

# Fungal diversity and deterioration in mummified woods from the ad Astra Ice Cap region in the Canadian High Arctic

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**Abstract** Non-permineralized or mummified ancient wood found within proglacial soil near the ad Astra Ice Cap (81°N, 76°W), Ellesmere Island, Canada was investigated to ascertain the identification of the trees, current morphological and chemical characteristics of the woods and the fungi within them. These woods, identified as *Betula*, *Larix*, *Picea* and *Pinus*, were found with varying states of physical and chemical degradation. Modern microbial decomposition caused by soft rot fungi was evident and rDNA sequencing of fungi obtained from the samples revealed several species including *Cadophora* sp., *Exophiala* sp., *Phialocephala* sp., as well as others. Analytical <sup>13</sup>C-labeled tetramethylammonium hydroxide thermochemolysis showed the lignin from the ancient wood was in a high degree of preservation with minor side chain alteration and little to no demethylation or ring hydroxylation. The exposure of these ancient woods to the young soils, where woody debris is not usually prevalent, provides carbon and nutrients into the polar environment that are captured and utilized by unique decay fungi at this Arctic site.

**Keywords** Non-permineralized wood · Ellesmere Island · Fungi · Wood decay · Biodeterioration · Lignin chemistry

## Introduction

The Canadian High Arctic contains several deposits of non-permineralized, ancient woody materials in various states of preservation (Francis 1988; Basinger et al. 1988; Wheeler and Arnette 1994). Perhaps the most well studied of these deposits is located on Axel Heiberg Island near the Geodetic Hills. These woods have been primarily dated to the Eocene period (34–55 million years ago) based on geomorphology and vertebrate fossils found in the same strata layers. However, other sites such as the region near the ad Astra Ice Cap, Ellesmere Island, Nunavut Province, Canada (Fig. 1) also have mummified woods that have not been adequately studied. Unlike the woods of Axel Heiberg that have been dated using features in the surrounding sedimentary strata layers, the woods near the ad Astra Ice Cap have been displaced from their original location to proglacial soils (Fig. 2) and their age cannot be determined with accuracy making dating difficult. However, they are likely to be of a similar age ranging from the Paleocene–Eocene transition period of ~55 million years ago when dramatic global warming occurred (Zachos et al. 2001) and the Eocene–Oligocene boundary ~33 million years ago when a pronounced cooling initiated (Zanazzi et al. 2007), as this period of time provided temperatures adequate to support flora of this type in the Arctic. This age is further supported by the proximity to the dated material on Axel Heiberg and species composition similarities between the two sites (Jahren 2007). The current environment, based on data collected by the Meteorological Service of Canada from 1971 to 2000 at the nearest weather station to the collection site (Eureka, Nunavut, Canada, latitude 79°58.800'N; longitude 85°55.800'W), has an average annual temperature of –19.7°C, with the highest average monthly temperature of 5.7°C occurring in July and the lowest of –38.4°C in

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**Fig. 1** Map of the Canadian High Arctic Region indicating mummified wood collection location near the ad Astra Ice Cap on Ellesmere Island



**Fig. 2** Valley at the base of the retreating ad Astra Ice Cap on Ellesmere Island, Nunavut, Canada where mummified wood was found

February. The average annual precipitation over the 30-year period was 75.5 mm, with the greatest amount falling in August (14.9 mm) and the least in February (2.6 mm). Investigations of these woods provide an excellent opportunity to gain a better understanding of the past landscape of this region, as well as an opportunity to study the microorganisms that utilize this labile food source, relative to glacial soils with sparse organic matter, in this harsh environment.

Much of the mummified wood that has been investigated in the Canadian High Arctic has been from the class gymnospermea, belonging primarily to *Metasequoia* (Visscher and Jangels 2003) or *Larix* (Jagels et al. 2001) genera. Additional evidence of members from Cupressaceae, Pinaceae and Taxodiaceae in the form of cones, leaves, needles, palynomorphs, twigs and wood have been identified and a

review of these studies has been presented by Basinger (1991) and Jahren (2007). Although, similar findings of fruit, leaves, nuts and palynomorphs of dicot angiosperms have been found in these same assemblages with the gymnosperms, limited evidence of wood from the angiosperm group persist. Only one example was found in the Geodetic Hills of Axel Heiberg Island as an in situ stump and tentatively identified by Jagels et al. (2005) as belonging to either, the Lauraceae, Euphorbiaceae or Flacourtiaceae families.

