Arctic driftwood reveals unexpectedly rich fungal diversity

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ABSTRACT

Arctic driftwood can provide unique insight into the diversity of colonizing and decaying fungi at the interface of extremely cold terrestrial and marine environments. Entering the Arctic Ocean via large boreal river systems and being transported by currents and sea ice, driftwood is finally deposited along shallow coastlines. Here, we sequence 177 fungal cultures in driftwood from Iceland, Greenland and the Siberian Lena Delta. Although some fungi may survive during ice drift, most species are not shared among the different sampling sites. Many indigenous Arctic fungi are generalists in their ability to colonize and decompose organic substrata, with massive effects on carbon cycling. Cadophora species are the most frequent Ascomycota, and soft rot is the most prevalent form of decay. Few Basidiomycota were found, with many of them having poor sequence matches to known species. Future research is warranted with a focus on the biology, ecology and taxonomy of Arctic driftwood inhabiting fungi.

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1. Introduction

Driftwood deposits along Arctic coasts where trees do not grow have been utilized in the past by indigenous people for cultural purposes (Alix and Brewer, 2004; Alix, 2005), and their origin has been a topic of interest since early Arctic explorers reported their occurrence (Graah, 1828; Agardh, 1869; Hellmann et al., 2013) Currently, the massive amount of Arctic driftwood is considered an extremely valuable resource for dendrochronological studies to better understand paleo-environmental changes over the past centuries to millennia (Eggertsson, 1994; Hellmann et al., 2015), and ideally even over the entire Holocene (Dyke et al., 1997; Funder et al., 2011). Arctic driftwood has been documented from archaeological sites in Greenland where it has survived with excellent preservation underground in permafrost (Matthiesen et al., 2014). Thawing of permafrost, however, in some Arctic areas due to climate change is accompanied by microbial degradation of this ancient material and concerns are mounting that increasing temperatures and thawing will accelerate the decay of these important archaeological remains (Matthiesen et al., 2014). Recent investigations utilizing Arctic driftwood for tree-ring analyses reported microbial decay in many of the samples studied (Hellmann et al., 2013). The decomposition complicates anatomical assessment for studying the origin of the material and interferes with any kind of dendrochronological and wood anatomical assessments. Although polar regions have extreme environments that may limit the rate of microbial decomposition, studies of relict driftwood and other woods brought into the Arctic or Antarctic by early explorers have shown that fungi in these cold ecosystems can cause considerable wood degradation over time (Blanchette et al., 2004, 2008, 2010; Matthiesen et al., 2014).
The origin of the driftwood in Greenland and Iceland has long been suggested to come from the boreal forests of Siberia arriving by circumpolar currents (Graah, 1828; Johansen and Hytteborn, 2001). Recently, Hellmann et al. (2013, 2015), using a large number of driftwood samples from East Greenland, Iceland and Svalbard, showed that species specific wood anatomical characteristics could be used to trace the origin of most driftwood to Siberia. The wood enters the Arctic Ocean through the large boreal river systems, moves out to the sea and freezes into the ice pack (Hellmann et al., 2015). The wood drifts in the ice following Arctic sea surface currents and as thawing occurs the wood is deposited along Arctic coastlines. The rafting of wood has been previously proposed as a mechanism for long distance dispersal of fungi in the Arctic (Stenlid, 2008), but detailed study of the fungi that colonize driftwood and their origin has not yet been carried out. A study of marine fungi in submerged driftwood along the northern coast of Norway was recently completed, showing diverse fungal communities to be present in waterlogged woods (R.A. Blanchette et al., 2010). Differences in fungal taxa between the eastern and western regions of the northern Norwegian coast were found, and the type of substrate as well as origin of the logs were suggested as key factors that influenced different microbial communities.

Table 1 Collection sites for Arctic driftwood used in this study.

