Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2021:71
Faculty of Forest Sciences

Diversity and functional potential of fungal communities in Arctic and boreal freshwaters

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DOCTORAL THESIS
Uppsala 2021

Acta Universitatis Agriculturae Sueciae
2021:71

Cover: Pathway to the sampling site at Stordalen mire in Abisko, Sweden.
(photo: Mariana Kluge)

ISSN 1652-6880
ISBN (print version) 978-91-7760-819-6
ISBN (electronic version) 978-91-7760-820-2
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Uppsala
Print: SLU Service/Repro, Uppsala 2021

Diversity and functional potential of fungal communities in Arctic and boreal freshwaters

Abstract

Fungi are important decomposers of the organic matter (OM) in terrestrial environments, but there is limited knowledge about their ecological roles in aquatic ecosystems. This thesis applies a variety of molecular techniques (e.g. metabarcoding, sequencing of genomes and metagenomics) to explore the diversity of freshwater fungi and their role in the recycling of organic matter. The aquatic fungal diversity was first assessed in boreal lakes in Scandinavia. The composition of the communities varied significantly across lakes, and was associated with organic carbon availability. Furthermore, the relationship between fungal diversity and OM was extensively explored in permafrost ecosystems. Climate change is causing the thawing and collapse of permafrost peatlands, creating water bodies named thermokarst ponds. These ponds receive a significant amount of carbon previously stored in the permafrost, and are considered hotspots for carbon cycling. To investigate the ecology of the fungi in these ponds, five regions across the Arctic were sampled, comprising a gradient of permafrost integrity: from sites not affected by thawing to degraded sites with thermokarst ponds. The quality of the dissolved organic matter (DOM) was strongly linked with the fungal community composition, and associated with a significant decline in beta-diversity towards the degraded sites. Also, the genetic functional potential of the fungal communities in the pond water and sediment was explored at one of the degraded sites. The results showed the highest potential for breaking down the OM in the sediment, associated with higher relative abundances of fungal isolates with greater potential for degradation of plant litter. On the contrary, in the water, the fungi had a high potential for growth. Correlations between genes and proxies for the water DOM quality were found, suggesting that the aquatic fungi are able to benefit from the freshly produced OM of microbial sources and old OM from terrestrial sources. These findings shed light onto the ecological roles of aquatic fungal communities in the carbon cycle of ecosystems affected by a warming climate.

Keywords: aquatic fungi, Arctic ponds, boreal lakes, thawing permafrost, carbon cycling, fungal diversity, functional potential, climate change.

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Mångfald och funktionell potential hos svampsamhällen i arktiska och boreala sötvatten

Abstract

Svampar är viktiga nedbrytare av det organiska materialet (OM) i markbundna terrestra miljöer, men det finns begränsad kunskap om deras ekologiska roller i vattenlevande akvatiska ekosystem. Denna avhandling undersöker mångfalden av svampar i sötvatten och deras roller vid omsättningen av organiskt material. Den akvatiska svampdiversiteten analyserades först i boreala sjöar i Skandinavien. Svampsamhällenas sammansättning varierade starkt mellan olika sjöar och påverkades av tillgängligt organiskt kol. Dessutom undersöktes förhållandet mellan svampdiversitet och OM i permafrostekosystem. Klimatförändringar orsakar upptining och kollaps av permafrost och bildar vattensamlingar som benämns termokarstdammar. Dessa dammar tar emot en betydande mängd kol som lagrats i permafrosten i årtusenden och de anses vara hotspots för koldioxidavgivning till atmosfären. För att undersöka svamparnas ekologi i dessa dammar togs prov från fem regioner över hela Arktis. Proverna omfattade en gradient av permafrostintegritet: från platser som inte påverkas av upptining till nedbrutna platser med termokarstdammar. Kvaliteten på det lösta organiska materialet (DOM) var starkt kopplat till sammansättningen av svampsamhället och förknippades med en betydande minskning av mångfalden av arter i platser med mer nedbruten permafrost. Dessutom undersöktes den funktionella potentialen för svampsamhällen i dammvattnet och sedimentet på en av platserna. Detta gjordes genom sekvensering av arvsmassan hos enskilda svamparter men också genom metagenomik där hela det genetiska materialet i miljön undersöktes. Resultaten visade den högsta potentialen för att bryta ner OM i sedimentet. Däremot, i vattnet från termokarsterna hade svamparna en hög tillväxtpotential och visade en genetisk profil för användning av enklare kolföreningar. Vattensvamparna drar sannolikt nytta av DOM från mikrobiell produktion och algbiomassa. Dessa fynd belyser de ekologiska rollerna för vattenlevande svampar i kolets kretslopp i ekosystem som påverkas av ett varmare klimat.

Keywords: vattensvampar, arktiska dammar, boreala sjöar, upptining av permafrost, kolcykling, svampdiversitet, funktionell potential, klimatförändringar.