Previous micromorphological and chemical investigations of mummified woods from different regions of the Canadian Arctic have shown that extensive alterations within these woods had taken place. In these studies, the residual wood had high but variable lignin content, thought to be controlled by the degree of vitrification (Kaelin et al. 2006) with the lignin from some samples being methoxyl group deficient, sidechain degraded and of a more condensed lignin state as compared to lignin in modern wood (Blanchette et al. 1991; Obst et al. 1991). Many of the woods from these investigations also had extensive carbohydrate degradation with complete loss of hemicellulose and very little crystalline cellulose remaining. Others, however, have been able to extract hemicellulose and cellulose from wood samples collected in this region (Jahren and Sternberg 2003; Jagels and Day 2004) indicating that some polysaccharides can survive in the residual cell wall layers. Ultrastructural studies of mummified wood samples removed from sedimentary layers at Axel Heiberg and other locations in the Arctic have shown no evidence of microbial degradation, paleo or modern (Blanchette et al. 1991). Although free of attack from fungi and bacteria, advanced stages of degradation were evident and deterioration appeared to be driven by nonbiological chemical hydrolysis mechanisms (Kaelin et al. 2006). The reasons for the lack of paleo biological degradation are uncertain but most likely involve a unique burial condition or conditions that limited microbial activity quickly. These conditions included rapid and deep burial (some reaching depths of 300 m) that restricted oxygen and possibly preserved an abundance of tannin-like compounds in some of the plant materials that may have helped to suppress microbial decomposition at early stages of burial (Schoenhut et al. 2004). Although microbial attack was inhibited, the wood appeared to be affected by a gradual hydrolysis and chemical attack (Obst et al. 1991). The loss of carbohydrates and conversion of lignin by abiotic processes, as well as the ultrastructural changes that were observed corresponded to the changes taking place during the formation of coalified wood (Blanchette et al. 1991).

This study was done to identify the type of ancient woods found at this high Arctic site and to ascertain their current morphological and chemical characteristics, as

well as to determine the fungal diversity within the woods after release from the glacier and exposure to the polar environment.

## Materials and methods

Mummified wood samples were initially found in the west valley below the retreating ad Astra Ice cap, Ellesmere Island, Nunavut, Canada (81°N, 76°W) by Parks Canada personnel from the Quttinirpaaq National Park (Figs. 2, 3). In cooperation with Parks Canada and under Park Permit # QQ-01-01 and Nunavut Research Institute Research License # 0100501 N-M, several sites near the ad Astra Ice Cap were visited and small sections of wood in contact with the ground were collected, placed in sterile bags and kept cool during transport to the University of Minnesota where they were investigated. Wood segments from the samples were cultured for microorganisms using three types of growth medium: 1.5% Difco malt extract agar (MEA), MEA with 2 ml of lactic acid added after autoclaving and a semi-selective media for Basidiomycetes that included 15 g of malt extract, 15 g of agar, 2 g of yeast, 0.06 g of benlate with 0.01 g of streptomycin sulfate and 2 ml of lactic acid added after autoclaving. All ingredients for each media type were added to 1 l of deionized water. Incubation was at 20–22°C since previous studies have shown fungi from Polar Regions are primarily psychrotrophs and grow well above 20°C (Robinson 2001). Plates were checked daily for fungal growth and pure cultures were transferred to individual plates. Fungi from pure cultures were identified using analysis of rDNA internal transcribed spacer sequences as previously described by Held et al. (2005).

Other segments from the same wood samples were used for micromorphological observations and chemical analyses. Samples of wood were prepared for scanning electron



**Fig. 3** Mummified wood found near the ad Astra Ice Cap, Ellesmere Island, Nunavut, Canada. The wood was separating along the annual rings and the surface in contact with the ground was soft and decayed

microscopy (SEM) using previously described techniques (Blanchette and Simpson 1992) and images taken using a Hitachi S3500 N SEM. Hand sections of samples were also made and viewed under a Nikon Eclipse E600 with images captured using ACT1 software connected to a Nikon DXM 1200F digital camera.

Lignin chemistry of the wood was assessed using <sup>13</sup>C-labeled tetramethyl ammonium hydroxide (TMAH) thermal chemolysis according to methods outlined in Filley (2003) and Filley et al. (2006). This procedure decomposes lignin into methylated monomers for gas chromatographic analysis and permits the assessment of the number of free hydroxyls on the lignin structure, thereby assessing microbial or chemical demethylation and the contribution of tannin and phenol components to the lignin monomers (Filley et al. 2006). Briefly, 100–300 µg of dried and powdered sample were added to a platinum cup along with 3 µl of a 25% w/v solution of <sup>13</sup>C-TMAH in water. The sample was then placed into an isothermally heated (350°C) Shimadzu Pyr4A pyrolyzer, interfaced to a GC17A gas chromatograph containing a Restek Rtx-5MS capillary column with helium flow. Quantification of fragmentation and structure was by mass spectrometry using a Shimadzu QP5050A mass spectrometer. Sixteen methylated phenols commonly released in the TMAH chemolysis/pyrolysis of natural organic matter comprising guaiacyl (G), syringyl (S) and cinnamyl (C) monomers (Table 1) were analyzed for their original aromatic methoxyl/hydroxyl content by determining

