

**An Isotopic  $^{15}\text{N}$  Analysis of Ectomycorrhizal Associations and the Effect  
of Soil Warming on Fungal Abundance and Fungal Nitrogen Storage**

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## Abstract

As the global climate warms, so do the soils where important organisms to ecosystem function and decomposition such as fungi, thrive. Using soil warming plots in the Harvard Forest, MA, symbiotic ectomycorrhizal fungi as well as the general fungi population were evaluated to determine the impact of heating. The nitrogen content was also determined based on previous work and total hyphal length calculated using epifluorescent microscopy techniques.  $^{15}\text{N}$  isotope analysis was also done on three types of ectomycorrhizal fungi and their corresponding roots to observe  $^{15}\text{N}$  fractionation. This fractionation was observed in two of the three ectomycorrhizal fungi types (1.9 - 4‰), evidence that these fungal types are hydrolyzing organic nitrogen in the soil and providing amino acids to their host plants. . My conclusion is that no significant difference appeared in the fungal abundance and nitrogen content of the hyphae in the soil warming plots. Ectomycorrhizal fungal root tip colonization ranged from 44 to 61%, total hyphal length ranged from 574 to 617 m hyphae/ g soil, and the nitrogen content of the hyphae ranged from 0.024 to 0.029 mg N/ g soil. The total hyphal length was higher than past findings but the rest of the results were similar to the literature values.

**Key Words and Phrases:** ectomycorrhizal fungi,  $^{15}\text{N}$ , soil warming plots, hydrolytic, nitrogen.

## Introduction

In our constantly warming global climate, terrestrial ecosystems are affected by many factors such as rising  $\text{CO}_2$  levels, more extreme weather, and a general climate warming that may increase the soil temperature. In the North American temperate forest, climate change is expected to increase the productivity of the forest over the next 50-100 years. However, modeled predictions regarding long-term or extreme climate change suggest the possibility of widespread forest decline (IPCC, 2001). In order to evaluate the impacts of climate change on the forest, Melillo et al. (2002) warmed the soils of a mid-latitude hardwood forest and found that “soil warming accelerates soil organic matter decay and carbon dioxide fluxes to the atmosphere” but that this is a small and transient result because of the limited labile soil carbon pool. In conjunction with these findings, 80% of the soil respiration is attributed to microbial respiration. Therefore, many of the changes in the soil associated with warming are attributed to microbial activity while the microbial details are still unknown.

One of the important contributors in the temperate forest to microbial processes are the symbiotic ectomycorrhizal fungi. The ectomycorrhizal fungi form sheaths of hyphae around plant fine roots in a symbiotic relationship with the plant. In most boreal and temperate forests, trees such as oak, beech, birch, willow, and most conifers tend to be symbiotic with ectomycorrhizal fungi (Hobbie and Hobbie 2006). These symbiotic fungi are thought to play an essential role in terrestrial ecosystems by increasing the root surface area of the associated plant for the uptake of mineral nutrient ions and also through the breakdown of organic nitrogen compounds such as protein or chitin using enzymatic processes (Read and Perez-Moreno 2002, Hobbie and Hobbie 2006). Organic nitrogen is then transferred to the host plant. In return, the fungi receive carbon in the

form of sugars from the plant that is vital for fungal growth. In a recent study the nutrient exchange occurring in this relationship has been quantified for the first time showing that mycorrhizal fungi in the Arctic provide 61-86% of the nitrogen plants require while plants give 8-17% of their photosynthetic carbon to the fungi (Hobbie and Hobbie 2006).

The recent work by Hobbie and Hobbie (2006) and others such as Zeller et al. (unpublished manuscript) makes use of stable isotopes to evaluate the extent of hydrolytic processes occurring in the fungi and tree roots. This concept of the ectomycorrhizal fungi as an active decomposer rather than simply a passive transporter has been proposed before by Read and Perez-Moreno (2002) and is supported by methods other than isotope work. Read's argument is based on the facts that mycorrhizal fungal hyphae compose much of the microbial biomass in many terrestrial systems, mycorrhizal fungi in culture can degrade a wide variety of organic nitrogen compounds, and genes encoding for hydrolytic enzymes are found in cultured fungi and in fungal DNA extracted from soil. Even though these arguments are valid, isotope analysis can present further evidence and provide information about the activities of different ectomycorrhizal fungal types.

As stated by Hobbie et al. (2005), "nitrogen isotopes ( $^{15}\text{N}/^{14}\text{N}$  ratios, presented as  $\delta^{15}\text{N}$ ) are useful markers of the mycorrhizal role in plant nitrogen supply because discrimination against  $^{15}\text{N}$  during creation of transfer compounds within mycorrhizal fungi decreases the  $^{15}\text{N}/^{14}\text{N}$  in plants (low  $\delta^{15}\text{N}$ ) and increases the  $^{15}\text{N}/^{14}\text{N}$  of the fungi (high  $\delta^{15}\text{N}$ )." This change in the isotopic composition of the nitrogen in mycorrhizal fungi and their plant hosts is not seen in all mycorrhizal fungi. Instead, this fractionation occurs in only some of the fungal species suggesting that not all mycorrhizal fungi have enzymes capable of breaking down soil organic nitrogen (Hobbie et al. 2005). Because so much is still unknown about many of the mycorrhizal fungi processes, it is important to try to link functions to the different species.