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To my family

À minha família

Contents

List of publications....................9

Abbreviations....................11

1. Introduction....................13
   - 1.1 A thawing Arctic....................14
   - 1.2 Biodegradation of the dissolved organic matter....................16
   - 1.3 Objectives of the thesis....................17

2. Methods for studying fungal communities in freshwaters....................19
   - 2.1 Methods for describing fungal communities in the environment....................19
     - 2.1.1 Metabarcoding....................19
     - 2.1.2 Metagenomics....................22
     - 2.1.3 Isolation, cultivation and full genome sequencing....................22
   - 2.2 Bioinformatic pipelines applied for fungal taxonomic and functional diversity....................23
     - 2.2.1 Taxonomic assignment of molecular markers....................23
     - 2.2.2 Processing of metagenomic reads....................23
     - 2.2.3 Functional annotation of genes....................25
     - 2.2.4 Obtaining the relative abundance of genes of interest....................26
   - 2.3 Characterization of the dissolved organic matter in freshwaters 27

3. Fungal communities in lakes....................29
   - 3.1 Composition of fungal communities across lakes....................29
     - 3.1.1 Composition of fungal communities across lakes....................29

4. Impact of permafrost thaw on fungal diversity....................33
   - 4.1 DOM quality linked with the composition of fungal communities in thermokarst ponds....................33
   - 4.2 Declining beta diversity towards degraded sites....................36
   - 4.3 OTUs correlated with DOM quality....................37

5. Functional potential of aquatic fungal communities 41
5.1 Different functional potentials for water and sediment 41
5.2 Correlations between CAZymes and DOM quality 42

6. General conclusions and future perspectives 45

References 47

Popular science summary 61

Populärvetenskaplig sammanfattning 65

Acknowledgements 69

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Sanyal, A.†, Kluge, M. †, Redondo, M. A., Buck, M., Mershahed, M., Garcia, S. L., Bertilsson, S., Peura, S. A metagenomic view on fungal diversity in freshwater lakes. (manuscript).

II. Kluge, M., Wurzbacher, C., Wauthy, M., Clemmensen, K. E., Hawkes, J. A., Einarsdottir, K., Stenlid, J., Peura, S. (2021). Community composition of aquatic fungi across the thawing Arctic. *Scientific Data*, 8 (221). Doi: 10.1038/s41597-021-01005-7.

III. Kluge, M., Wauthy, M., Clemmensen, K. E., Wurzbacher, C. Hawkes, J. A., Einarsdottir, K., Rautio, M., Stenlid, J., Peura, S. (2021). Declining fungal diversity in Arctic freshwaters along a permafrost thaw gradient. *Global Change Biology*. Doi: 10.1111/gcb.15852.

IV. Kluge, M., Simone, D., Clemmensen, K. E., Wurzbacher, C., Stenlid, J., Peura, S. Fungal communities of thermokarst ponds share different potentials for carbon cycling in water and sediment. (manuscript).

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† Contributed equally

The contribution of Mariana Kluge to the papers included in this thesis was as follows:

I. Participated in the data analysis (bioinformatics and statistics) and is one the main writers of the manuscript.

II. Contributed with the experimental design, participated in most of the samplings, performed the laboratory experiments and data analyses, was one of the main writers of the paper, and was the responsible for the correspondence with the journal.

III. Participated in most of the samplings and experimental design, performed all laboratory experiments and data analyses (except for the carbon data), was the main writer of the manuscript and corresponding author.

IV. Performed the sampling and all molecular analyses, conducted all the data analyses (except quality trimming and assembly of the sequences and annotation of fungal genomes) and was the main writer of the manuscript.

Abbreviations

OTU	Operational taxonomic unit
ITS	Internal transcribed spacer
SSU rRNA	Small subunit ribosomal RNA
LSU rRNA	Large subunit ribosomal RNA
PCR	Polymerase chain reaction
PacBio	Pacific BioSciences
GHG	Greenhouse gasses
CAZymes	Carbohydrate-active enzymes
GT	Glycosyltransferases
GH	Glycoside hydrolases
PL	Polysaccharide lyases
CBM	Carbohydrate-binding modules
AA	Auxiliary activities
CE	Carbohydrate esterases
DOM	Dissolved organic matter
OM	Organic matter
DOC	Dissolved organic carbon
TOC	Total organic carbon
TN	Total nitrogen
HTS	High-throughput sequencing
HS	Humic substances

1. Introduction

It is estimated that the fungal kingdom contains between 2.2 - 3.8 million species, of which less than 10% are formally described (Hawksworth & Lücking 2017). The development of high-throughput sequencing (HTS) technologies in the past two decades has allowed us to uncover many of these unknown fungi by enabling us to cost-effectively sequence genetic material of whole communities (Logares *et al.* 2012), although the formal species descriptions are still lagging behind. Yet, there are groups of fungi that are particularly underrepresented and undersampled, and aquatic fungal communities are one of the least explored groups (Grossart *et al.* 2019). Little is known about the aquatic fungal diversity and ecological roles, and current databases still miss a vast majority of aquatic fungi.

Fungi are known for their ability to degrade a variety of organic compounds (Phillips *et al.* 2013; Frey 2019). Many fungi possess enzymes specialized in breaking down plant derived molecules such as cellulose and hemicellulose, and less labile and more complex organic molecules such as lignin and humic substances (HS) (Osono 2007; Hanson *et al.* 2008; Collado *et al.* 2018). However, most of our knowledge is based on studies of terrestrial ecosystems. When it comes to aquatic environments, knowledge becomes scarcer. The aquatic hyphomycetes, a heterogenous group of aquatic fungi first described by Ingold (1942), are known for being saprotrophs that degrade plant litter, mainly leaves in streams. Another group, the chytrids, are degraders of the dissolved organic matter (DOM), but may also live as parasites (Comeau *et al.* 2016; Longcore & Simmons 2020). Basidiomycetes are mostly found in waters as yeast forms, and have been found to dominate marine sediments (Bass *et al.* 2007; Grossart *et al.* 2019). The exception is one true gilled mushroom (order Agaricales) described in a river (Frank *et al.* 2010). Other groups of fungi such as the

Mucoromycota have also been reported in marine and freshwater environments, though our knowledge about their ecology in water habitats is limited (Nguyen *et al.* 2019; Banos *et al.* 2020). Despite the recent efforts to unravel the diversity of fungi in aquatic ecosystems, more research is needed, especially to understand their functional roles in organic matter degradation.