**Table 1** Methylated lignin phenols analyzed using TMAH thermochemolysis

TMAH thermochemolysis products	
G4	3,4-Dimethoxybenzaldehyde
G5	3',4'-Dimethoxyacetophenone
G6	3,4-Dimethoxybenzoic acid, methyl ester
S4	3,4,5-Trimethoxybenzaldehyde
G7	<i>cis</i> -2-(3,4-Dimethoxyphenyl)-1-methoxyethylene
G8	<i>trans</i> -1-Methoxy-2-(3,4-dimethoxyphenyl) ethylene
S5	3',4',5'-Trimethoxyacetophenone
P18	<i>trans</i> -4-(4-Methoxyphenyl) acrylic acid, methyl ester
S6	3,4,5-Trimethoxybenzoic acid, methyl ester
G14	1-(3,4-Dimethoxyphenyl)-1,2,3-trimethoxypropane ( <i>erythro</i> or <i>threo</i> )
S7	<i>cis</i> -2-(3,4,5-Trimethoxyphenyl)-1-methoxyethylene
G15	1-(3,4-Dimethoxyphenyl)-1,2,3-trimethoxypropane ( <i>erythro</i> or <i>threo</i> )
S8	<i>trans</i> -1-Methoxy-2-(3,4,5-dimethoxyphenyl) ethylene
G18	<i>trans</i> -4-(3,4-Dimethoxyphenyl) acrylic acid, methyl ester
S14	1-(3,4,5-Trimethoxyphenyl)-1,2,3-trimethoxypropane ( <i>threo</i> or <i>erythro</i> )
S15	1-(3,4,5-Trimethoxyphenyl)-1,2,3-trimethoxypropane ( <i>erythro</i> or <i>threo</i> )

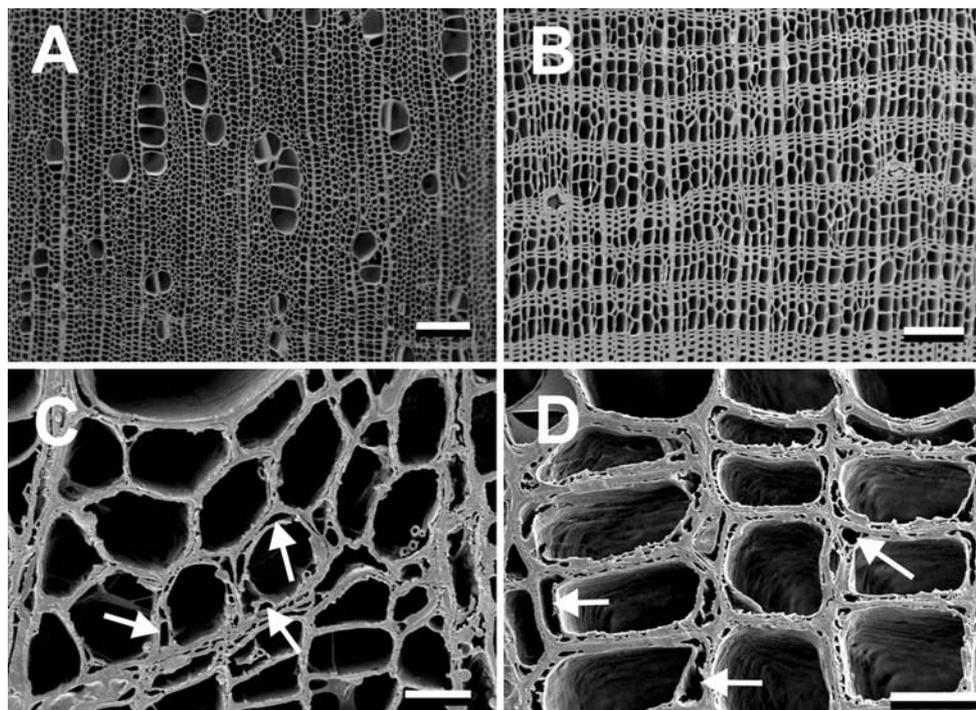
the number of  $^{13}\text{C}$ -labeled methyl groups added during the procedure using mass spectral methods modified from Filley et al. (2006). Commercially available standards and unlabeled TMAH thermochemolysis of the samples investigated herein were used to obtain the appropriate baseline ion fragment ratios to permit accurate calculation of the  $^{13}\text{C}$ -labeled methyl groups added. Carbon elemental analysis was performed using a Carlo Erba 1108 C/H/N analyzer. In addition to the Arctic wood samples, modern samples of birch, pine and spruce were assessed and used for comparison.

