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**Report to  
Pacific Northwest Cooperative Ecosystem Studies Unit  
National Park Service**

**COOPERATIVE AGREEMENT NO. H8W07060001**

**TASK AGREEMENT NO. J8W07070012**

**PROJECT TITLE:** Diversity of high elevation soil fungi in North Cascades National Park

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**INTRODUCTION**

Fungi play a pivotal role in all ecosystems. They mediate plant establishment and growth through mycorrhizal associations, maintain soil structure, are essential for decomposition, and can be devastating pathogens. Certain fungal groups and fungal associations are extremely sensitive to changes in temperature, moisture and soil mineral concentrations. Global warming as a result of CO<sub>2</sub> increases in the atmosphere from fossil fuel burning is now widely accepted, and alpine areas are expected to be drastically effected. Recent studies have documented that changes in alpine and subalpine floral communities are occurring. Despite the documented changes in alpine flora, and the importance of fungi on plants, very little study has been devoted to alpine fungi.

This study attempted to identify and characterize the fungi of alpine and subalpine environments in North Cascades National Park (NOCA) using molecular techniques. Targeted fungi were Basidiomycota, Ascomycota, Glomeromycota, Zygomycota, and Chytridiomycota and included endomycorrhizal, ectomycorrhizal, and saprophytic fungi. Mycorrhizal fungi provide plants with essential nutrients, water, and enhanced defenses. Saprophytic fungi are essential in nutrient cycling because they are the only organisms that can decompose cellulose. Fungi also affect ecosystems by maintaining stable soil structure (particularly on slopes). Studies have shown a large disjunct between above-ground (epigeous) and below-ground (hypogeous) fungal communities, and comparisons using molecular techniques have shown never more than a 25% overlap between the two communities (Peter et al. 2001). Hypogeous studies result in a more robust data sample of the all fungi within an ecosystem. The few studies of alpine and subalpine environments have detected functionally and taxonomically diverse fungal groups, based on epigeous fungi (Cripps and Eddington 2005). Only one study on hypogeous alpine soil fungi has been performed using molecular techniques (Schadt et al. 2003) and it showed the presence of an uncharacterized taxonomic group of fungi from alpine soils. There are currently no comprehensive studies of fungi in the NOCA.

Given the current global trends of temperature and CO<sub>2</sub> increases, this research is particularly timely. Recent studies indicate recent treeline advances and drastic shifts in alpine floral communities in response to temperature and CO<sub>2</sub> shifts (Walther et al., 2005). These studies report the disappearance of certain alpine floral species and the increased dominance of other species, and the rates of this change are increasing (Walther, 2005). Although several studies suggest fungi drive plant establishment and growth, and that fungi respond to changing climatic variables (Treseder, et al. 2007), no research exists on the response of alpine fungi to climate change. As it is likely that fungi will react to changing climatic conditions, it is important that we establish accurate baseline information on alpine and subalpine fungi.

## OBJECTIVES

The goals of this study were to: 1) survey alpine areas of NOCA to create and inventory of fungi using molecular techniques; 2) document alpine macrofungi; 3) provide college credit through field and laboratory research to graduate and undergraduate UW students in a National Park and expose students to NPS management perspectives; 4) develop a PhD research proposal to monitor alpine fungal species responses to climate change based on the data collected; 5) present a PowerPoint presentation to the NPS at NOCA and provide the PowerPoint presentation for use by NPS interpreters in public outreach; and 6) develop a webpage describing this research and results for the official NOCA website.

## STUDY SITES AND METHODS

### Study sites

Three transect sampling sites were established on June 28, 2007 west of Twisp pass on the southeastern arm of Stiletto Peak in NOCA. The three transects were along an elevational gradient, each with similar slope, aspect, and vegetation. Sites are typical Cascadian subalpine meadows with established forest beyond the meadow and have many saplings of *Abies lasiocarpa* (Hook.) Nutt. that are establishing within the meadow. Sapling encroachment on meadows is common in many of the subalpine meadows in the area.

Site Coordinates as follows:

Site 1: 48°28'31" N, 120°38'54" W, 1929 m

Site 2: 48°28'32" N, 120°38'55" W, 1932 m

Site 3: 48°28'33" N, 120°39'08" W, 1923 m

Transects extended from the closed canopy forest, through the subalpine parkland to the alpine zone and representative photos are shown below.



Site 1. Forest, June 28, 2007



Site 1. Meadow is upper right, saplings are middle left, and forest upper left, September 29, 2007



Site 1. Amy Honan collecting soil samples from meadow, September 29, 2007



Site 2, Meadow is in foreground, saplings are beyond meadow, and forest is in distance, June 28, 2007



Site 2. Meadow is right off frame, saplings are in center, and forest beyond, Sept. 29, 2007





Site 3. Meadow is in distance, saplings are in foreground, and forest is to left and out of frame, June 28, 2007



Site 3, meadow left and out of frame, saplings center and right, Sept 29, 2007



Site 3. Saplings are left, meadow is far distance, Sept. 29, 2007

### **Sampling**

Samples were taken in the spring, on June 28, 2007 and on September 29, 2007. Twelve soil samples were taken on each sampling date from each of the meadow sites. Four samples were taken in the meadow with no conifer species, four adjacent to saplings within the meadow, and four from within established trees. Soil samples were sieved to separate out roots. Roots were examined for the presence of mycorrhizae and mycorrhizal root tips retained for genetic analyses.

DNA was extracted from 0.5 g of each soil sample using Ultra Clean Soil DNA Isolation Kit. The ITS region of fungal DNA was amplified using fungal specific primers using standard PCR techniques. PCR products were separated using denaturing gradient gel electrophoresis (DGGE). Target bands were to be excised, reamplified using PCR, and cloned. The clones were to be sequenced and identified using GenBank sequence data. Total genomic DNA was extracted from the soil samples and amplified with fungal specific primers to determine the fungi present in the soil. DNA was extracted from mycorrhizal root tips to determine both the plant and fungal components of the mycorrhizal association.

### **Epigeous fungi**

Any fungus fruiting bodies encountered as result of the field sampling were documented. These fungi were photographed, their GPS location recorded, and the specimen collected. Macromorphological characters of these fungi, including size, shape, color, substrate, odor, and taste will be recorded. The specimens will be identified by Amy Honan, dried, and were to be deposited in the NOCA herbarium with their notes.

### **RESULTS**

Amplification of DNA extracts from soil and mycorrhizas were unsuccessful so no data on fungi present in soil or mycorrhizal fungi were obtained. New techniques with additional novel primers were utilized in an attempt to recover DNA sequences of the fungal community

Several fruiting bodies were photographed. See below.



*Chalciporus attenuatus*





*Coltricia perennis*



*Hydnellum caeruleum*



*Macowantes subalpinus*



*Pholiota populnea*

## LITERATURE CITED

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