Besides classification by species, work has been done to classify ectomycorrhizal fungi by exploration types (Agerer 2001) as well as through descriptive terminology (Brundrett et al. 1996). These are valid attempts to understand and differentiate the types, the species, and the functions of ectomycorrhizal fungi.

In this study I attempted to understand some basic ectomycorrhizal associations through observation, through the exploration of methods and quantitative techniques, and through isotope analysis. This experiment has several components that are designed to look at ectomycorrhizal fungi as well as fungal abundance in general in soil that has been heated to simulate the results of global warming. The study sites used were established by Melillo et al. in the Harvard Forest and have been heated for different amounts of time (5 and 15 years). Samples from control and heated plots were compared by calculating the percent colonization of the root tips with mycorrhizal fungi, the total hyphal length of all fungi in the soil, and the total nitrogen content of the fungi. Isotope analysis was also done on samples of mycorrhizal fungi and the roots they inhabit. The type of mycorrhizal fungi tested were documented through photographs.

## **Methods**

### *Study Sites*

This study took place at the Harvard Forest in central Massachusetts (42.54°N, 72.18°W) at two soil warming experimental sites (Prospect Hill and Barre Woods)

established by Melillo et al. In the Harvard Forest the dominant trees include paper birch (*Betula papyrifera* Marsh.), red maple (*Acer rubrum* L.), black oak (*Quercus velutina* Lam.), and striped maple (*Acer pennsylvanicum* L.). The soil has a surface pH of 3.83 with a sub-surface pH of 4.85. The average bulk density of the first 15 cm of the soil is  $0.64\text{g/cm}^3$ . The area shows signs of past cultivation with a distinct soil plow layer. The mean weekly air temperature ranges from  $20^\circ\text{C}$  in July to about  $-6^\circ\text{C}$  in January and the precipitation is distributed evenly with an annual average of 108cm (Melillo et al. 2002).

### *Experimental Design*

The soil at the Prospect Hill site has been warmed by  $5^\circ\text{C}$  above ambient since April 1991 through the use of buried heating cables placed at a depth of 10cm and spaced 20cm apart. The site consists of 18 6m x 6m plots grouped into six blocks. The three plots within each block are heated, disturbed control (cables are buried but the heat is not turned on), and undisturbed control plots. In this experiment only the heated and undisturbed control plots were sampled as no significant difference has been found between the disturbed and undisturbed control plots (Melillo et al. 2002).

The Barre Woods site has been warmed using the same system as at the Prospect Hill site since the fall of 2001. At this site, the experiment is divided into both a 30m by 30m heated plot and a 30m x 30m control plot. The plots are divided into 5m x 5m subplots and the heated plot is warmed  $5^\circ\text{C}$  above the control plot (Melillo and Steudler unpublished 2004).

### *Soil Sampling*

From both the Prospect Hill and Barre Woods sites five organic soil layer samples from the heated and control plots were collected on November 15<sup>th</sup>, 2006 (total of 20 samples). The litter layer was brushed away in the field and 12cm x 12cm soil squares ranging from a depth of 1.1cm to 3.4cm based on the depth of the organic layer, were collected in random subplots. The samples were placed on ice until they could be permanently stored in the refrigerator at  $4^\circ\text{C}$ .

### *Isotope Analysis*

Ectomycorrhizal samples were collected for isotope analysis using fine forceps and needle points to extract the mycorrhizae from the fine root tips of the collected soil samples. The fine roots from which the fungi were collected were also cleaned of all fungi and analyzed for their  $^{15}\text{N}$  content. Samples of mycorrhizae types A and B along with their respective roots were collected from the Barre Woods heated and control plot samples (Image Appendix). Mycorrhizal fungi and root collections were taken and combined from each of the five samples per plot type. Type C roots and mycorrhizae were also collected from the Barre Woods control plot (Image Appendix). Soil samples from the Barre Woods heated and control plots composed of a mix of soil from each sample per plot were also analyzed for a baseline comparison.

Samples were prepared for isotope analysis by drying at  $60^\circ\text{C}$  for 24 hours. The soil samples were then ground with a mortar and pestle. The samples were submitted to the isotope lab at the Marine Biological Laboratory (MBL) where they were evaluated for  $^{15}\text{N}$  content with a PDZ Europa 20-20 continuous-flow isotope ratio mass spectrometer.

The percent nitrogen of the heated and control soils were also determined using the mass spectrometer and compared to the nitrogen content of the fungal hyphae.

To differentiate the types of mycorrhizae analyzed, photographs were taken with a Zeiss Axiocam MRc and labeled types A through G. Based on descriptions from Agerer (2001), the exploration type for each morphotype was predicted. This classification is neither adequate nor is it intended for taxonomic purposes.

### *Mycorrhizal Colonization*

The percent of the root tips colonized by mycorrhizal fungi was determined simply by comparing the number of potentially colonized root tips to the number of root tips covered with mycorrhizal fungi. The roots were separated from the soil using running water and a 0.5mm sieve. Once cleaned, the fine roots were stored in damp paper towels while processed. Small subsections of the roots were carefully removed and observed under the dissecting microscope to determine colonization. Three hundred root tips were counted per sample (Bundrett et al. 1996).