## Results

Wood examined on the ground or partially buried in soil at the ad Astra Ice Cap had obvious weathering and a bleached appearance. Some wood surfaces had defibrated zones and a delamination that was evident at the early-wood/latewood transition within the annual rings (Fig. 3). In addition to surface weathering and delamination at the edges of exposed wood pieces, some wood in contact with the ground appeared soft and decayed. Light microscope and SEM observations of anatomical characteristics

allowed identification and readily displayed the exceptional preservation of these woods (Fig. 4a, b). The woods were identified as *Pinus* (subgenus *Strobus*) representing nearly 39% of the collection, *Picea* at just over 33%, *Betula* with nearly 14% and *Larix* consisting of 11% (Table 2). Several micromorphological characteristics were utilized to identify the wood samples to the generic level (Hoadley 1995; Schweingruber 1990). The gymnosperm samples were separated on the bases of the regular presence (*Pinus*) or absence (*Picea* and *Larix*) of resin canals, pitting in the rays and the type of walls and pitting in the ray tracheids. Ray or cross-field pitting separated the *Pinus* samples having window pits, from the *Larix* and *Picea* samples which had piceoid pitting. Further separation was based on features in the ray tracheids which included smooth walls in the *Pinus* subgenus *Strobus*, while *Picea* type I bordered pitting was present in the *Picea* samples and *Larix*-type in the *Larix* samples. The *Betula* samples were identified based on their diffuse-porous vessel arrangement and grouping, very small, alternate intervessel and ray-vessel pitting, as well as the presence of scalariform perforation plates.

To evaluate the modern biological degradation taking place in the various collections of wood, sections were examined with light and scanning electron microscopy.



**Fig. 4** Scanning electron micrographs of mummified wood collected near the ad Astra Ice Cap, Ellesmere Island, Nunavut, Canada. **a** Section of birch (*Betula* sp.) wood from the inner part of a sample showing an excellent state of preservation with all cell wall layers intact and free of degradation (Bar 100  $\mu\text{m}$ ), **b** section of pine (*Pinus* subgenus *Strobes.*) (Bar 150  $\mu\text{m}$ ) wood from the inner region of a sample showing cell walls with no defect or alterations. **c** Soft rot attack in birch

(*Betula* sp.) from the outer region of a wood sample. The degradation patterns of soft rot consist of cavities formed in the  $S_2$  layer of the cell wall (arrows). Some cell walls are so extensively degraded that the entire secondary wall has been removed by the fungal attack (Bar 10  $\mu\text{m}$ ), and **d** soft rot attack from the surface of a pine (*Pinus* subgenus *Strobes.*) sample. Cavities in the  $S_2$  layer of the cell wall (arrows) can be seen and fungal hyphae is present within the wood cells (Bar 25  $\mu\text{m}$ )

**Table 2** Non-mineralized, Eocene aged wood identification from the ad Astra Ice Cap region, Ellesmere Island, Nunavut Province, Canada

Sample identification	Number of samples
<i>Betula</i> sp.	6
<i>Larix</i> sp.	4
<i>Picea</i> sp.	12
<i>Pinus</i> subgenus <i>Strobus</i>	14

Many wood sections had cells that appeared sound, primarily from the interior portion of the sample, and no evidence of biological attack was observed. However, some samples had fungal hyphae present and displayed distinct wood decay characteristics of attack by soft rot fungi. Cell walls contained chains of cavities typical of type 1 soft rot activity. These cavities formed in the S<sub>2</sub> layer of the secondary wall in cells of the angiosperm and gymnosperm woods. Scanning electron micrographs of transverse sections of the decayed regions in both wood types displayed extensive cavities (Fig. 4c, d) characteristic of recent decay and similar to soft rot decay described by Blanchette et al. (2004) and Eriksson et al. (1990) present in modern woods. No other forms of fungal or bacterial degradation were found.

Culturing of wood segments on the various growth media yielded several fungal cultures, however, no growth was obtained on the semi-selective media used to preferentially isolate Basidiomycetes but a Basidiomycete was isolated from malt extract agar. Fungi that were isolated in pure culture were identified by sequencing the ITS region of the rDNA. Blast search results of these sequences with GenBank accessions with a greater than 97% similarity revealed several fungi based on best Blast matches including

*Acephala* sp., *Exophiala* sp., *Phialocephala* sp., Fungal sp. AB10 (*Cadophora* sp.), Fungal sp. AB29—isolated from Antarctic soil, cf. *Pyrenopeziza revincta* and Uncultured fungus clone N3—DNA from Alaskan soil (Table 3). A Basidiomycete with only an 88% match to an uncultured fungus was also isolated.

All wood samples showed excellent preservation of lignin with only minor modifications apparent with respect to the modern analogs analyzed for comparison (Table 4). Our results were consistent with previous chemical studies on mummified, non vitrified, wood from this region using TMAH thermochemolysis in that proxies for lignin side chain oxidation and presence of intact lignin monomers showed the wood to be remarkably “fresh” (Kaelin et al. 2006). For example, the G6/G4 ratios, which are frequently used as a proxy for the degree of side chain oxidation and increases with increasing microbial decay, are only mildly higher than the fresh counterparts with the exception of sample FW 2. The modern samples range from 0.31 to 0.61 and the mummified woods range from 0.45 to 0.67, while FW 2 had a value of 1.02 indicating a greater degree of biological degradation than the other samples. Additionally, the S6/S4, a proxy for syringyl based lignin decay, values for the angiosperm woods shows only minor elevation when compared to the control. An additional proxy for the lignin side chain alteration,  $\Gamma_{(S,G)}$ , which is defined as [G6/(G14 + G15)], showed that there is loss of extractable monomers with the intact glycerol side chain as this value for both the guaiacyl and syringyl analogs is substantially higher in the mummified than fresh woods (Table 3). It is thought that the increase in this ratio can be promoted by chemical condensation processes or loss of side chain hydroxyl groups.

