Ectomycorrhizal Diversity in *Picea mariana* for burned and unburned forests

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Introduction

Mycorrhiza is a type of symbiotic relationship between a fungus and a plant root. The primary function of ectomycorrhiza, a form of mycorrhiza, is to provide mineral nutrients from the soil to its host plant and aids it by preventing certain diseases from infecting the roots (8). The fungus sustains its symbiosis by the sugar that it accumulates from its host plant. In a burned habitat studies have found low levels of mycorrhizal propagules meaning that there is a less diverse communites of mycorrhiza after a fire (2).

Studies have agreed that there is a dramatic increase in fires in the north (4). The ectomycorrhiza on the trees and plants may be the gateway to predicting what type of environment may develop after a fire.

By studying the ectomycorrhiza that appear after a fire, called resistant propagules, scientists may better predict the kind of forest that transpires afterwards (3).

The objective to my study is to compare the diverse sites of ectomycorrhizal communities on Picea mariana seedlings of a burned versus a mature forest. We suggest that there may be more diverse ectomycorrhiza in a mature forest than in a burned forest. Case studies in other sites have also suggested this pattern (6).

Restriction Fragment Length Polymorphism (RFLP) is when a restriction enzyme digests a DNA sequence into fragments, which can later be distinguished by electrophoresis. When the fragments are separated it will reveal a pattern for a specific genotype used to find the measure of diversity for molecular analysis (1).

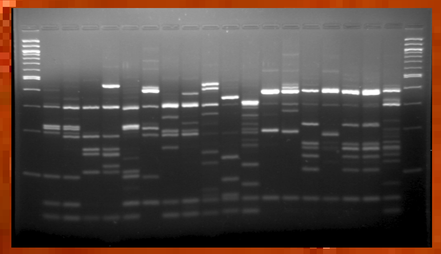
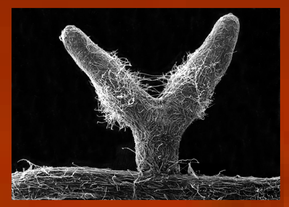


Table 1: Fungal types of root tips

Seed-ling	Sample	Fungi	Site	BLAST % Identity
3	E	Wilcoxina (Ascomycota)	burned	98%
3	D	Unknown 9	burned	
4	F	Wilcoxina + another fungus	burned	
4	H	Unknown 1	burned	
4	G	Unknown 1	burned	
8	N	Unknown 1	burned	
8	O	Wilcoxina	burned	
11	X	Tylospora (Basidiomycota)	burned	83%
11	W	Unknown 5	burned	
12	Y	Rhyzoscyphus + another fungus	burned	
13	AA	Rhizoscyphus (Ascomycota)	burned	86%
5	I	Unknown 2	unburned	
5	J	Tomentellopsis (Basidiomycota)	unburned	97%
6	K	Really mixed	unburned	
6	L	Amphinema (Basidiomycota)	unburned	99%
6	M	Unknown 7	unburned	
9	T	Unknown 6	unburned	
9	U	Unknown 6	unburned	
9	V	Unknown 2	unburned	
10	Q	Cortinarious (Basidiomycota)	unburned	99%
10	S	Unknown 6	unburned	
10	R	Unknown 4	unburned	
14	BB	Unknown 3	unburned	
14	CC	Unknown 4	unburned	
14	DD	Unknown 3	unburned	
14	EE	Unknown 3	unburned	
14	GG	really mixed	unburned	
14	FF	Unknown 8	unburned	



Discussion and Conclusion

We found through our methods that there were more species present than we could identify morphologically. The unknowns could not be sequenced because of mixed DNA samples. The taxa that were sequenced showed that Ascomycota colonized the burned seedlings rapidly compared to the unburned seedlings. Being the first to experiment on ectomycorrhizal diversity for burned and unburned areas in Alaska, it is interesting that we found that we had similar outcomes to other studies much further south (Molecular diversity and phylogenetic affinities of symbiotic root-associated ascomycetes of the Heliotiales in burnt and metal polluted habitats. 2002). The family for Wilcoxina is a Pyronemataceae meaning it grows after fires, which is consistent with our results on burned seedlings.

In our results we found a basidiomycota that was never sequenced internationally. There is a possibility that this is a specific basidiomycota that develops after fires or that the forests are beginnining to stabilize.

There are more types of fungi in unburned forests meaning the soil that is burned can affect the quality of Black Spruce. By quality we mean the black spruce can have an altered growth and health effects due to differences in mycorrhiza.

Methods and Materials

The sites investigated were within Caribou Poker Creek Watersheds 30km North of Fairbanks in Alaska. The *Picea mariana* seedlings found were either roadside, mature, or burned habitat. We collected a total of 13 seedlings. In order to sample the ectomycorrhiza, I washed the dirt off of each seedling, which is required for it to be inspected under a dissecting microscope. I characterized different morphotypes using size, color, and how the hyphae were arranged. We cleaned and extracted the fungal DNA, using the Qiagen Plant DNeasy Kit, from the plant tissue in order to perform PCR. 18 samples, after PCR, underwent RFLP using the Mbo1 restriction enzyme. The DNA left plus the next set of sample we did not run where subjected to RFLP.

The rest of the samples that have been amplified were run under electrophoresis. Once the results came we chose the samples that did not have double-bands and ran a cycle sequence. We used primers ITS1F and ITS4. Next we performed sequence corrections on the computer. The sequences were compared to known fungi from an international database using BLAST (basic local alignment and sequence analysis tool).

Results

DNA extraction and amplification were successful (28 root tip samples). We sequenced 8 of our clean PCR products and found 2 undecipherable. The remaining 6 were run on BLAST. The traceable 4 fungi were *Wilcoxina mikolae*, *Tomentellopsis submollis*, *Amphinema byssoides*, and *Cortinarious neofurvolaesus*. A short region of sample X. was 83% similar to *Tylospora asterophora* meaning its taxonomic group has not been sequenced internationally. Sample AA. entire sequence was 86% similar to *Rhizoscyphus ericae*, which suggests its genus is uncertain. The ectomycorrhiza for burned habitats had a high ratio of unsure taxa. We found 15 different types of fungi out of our 28 samples from our RFLP results. There are 6 different types of fungus out of 6 seedlings in burned. There are 5 unburned seedlings, which were found to have 9 forms of ectomycorrhiza in unburned (Table 1).

In the burned areas there is a higher ratio (5:1) of ascomycota than there are basidiomycota for the morphotypes we could identify. For the unburned habitats all the identified phyla are basidiomycota (0:3).

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