Studying the microbial contributions to carbon cycling and degradation of organic matter is particularly important in areas highly affected by the warming climate, such as the Arctic. The increasing temperatures in northern permafrost areas are causing a collapse of the landscape as permafrost starts to thaw (Schuur *et al.* 2015). From such collapse, known as thermokarst, the so-called thermokarst ponds originate. These ponds are small and shallow waterbodies that emerge when ice-rich permafrost soils thaw (Vonk *et al.* 2015). They receive considerable input of organic matter (OM) that had been stored in the frozen soil for millennia. This OM can be recycled by microbial communities, generating carbon dioxide (CO_2) and methane (CH_4) as end products, which turns these ponds into hotspots of GHG emissions (in ’t Zandt *et al.* 2020). Despite the relevance of such water bodies, little is known about the aquatic fungal communities in these ponds, their ecological role and their contributions to the degradation of OM that comes from the thawing permafrost.

1.1 A thawing Arctic

Northern permafrost (0-3 m depth) is estimated to store about 1035 gigatons (Gt) of carbon - double the amount of carbon present in the atmosphere (Hugelius *et al.* 2014). By 2100, 20-58 Gt of this stored carbon is expected to thaw and may be released to the atmosphere as greenhouse gases (GHGs), representing a 0.1 °C increase in the average global surface temperature (Schneider Von Deimling *et al.* 2015). Permafrost is defined as ground (soil or rock and its ice content) that remains frozen (≤ 0 °C) for at least two consecutive years (Osterkamp & Burn 2003). The permafrost topsoil that is subjected to seasonal freezing and thawing is called the active layer (Osterkamp & Burn 2003). Depending on the extension of frozen ground over a landscape, the region can be classified as a continuous permafrost zone (over 90% of underlying ground is permafrost), discontinuous (between 50-90%) or sporadic (up to 50%) (Osterkamp & Burn 2003).

Warming temperatures can lead not just to the thickening of the active layer, but can also start a process known as thermokarst, which is when ice-rich permafrost thaws cause land subsidence (Olefeldt *et al.* 2016) and form depressions that allow the emergence of ponds and lakes (Dixon 2020). The permafrost landscape can present different features such as peat plateaus, ice-wedge polygons (patterned ground with geometric shapes) and palsas (peat mounds with a frozen core) (Svensson 1986). In permafrost peatlands, thawing causes the collapse of palsas, which also can result in the formation of thaw ponds (Olvmo *et al.* 2020). The impacts of permafrost thaw rely not only on the quantity of organic matter that is released in the process, but also on its quality (Kuhry *et al.* 2020), i.e., how much of it is subjected to degradation, and on the availability of nutrients (Reyes & Lougheed 2015). Studies have been conducted to evaluate the functionality of microbial communities in permafrost. Permafrost microbial communities submitted to warming experiments were shown to be less responsive than communities from the active layer, particularly for fungi (Luláková *et al.* 2019). However, upon the thaw, with the *in situ* colonization of the previously frozen soil from the active layer or by airborne microorganisms, the overall functional potential of the permafrost communities has been suggested to expand (Monteux *et al.* 2020). This could favor the degradation of the OM by certain groups of fungi. Interestingly, it has been shown that an expansion in functional potential of microbial communities in permafrost soils are not always related to an increase in taxonomic diversity. In fact, it has been observed that while taxonomic diversity of fungi in thaw soils has decreased compared to pristine soils, the functional gene diversity has increased (Chen *et al.* 2020).

The quality of permafrost organic matter is heterogeneous and without a clear depth pattern, as it depends on the decay state of the compounds at the time it was incorporated into the frozen ground (Uhlířová *et al.* 2007; Graham *et al.* 2012; Strauss *et al.* 2015). Many studies have reported how permafrost OM can be labile (Abbott *et al.* 2014; Mueller *et al.* 2015), with low molecular weight DOM and low aromaticity (Fouché *et al.* 2020). Also, permafrost labile OM was shown to be present even in higher amounts than in the active layer (Chen *et al.* 2016). This labile OM can rapidly go through bio- and photodegradation (Drake *et al.* 2015; Panneer Selvam *et al.* 2017). However, other studies also point to the low lability of permafrost OM (Kuhry *et al.* 2020) and low biodegradation of active layer OM in bogs

(AminiTabrizi *et al.* 2020). As for OM quantity, while some studies show that the OM concentration in the active layer is lower than in the organic permafrost (Fouché *et al.* 2020), others point to a higher concentration of organic carbon in the topsoil (Mueller *et al.* 2015; Wu *et al.* 2018). These divergent results reflect the complexity of such dynamic systems.