**Table 3** Fungi cultured from mummified wood collected near the ad Astra Ice Cap, Ellesmere Island, Nunavut, Canada and identified based on BLASTn search of the ITS region of rDNA

Sample	Best BLAST match (accession #)	Match (%)	Overlap <sup>a</sup>	Accession #
FW 1	<i>Exophiala</i> sp. (DQ317336)	100	468/468	FJ457766
FW 2	<i>Phialocephala</i> sp. (EF485233.1)	98	428/433	FJ457767
FW 3	<i>Acephala</i> sp. (EU434829)	99	436/437	FJ457768
FW 4	Uncultured fungus (AM999656) (unknown Basidiomycete)	88	466/529	FJ457769
FW 5	<i>Acephala</i> sp. (EU434829)	99	403/404	FJ457770
FW 13	Fungal sp. AB10 (FJ235943) ( <i>Cadophora</i> sp.) <sup>b</sup>	99	439/440	FJ457771
FW 14	Fungal sp. AB29 (FJ235962) (isolated from Antarctic soil) <sup>c</sup>	99	421/423	FJ457772
FW 16	Uncultured fungus clone N3 (EF434036) (DNA from Alaskan soil) <sup>c</sup>	97	494/509	FJ457773
FW 17	cf. <i>Pyrenopeziza revincta</i> (AJ430226)	99	456/458	FJ457774
FW 18	Uncultured fungus clone N3 (EF434036) (DNA from Alaskan soil) <sup>c</sup>	97	496/510	FJ457775

<sup>a</sup> Overlap of ITS alignment of best BLAST match in base pairs

<sup>b</sup> Based on authors unpublished phylogenetic neighbor joining analysis

<sup>c</sup> Provenance of fungal isolate or DNA

**Table 4** Tetramethylammonium hydroxide lignin concentration proxies from mummified wood collected near the ad Astra Ice Cap, Ellesmere Island, Nunavut, Canada

Sample and wood identification	Guaiacyl Ac/Al (G6/G4)	Syringyl Ac/Al (S6/S4)	Gamma-G	Gamma-S	Demethylation <sup>a</sup> (monomers of full lignin side chain)	Demethylation <sup>b</sup> (low molecular weight monomer)
FW 1 ( <i>Picea</i> )	0.67		1.05		0.96	0.92
FW 2 ( <i>Larix</i> )	1.02		0.34		0.97	0.93
FW 3 ( <i>Pinus</i> <sup>c</sup> )	0.52		0.60		0.99	0.92
FW 4 ( <i>Pinus</i> <sup>c</sup> )	0.45		0.65		0.99	0.94
FW 5 ( <i>Pinus</i> <sup>c</sup> )	0.47		0.54		0.99	0.93
FW 13 ( <i>Picea</i> )	0.57		0.57		0.98	0.92
FW 14 ( <i>Pinus</i> <sup>c</sup> )	0.54		0.50		0.99	0.94
FW 15 ( <i>Betula</i> )	0.57	0.74	0.46	1.91	0.95	0.93
FW 16 ( <i>Pinus</i> <sup>c</sup> )	0.67		0.94		0.98	0.95
FW 17 ( <i>Pinus</i> <sup>c</sup> )	0.60		0.65		0.99	0.94
FW 18 ( <i>Pinus</i> <sup>c</sup> )	0.49		0.53		1.00	0.94
AF 1 ( <i>Betula</i> )	0.43	0.65	0.23	0.66	0.97	0.92
AF 2 ( <i>Picea</i> )	0.60		0.70		1.00	0.94
<i>Betula</i> <sup>b</sup>	0.61	0.56	0.11	0.18	0.98	0.88
<i>Picea</i> <sup>d</sup>	0.31		0.13		0.99	0.91
<i>Pinus</i> <sup>d</sup>	0.33		0.14		0.99	0.91

<sup>a</sup> Full lignin structure monomers (G14, G15, S14, S15)

<sup>b</sup> Lower molecular weight monomers (G4, G6, G7, G8, S4, S6, S7, S8)

<sup>c</sup> Subgenus *Strobus*

<sup>d</sup> Modern wood samples used for comparison

As both the abiotic hydrolysis reaction chemistry, the generally accepted slow process which transformed the mummified Arctic woods, and soft rot fungal decay may cause lignin demethylation, we investigated the degree of aromatic hydroxyl content on the thermochemolysis products. The data (Table 4) indicate that biotic or abiotic demethylation in the remaining lignin in the mummified woods was limited. The compounds indicative of the full lignin structure (G14, G15, S14, S15) have methoxyl contents indistinguishable from the modern analogs (Table 4). The lower molecular weight monomers (G4, G6, G7, G8, S4, S6, S7, S8) which are a combination of analytical breakdown products and environmental decay products show greater demethylation (or hydroxyl content) in the modern woods than the mummified counterparts. No relationship was evident between demethylation extent and the proxies for side chain oxidation.