This method gives a rough estimate about the prevalence of the mycorrhizal fungi and has been used in other studies such as Urcelay et al. (2002) to “investigate whether [arctic plant] species interactions and/or nutrient availability affect mycorrhizal colonization.”

### *Total Hyphal Length*

To determine the amount of fungal mycelium in the soil, 10 grams of soil from each sample and 95ml of distilled water were blended in a standard blender at top speed for one minute. Nine ml of the soil suspension were fixed with 1ml of Formalin and vortexed for 30 seconds. The 10ml solution was diluted three times with a 1:5 soil to distilled water ratio. The samples were then stained for 2 hours at room temperature in the dark with 1 ml of Calcofluor White M2R Fluorescent Brightener at a dilution of 0.1g per 50ml of water. Once stained, the samples were stored in the dark at 4°C until processing.

Slides were prepared by filtering 4ml of the stained solution (1.44mg soil) through a black 1µm pore size polycarbonate filter and were rinsed three times with 3ml of distilled water. The slides were prepared with immersion oil and viewed in a dark room with an epifluorescence microscope with UV illumination (DAPI filter) at 100x magnification. In one transect across the filter 70 gridded fields were observed while counting the number of times the hyphae intersected the grid lines.

The hyphal length (H, (µm/grid)) is calculated using the equation  $H = (I \pi A) / (2L)$  where I is the average number of intersections per grid, A is the grid area (  $10^4 \mu\text{m}^2$ ) and L is the total length of the lines in the grid (22 x 100 = 2,200µm). The total length of fungal hyphae (F, m/g) is then calculated as  $F = H 10^{-6} (A/B) (1/S)$  where H is the hyphal length from the previous equation, A is the area covered by the filter ( $8.5^2 \pi = 226.98 \text{ mm}^2$ ), B is the area of the grid ( $0.01 \text{ mm}^2$ ) and S is the amount of soil on the filter (1.44 mg) (Bloem et al. 1995).

This method provides a quantitative estimate of the amount of fungal hyphae in the soil and has been used by others such as Frey et al. (1999) to determine differences in the amount of fungal hyphae present in agricultural soils.

Pictures were taken with a Zeiss AxioCam MRm in order to document the appearance of the fungal hyphae under the compound microscope.

### *Fungal Nitrogen Content*

Based on the observed fungi content in the soil, the nitrogen content of the fungal mycelium was calculated based on previously determined typical nitrogen and carbon content values in fungal mycelium. First, the biovolume is calculated assuming an average hyphal width (W) of 4 $\mu\text{m}$  (Rublee 1982) and using the hyphal length (L,  $\mu\text{m}$ ) based on the previous calculations. The equation used is  $V (\mu\text{m}^3) = (\pi/4) W^2 (L-W/3)$ .

The amount of carbon is estimated from the biovolume using a specific carbon content of  $1.3 \times 10^{-13} \text{ g C } / \mu\text{m}^3$  (Van Veen and Paul 1979; Bakken and Olsen 1983). The average C:N ratio of 12.7 was used to calculate the nitrogen content in the hyphae based on the average nitrogen and carbon content of vegetative mycelium from leaf litter as determined by Lodge (1987).

## **Results**

### *Isotope Analysis*

Isotope analysis of the  $\delta^{15}\text{N}$  signature of three types of mycorrhizae and their corresponding roots from Barre Woods showed differing degrees of fractionation between the types of fungi and their roots (Fig. 1 and 2). In the heated and control plots, no fractionation occurs for fungi type A. In contrast, both fungal types B and C seem to be  $^{15}\text{N}$  enriched while their roots are depleted in  $^{15}\text{N}$ . This fractionation between the roots and the fungi for type B is 4‰ for the control sample and 2.6‰ for the heated sample. Fungi type C is also enriched in  $^{15}\text{N}$  while its roots are depleted in  $^{15}\text{N}$  but the fractionation is less than with type B (1.9‰).

Compared to the  $\delta^{15}\text{N}$  signature of the soil in the control sample (0.29‰), fungi types B and C are enriched while their roots are depleted. The soil in the heated plot is more  $\text{N}^{15}$  enriched (1.5‰) than either the roots or the fungal mycelium.

### *Mycorrhizal Colonization*

In a comparison of the heated and control plots within each site, the mean percent of the root tips colonized by ectomycorrhizal fungi in the Barre Woods and Prospect Hill sites does not differ significantly (Table 1). In the Barre Woods the heated plot is slightly more colonized while at Prospect Hill the control site has slightly more ectomycorrhizal fungi. Nevertheless, there is no significant difference between treatments once the standard error is considered. The control plots in each site showed similar percent colonization (52.7% and 51.1%) while the heated Barre Woods site was significantly higher than the Prospect Hills heated site (61.3% and 43.7%, respectively).

### *Total Hyphal Length*

The mean hyphal length in the soils (m hyphae/g soil) of the Barre Woods and the Prospect Hill sites shows no significant difference between heated and control plots (Table 2). Slightly more hyphae were measured in the control Barre Woods plots than in the heated plots but in the Prospect Hill site, this trend was reversed. When the standard error is considered, neither of these trends are significantly different. Overall, the

Prospect Hill site tends to have more hyphae than the Barre Woods site but once again, this trend does not appear as a significant difference.