<table>
<thead>
<tr>
<th>Country</th>
<th>Region</th>
<th>Latitude</th>
<th>Longitude</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenland</td>
<td>Scoresbysund</td>
<td>70° 30' N</td>
<td>25° 00' W</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Kulusuk</td>
<td>65° 57' N</td>
<td>37° 18' W</td>
<td>8</td>
</tr>
<tr>
<td>Iceland</td>
<td>Westfjords</td>
<td>65° 38' N</td>
<td>21° 38' W</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Grímsey Island</td>
<td>66° 55' N</td>
<td>18° 00' W</td>
<td>6</td>
</tr>
<tr>
<td>Russia</td>
<td>Lena Delta</td>
<td>72° 31' N</td>
<td>127° 03' E</td>
<td>12</td>
</tr>
</tbody>
</table>

A study of wood colonizing fungi (Cavaliere, 1968; Elburne and Bard, 2004) showed that species specific fungi occur in driftwood from Greenland and Iceland and has been presented in checklists of macrofungi which include several taxa of wood colonizing fungi (Cavaliere, 1968; Elburne and Knudsøn, 1990; Hallgrimsson and Hauerslev, 1995; Jensen, 2003; Hallgrimsson and Eyjolfsdottir, 2004; Borgen et al., 2006). It was thought, however, that many of these fungi were introduced by planted conifers, birch and other trees, as well as imported timber. Although relatively little research has been completed on the organisms responsible for microbial decomposition across the Arctic, there is currently a need to better understand microbial diversity and decomposition in Arctic ecosystems. Fungal activity in the Arctic has been suggested to be exceedingly important to the future of the biosphere (Timling and Taylor, 2012). The Arctic stores large amounts of organic carbon and considerable microbial activity occurs in Arctic soils under snow packs (Sturm et al., 2005; Timling and Taylor, 2012). As climate changes and the Arctic warms, microbial decomposition of organic carbon is expected to release greenhouse gases into the atmosphere that could influence the Earth's climate. Although microbial decay is exceedingly important in this process, the microorganisms responsible for decomposition of organic materials in the Arctic are poorly understood (Timling and Taylor, 2012). A recent study of mumified wood from forests that once grew in the high Arctic of Canada has shown this ancient wood being released onto soil surfaces after glacier retreat. Once on the soil surface, indigenous fungi were found to be important pioneer colonists capable of growing in the exposed woody material and decomposing it (Jurgens et al., 2009). The influx of driftwood into Greenland and Iceland over the past millennia has brought and continues to bring large amounts of woody biomass into the Arctic. This material provides a resource to study Arctic fungal diversity and ecology, as well as to get further insights into their possible origin and to obtain new knowledge on the microbial diversity of the region and how these fungi contribute to ecosystem functioning.

Here we explore the diversity of wood inhabiting fungi in Arctic driftwood from East Greenland and Iceland. We also compare these fungi with those found in driftwood from the Lena Delta in northeast Siberia, the world's largest delta that delivers endless amounts of wood into the Arctic Ocean (Büntgen et al., 2014). A culture-based approach was used to obtain study materials from which rDNA was used for sequencing to identify the fungi obtained. The fungal cultures provide opportunities for more in-depth studies on the taxonomy, physiology and ecology of these fungi.

