Climate Change Effects on the Rhizosphere: A Comparative Study of the Enzyme Activity of Mycorrhizae in Heated and Unheated Forest Soils at Harvard Forest, Petersham MA

Alana Marie Thurston
Haverford College
Haverford, PA 19041

Advisors: John Hobbie and Jerry Melillo
Ecosystems Center, Marine Biological Laboratory
Woods Hole, MA 02543
Abstract:

Climate change models predict that the annual average temperature could increase up to 5°Celsius within the next century, which has serious implications for nutrient cycles, gas fluxes, and species populations. Experiments carried out in the Prospect Hill and Barre Woods soil plots in Harvard Forest have already shown that heating soils affects mineralization rates, soil respiration, and tree growth, which led me to predict that heating will also impact mycorrhizal relationships. I made measurements to see how heating would affect the species abundance and diversity of soil fungi and also the enzymatic activity of soil fungi. I identified and counted the fungi on root tips in a series of soil cores taken from Barre Woods and also isolated these root tips to evaluate the enzymatic activity for chitobiase, endopeptidase, phosphatase, and cellobiase. I observed a shift in the fungal community in which there was an increase in the abundance of *Russula* and a decrease in the abundance of *Cortinarius* in response to soil warming. Additionally, between these two species, I saw that both had differences in enzymatic activity in endopeptidase and cellobiase with higher activity observed in the control plots. The species shifts I observed do not match those observed in tundra heating experiments at the LTER site in the Brooks Range, Alaska, where there was a significant increase in *Cortinarius* over *Russula*. For the Harvard Forest soil warming studies, I predict that if nutrient pools are depleted over time due to the increased rates of soil processes that trends similar to those observed in Alaska might surface.

Key Words: Mycorrhizae, Ectomycorrhizae, Enzyme Activity, Climate Change, Nutrient cycling.
Introduction:

Mycorrhiza refers to the mutualistic relationship between fungi and plant roots. The fungal hyphae form in a sheath around the roots, and the ends of the hyphae move out into the surrounding soil. This enables the plant to take up a variety of nutrients, such as phosphorus, nitrogen, zinc, sulfur, calcium, and potassium, better than plants without mycorrhizal roots (Allen et al. 2003). These fungal hyphae also secrete enzymes that break down organic material and thus increase the available nutrients in the ecosystem. In return, the plant provides the fungi with photosynthetically derived carbohydrates (Brundrett et al. 1996). These relationships can be extremely complex, especially because a single host plant can form mycorrhizal associations with a variety of different fungal species (Allen et al. 2003).

Studies of the interactions between host plants and soil fungi have identified seven types of mycorrhizal relationships, each of which interacts with root tips in a unique manner. Ectomycorrhizae are a type of mycorrhizae that characteristically form a surrounding sheath around a root and forms a Hartig net, which is hyphae that have penetrated into the root but remain between the root cells. This arrangement provides a location where nutrient and carbon exchange can occur between the plant and fungi. Ectomycorrhizae tend to form on fine root tips and closer to surface soil than in the mineral layer (Brundrett et al. 1996).

Different species of ectomycorrhizae have different nutrient uptake capabilities, abilities to break down organic material, and physical appearances. One characteristic that has been used to categorize ectomycorrhizae is the distance the hyphae extend into the soil. Some ectomycorrhizae, such as *Russula*, have only short hyphae and form a close-contact relationship with an organic source. The ectomycorrhizae that do form hyphae can
be classified as short-distance exploration, medium-distance fringe, mat, or smooth exploration, and long-distance exploration (Agerer 2001).

While there is still much to learn about both the structure and function of mycorrhizal hyphae, one proposed mode of function is that the hyphal tip produces a mucopolysaccharide exudate that surrounds both the hyphal tip and organic particles. This keeps secreted fungal enzymes from being lost into the soil and also keeps the hyphal tip in contact with organic compounds produced by enzymatic action. Enzymes, such as proteases, chitinases, and peroxidases, are released from the hyphal tip to break down plant litter and large organic matter compounds into smaller ones, such as amino acids. These smaller compounds can enter the hyphal tip and then be transported back to the host plant (Hobbie and Hobbie 2008).