### *Fungal Nitrogen Content*

Because the calculation of the nitrogen content in the fungal hyphae is based on the results from the hyphal length counts, the nitrogen content of the hyphae follows the same trend as the hyphal length data (Table 3). The nitrogen content varies only slightly between plots and sites (from 0.024 to 0.029 mg N/g soil).

Compared to the nitrogen content of the soil at the Barre Woods heated and control sites (7.1 and 13 mg N/g soil), the nitrogen in the hyphae makes up only 0.34% of the nitrogen at the heated site and 0.22% of the nitrogen at the control site. At the Prospect Hill site the nitrogen in the hyphae for the heated and control plots made up 0.18% and 0.14%, respectively, of the total soil nitrogen content (15 and 19 mg N/g soil). In both sites the control soil nitrogen content was higher than the heated soil nitrogen content.

## **Discussion**

### *$\delta^{15}\text{N}$ Isotope Analysis*

The isotopic results from this study provide more evidence in support of the proposition that mycorrhizal fungi are active in hydrolysis of organic nitrogen compounds. The  $^{15}\text{N}$  fractionation occurring between the roots and the fungal types B and C shows that the trees selectively receive the lighter  $^{14}\text{N}$  from the mycorrhizal fungi and while the heavier  $^{15}\text{N}$  remains in the fungal mycelium. This has been observed in the Arctic as well as in France (Hobbie and Hobbie 2006, Zeller et al. manuscript). Högberg et al. (1996) found that the ectomycorrhizal fungal mycelium of beech and Norway spruce trees in Europe were 2.4 to 6.4‰ enriched in  $^{15}\text{N}$  relative to the encased root. These fractionations are similar to those found in my study (1.9 - 4.0 ‰).

Nevertheless, the fractionation occurring in the Harvard Forest is much less than in the Arctic where fractionations as large as 12‰ occur within the ectomycorrhizal associations (Hobbie and Hobbie 2006). The higher fractionation in the Arctic may be due to less nitrogen availability; there is less than 1 kg ha<sup>-1</sup> yr<sup>-1</sup> of nitrogen deposition in the Arctic (McKane et al. 1997) compared to an average of 12 kg ha<sup>-1</sup> yr<sup>-1</sup> of nitrogen deposition in New England (Bowen and Valiela 2001). Because of this difference in the annual nitrogen addition to the New England area compared to the Arctic, John Hobbie (personal communication) thought that there would be no hydrolytic activities of mycorrhizal fungi at all. It has been suggested that mycorrhizal fungi play a key role in nitrogen cycling and providing nitrogen to plants in more nitrogen limited systems but is there so much nitrogen available in New England that mycorrhizas are unnecessary? Others have explored the effects of acid deposition on mycorrhizas and my work tends to support the conclusion that the mycorrhizal fungi still depend on enzymes to supply nitrogen to their symbionts even in systems not limited or only slightly limited by nitrogen (Ruhling and Tyler 1991, Wallenda and Kottke 1998). Nevertheless, the plants seem to depend less on the mycorrhizal fungi in the Harvard Forest based on smaller fractionation values compared to the fractionations seen in the Arctic. It is also important to note that the smaller fractionation values observed in this study may be due to impure samples that contain small fragments of the fine roots.

In a recent attempt to quantify the percent of the nitrogen in the plant that comes from the mycorrhizal fungi, Hobbie and Hobbie (2006) have developed a model using the  $\delta^{15}\text{N}$  signatures of the soil, fungi, and roots in a simple mass balance equation. In the Arctic they found that mycorrhizal fungi provide 61-86% of the nitrogen in the plants. When the  $\delta^{15}\text{N}$  values determined for the control type B fungi in this study are run through this model, 21% of the nitrogen in the plant appears to be supplied by the mycorrhizal fungi. Again this provides more evidence that despite higher acid deposition rates in New England compared to the Arctic, an important portion of the tree's nitrogen supply appears to be coming from its ectomycorrhizal association.

Another interesting finding from the isotope analysis is the occurrence of  $^{15}\text{N}$  fractionation in fungi types B and C and not in type A. This shows that not all species of ectomycorrhizal fungi are capable of hydrolytic activities. This has been noted before by Hobbie et al. (2005) as different mycorrhizal species show a dramatic range of  $^{15}\text{N}$  signatures. Only some of the ectomycorrhizal fungi found on the tree roots are involved in hydrolytic activities. Therefore, the ectomycorrhizal fungi must also be providing the plants with other nutrients, water, or other benefits.

#### *Fungal Reactions to Warming*

Since the soil warming had no significant effects on the ectomycorrhizal fungi or general fungal population in either the Barre Woods or Prospect Hill sites, more work needs to be done to determine the impact of soil warming. It is possible that soil warming does not significantly affect the ectomycorrhizal population but more likely, the sample size and techniques used in this study do not provide enough evidence for any accurate conclusions. Findings by Clemmensen et al. (2006) show some change in fungal biomass in heated plots but their results are not conclusive and leave lots of room for further study.

#### *Fungal Abundance and Nitrogen Content*

Despite the insignificant impact of soil warming on the fungi, the results regarding the percent colonization by ectomycorrhizal fungi found in this study (61% - 44%) are in the range of the percent colonization presented by Urcelay et al. in the Arctic tundra (60% - 32%).