## Discussion

In contrast to past research completed in the Arctic on mummified woods that found *Metasequoia* to be the dominant genera (Jahren 2007), our study revealed an absence of this type of wood at the site based on morphological characteristics of the samples collected. This, however, may simply be related to the limited number of samples collected (36) or perhaps gives some suggestion of the topography of the site when the burial event occurred. This assemblage of species including *Betula*, *Larix*, *Picea* and *Pinus* and the lack of *Metasequoia*, which is considered more of a swamp

or floodplain species, indicate that the collection site was most likely a foothill of montane region (Richter and LePage 2005; Williams et al. 2003). As the original context of these samples has been disturbed by both glaciations and water movement it is also possible that the wood has been washed down from these higher elevation sites. The presence of *Betula* wood is very unique in this region as angiosperm wood appears to be exceedingly rare with only one published example and its identity was not determined but was tentatively suggested to belong to one of three families (Jagels et al. 2005).

Although Wheeler and Arnette (1994) noticed fungal hyphae in mummified *Picea* sp. wood collected in Alaska, this article is the first report of modern biological degradation and identification of the decay fungi associated with ancient mummified woods after their release into the Arctic environment. Many of the fungi isolated from the mummified woods that exhibited soft rot decay when examined microscopically have previously been shown to cause this same type of degradation in laboratory studies. *Phialophora finlandia*, recently renamed *Cadophora finlandia* based on a molecular taxonomic study (Harrington and McNew 2003) and *Phialocephala fortinii* have shown the ability to degrade various polysaccharides including cellulose, laminarin, starch and xylan (Caldwell et al. 2000). Also, Blanchette et al. (2004) and Held et al. (2006) conducted laboratory decay studies on *Betula* wood using *Cadophora* species isolated from Antarctica which revealed extensive type 1 soft rot after 16 weeks. In other laboratory studies, Worrall et al. (1997) found that isolates of *Phialocephala* sp. and *Phialophora* (*Cadophora*) sp. were capable of

causing seven to thirty times greater weight loss in birch (*Betula*) wood as compared to pine (*Pinus*) after 12 weeks. Since birch is very susceptible to soft rot attack it is not surprising that this mummified wood was affected more severely during the relatively short time of exposure. Fungi that cause soft rot appear to be the predominant decay fungi in Polar Regions. In other studies done in the Antarctic, as well as the Arctic, soft rot were the only type of decay fungi observed (Arenz et al. 2006; Blanchette et al. 2004; Blanchette et al. 2008). These fungi appear endemic to the area and well established in soils. They will colonize carbon and nutrient resources introduced into the environment and the ancient mummified woods, with their chemical signatures resembling modern woods, are readily attacked and degraded by these fungi.

There are few direct chemical studies on the nature of soft rot decay on lignin chemistry. Studies of both laboratory inoculations (Shary et al. 2007) and field sampled woods (Nelson et al. 1995; del Rio et al. 1998) indicate that some soft rot fungi are capable of lignin side chain oxidation to produce low molecular weight aromatic acids. Their chemistry is thought to most resemble the chemical action of white rot decay (Shary et al. 2007) and there is no direct evidence for extensive demethylation chemistry such as would be evident after brown rot decay.

Comparing the state of lignin chemistry of the mummified Arctic woods with the modern analogs demonstrates that the wood preservation mechanisms at this Arctic site did not result in any observable demethylation of the remaining lignin. In fact, the mummified woods exhibited slightly higher methoxyl contents for the low molecular weight lignin products than the modern analogs. The source of the higher phenol containing monomers in modern lignin can be attributed to non-core lignin associated with carbohydrates that are lost upon decay or in this case possibly hydrolysis (Fillee et al. 2006). The lack of measurable demethylation in the mummified woods also suggests that there are no hydrolysable tannin decomposition products (Yang et al. 2005) and that the hydrolysis chemistry thought to be responsible for sugar decomposition, as well as changes to lignin chemistry (Kaelin et al. 2006) was incapable of methoxyl carbon removal. Alternatively, demethylated lignin fragments may have further reacted to produce material not released by this chemolytic procedure.

Both measures of side chain oxidation (Ac/Al and Gamma for both G and S monomers) in the mummified woods demonstrate a wide range of values, consistent with the findings of Kaelin et al. (2006) but show only mild oxidation as compared to the modern samples. Although birch samples FW 15 and AF 1 had only slightly higher levels of the syringyl lignin compared to the control, the dominant expression of soft rot was exhibited by the gamma-S values which were 3.5–10 times greater than the control sample

(Table 3). The mummified gymnosperm samples with soft rot displayed a similar expression of these chemical proxies with most having significantly higher gamma-G values compared to the controls with slightly higher Guaiacyl levels. As the soft rot decay in the samples were observed to be primarily a surface phenomenon, it is possible that the chemical signature of this relatively limited decay was diluted with nondegraded interior lignin when the wood samples were ground.