Three important enzymes that work to make either nitrogen or phosphorus available are chitinases, endopeptidases, and phosphatases. Chitin is an amide-sugar polysaccharide that is found in many natural sources, including the cell walls of fungi and arthropod shells. Chitinases act to break glycosidic bonds in chitin, which makes nitrogenous compounds available (Cohen 1987). Similarly, endopeptidase is a protease that also makes nitrogen available by breaking peptide bonds in amino acids. Phosphatases on the other hand remove phosphate groups from organic compounds to make phosphorus available. Another common enzyme found in soils is the cellulases, for example cellobiase, which breaks cellulose into smaller polysaccharides.

The Harvard Forest soil warming experiment was initiated in 1991 and was designed to test the response of soil processes under a 5° C increase in temperature. This temperature was chosen because it is at the upper end of climate-change projections of a
soil temperature rise of 2° to 5° C over the next century (Cox et al. 2000). Current research in the Prospect Hill 6 by 6-meter plots has measured CO₂, CH₄, and N₂O gaseous fluxes and nitrogen mineralization to determine how heating will affect soil processes (Melillo et al. 1999). In 2003, another set of soil warming plots was established in Barre Woods, but these plots were significantly larger, 30 by 30 meters. The size of these larger plots captures entire tree and root systems, which has allowed research to focus on the effect of soil warming on root processes and tree growth.

In both soil warming experiments, it was noted that heated plots had increased nitrogen availability within the first few years of the project, but this increase has declined steadily in recent years (Melillo et al. 2001, Burton et al. 2011). Additionally, in studying root biomass in the large heated plots, researchers have found that while there has been an increase in respiration rates of roots that there has been a substantial decrease in the fine root biomass (Zhou et al. 2011). Specifically, it was estimated that over the course of a 7-year study the fine root biomass in the top ten cm of soil decreased by 62%. The current hypothesis for this observation is that because soil warming has increased nitrogen availability in soils, trees are not allocating as much carbon and energy into forming fine roots for nitrogen acquisition (Melillo et al. 2011).

Decreasing fine root biomass could be correlated to changes in soil fungi species diversity and abundance, as it may not be energetically favorable for plants to form mutualistic relationships with certain species of fungi if there are excess nitrogen and nutrients readily available in the ecosystem. This could have further implications on the enzymatic activity in the soil, as fungi secrete enzymes to break down organic molecules so that plants can take them up.
In my project, I tested the hypothesis that there would be a shift in soil fungi communities between the heated and control soil plots. While I was not able to perform DNA sequencing analysis of soil fungi in Barre Woods, I was able to microscopically identify some fungal lineages by looking at photographs of DNA-identified root tip hyphae from chronic nitrogen addition plots at Harvard Forest. Additionally, I analyzed the potential enzymatic activity of both the soil and the root tips for chitobiase, endopeptidase, phosphatase, and cellobiase through the use of fluorogenic substrates to determine if soil heating has implications for the activity levels of the fungal and microbial community.

Methods:

Field Sampling:
Barre Woods contains two 30 by 30 meter experimental plots that contain nine different species of trees, including black birch (*Betula lenta*), red oak (*Quercus rubra*), white ash (*Fraxinus americana*), and red maple (*Acer rubrum*) (Melillo et al. 2011). One plot has been heated 5° C above ambient temperature since 2003, which was achieved by embedding wires beneath the surface of the soil, while the other is an undisturbed control plot. Within these larger plots, a series of smaller subplots had been established. I selected four subplots in the heated and control plots to sample; two that contained *Betula lenta* and two that contained *Quercus rubra*. I excluded any plots that were in close proximity to *Acer rubrum*, as these trees are known to have arbuscular mycorrhizae and not ectomycorrhizae. In each subplot, I collected two soil cores that were five cm in diameter and 15 cm deep and as close to the base of the tree of interest as possible, <15 cm away, in an attempt to ensure that the roots collected would be from the species I identified.
In addition to collecting soil cores, I photographed and collected nine mushrooms that were present within the plots (Image 2).

**Laboratory Analysis:**

**Core Analysis:**

The soil cores and mushroom samples were stored on ice and transported back to Woods Hole. Upon returning to the lab, I separated the undecomposed organic material into soil, root, and debris portions. Tree bark, branches, and leaves were considered to be debris. I separated the soil cores by hand and not through a mesh sieve, so that I did not damage the root tips. Approximately one hour was spent sifting through each core, after which the soil, root, and debris portions were weighed and recorded. Soil and roots were stored in the refrigerator in plastic bags with a damp paper towel to ensure that the samples did not dry out and that the roots would remain healthy.