The DOM released with permafrost thaw can suffer different fates until it reaches thermokarst ponds, as part of the DOM can be degraded during its transportation or be filtered through the soil (Vonk *et al.* 2015). Once in the water, the DOM may sink and be stored in the sediment or be recycled in the microbial loop, resulting in the emissions of GHGs, such as carbon dioxide (CO_2) and methane (CH_4) (Negandhi *et al.* 2013). Whereas the fast biodegradation of DOM originating from the permafrost and active layer have been shown in streams (Textor *et al.* 2019), some studies point to DOM being resistant to bio- and photodegradation in ponds (Shirokova *et al.* 2019; Laurion *et al.* 2021).

1.2 Biodegradation of the dissolved organic matter

Prokaryotes are the main degraders of labile/low molecular weight DOM in aquatic environments (Berggren *et al.* 2010; Mostofa *et al.* 2013). However, they are not efficient decomposers of semi-labile terrigenous DOM (Herlemann *et al.* 2014). In terrestrial ecosystems, fungi are known to play an important role in recycling of the organic matter, including recalcitrant substrates such as lignin (Osono 2007). Their role in aquatic systems, however, is still not fully elucidated. It is known that aquatic fungi are key players in the decomposition of plant debris, with most of the studies focusing on streams (Gulis *et al.* 2008; Canhoto *et al.* 2016). Moreover, aquatic fungi were shown to provide carbon substrates for the microbial loop (Fabian *et al.* 2017). For instance, it has been suggested that the Chytridiomycota may play a role as facilitators by converting particulate organic matter into DOM, which, in turn, becomes available for the bacterial communities (Roberts *et al.* 2020). Studies on freshwater fungi have revealed their ability to degrade HS, and also to transform the HS into more aromatic compounds (Claus & Filip 1998; Rojas-Jimenez *et al.* 2017; Masigol *et al.* 2019). Therefore, aquatic fungi may have an important role as recyclers of the DOM in thermokarst ponds, which are dominated by allochthonous organic carbon (Wauthy *et al.* 2018).

1.3 Objectives of the thesis

The aim of this thesis is to explore the diversity of aquatic fungal communities in freshwater ecosystems, and find which possible associations the composition of fungal communities could have with the quality and quantity of DOM. The thesis mainly focuses on permafrost areas, looking at fungal communities from thermokarst ponds and also from ponds in permafrost sites that are not affected by thawing. The aim is to investigate how the fungal diversity is impacted by thawing and what is the functional potential of the community for carbon recycling.

As a starting point to survey aquatic fungal communities, we have investigated the fungal diversity in a large dataset containing several boreal lakes in Scandinavia and a tropical reservoir, which is shown in **paper I**. In this exploratory study, we looked at the spatial variation in the composition of fungal communities across different lakes. Additionally, we analyzed the community composition in different depth layers of the lakes and evaluated whether the community composition was related to carbon, oxygen, or nitrogen concentrations.

The study of fungal communities in thermokarst ponds is introduced in **paper II**. It describes the sampling campaigns, including the sites, and the methods applied for investigating the diversity and functional potential of aquatic fungal communities from permafrost areas. This paper compiles data from five regions across the Arctic, which represent a gradient of permafrost integrity: from sites that are least affected by thawing, with unaffected landscape (Alaska and Greenland - here called pristine sites), to sites with clear collapse of the permafrost resulting in thermokarsts (Canada, Sweden and Russia – here named degraded sites). The site in Canada also presented ponds in different developmental stages: from emerging ponds to more mature ones, which enabled us to investigate the succession of the fungal communities as the ponds develop. We analyzed samples for carbon quality and quantity as well as nutrients and pH to examine which environmental factors would change in concert with the community composition. The fungal communities were surveyed with molecular analyses, by amplifying molecular markers that allowed the identification and relative quantification of fungal species.

In paper **III**, the results from the sampling campaigns presented in **paper II** are shown. Here, we hypothesized that the DOM quality and availability, as reflected by the permafrost integrity and pond developmental stage, are

tightly interlinked with the composition of aquatic fungal communities across the thaw gradient.

In **paper IV**, we investigate the functional potential related to the carbon cycling of fungal communities in thermokarst ponds situated in one of the degraded sites affected by thawing permafrost. For that, we have conducted a metagenomic analysis to investigate the genes involved in the carbon metabolism of fungi. We also analyzed the genomes of six fungi isolated from the ponds, to look into their functional potential. We looked at whether sediment and water samples shared similar functional potentials and we also hypothesized that genes involved in the carbon metabolism would correlate with the quality of the DOM across the ponds.

2. Methods for studying fungal communities in freshwaters

2.1 Methods for describing fungal communities in the environment

The advance of HTS technologies has completely changed the field of fungal ecology. The sequencing of fungal genomes at a lower cost and with high accuracy has enabled the discovery and characterization of new species, and provides deeper insights into the functional roles of fungi. The HTS technologies differ in their ability and accuracy to sequence short and long fragments of DNA. In this thesis, to study the fungal diversity and functional potential, we have used different HTS platforms which will be briefly introduced in this chapter. However, it is out of the scope of this thesis to discuss the advantages and drawbacks of each platform in detail. Nevertheless, more information about the current state of sequencing platforms used in fungal ecology is found in Nilsson et al. (2019).

2.1.1 Metabarcoding

DNA metabarcoding has been widely used for studying microbial communities in environmental samples (Günther *et al.* 2018; Bush *et al.* 2019). This method comprises the following steps: a) the extraction of genetic material from a sample, b) the amplification of molecular markers through polymerase chain reactions (PCR) and c) the use of HTS for the simultaneous identification of multiple taxa within a sample (Taberlet *et al.* 2012; Ji *et al.* 2013). For fungi, the most used molecular markers are the internal transcribed spacer (ITS) region and small and large subunits of the ribosomal RNA (SSU/LSU rRNA) (Nilsson *et al.* 2018) (Figure 1).