In contrast to past studies, total hyphal length was substantially more in the Harvard Forest. Using the same techniques implemented in this study, Frey et al. found that hyphal length ranged from 19 to 292m hyphae/g of soil while my results ranged from 574 to 646m of hyphae/g of soil. This difference may be attributed to different ecosystem and soil types and the location of the studies. In the Frey et al. (1999) study, they were evaluating disturbed agricultural land while I am looking at forested land that has not been recently disturbed. Therefore, a higher fungal hyphae count is not all together unexpected. Read (1992) also found a total hyphal length of 200m per g of dry soil of *Suillus bovinus* (a type of mycorrhizal fungi) in forest soils of root chambers but it is difficult to compare my results to experiments done in unnatural laboratory settings.

Another factor that may have contributed to my high hyphal counts is my definition of hyphae under the microscope. I considered all fluorescing, stringy, hyphae-like structures to be hyphae even when identifying factors such as septated cells were not visible. Nevertheless this may not be the issue here because it is possible to have

unseptated hyphae as seen in the images taken from actual samples (Image Appendix) (Brundrett et al. 1996).

The nitrogen content calculated in this study is based on findings by Lodge (1987) that the nitrogen content of the mycelium is around 34.64 mg N/g dry mycelium. Despite this high nitrogen concentration in the mycelium, fungi make up only a small percentage of the total soil nitrogen. In the Harvard forest I found the fungi to make up only 0.14% to 0.34% of the total soil nitrogen. The higher nitrogen content in the control soil versus the heated soil may be due to an excess amount of nitrogen in the heated plots that is possibly being denitrified or leaching out of the soil.

### **Conclusions**

Despite the tremendous amount of research done on mycorrhizal fungi and their symbiotic relationship with plants, a great deal remains to be learned about the processes involved. Many studies have looked at mycorrhizas in the laboratory but more field studies need to be done in order to observe the mycorrhizal associations in their natural environment. In this study I have done some basic analyses to look at the effect of soil warming but more importantly I have been able to provide further evidence toward the on-going dispute regarding the functioning of mycorrhizas. My findings seem to prove that some mycorrhizal fungi partake in hydrolytic activity based on isotopic  $^{15}\text{N}$  fractionation even in a region of relatively high acid deposition. Nevertheless, this is just a start and more samples need to be analyzed in order to conclude anything further.

### **Acknowledgments**

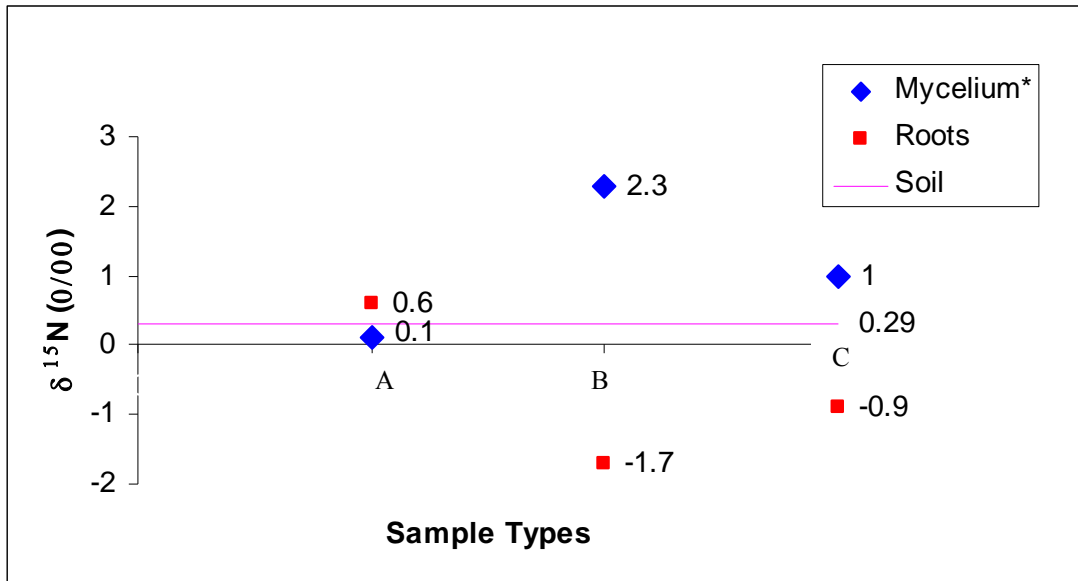
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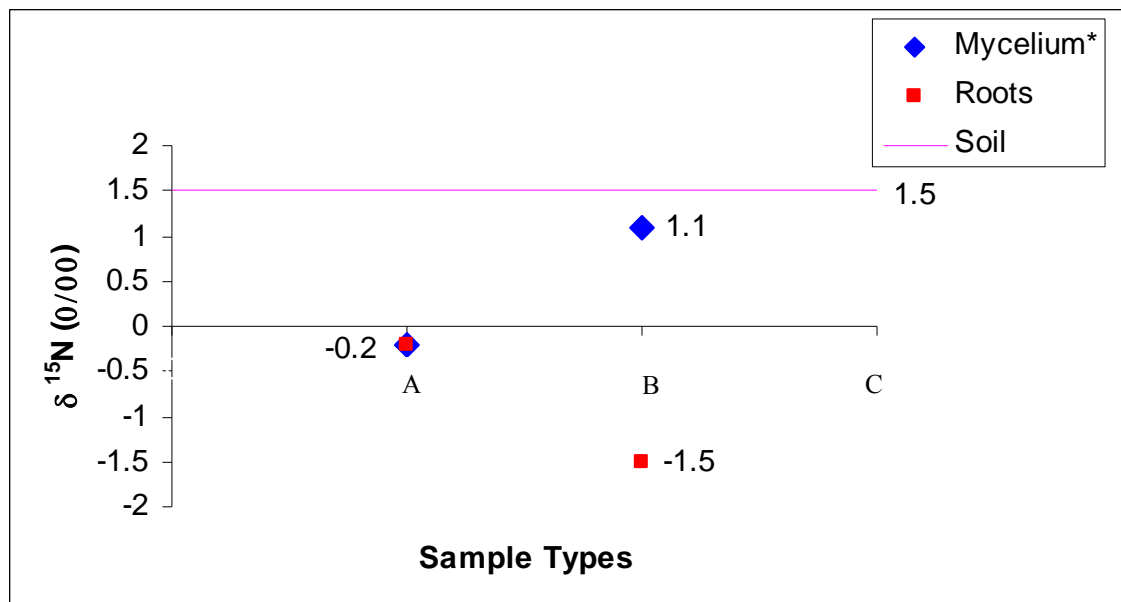
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**Fig. 1:**  $\delta^{15}\text{N}$  isotope data from Barre Woods control plots of fungal mycelium, fine roots, and soil.