**Root Tip Identification:**

The root portion of the soil cores were further separated by mycorrhizae type through a visual comparison between the appearance of the root tips and images taken by Jesse Sadowsky of fungi from the Harvard Forest chronic nitrogen addition plots that had been identified through genetic sequencing (Sadowsky and Frey 2014). Even with guidance from these images, I only felt comfortable distinguishing between *Russula*, *Cortinarius*, *Tomentella*, and *Cenococcum*. After I had made my species identifications, I photographed examples of each root tip and sent them to Jesse Sadowsky for confirmation.

In addition to separating the roots tips by species, I made counts for each species based on the number of root tips observed. Roots were stored in Petri dishes with a moist paper towel in the refrigerator.
Enzyme Activity of Root Tips:

For my study of mycorrhizal enzyme activity, I adapted methods of Pritsch et al. (2011) and Higgins and Cohen (2012). The resulting protocol optimized conditions for the enzyme assays.

Roots were thoroughly cleaned and the root tips with previously identified mycorrhizae were severed with a scalpel so that all root tips were approximately the size of a single *Cenococcum* root tip, 0.5 to 2 mm long. Because there were not enough root tips to run separate analysis on the *Betula lenta* and *Quercus rubra* plots, root tips from the two treatments were combined.

A total of ten root tips for each species from both plots were tested for enzymatic activity. Each root tip was placed into an individual well on a 96-microwell plate with 150 μL of deionized water. Two of these root tips were reserved as deionized water controls, while the other eight were tested for the enzymatic activity of chitobiase, endopeptidase, phosphatase, and cellobiase in replicate. Different substrates were used to test the activity of each enzyme: MUF-N-acetyl-D-glucosaminide for chitobiase, a chitinase; MUF-phosphate for phosphatase; L-Leucine-7-amido-4-methylcoumarin HCl for endopeptidase; and MUF-D-glucoside for cellobiase, a cellulase. I added 50 μL of an enzyme substrate to the corresponding well and inserted the plate into a Biotek reader. The plate was set to a shake cycle so that the plate would shake for four minutes and a reading of fluorescence would be performed every fifth minute for a total of 30 minutes. Plates were read at wavelengths of 364 nm excitation and 445 nm emission.

Because this assay yielded overflowing fluorescence response from some of the more active root tips, I set up a second run for enzyme activity that used less enzyme...
substrate, only 25 μL. For the second run, the plates were read every minute for the first six minutes for fluorescence, and after that they were only ready once every five minutes for a total of 30 minutes.

For both experiments, 0.01, 0.05, 0.1, 0.3, 0.4, and 0.5 μM of MUF substrate was added to a series of wells to create a standard curve. Additionally, enzyme substrates and deionized water were added as controls.

To analyze the data, the absorbance of deionized water was subtracted from all of the experimental wells and the absorbance of enzyme substrate was subtracted from their respective wells. After deriving an equation from the standard curve (R²=0.94342), the absorbance of each well was entered into the equation to calculate potential enzyme activity. I adjusted the activity to account for differences in surface area of the root tips by photographing subsamples of each root tip type using an Axio View microscope and measuring the length and width of each root tip. I estimated surface area based on the equation for surface area of a rectangular prism, in which I assumed that the width and the height were the same for all species except Cortinarius, which was very thin. For Cortinarius, I set the height equal to 0.05 mm. I divided the enzymatic activity by the average surface area of each species of root tip to determine the activity per mm² of root tip (Image 1).

**Soil Potential Enzyme Activity:**

I also sieved the soil portion of each core through a 1.00 mm mesh screen to ensure that all root had been separated from the sample. Because the fluorometer can only make measurements on liquid samples, I followed Yangtsho Gyaltshen’s (2013) procedure for making soil slurries. To do this, I collected 0.5 g of the sifted soil and transferred it to a 50
mL Falcon tube with 25 mL of deionized water. To test for enzymatic activity, I scaled up the procedure written by Joseph Vallino (SES Microbial Ecology Course, unpublished) for microbial methods. After the soil slurries had been shaken and well mixed, four mL of liquid sample was transferred into four 15 mL Falcon tubes. To each set of four tubes, 400 μL substrate of enzyme substrate – to test chitobiase, endopeptidase, phosphatase, and cellobiase – was added, and the tubes were vortexed and stored in the dark.