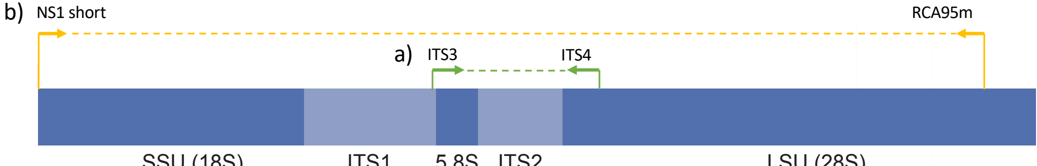


Figure 1. Schematic diagram of the fungal ribosomal operon, including the set of primers used in a) **papers II** and **III** and b) **paper IV**.

The ITS region comprises the ITS1 and ITS2 spacers and the 5.8S gene (Schoch *et al.* 2012). This is the most used region for species-level identification of fungi, as it is highly variable among species (Schoch *et al.* 2012). The flanking regions of the ITS are conserved and, for this reason, primers target the genes flanking ITS regions (e.g. SSU-ITS1-5.8S or 5.8S-ITS2-LSU). Either the ITS1 or ITS2 region can be used for the identification of fungal species, although the 5.8S-ITS2-LSU has been recommended for wider taxonomic coverage (Tedersoo *et al.* 2015). Despite its use for species identification, the ITS region is too variable for phylogenetic studies at higher taxonomic ranks (Schoch *et al.* 2012; Lindahl *et al.* 2013; Tedersoo *et al.* 2015). The SSU (18S) region is more conserved among fungi, and can only be used for higher taxonomic ranks (e.g. orders), as its variation is not high enough for species-level identification (Schoch *et al.* 2012). The LSU (28S) is also more conserved than the ITS region and it can be used to compare taxa that are more distantly related (Lindahl *et al.* 2013). Also, the use of SSU and LSU can be an interesting alternative for some groups of fungi with high genetic variability and/or which lack representatives in current databases. This is the case for, for example, Glomeromycota and Chytridiomycota (Tedersoo *et al.* 2015).

In **papers II** and **III**, we have used the IST2 region for the detection of the fungal communities in Arctic thaw ponds (Figure 1). The DNA from water samples (extracted from filter membranes) and sediments was

extracted using available commercial kits, as described in **paper II**. PCRs were then conducted to amplify the ITS2 region, using primers originally designed by Tedersoo *et al.* (2015) and modified by Wurzbacher *et al*. (2017) for wider taxonomic coverage and to capture aquatic fungal groups such as Chytridiomycota. The ITS2 region shows a variation in size across different fungal groups (Yang *et al.* 2018). This size variation can be challenging for certain HTS technologies such as Illumina, which is sensitive to fragment length variation and can result in sequencing biases towards shorter sequences (Castaño *et al.* 2020). To overcome this challenge, we have sequenced ITS2 amplicons with the Pacific Biosciences technology (PacBio), which has been shown to result in much less biased communities than the Illumina technology, when amplicons with variable fragment size are used (Castaño *et al.* 2020).

The use of one set of primers, however, is not always enough to capture the total fungal diversity. This is due to lack of primer coverage (e.g. caused by genetic variation across fungal genomes) and unequal representation of certain groups of fungi in databases of different markers, hindering the development of universal fungal primers (Tedersoo & Lindahl 2016). For the first, a mix of primers with different substitutions can be used to amplify the same region. For the latter, the use of multiple primers that target different ribosomal regions is recommended (Tedersoo & Lindahl 2016). Alternatively, the amplification of the nearly complete ribosomal operon, i.e. covering the whole region from the SSU to most of the LSU, including the variable ITS region, has been suggested (Heeger *et al.*; Wurzbacher *et al.* 2019). The latter approach only uses one set of primers, typically generating a ~5kb fragment that can be sequenced in platforms that can deal with long reads, such as Oxford Nanopore or PacBio (Wurzbacher *et al.* 2019). In this way, it is also possible to increase the taxonomic resolution and the chances of successfully assigning a sequence to a certain taxonomic level. This is particularly interesting when investigating communities in underexplored environments, such as freshwaters. This method was applied in paper **IV** for the sequencing of the nearly complete ribosomal operon (Figure 1) of 32 fungal isolates from thermokarst ponds. Each individual isolate had their ribosomal operons sequenced with Nanopore, similarly as in Wurzbacher et al. (2018). The sequencing of the ribosomal operon allowed us to use different databases to identify the fungal isolates, and obtain the best possible taxonomic assignment. It is important to notice that Nanopore sequencing

typically leads to a sequencing error rate of 10-15% (Maestri *et al.* 2019). However, protocols such as the $1D^2$ allows that a both DNA strands of the same fragment are sequenced one after the other, which promises up to 99% accuracy (Leggett & Clark 2017; Calus *et al.* 2018). In our study, the use of the $1D^2$ protocol allied with the sequencing of pure cultures allowed us to obtain good quality consensus sequences of the ribosomal operon of our isolates.

