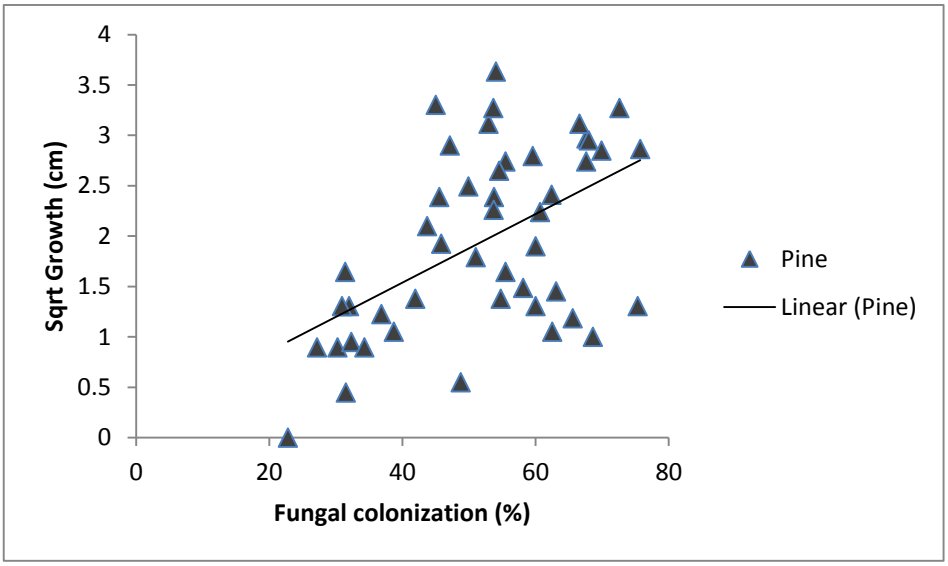


Fig. 4.7 Pine growth vs. colonization. Growth of loblolly pine seedlings influenced by arcsine-transformed percent colonization with regression line. R^2=0.593. n=45