While the samples were incubating, I prepared borosilicate tubes for each Falcon tube with four mL of 200 uM glycine buffer (10.5 pH). After one hour, 1.5 mL of enzyme-sample solution was transferred from the incubation tube to a microcentrifuge tube. Each sample was centrifuged for two minutes to ensure that all sediment particles were separated from the liquid portion. From these tubes, one mL of liquid was transferred to the borosilicate tubes to stop the reaction. This was repeated again four hours after the enzyme substrate was initially added, and both time points were read on a fluorometer.

I made a standard curve by reading the fluorescence of MUF substrate at 0, 0.1, and one μM (R²=0.99993), and I used this equation to convert absorbance to concentration. Standard error was calculated so that error bars could be added to the bar graph.

**Isotope Analysis of Mushrooms:**

Out of the nine mushrooms initially collected from Barre Woods, only eight had enough biomass in the cap to run for isotope analysis. I separated the mushroom cap from the stalk, and placed them each in a 20 mL glass scintillation vial in the drying oven overnight. After they had been thoroughly dried, I ground them using a mortar and pestle and between 2.7 and 3.4 mg of each sample was run for δ¹³C and δ¹⁵N analysis on the mass spectrometer in the Ecosystems Center, Woods Hole.
Phosphorus Content of Harvard Forest Leaf Samples:

Leaf samples taken in 2012 and 2013 from the three species of trees that are most abundant in Harvard Forest plots – *Acer rubrum*, *Quercus rubra*, and *Fraxinus americana* – were analyzed for both total and inorganic phosphorus content. A modification of the method of Murphy and Riley (1962) was used to analyze the samples. Approximately 0.1 gram of dried, ground sample was added to 20 mL glass scintillation vials. For the samples that were being analyzed for total phosphorus content, 0.5 mL of 50% w/v Mg(NO$_3$)$_2$ was added to each vial and they were allowed to ash for two hours at 550° C. After the total phosphorus samples had cooled, ten mL of 1N HCl was added to both the total and inorganic phosphorus samples and they were placed on a shaker for 16 hours. Total phosphorus samples were diluted 30:1 with deionized water, while the inorganic phosphorus samples were diluted 20:1 so that each borosilicate tube had a total of three mL of sample. To each vial, 0.3 mL of PO$_4^{3-}$ was added, and samples were allowed to incubate for 30 minutes in the dark to develop. After color had developed in the tubes, absorbance was measured at 885 nm on a spectrophotometer.

I made a standard curve with 0, 0.5, 2.5, 5, 10, 15, 25 μM PO$_4^{3-}$ in 1 N HCl (R$^2$=0.99896) to convert from absorbance to concentration.

Results:

Core Contents:

For the control and heated plots, the shift between soil, root, and debris content of the cores was graphed as percent composition. While the data suggested that there was an increase in the soil content in the heated cores and a decrease in both the roots and debris, the standard deviation was too large to determine if these shifts were significant (Figure 1).
The distribution of core elements was also broken down between the Betula lenta and Quercus rubra sites. On a species to species basis, the Betula lenta plots had the same high standard deviation as previously observed. The Quercus rubra sites on the other hand had lower standard deviation and showed a 7% increase in soil mass and a 2.4% decrease in root biomass in the heated plots (Figure 2).

**Root Tip Distribution:**

From the root tips identified and quantified from the 16 soil cores, there seems be a shift in the abundance of fungal species when soils are heated. Two species experienced an increase with heating – *Russula* by 14% and *Cenococcum* by 7.2% – while all other species experienced a decrease in abundance – most notably *Cortinarius* with a 15.6% decrease (Figure 3). The total mean between the treatments was also analyzed on a species basis. In the control plots there was relatively little difference between the Betula lenta and Quercus rubra plots, but the shifts were greater in the heated plots. The Betula lenta plots had a higher increase in *Cenococcum* – 11% compared to just 3.5% in the Quercus rubra – while the Quercus rubra had a slightly higher increase in *Russula* and a larger decrease in *Cortinarius* (Figure 4).