2.1.2 Metagenomics

Another way of exploring the diversity of environmental microbial communities is through metagenomics (Orellana, 2013). This method consists of extracting the total DNA from a sample and sequencing it with HTS technologies, without targeting a specific genetic region (Wooley *et al.* 2010). This generates the so-called shotgun metagenomic reads and, by sequencing the total pool of genes of a sample, we are able to explore not only the taxonomic diversity of a community, but also its functional potential. Moreover, metagenomics can offer an alternative way to explore the microbial community composition without having to rely on molecular markers and potentially biased PCR (Orellana, 2013). This approach was employed in **paper I**, where a large set of metagenomic data, sequenced on Illumina platform, was used to explore the taxonomic diversity of fungal communities from several lakes. Metagenomic reads from Illumina sequencing were also used in **paper IV**, although for a different purpose than taxonomic diversity. In this study, the goal was to look at the functional potential of the fungal communities in the water and sediments of thermokarst ponds. More specifically, we investigated the genes related to carbon metabolism, in terms of abundances and composition of enzymes involved in the degradation and synthesis of organic carbon compounds. We have also investigated whether different habitats (e.g. pond water and sediment) would share the same functional potential.

2.1.3 Isolation, cultivation and full genome sequencing

The isolation and full genome sequencing of fungal pure cultures is a powerful way to study their functional traits and ecological roles (Sharma 2016; Stajich 2017; Miyauchi *et al.* 2020). In **paper IV**, I have isolated 32 fungal specimens from the sediment of thermokarst ponds to investigate their functional potential towards the carbon metabolism. The DNA from the

mycelia was extracted from pure cultures and their ribosomal operon was amplified and taxonomically assigned, as described in section 2.1.1. I have selected 13 out of the 32 fungal isolates to be fully sequenced by the Joint Genome Institute (JGI) in Berkeley, CA, USA. The criterion was to select the fungal isolates with the least similarities to current databases. Six of these isolates have been fully sequenced and are included in **paper IV**. These isolates were fully annotated, enabling us to investigate their functional potential towards the carbon metabolism and their abundances in the water and sediments of the thaw ponds.

2.2 Bioinformatic pipelines applied for fungal taxonomic and functional diversity

2.2.1 Taxonomic assignment of molecular markers

The amplicons generated using PacBio sequencing and used in the analyses in **papers II** and **III** were processed using the SCATA pipeline (https://scata.mykopat.slu.se/), which includes the quality filtering and clustering of the sequences into operational taxonomic units (OTUs - i.e., species level molecular types). The preprocessing and taxonomic assignment of these sequences is explained in detail in **paper II**. Briefly, the sequences were searched against the UNITE database (https://unite.ut.ee/) via the Pluto-F platform (https://plutof.ut.ee/). Additionally, BLASTn searches were done against the NCBI nucleotide database. The final taxonomic assignment was based on a consensus of the different searches.

As for the sequences generated in **paper IV**, we have used the ITSx tool (Bengtsson-Palme *et al.* 2013) to separate the different regions of the ribosomal operon of the fungal isolates. Each region was submitted to a different database: the ITS regions were submitted to UNITE, the SSU and LSU to SILVA (https://www.arb-silva.de/), and the entire operon to BLASTn searches against the NCBI non-redundant nucleotide database. The final taxonomic assignment was based on a consensus of these searches.

2.2.2 Processing of metagenomic reads

The analysis of the shotgun metagenomic reads of **papers I** and **IV** followed several steps in a pipeline that is summarized in Figure 2 and described in the following sections.