\*The mycelium sample may also include a small amount of fine roots.



**Fig. 2:**  $\delta^{15}\text{N}$  isotope data from Barre Woods control plots of fungal mycelium, fine roots, and soil.

\*The mycelium sample may also include a small amount of fine roots.

**Table 1:** Percent colonization with ectomycorrhizal fungi in heated and unheated plots in the Harvard Forest.

	%					Mean ± SE
	1	2	3	4	5	
<b>Barre Heated</b>	56.7	51.0	67.0	68.0	64.0	61.3 ± 3.26
<b>Barre Control</b>	64.0	65.3	40.0	33.3	61.0	52.7 ± 6.68
<b>Prospect Heated</b>	45.0	51.0	42.3	32	48.3	43.7 ± 3.28
<b>Prospect Control</b>	50.3	62.0	53.3	34.0	55.7	51.1 ± 4.68

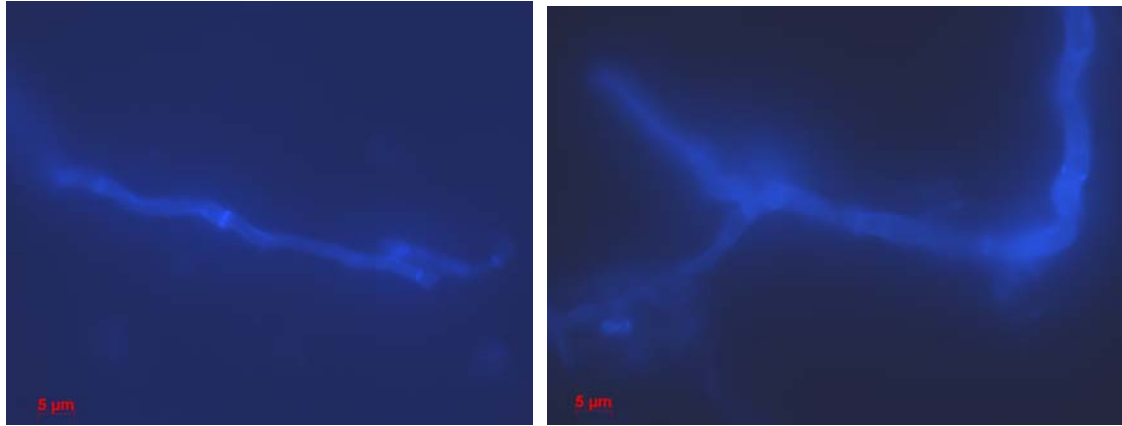
**Table 2:** Total hyphal length in the soil of heated and unheated plots in the Harvard Forest.

	<b>m hyphae/g soil</b>					<b>Mean ± SE</b>
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	
<b>Barre Heated</b>	813.5	733.1	336.5	393.9	592.0	573.8 ± 92.7
<b>Barre Control</b>	779.7	622.2	578.8	653.9	363.5	599.6 ± 67.8
<b>Prospect Heated</b>	847.5	408.5	715.8	727.0	532.3	646.2 ± 77.9
<b>Prospect Control</b>	704.5	462.6	809.2	667.4	443.4	617.4 ± 71.1