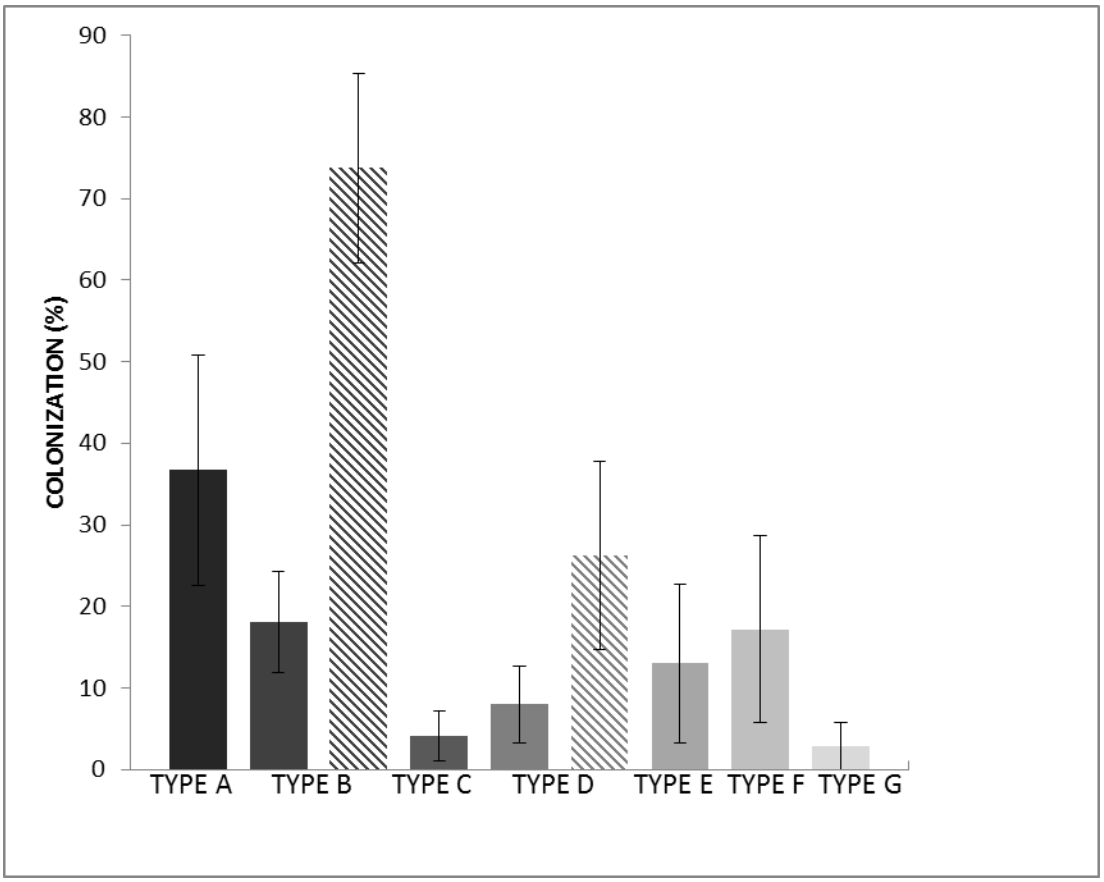
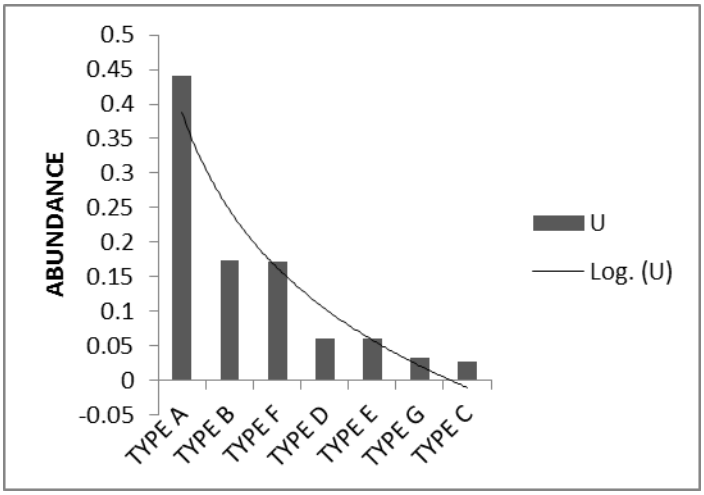


Fig. 4.8 Abundance of fungal morphotypes colonizing oak seedling roots with SE bars. Solid bars indicate types found in the understory and striped bars indicate types found in the gap.

A.



B.

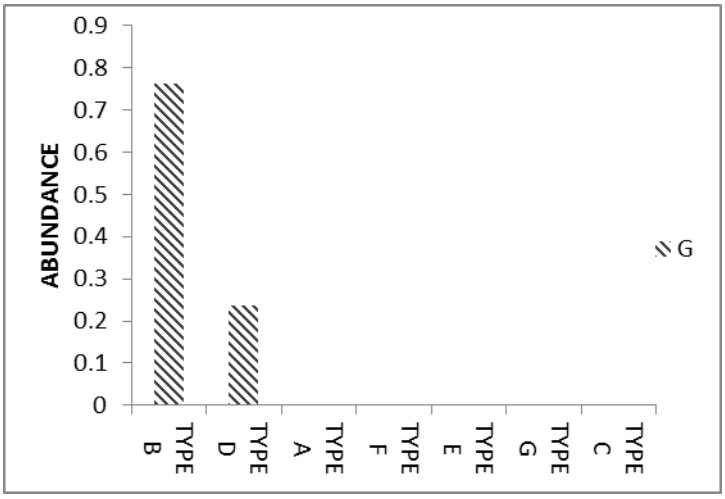


Fig. 4.9 Dominance and diversity curves of morphotypes found on oak seedling roots. A) depicts types found in the understory and B) gap.