2. Materials and methods

Driftwood from the supralittoral zone and just above this area along terrestrial coastlines in Scoresbysund and Kulusuk, East Greenland; Westfjords north of Holmavik, Eyjafjörður and Grímsey Island, Iceland and the Lena Delta in Siberia was sampled (Table 1, Fig. 1). Eighty logs were sampled (25, 43 and 12 from Greenland, Iceland and Siberia respectively) by cutting wood disks from logs and sampling smaller segments from below the surface of the wood or by removing segments of wood directly from the circumference of the driftwood to a depth of 5–10 cm (Fig. 2). One wood disk or partial wood disk was used per log. Wood samples were placed into separate sterile bags and kept cool until processed. Five subsamples were obtained from each of the log samples and small segments were cut from each subsample using aseptic techniques and incubated on four different culture media: 1.5% Difco malt extract agar (MEA), MEA with 2 ml of lactic acid added after autoclaving, a semi-selective media for Basidiomycota that included 15 g of malt extract, 15 g of agar, 2 g of yeast extract, 0.06 g of Benlate with 0.01 g of streptomycin sulfate, and 2 ml of lactic acid added after autoclaving, and a selective media for the isolation of ophiostomatoid fungi that cause blue stain consisting of MEA amended with 0.01 g cyclohexamide and 0.05 g chloramphenicol added after autoclaving. All ingredients for each media type were added to 1 L of deionized water. These types of media were used because of previous success obtaining diverse taxa in other investigations at terrestrial polar sites (Blanchette et al., 2004, 2010; Arenz and Blanchette, 2009; Arenz and Blanchette, 2011). Incubation was at 20 °C–22 °C since previous studies have shown filamentous fungi from polar environments are primarily psychrotrophs or mesotrophs and can grow above 20 °C (Robinson, 2001; Arenz and Blanchette, 2009; Blanchette et al., 2010). Cultures of fungi were transferred to new individual plates, and pure cultures were obtained.

DNA was isolated from pure cultures grown on malt agar (15 g malt extract, 15 g agar and 1 L deionized water) using a CTAB extraction procedure. Fungal hyphae from approximately ½ of a Petri dish were scraped from the surface of an actively growing culture and suspended in 500 ml of cetyltrimethylammonium bromide (CTAB) lysis buffer and glass beads and vortexed for 1 min and centrifuged briefly to aggregate hyphal material. Supernatant was transferred to a new microcentrifuge tube and placed in a hot water bath (65 °C) for 20 min. Following the hot water bath, 500 ml of chloroform/phenol/isoamyl alcohol (25:24:1) was added to each tube and mixed vigorously and centrifuged for 5 min at 15,871 rcf. The supernatant was then removed and transferred to a clean microcentrifuge tube and isopropanol (stored at −20 °C) was added (2/3 the amount of supernatant), gently mixed and incubated at room temperature for 5 min. Tubes were then centrifuged for 7 min at 21,130 rcf and isopropanol was carefully removed. The DNA pellet remaining was washed with 500 ml of 70% ETOH (stored at −20 °C) followed by centrifuging for 3 min at 21,130 rcf after which the ETOH was removed and tubes were left in a sterilized bio-safety cabinet to air dry. DNA was rehydrated with 100 ml of sterile water. The internal transcribed spacer (ITS) region of rDNA was amplified using the primer combination ITS1F/4 (Gardes and Bruns, 1993) via PCR. One ml of DNA template was used in each
PCR reaction that contained 1 µl of each primer (5 µM), 0.5 µl BSA, 12.5 µl of GoTaq® Green Master Mix and 9.5 µl of sterile water in a thermocycler with the following program: 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, followed by a final extension step of 72 °C for 5 min. Amplicons were verified by electrophoresis on a 1% agarose gel with a SYBR green 1 prestain and transilluminated with a Dark Reader DR45 (Clare Chemical Research, Denver, Colorado). Sequencing was carried out using both forward and reverse primers using an ABI 3730xl DNA sequencer (Applied Biosystems, Foster City, CA, USA). A consensus sequence was assembled using Geneious 7.0 (Kearse et al., 2012). ITS sequences were obtained and the best BLAST match to GenBank sequences was determined, if possible, accessions from taxonomic publications. For all taxa with a sequence match less than 97%, a notation, **, was made following the name of the fungus in Fig. 3 and in Table 1 of the supplemental data. Species accumulation (rarefaction) curves were made using EstimateS v9.1.0 (Colwell, 2013). 100 randomizations were used and rarefaction was extrapolated to 100 samples.