This paper addresses the first documented occurrence of modern colonization and utilization of this ancient substrate by microorganisms. The effect of fungi on mummified woods exposed to the environment at other locations has not been investigated but results from this study suggest that degradation, although slow under Arctic environmental conditions may be occurring more widely than previously suspected. With the addition of this ‘fresh’, relatively easily metabolizable plant material, rates of stabilized soil organic matter should dramatically increase (Fontaine et al. 2007), and this Eocene wood may contribute to the creation of microbial and nutrient hot spots. The role of these fungi in the Arctic has received little attention and most isolates that were found with poor sequence similarity to Genbank accessions or isolates that match unclassified and unknown species may be new taxa and warrant additional investigation.

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## References

- Arenz BE, Held BW, Jurgens JA, Farrell RL, Blanchette RA (2006) Fungal diversity in soils and historic wood from the Ross Sea Region of Antarctica. *Soil Biol Biochem* 38:3057–3064. doi:10.1016/j.soilbio.2006.01.016
- Basinger JF (1991) The fossil forests of the Buchanan Lake Formation (Early Tertiary), Axel Heiberg Island, Canadian High Arctic Archipelago: preliminary floristics and paleoclimate. In: Christie RL, McMillan NJ (eds) Tertiary fossil forests of the Geodetic Hills, Axel Heiberg Island, Arctic Archipelago. Geological Survey of Canada Bulletin 403, pp 39–65
- Basinger JF, McIver EE, LePage BA (1988) The fossil forest of Axel Heiberg Island. *Musk Ox J* 36:50–55
- Blanchette RA, Simpson E (1992) Soft rot decay and wood pseudomorphs in an ancient coffin (700 BC) from tumulus MM at Gordion, Turkey. *Int Assoc Wood Anat J* 13:201–213
- Blanchette RA, Cease KR, Abad AR, Burnes TA, Obst JR (1991) Ultrastructural characterization of wood from a Tertiary fossil forest in the Canadian Arctic. *Can J Bot* 69:560–568. doi:10.1139/b91-076
- Blanchette RA, Held BW, Jurgens JA, McNew DL, Harrington TC, Duncan SM, Farrell RL (2004) Wood-destroying soft rot fungi in the historic expedition huts of Antarctica. *Appl Environ Microbiol* 70:1328–1335. doi:10.1128/AEM.70.3.1328-1335.2004