**Potential Enzyme Activity of Root Tips:**

Out of the four root tips that were tested, only *Russula* and *Cortinarius* showed differences in enzyme activity levels between the two treatments. Both *Cortinarius* and *Russula* showed low chitobiase activity and relatively minimal differences between the heated and control samples (Figure 5a & 5b).

When testing for endopeptidase, I found that *Russula* data showed low activity in the control plots and higher activity in the heated plots. The *Cortinarius* data showed the
opposite trend, although even in the heated plots there was high activity. In comparing the *Russula* and *Cortinarius* activity rates for endopeptidase, the *Cortinarius* overall had higher activity (Figure 5c & 5d).

*Cortinarius* samples also showed similar trends in the phosphatase activity, where the activity rates were higher than that of the *Russula* (Figure 5e & 5f). For both species, there was a slight increase in activity in the heated plots.

Both *Russula* and *Cortinarius* had higher cellobiase activity in the control plots, more than three times the activity rate of the heated plots. As seen in the other activity measurements, there was higher overall activity in the *Cortinarius* (Figure 5g & 5h).

**Soil Enzyme Activity:**

Out of the four enzymes that were being tested, phosphatase had the highest activity and endopeptidase had the lowest. For these two enzymes, there was no difference in the activity rates between the two treatments. While both chitobiase and cellobiase had roughly half the activity of phosphatase, they showed differences in the activity between the heated and control plots. For chitobiase, the heated plots had higher activity, while for cellobiase the control plots had higher activity (Figure 6).

**Isotope Analysis of Mushroom Caps:**

Five out of the eight samples collected had a $\delta^{13}$C of $\sim$-28 and a $\delta^{15}$N of $\sim$12 while the remaining samples were much lower with a $\delta^{13}$C of $\sim$-23 and a $\delta^{15}$N of $\sim$1.5 (Table 1). Despite these differences, there was no apparent trend between the heated and control plots and I was not able to draw any conclusions.
**Phosphorus Analysis of Harvard Forest Leaf Samples:**

The total phosphorus concentration was observed to be slightly higher in control plots, with the exception of *Quercus rubra* in 2012 (Figure 7). The inorganic phosphorus analysis reflected the same trend, in which there was higher inorganic phosphorus content in all samples, except *Fraxinus americana* in 2013, which consequently indicated that the heated plots had a higher proportion of organic phosphorus (Figure 8). In general, the *Fraxinus americana* had higher phosphorus concentrations than the other two species.

While this trend was seen across an annual and species basis, due to overlapping of error bars it is difficult to determine if this trend is significant.

**Discussion:**

The difference I observed in enzymatic activity between the heated and control plots suggests that warming of soils does impact the production of enzymes by soil fungi. The decrease in endopeptidase activity in the heated samples, most prominently seen in *Cortinarius* samples, may be attributed to the effect heating has on net nitrogen mineralization rates. Previous studies have shown that heating soils 5°C above ambient temperature has resulted in a 45% increase in the average net mineralization rate (Butler et al. 2012). The increase in net mineralization would result in increased nitrogen availability to plants. Increased nitrogen availability may decrease both fine root biomass and the amount of mycorrhizae and could also decrease the production of enzymes related to the breakdown of nitrogenous compounds by fungi. Because enzymes are energetically expensive to produce, if there are nutrients readily available to the plants, the fungi may not expend resources to make endopeptidase.

Similarly, soil warming has been shown to increase the decomposition rate of woody debris. A 2-year study showed that soil warming increased the mass loss of woody
debris by as much as 30%, but that lignin degradation did not follow the same mass loss trend (Berbeco et al. 2012). Thus, soil warming appears to deplete labile rich carbon sources, which consequently increases the proportion of lignin. This would explain the trend I observed in both the *Russula* and *Cortinarius* data, in which the control plots had higher activity rates than their heated counterparts. The heated plots may have lower cellobiase activity rates because there is an overall decrease in the amount of cellulose and woody biomass present in the soil, and so there may be a shift in enzyme production in which cellulases are less active and enzymes that act on lignified compounds may be more active.