3. Results

3.1. Fungal identity

Culturing of Arctic driftwood samples on the four different media resulted in 177 pure cultures of filamentous fungi. Since several types of media were used, if the same taxon was found growing on different media from the same log only one was reported in Fig. 3 and in Supplemental Data Table 1. Of the 177 fungi obtained, 103 were different taxa. Many of these (85 taxa) were single isolations from each location. However, a few taxa were repeatedly isolated from multiple logs at all locations. Ascomycota dominated at all locations (150 isolates) and only a few Basidiomycota (16 isolates) and Zygomycota (11 isolates) were cultured (Fig. 3). The most frequently isolated taxa were Cadophora species (32 isolates) followed by Lecythophora sp. and closely related Coniochaeta sp. (15 isolates) and Penicillium sp. (13 isolates). Fifteen isolates matched sequences of previously investigated taxa at less than 97%, a notation, **, was made following the name of the fungus in Fig. 3 and in Table 1 of the supplemental data. Species accumulation (rarefaction) curves were made using EstimateS v9.1.0 (Colwell, 2013). 100 randomizations were used and rarefaction was extrapolated to 100 samples.

Fig. 1. Driftwood sampling sites across the Arctic. Wood affected by fungi was collected on Iceland (orange), East Greenland (red), and in the Siberian Lena Delta (blue).

Fig. 2. Sampling of Arctic driftwood for fungal investigations. (A) Sampling with the chainsaw to obtain discs with a full radius. (B) Wood affected by blue stain fungi showing dark staining in the sapwood. (C) Samples were labeled and packed in sterile plastic bags directly after cutting.
3.2. Fungal communities

Fungal communities differed between Greenland, Iceland and Siberia. Most isolates obtained from each country represented different taxa with 31 isolates found only in Greenland, 26 from Iceland and 27 from Siberia (Fig. 3, Supplemental data Table 1). Few isolates were found at more than one location with only 3 taxa found in Greenland, Iceland and Siberia, 7 from Greenland and...
Iceland, 3 from Iceland and Siberia and 4 from Greenland and Siberia. Species abundance curves indicate that more sampling in all locations would yield more fungal species (Fig. 4). Sampling from 80 or more logs would capture most of the species richness from the Siberia site but for Iceland and Greenland even greater numbers of samples would be needed for a complete assessment of the fungi colonizing the driftwood.

Driftwood logs sampled in Greenland, Iceland and Siberia frequently exhibited blue stain in the sapwood and a selective medium for ophiostomatoid fungi was used for culturing as well as malt extract agar and an acidified malt extract agar that would allow all types of blue stain fungi to be isolated. Cultures of Ophiostoma were obtained only from Siberian samples and no blue staining fungi were isolated from any of the samples from Greenland and Iceland.

Various stages of wood decomposition were evident in the samples collected. The most common type of decay was a soft rot (Fig. 5) which was associated with the large numbers of soft rot Ascomycota that were isolated from the driftwood samples such as the Cadophora species. Since it is very difficult to visually detect incipient to moderate stages of soft rot in wood, quantification of the amount of decay in the logs at field sites was not attempted.