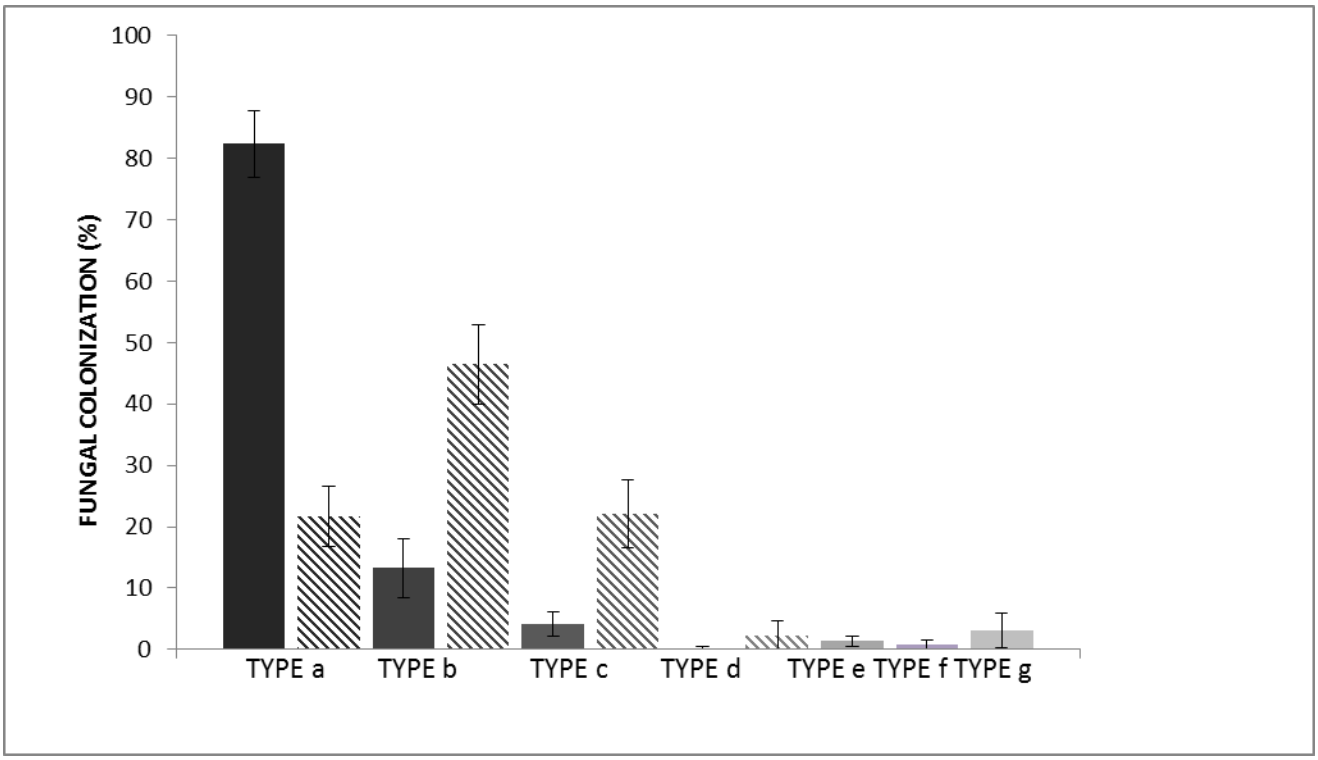
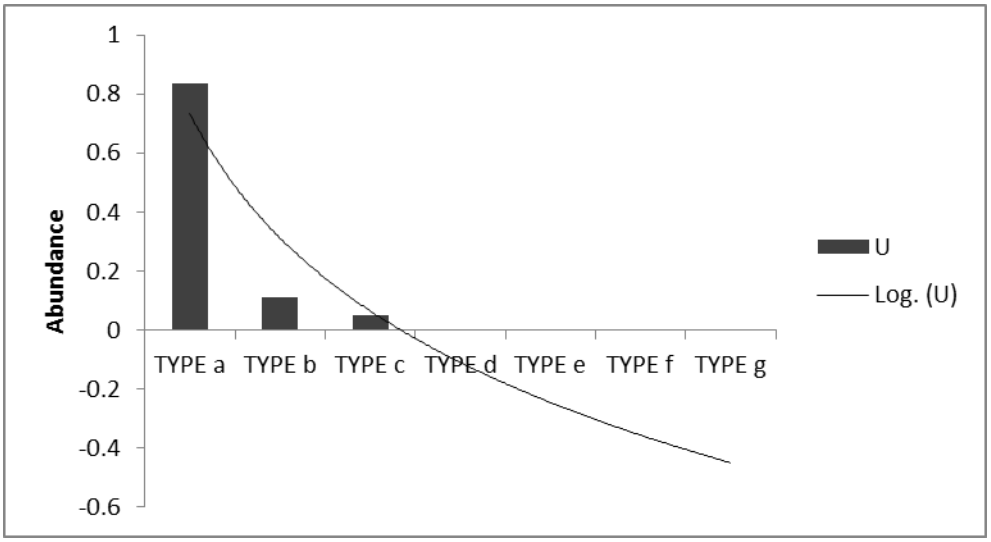