- Blanchette RA, Held BW, Jurgens JA (2008) Northumberland House, Fort Conger and the Peary Huts in the Canadian High Arctic: current condition and assessment of wood deterioration taking place. In: Barr S, Chaplin P (eds) Historical Polar Bases—preservation and management. ICOMOS monuments and sites No. XVII. International Polar Heritage Committee, Oslo, p 96
- Caldwell BA, Jumpponen A, Trappe JM (2000) Utilization of major detrital substrates by dark-septate root endophytes. *Mycologia* 92:230–232. doi:10.2307/3761555
- del Rio JC, McKinney DE, Knicker H, Nanny MS, Hatcher PG (1998) Structural characterization of bio- and geo-macromolecules by off-line thermochemolysis with tetramethylammonium hydroxide. *J Chromatogr A* 823:433–448. doi:10.1016/S0021-9673(98)00268-4
- Eriksson KE, Blanchette RA, Ander P (1990) Microbial and enzymatic degradation of wood and wood components. Springer, Berlin, p 407
- Filley TR (2003) Assessment of fungal wood decay by lignin analysis using tetramethylammonium hydroxide (TMAH) and <sup>13</sup>C-labelled TMAH thermochemolysis. ACS Series 845. In: Goodell B, Nicholas DD, Schultz TP (eds) Wood deterioration and preservation: advances in our changing world. ACS Symposium Series 845. Oxford University Press, Oxford, pp 119–139
- Filley TR, Nierop KGJ, Wang Y (2006) The contribution of polyhydroxyl aromatic compounds to tetramethylammonium hydroxide lignin-based proxies. *Org Geochem* 37:711–727. doi:10.1016/j.orggeochem.2006.01.005
- Fontaine S, Barot S, Barre P, Bdioui N, Mary B, Rumpel C (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* 450:277–281. doi:10.1038/nature06275
- Francis JE (1988) A 50-million-year-old fossil forest from Strathcona Fiord, Ellesmere Island, Arctic Canada: evidence for a warm polar climate. *Arctic* 41:314–318
- Harrington TC, McNew DL (2003) Phylogenetic analysis places *Phialophora*-like anamorph genus *Cadophora* in the Helotiales. *Mycotaxon* 87:141–151
- Held BW, Jurgens JA, Arenz BE, Duncan SM, Farrell RL, Blanchette RA (2005) Environmental factors influencing microbial growth inside the historic huts of Ross Island, Antarctica. *Int Biodeterior Biodegradation* 55:45–53. doi:10.1016/j.ibiod.2004.06.011
- Held BW, Jurgens JA, Duncan SM, Farrell RL, Blanchette RA (2006) Assessment of fungal diversity and deterioration in a wooden structure at New Harbor, Antarctica. *Polar Biol* 29:526–531. doi:10.1007/s00300-005-0084-3
- Hoadley BR (1995) Identifying wood: accurate results with simple tools. The Taunton Press, Newtown, p 223
- Jagels R, Day ME (2004) The adaptive physiology of *Metasequoia* to Eocene high-latitude environments. In: Hemsley AR, Poole I (eds) The evolution of plant physiology: from whole plants to ecosystems. Elsevier, London, pp 401–425
- Jagels R, LePage BA, Jiang M (2001) Definitive identification of *Larix* (Pinaceae) wood based on anatomy from the Middle Eocene, Axel Heiberg Island, Canadian High Arctic. *Int Assoc Wood Anatomists J* 22:73–83
- Jagels R, Visscher GE, Wheeler EA (2005) An Eocene high Arctic Angiosperm wood. *Int Assoc Wood Anat J* 26:387–392
- Jahren AH (2007) The Arctic forest of the Middle Eocene. *Annu Rev Earth Planet Sci* 35:509–540. doi:10.1146/annurev.earth.35.031306.140125
- Jahren AH, Sternberg LSL (2003) Humidity estimates for the middle-Eocene Arctic rainforest. *Geology* 31(5):463–466. doi:10.1130/0091-7613(2003)031<0463:HEFTME>2.0.CO;2
- Kaelin PE, Huggett WW, Anderson KB (2006) Comparison of vitrified and unvitrified Eocene woody tissues by TMAH thermochemolysis—implications for the early stages of the formation of vitrinite. *Geochem Trans* 7(9). doi:10.1186/1467-4866-7-9
- Nelson BC, Goni MA, Hedges JJ, Blanchette RA (1995) Soft-rot fungal degradation of lignin in 2700 year old archaeological woods. *Holzforschung* 49:1–10
- Obst JR, McMillan NJ, Blanchette RA, Christensen DJ, Faix O, Han JS, Kuster TA, Landucci LL, Newman RH, Pettersen RC, Schwabdt VH, Wesolowski WF (1991) Characterization of Canadian Arctic fossil woods. In: RL Christie, McMillan NJ (eds) Tertiary fossil forests of the Geodetic Hills, Axel Heiberg Island, Arctic Archipelago. Geological Survey of Canada Bulletin 403, pp 123–146
- Richter SL, LePage BA (2005) A high-resolution palynological analysis, Axel Heiberg Island, Canadian High Arctic. In: LePage BA, Williams CJ, Yang H (eds) The geobiology and ecology of *Metasequoia*. Springer, Berlin, pp 137–156
- Robinson CH (2001) Cold adaptation in Arctic and Antarctic fungi. *New Phytol* 151:341–353. doi:10.1046/j.1469-8137.2001.00177.x
- Schoenhut K, Vann DR, LePage BA (2004) Cytological and ultrastructural preservation in Eocene *Metasequoia* leaves from the Canadian High Arctic. *Am J Bot* 91:816–824. doi:10.3732/ajb.91.6.816
- Schweingruber FH (1990) Anatomy of European woods. Haupt, Bern, p 800
- Shary S, Ralph SA, Hammel KE (2007) New insights into the ligninolytic capability of a wood decay Ascomycete. *Appl Environ Microbiol* 73:6691–6694. doi:10.1128/AEM.01361-07
- Visscher GE, Jangels R (2003) Separation of *Metasequoia* and *Glyptostrobus* (Cupressaceae) based on wood anatomy. *Int Assoc Wood Anat J* 24:439–450
- Wheeler EA, Arnette CG (1994) Identification of Neogene woods from Alaska-Yukon. *Quat Int* 22/23:91–102. doi:10.1016/1040-6182(94)90008-6
- Williams CJ, Johnson AH, LePage BA, Van DR, Sweda T (2003) Reconstruction of Tertiary *Metasequoia* forests. II. Structure, biomass and productivity of Eocene floodplain forests in the Canadian Arctic. *Paleobiology* 29:271–292. doi:10.1666/0094-8373(2003)029<0271:ROTMFI>2.0.CO;2
- Worrall JJ, Anagnost SE, Zabel RA (1997) Comparison of wood decay among diverse lignicolous fungi. *Mycologia* 89:199–219. doi:10.2307/3761073
- Yang H, Huang Y, Leng Q, LePage BA, Williams CJ (2005) Biomolecular preservation of Tertiary *Metasequoia* fossil Lagerstätten revealed by comparative pyrolysis analysis. *Rev Palaeobot Palynol* 134:237–256. doi:10.1016/j.revpalbo.2004.12.008
- Zachos J, Pagani M, Sloan L, Thomas E, Billups K (2001) Treads, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292:686–693. doi:10.1126/science.1059412
- Zanazzi A, Kohn MJ, MacFadden BJ, Terry DO (2007) Large temperature drop across the Eocene–Oligocene transition in central North. *Am Nat* 445:639–642