4. Discussion

Samples of driftwood from the Arctic revealed a diverse assemblage of fungi with over 60 different genera and many had poor matches (below 97%) to described taxa. Others matched sequences of fungi to only broad classification categories such as class and order. Additional phylogenetic and taxonomic studies are needed to determine the identification of these isolates. Most of the fungi identified were Ascomycota with relatively few Basidiomycota found. From all sampling sites, only 12 different taxa of Basidiomycota were found and these include 4 from Greenland, 3 from Iceland and 7 from Siberia. From these 12 taxa, 6 of them did not match known sequences at above 97% indicating the Basidiomycota from Arctic Regions have been poorly represented in previous studies and many of the Arctic Basidiomycota found likely represent new species. Only 2 of the Basidiomycota found in Greenland and Iceland were also found in Siberia. Basidiomycota in polar regions have been noted in many previous investigations to be found in lower numbers than Ascomycota and when found they are usually basidiomycetous yeasts (Tosi et al., 2002; Connell et al., 2006; Malosso et al., 2006; Ludley and Robinson, 2008; Blanchette et al., 2010; Arenz and Blanchette, 2011; Arenz et al., 2014). In contrast to the situation in the Arctic, Basidiomycota are a key component of the boreal forests, ecosystems and a check list of aphyllorophor fungi from the Pinega Reserve in northeast Europe and north Russia contained 328 species from 158 genera (Ezhov and Zmitrovich, 2015). Other studies have also shown large diverse populations of Basidiomycota that colonize wood and cause decay in wood from boreal forests (Hansen and Knudsen, 1992, 1997; Dai and Penttila, 2006; Stenlid et al., 2008). In the study presented here, few Basidiomycota were found in driftwood from coastal regions of the Lena River in Siberia or from Greenland and Iceland as compared to what would be found on wood in the boreal forests where these logs originated. In a study by Hellmann et al. (2013) nearly half of the driftwood was from logging operations. Since most of this wood appears to come from the cutting of living trees, they apparently enter into the river systems in a sound state without prior colonization by wood decay fungi. Some heartwood colonizing fungi may be present in these trees before they are cut, but cultures of these types of Basidiomycota were not found in Greenland and Iceland. Environmental conditions such as high moisture as well as salts from the marine coastlines are likely to exert strong selection pressure on the microorganisms that survive and tolerate these conditions. Although the logs originated in Siberian boreal forests, the large diverse population of wood destroying Basidiomycota that are present in the boreal forest ecosystem were not found in driftwood studied in this investigation.

A large diverse group of Ascomycota was evident in driftwood

Fig. 4. Species accumulation curves of samples from Siberia (blue), Greenland (red) and Iceland (orange). Solid line represents actual number of samples and dotted lines represent extrapolation to 100 samples.
from all locations sampled and differences in taxa were found among sites with few taxa shared between sites. Even with over 100 taxa identified from the samples obtained in this study (25, 43 and 12 from Greenland, Iceland and Siberia respectively), species abundance curves indicate that many more samples would be needed to fully document species richness from the sites. The southernmost Arctic sampling site at the Lena River Delta in Siberia had more diversity present in the smaller number of samples investigated, and the species accumulation curve (Fig. 4) suggests that nearly 100 samples would be needed to fully document the fungi in driftwood at this site. Greater numbers would be needed at the Iceland and Greenland sites. The extreme conditions of the Arctic and other growth limiting conditions found at these coastal locations undoubtedly have an influence on the fungi that are able to colonize and decompose driftwood. However, despite these harsh conditions there appears to be a diverse group of fungi that are present. With so many different taxa found at each location and few of them shared among the three countries we can expect to find many more taxa with additional sampling (Fig. 4). Observations made at the collection locations indicate that site differences may be responsible for the varied taxa found at each site. In Greenland, in addition to having a much harsher climate, sampling locations were sandy, flat beaches whereas in Iceland the coasts were mostly stony and in areas above the supralittoral zone, the wood was among grasses and some logs were partially buried (Fig. 1). The collection locations at the Lena River Delta also had different conditions as compared to Iceland and Greenland with steep slopes along the banks and driftwood logs on or partially buried in sand within a freshwater delta system. These different environments appear to support different indigenous microflora, which in turn colonizes driftwood logs after they enter the ecosystem.

Driftwood logs from all locations commonly exhibited some degree of blue stain or other discoloration in the sapwood and despite efforts to isolate blue stain fungi, including a selective medium for Ophiostomatoid fungi, blue stain fungi were not isolated from Greenland and Iceland. In contrast, Ophiostoma canum, was isolated from several logs in Siberia. This fungus has been reported from Russia on conifers and bark beetles and is one of the more commonly encountered species of blue stain fungus on spruce and pine (Linnakoski et al., 2010). The lack of blue stain fungi being cultured from blue stained wood in Greenland and Iceland indicates that the fungus does not remain viable after transport in the Arctic Ocean. Likely the saturated environment, permeability characteristics of the sapwood and/or influence of salts from sea water as well as salts accumulating in the wood on coastal beaches limits survival of the blue stain fungi.