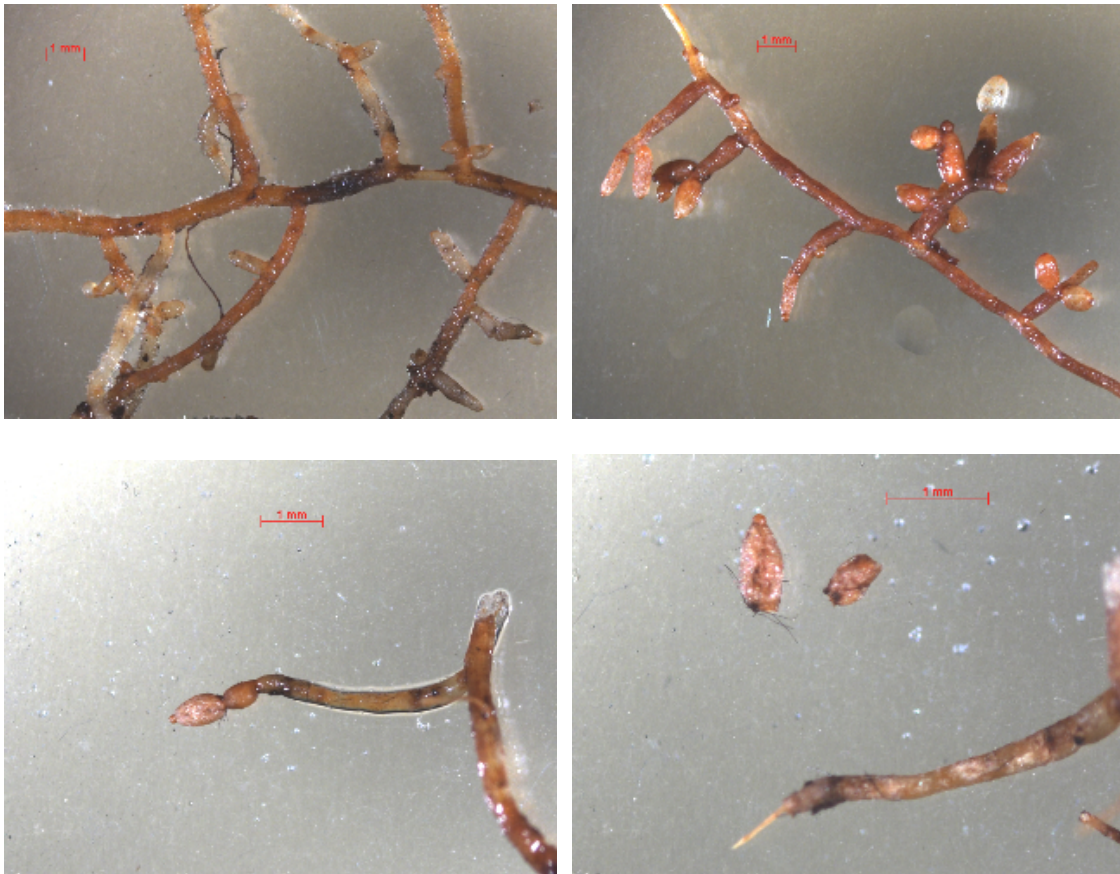
**Table 3:** Total nitrogen content in the fungal hyphae and in the soil in heated and unheated plots in the Harvard Forest. The fungal hyphae nitrogen content is based on the total hyphal length data while the soil nitrogen content comes from the percent nitrogen determined through isotope analysis.

	mg N in hyphae/ g soil					Mean ± SE	mg N/ g soil
	1	2	3	4	5		
<b>Barre Heated</b>	0.035	0.031	0.014	0.017	0.025	0.024 ± 0.0040	7.1
<b>Barre Control</b>	0.033	0.027	0.040	0.028	0.016	0.029 ± 0.0041	13
<b>Prospect Heated</b>	0.036	0.018	0.031	0.031	0.023	0.028 ± 0.0032	15
<b>Prospect Control</b>	0.030	0.020	0.035	0.029	0.019	0.027 ± 0.0031	19

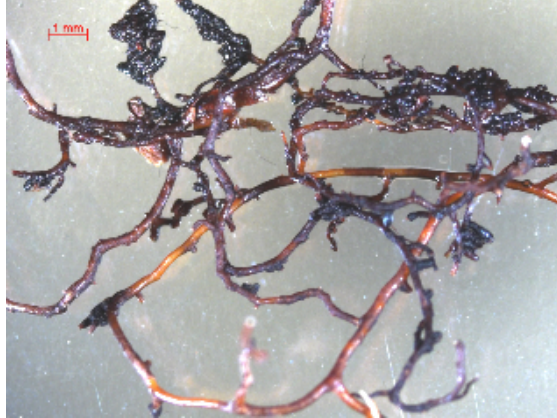
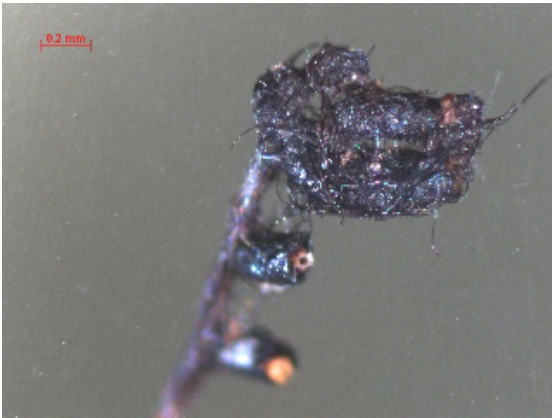
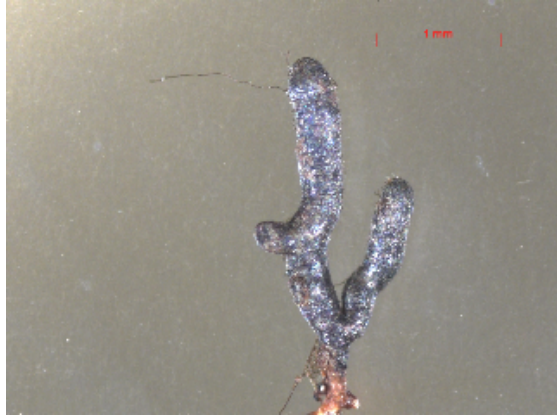
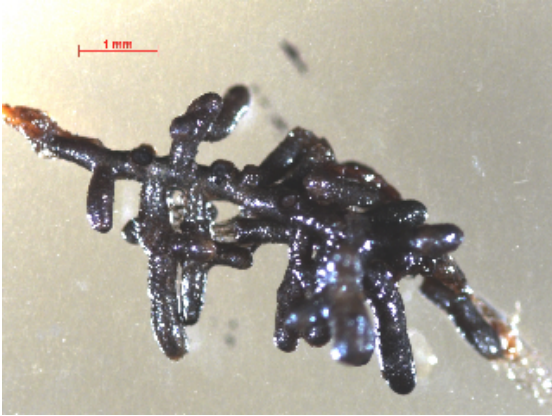
## Image Appendix