Further studies could test this hypothesis by measuring chemical soil fractionations with hot water and strong acids to quantify the starches, cellulose, and lignified carbon pools (Ryan et al. 1990). Additionally, while my data from core separation showed that the heated plots had increased soil mass and a decrease in both root and woody debris, the standard deviation was so high that it cannot be determined if this is a significant trend. By taking more replicates between the plots it would be possible to see if this trend is upheld, which would also have implications on the decomposition rate of woody biomass.

The trends that I found for both endopeptidase and cellobiase on the root tips are supported by the trends in enzyme activity that I observed in the soil. By measuring the enzymatic activity of the root tips, I was focusing on the activity of the fungi, whereas the enzymatic activity of the soil represents the activity of both the fungi and soil bacteria. Previous findings from Harvard Forest show that soil warming increases nitrogen mineralization; therefore the increase in chitobiase activity in the soil may be a reflection of the increased activity of soil microbes (Melillo et al. 1999).
For cellobiase activity in both the root tips and the soil, it was found that the control plots have higher activity than that in the warming plots. These findings, like the enzyme data for the root tips, suggests that over the ten years that the Barre Woods experiments have been running, the labile carbon of the organic matter in the soil has become depleted.

Because I had observed slightly lower phosphatase activity in the heated plots, I thought this might suggest that there was increased organic phosphorus available in the heated plots. To test this hypothesis, I measured the total and inorganic phosphorus content of leaves from the three most abundant species observed in the Harvard Forest warming experiment. My hypothesis was not supported, and I observed that the leaves from the heated plots were instead slightly nitrogen depleted. However, due to the large standard deviation and small sample size, it is likely that there was no significant difference. This indicates that perhaps there is no difference in phosphatase activity between the heated and control plot, or if there is increased phosphate availability in the heated plots, that it is coming from a different source or input to the system.

I observed consistent differences in cellobiase activity, which I thought could lead to shifts in the $\delta^{15}$N, which may be observed in mushrooms. In general, over time there are shifts to greater $\delta^{15}$N soil content, as it is a heavier isotope; consequently soil fungi and microbes more readily take up amino acids and compounds with $^{14}$N. In the heated plots where there is an increase in net mineralization and organic matter decomposition, there is an increase in the uptake of nitrogenous compounds by trees, which leads to higher growth rates (Butler et al. 2011). The increase in nitrogen uptake has the potential to cause the roots and fungi in the heated plots to leave more compounds with a high $\delta^{15}$N content and thus increase the $^{15}$N content of the control plots. If this process were present, then it might
be observed in the fruiting bodies of the mushrooms. However, the mushroom caps collected from Barre Woods did not show this trend.

There was no significant change in $\delta^{15}N$ across treatments and differences in $\delta^{15}N$ instead seemed to be more a function of the mushroom species. I think that this experiment may have failed to generate a trend in part due to the extremely small sample size and the fact that I was not able to collect species replicates between the two plots. There is also a possibility that the *Cortinarius* fruiting bodies have a much higher $\delta^{15}N$ than those of the *Russula* (Hobbie and Agerer 2010). If this experiment were to be carried out again in Barre Woods, I would suggest that mushroom samples be collected throughout the year and that there be a focus to identify species that are present in both plots.

While *Cortinarius* on the root tips showed higher enzyme activity, there was an overall decrease in *Cortinarius* abundance in the heated plots in comparison to *Russula*. The average length of *Cortinarius* that I measured was 7.57 mm while the average length of *Russula* was only 1.99 mm. The difference in fungi size on the root tip may be an indication that *Cortinarius* are more carbon expensive than *Russula*, and while they appear to have much higher activity rates, that they may be energetically unfavorable to sustain in the heated plots due to the increased accessibility of nutrients.

The fungal species shift I observed in Barre Woods was different from that of the Alaskan heated plots. These plots favored species like *Cortinarius*, which form rhizomorphs and relatively long hyphae; these are categorized as medium fringe exploration types (Deslippe et al. 2010). This difference may be explained by the fact that the tundra is a more nitrogen-limited system than Harvard Forest. Thus by heating the Alaskan soils it is possible that increased mineralization rates and nitrogen uptake by plants may have
caused the heated experimental plots to become even more nitrogen depleted than the control plots. If this is the case, then plants may be investing in *Cortinarius* because while it is more carbon expensive, it has the potential to explore further in the soil to access less recalcitrant material. With this Alaskan example in mind, the Barre Woods may not be showing this trend because there is still an abundance of available organic material. However if over time the mineralization and decomposition rates remain high, these resources may too become depleted and a similar shift in soil fungi could be observed. I think it would be useful for subsequent experiments to monitor soil fungi abundance over time, especially in regard to how nutrient cycles and concentrations change, to see if this trend presents itself.
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Figures, Tables and Images:

Figure 1: Soil sores were separated into soil, root, and debris portions. These portions were weighed and percent content of each core was determined. All eight cores from each treatment plot were averaged to show the shift in core content.