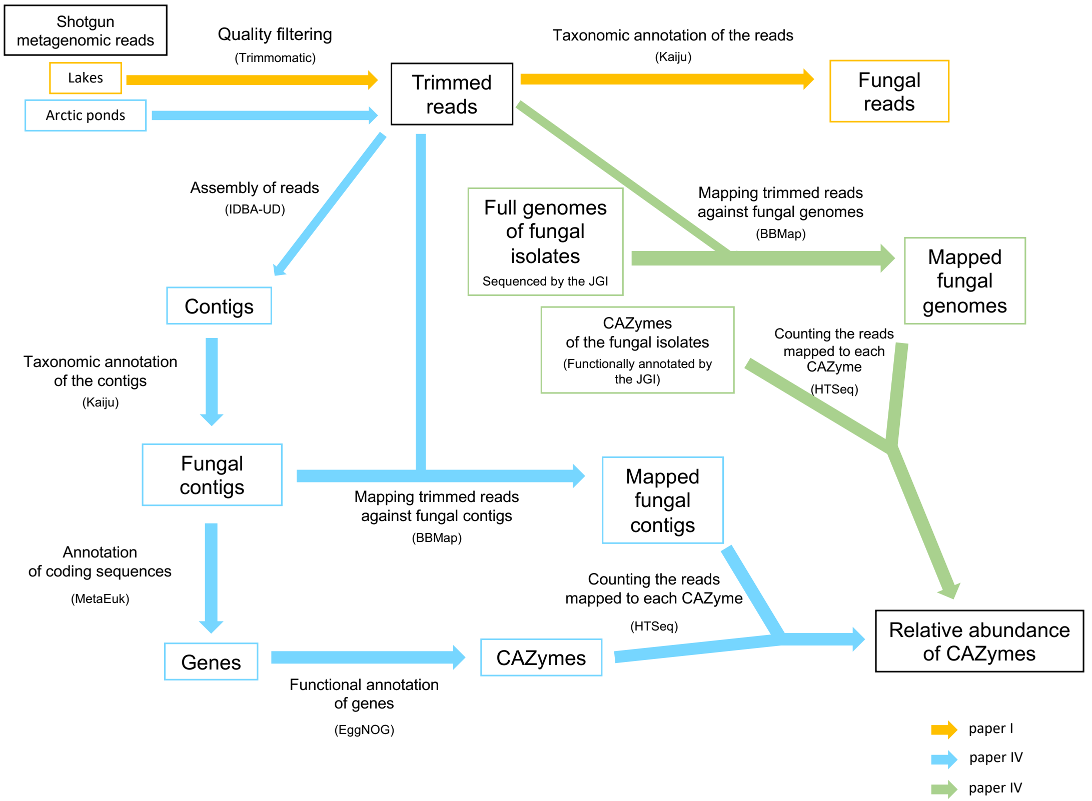


Figure 2. Schematic representation of the bioinformatic pipelines used to process the metagenomic reads for **papers I** and **IV**. The tools used for each step are informed in parenthesis and referenced in the text.

Initially, the shotgun metagenomic reads generated in **papers I** and **IV** were quality filtered with Trimmomatic (Bolger *et al.* 2014), which removes sequencing adapters used in the preparation of the sequencing libraries. The trimmed reads were analyzed in this thesis using two different methods: direct taxonomical annotation (**paper I**) and assembly into larger fragments,

so-called contigs, which are then taxonomically and functionally annotated (**paper IV**).

For the taxonomic annotation of the reads, we used a tool named Kaiju (Menzel *et al.* 2016). This tool applies BLASTx searches of the shotgun sequences against the NCBI databases. In **paper I**, we have used Kaiju for the taxonomic classification of the metagenomic reads to investigate the fungal diversity in lakes. For the Kaiju searches, a NCBI database of the non-redundant viral, prokaryotic and microeukaryotic protein sequences was combined with all the fungal protein sequences available at the JGI Mycocosm.

For **paper IV**, the metagenomic reads were assembled into contigs, i.e., consensus sequences of overlapping reads. To improve assembly efficiency, the reads were first normalized to an equal sequencing depth using BBNorm (sourceforge.net/projects/bbmap/). The assembly of the reads into contigs was done with the IDBA-UB software (Peng *et al.* 2012). In **paper IV**, we have used Kaiju to identify the contigs that belong to the fungal kingdom. Similar to **paper I**, I have created a customized database which included all the non-redundant protein sequences of microeukaryotes and prokaryotes from NCBI and all the protein sequences available at the JGI Mycocosm – which included our fungal isolates from the thermokarst ponds.

2.2.3 Functional annotation of genes

Annotation was performed on the contigs classified as fungi in **paper IV**, using the software MetaEuk (Levy Karin *et al.* 2020), which was designed to identify eukaryotic coding sequences. Once the coding sequences were annotated, they were functionally assigned to genes using eggNOG (Huerta-Cepas *et al.* 2019), which uses different databases, such as KEGG (Kanehisa & Goto 2000) and CAZy (Lombard *et al.* 2014). For **paper IV**, I have chosen the CAZy database to explore the functional potential of the fungal communities in thaw ponds. This database comprises different families of carbohydrate-active enzymes (CAZymes) involved in the degradation, synthesis or modification of carbohydrates (Cantarel *et al.* 2009). The CAZymes are classified in different families, according to their functional role. Glycoside hydrolases (GHs) are enzymes involved in lignocellulotic degradation, breaking down complex molecules such as cellulose, hemicellulose and starch (Andlar *et al.* 2018). Glycosyltransferases (GTs) catalyze the biosynthesis of glycosides, oligo- and polysaccharides (Breton

et al. 2006). Polysaccharide lyases (PLs) are involved in the cleavage of polyssacharides (Sutherland 1995). Auxiliary activities (AAs) are catalytic modules that are involved in the breakdown of complex plant cell components, such as lignin (Levasseur *et al.* 2013). Carbohydrate esterases (CEs) are hydrolases that act on ester bonds and can facilitate the breakdown of saccharides by providing easier access of GHs (Nakamura *et al.* 2017). The carbohydrate-binding modules (CBMs) do not have catalytic activity, but help bringing together the enzymes and their substrates and thus enhancing the breakdown of carbohydrates (Sidar *et al.* 2020). This includes, for example, enzymes that act on degradation pathways and on the biosynthesis of saccharides.

2.2.4 Obtaining the relative abundance of genes of interest

The relative abundances of genes of interest (in our case, CAZymes) were used to investigate the functional potential of fungal communities in **paper IV**. The relative abundances of CAZymes in each sample were used to compare functional potentials of different sample types (e.g. water and sediment) in **paper IV**. In **paper IV**, we have also looked into the relative abundances of CAZymes in the genomes of the fungal isolates. Finally, the relative abundances of each of the fungal isolates in each of the samples was also obtained by mapping the shotgun data to the ribosomal sequences and full genomes of the isolates.

In order to obtain the relative abundances of CAZymes for each sample, we first mapped all the shotgun metagenomic reads against the fungal contigs. The mapping was done using BBMap (sourceforge.net/projects/bbmap/). Then, the tool HTSeq (Anders *et al.* 2015) was used to count how many reads have mapped to the annotated CAZyme-containing sequences. For the fungal isolates, a similar approach was used. All the shotgun metagenomic reads were mapped against their genomes, and HTSeq was used to obtain the read counts for each annotated CAZyme, per sample (Figure 2).