Fig. 4.10 Abundance of fungal morphotypes colonizing pine seedling roots with SE bars. Solid bars indicate types found in the understory and striped bars indicate types found in the gap.

A.



B.

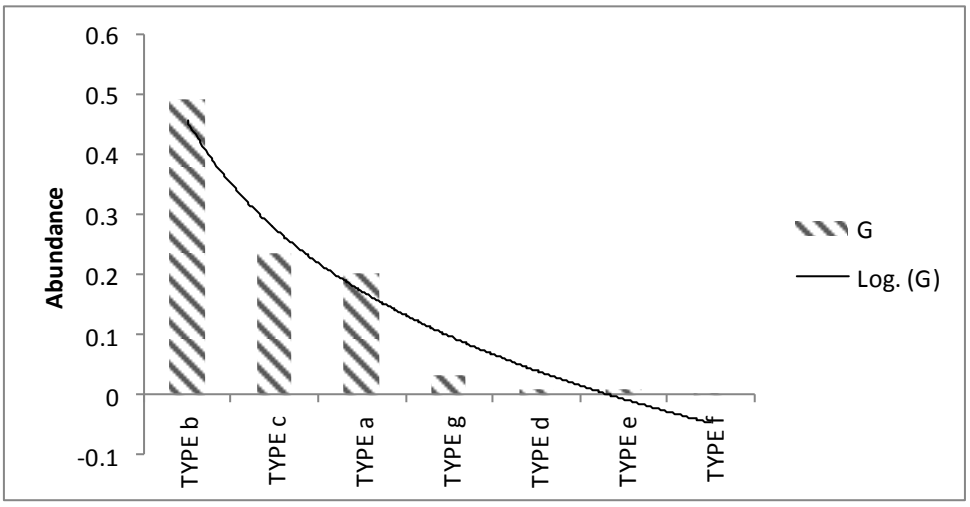


Fig. 4.11 Dominance and diversity curves of morphotypes found on pine seedling roots. A) depicts types found in the understory and B) gap.