Figure 2: The soil core distribution from the four core sets – Betula lenta control, Betula lenta heated, Quercus rubra control, and Quercus rubra heated – were averaged and plotted as percent composition.

Figure 3: Root tips were compared to images captured by Jesse Sadowsky from the chronic nitrogen addition plots that had been identified through DNA sequencing in order to determine their family. Four categories of fungi – Tomentella, Cortinarius, Cenococcum, and Russula – were identified, and counts were made for the abundance of each. Relative abundance of each species was determined to compare the shift in species composition between the two treatments.

Figure 4: The root tip distribution from the four core sets – Betula lenta control, Betula lenta heated, Quercus rubra control, and Quercus rubra heated – were averaged and plotted as percent composition.

Figure 5: Comparisons of the potential enzyme activity for chitobiase, endopeptidase, phosphatase, and cellobiase between Russula and Cortinarius. The heated data sets are expressed in red and the control are blue.
Figure 6: Potential enzyme activity was measured in all 16 soil cores taken from Barre Woods. To compare the difference in activity, the eight data points collected after the four hour incubation for the heated and control plots were averaged.

Figure 7: Leaf samples from Acer rubrum, Quercus rubra, and Fraxinus americana that had been collected in 2012 and 2013 were tested for total phosphorus content. Each data point represents an average of four leaf samples of the same species from a given year that was tested for total phosphorus content.

Figure 8: Leaf samples from Acer rubrum, Quercus rubra, and Fraxinus americana that had been collected in 2012 and 2013 were tested for total phosphorus content. Each data point represents an average of four leaf samples of the same species from a given year that was tested for inorganic phosphorus content.

Table 1: The isotope data from three mushroom caps in the heated plots and five from the control plots. Samples were run in duplicate if there was enough biomass in the cap. d_{13}C and d_{15}N shifts are shown as well as the C:N molar ratio.

Image 1: Root tips that had been cut down for the enzymatic activity assay were later imaged under a microscope and analyzed for the length and width of the root tip. Additionally, rough outlines were drawn around each root tip to estimate the area.
Image 2: The mushrooms collected from Barre Woods. No species identifications were made, but images were taken of each mushroom collected. The photographs with blue notation in the upper right corner are from the control plots, while those with red notation on the upper left corner are from the heated plots.
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Figure 7: Leaf samples from *Acer rubrum*, *Quercus rubra*, and *Fraxinus americana* that had been collected in 2012 and 2013 were tested for total phosphorus content. Each data point represents an average of four leaf samples of the same species from a given year that was tested for total phosphorus content.
Figure 8: Leaf samples from *Acer rubrum*, *Quercus rubra*, and *Fraxinus americana* that had been collected in 2012 and 2013 were tested for total phosphorus content. Each data point represents an average of four leaf samples of the same species from a given year that was tested for inorganic phosphorus content.
<table>
<thead>
<tr>
<th>Sample ID</th>
<th>d\textsuperscript{13}C (o/oo vs. PDB)</th>
<th>d\textsuperscript{15}N (o/oo vs. AIR)</th>
<th>Mole C:N</th>
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<tr>
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</table>

Table 1: The isotope data from three mushroom caps in the heated plots and five from the control plots. Samples were run in duplicate if there was enough biomass in the cap. d\textsuperscript{13}C and d\textsuperscript{15}N shifts are shown as well as the C:N molar ratio.
Image 1: Root tips that had been cut down for the enzymatic activity assay were later imaged under a microscope and analyzed for the length and width of the root tip. Additionally, rough outlines were drawn around each root tip to estimate the area.
Image 2: The mushrooms collected from Barre Woods. No species identifications were made, but images were taken of each mushroom collected. The photographs with blue notation in the upper right corner are from the control plots, while those with red notation on the upper left corner are from the heated plots.