Cadophora species were the most common taxa found in Greenland and Iceland and diversity in species was greater than those found in Siberia where only 3 isolates of Cadophora fastigiata were found. Cadophora species have been previously reported to be dominant organisms in other polar regions. In Antarctica, Cadophora was first found to be the most prevalent fungus colonizing wood in the historic huts built by Scott and Shackleton in the Ross Sea Region of Antarctica (Blanchette et al., 2004, 2010). Subsequent studies showed Cadophora to be one of the most common taxa in soils in the Ross Sea Region as well as at many sites on the Antarctic Peninsula (Arenz and Blanchette, 2009, 2011; Blanchette et al., 2010). It recently was also found associated with Antarctic lakes (Goncalves et al., 2012). The prevalence of this fungus and diverse species present indicates it is well adapted to polar conditions and likely indigenous to these polar regions. Recent studies are also finding Cadophora in Arctic ecosystems including the Canadian High Arctic on mummified wood, on submerged driftwood in the Arctic Sea, and on lichens, bryophytes and historic woods that were introduced into the Arctic (Blanchette et al., 2008; Jurgens et al., 2009; Rámá et al., 2014; Zhang et al., 2015). These reports and the results presented in this paper suggest that Cadophora are common circumpolar fungi capable of colonizing many different substrata and tolerating extreme environmental conditions.

Investigations of Arctic and Antarctic plants have also found that Cadophora as well as Lecyphthora, Leptodontium, Phialocephala and other genera occur as endophytes in association with roots (Rosa et al., 2010; Zhang and Yao, 2015). Commonly referred to as dark septate endophytes (DSE), they occur in a quasi-mycorrhizal association with Arctic vascular plants (Day and Currah, 2011). Our results indicate that many of the most common fungi found in driftwood are similar taxa to DSE fungi. These fungi have much broader saprotrophic abilities than previously realized and while able to live asymptomatically in the roots of live plants, they also may be primary decomposers of wood and other organic materials. Interestingly, Jurgens et al. (2009) showed Cadophora, and Phialocephala were pioneer organisms able to colonize ancient mummified wood that was released from a receding glacier in the High Arctic of Canada. These fungi also appear to successfully colonize other substrata and sources of carbon, such as driftwood, that enter the ecosystem. The number of isolates obtained in our study of driftwood suggest that they are generalists in their ability to capture nutrient substrates and rapid colonizers of this material. Their capacity to produce soft rot and significant decomposition in driftwood samples demonstrates they employ a wide range of enzymes that function in cold climates. Genomic and proteomic studies of these unique soft rot fungi are needed to better

Fig. 5. Scanning electron micrographs of transverse sections of Arctic driftwood (A and B) with a soft rot form of wood decay. Cavities form within secondary walls of tracheids (arrows). Bar = 25 μm.
understand the decomposition processes and mechanisms they utilize for saprotrophic survival.

From previous study of a large number of Arctic driftwood samples (Hellmann et al., 2013), we can assume that the species composition and origin of most if not all of the driftwood we encountered was from a broad region of boreal forests in Siberia. Since the substratum and wood species were similar at the sites sampled, differences in the microbial community appear primarily due to the indigenous population of fungi at each site. Some fungi may persist during transport from Siberia to Greenland and Iceland, due to the indigenous population of fungi at each site. Some fungi encountered was from a broad region of boreal forests in Siberia. Many of the indigenous fungi represented appear to be generalists in their ability to colonize and decompose organic substrates and important for their role in carbon recycling. Many unique taxa occur in this extreme environment that warrant future study of their biology, ecology and proper taxonomic placement.

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Supplementary data

Supplementary data related to this article can be found at http:// dx.doi.org/10.1016/j.funeco.2016.06.001.

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