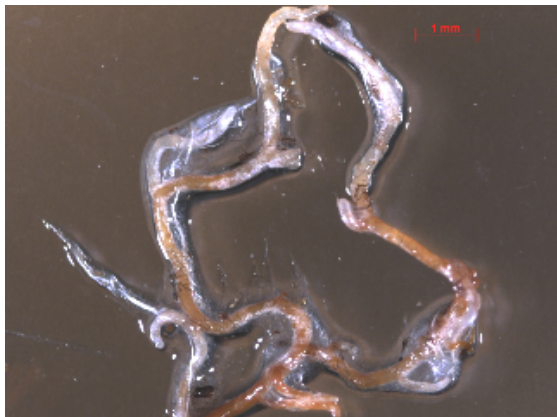
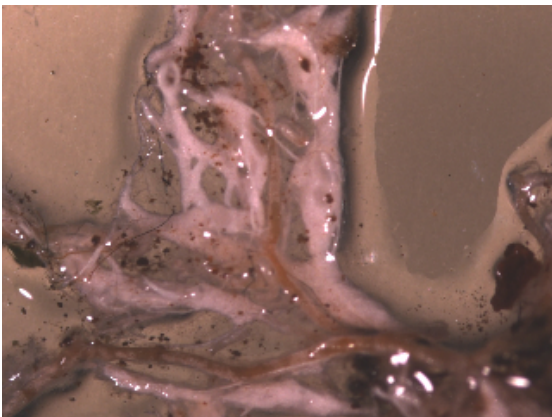
Fungal hyphae under a compound microscope viewed at 100x.



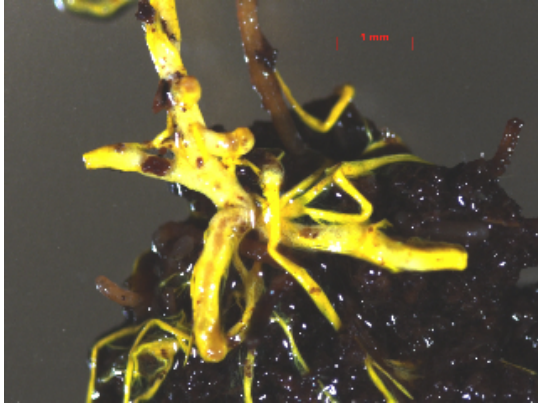
Type A (Medium-distance smooth exploration)



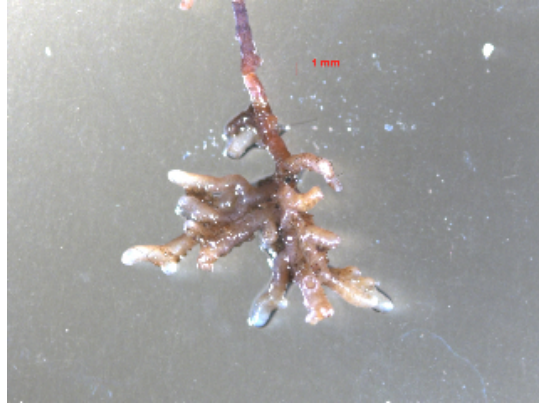
Type B (Short-distance exploration)



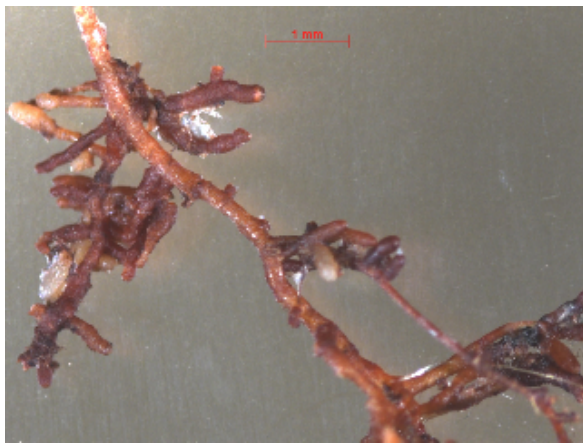
Type C (Medium-distance fringe exploration)



Type D (Medium-distance fringe exploration)



Type E (Contact Exploration)



Type F (Contact Exploration)



Type G