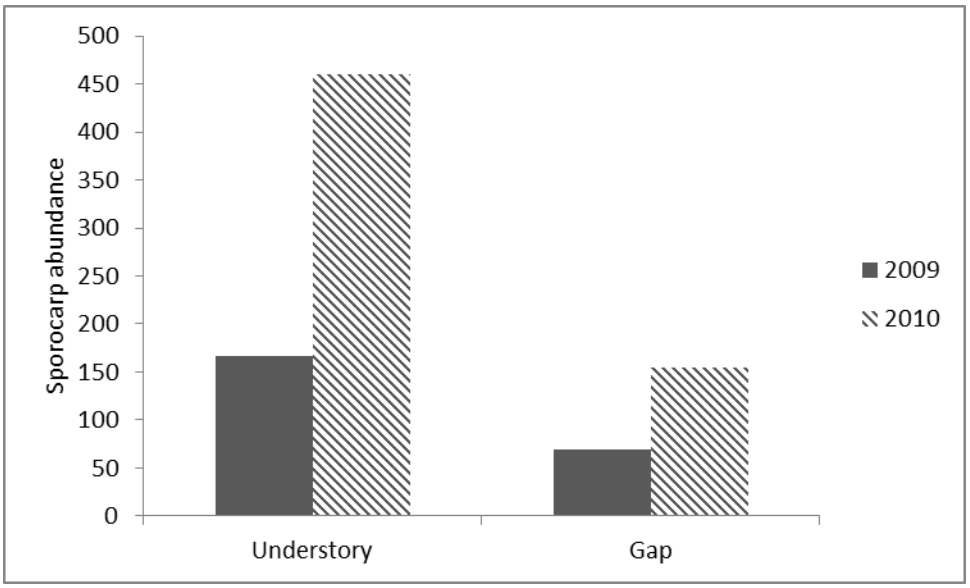


Fig. 4.12 Sporocarp abundance. Sporocarp abundance in the understory and gap conditions in 2009(solid) and 2010(striped).

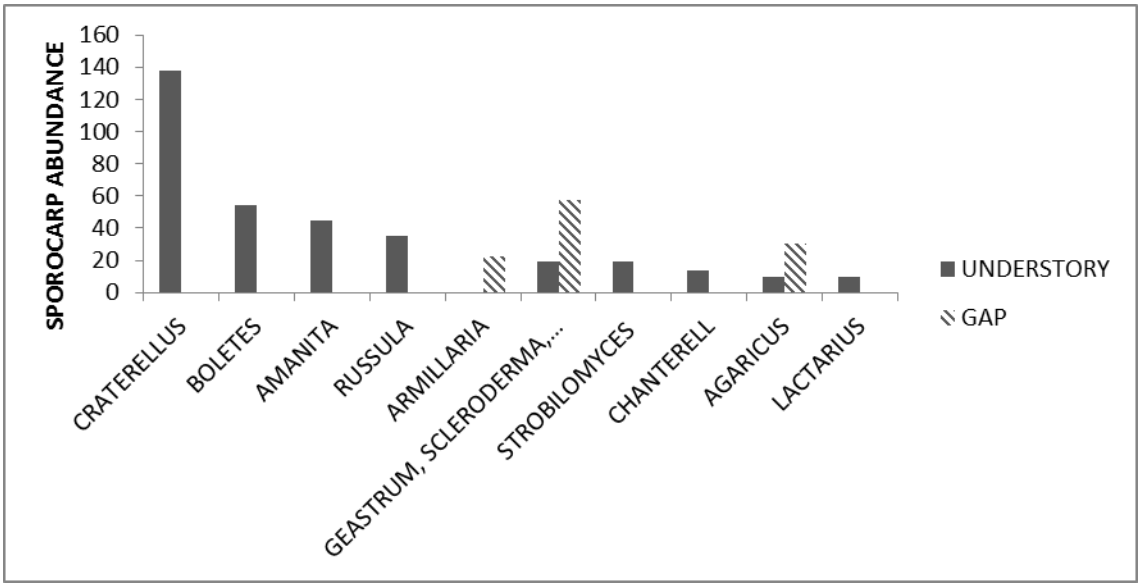


Fig. 4.13 Diversity of sporocarps. Diversity of sporocarps (genii) found in the understory (solid) and the gap (striped).