Lastly, to obtain the relative abundances of our isolates in our samples, I have mapped all the shotgun metagenomic reads against the ribosomal sequences and the full genomes of the isolates. This was to obtain the number of reads that were mapped against each isolate, per sample. The read counts were then normalized to account for different library sizes and rescaled to read counts per million, as described in paper **IV**.

2.3 Characterization of the dissolved organic matter in freshwaters

The quality of DOM in freshwaters can be assessed through optical analyses, with absorbance and fluorescence measurements. As described in **paper II**, the DOM from thaw ponds was characterized using different proxies for its content or origin. The spectral slope at 289 nm (S289) reflects the lignin-free content of DOM, and it is used as a proxy for DOM derived from primary producers (Loiselle *et al.* 2009). The humification index (HIX) is a proxy for the humic content of DOM. More complex and higher molecular weight DOM (e.g. aromatic compounds) would show higher values of HIX (Ohno *et al.* 2007; Huguet *et al.* 2009). $SUVA_{254}$ is a proxy of DOM aromaticity (Weishaar *et al.* 2003). The freshness index (BIX) is a proxy of the relative freshness of the DOM. DOM that is recently derived mainly from autochthonous production presents high BIX values (> 1), whereas older DOM would present lower values (0.6–0.7) (Huguet *et al.* 2009; Fellman *et al.* 2010). The fluorescence index (FI) relates to the source of DOM. Microbial derived DOM shows higher values (~1.8), whereas allochthonous DOM presents lower values (~1.2) (Fellman *et al.* 2010). Another method used for characterizing the DOM is high resolution mass spectrometry. This allows us to obtain the hydrogen-to-carbon ratio (H/C), which can be used to infer the aliphatic content of DOM. H/C values above 1 indicate more saturated (aliphatic) compounds, whereas values below 1 indicate more unsaturated, aromatic molecules (Riedel *et al.* 2016; Seidel *et al.* 2017; Wilske *et al.* 2020). All these proxies were applied in **papers III** and **IV** to infer DOM quality in thermokarst ponds.

3. Fungal communities in lakes

In **paper I**, the fungal diversity was explored across 26 different boreal lakes in Scandinavia and in a tropical reservoir in Puerto Rico. For most of the lakes, samples from different depth layers were taken, including the epi-, meta- and hypolimnion. When a lake is deep enough, it can stratify during the warmer season, typically into three layers. The epilimnion consists of the top layer, with the highest oxygen concentration and warmest temperature. The densest layer at the bottom consists of the hypolimnion, which has the lowest oxygen concentration (often anoxic). The middle layer is called metalimnion, where there is a drastic decrease of temperature from top to bottom (Boehrer & Schultze 2008). The effects of depth layers into the fungal community composition were analyzed.

We have opted to analyze the diversity at the order level, using shotgun metagenomics data. We have not used lower taxonomic ranks, such as genus or species, because of the limitation of the current databases to correctly assign fungal sequences at finer levels, especially for poorly known taxa. This paper also included the group of oomycetes, given their similar life-style to fungi and how poorly known they are in freshwaters. However, these are out of the scope of this thesis and will be left out of the discussion.

3.1 Composition of fungal communities across lakes

3.1.1 Composition of fungal communities across lakes

The proportion of fungal orders was obtained for each lake and we observed that the composition across lakes differed significantly. All lakes were dominated by Ascomycota and Basidiomycota. At the order level, the Saccharomycetales were one of the orders with the highest variation in proportion across the lakes (Figure 3). Ascomycetous yeasts are commonly found in lakes, including lakes in Scandinavia, and their activity has also been reported (Brandão *et al.* 2011; Khomich *et al.* 2017; Monapathi *et al.* 2020). We have correlated the proportion of Saccharomycetales to all environmental variables, however, no correlation was found. The observed sudden increases in the relative abundances of Saccharomycetales would be easier to understand provided that better databases were available. This would allow the classification at genus or species levels, making possible the

investigation of environmental effects on specific taxa of this group. Interestingly, the order Agaricales was one of the most dominant ones. This order is known for terrestrial gilled mushrooms, often saprotrophs or mycorrhizal that would not develop without their plant hosts (Halling 2001). Although Agaricales have been reported in freshwaters, it is likely that part of the members found in the lakes represent spores that were introduced in the lakes from the surrounding forests, as suggested by Wurzbacher et al. (2016). Nonetheless, a study in several lakes in Scandinavia found that Agaricales was the most dominant order of basidiomycetes (Khomich *et al.* 2017).

The differences in alpha diversity between depth layers were not significant, but lake depth was a significant factor driving the community composition, especially for the hypolimnion. This could be explained by the anoxic condition of the hypolimnion, as oxygen significantly correlated with the community composition (Figure 4). The effects of environmental variables on the fungal community composition were tested in a subset of 52 samples, for which total organic carbon (TOC), oxygen concentration and total nitrogen (TN) were measured. Among these variables, TOC was the one which explained the highest variation, being the only significant variable in the Mantel test. The effects of TOC in fungal lake communities composition has been also detected in Scandinavian lakes (Khomich *et al.* 2017).

Overall, lakes presented specific communities, which likely reflect the variation of environmental conditions of each lake and site-specific effects, with possible influence of TOC. This suggests that the carbon availability (and perhaps quality) may have an impact on the community composition - a topic that will be explored in **paper III**, where thermokarst ponds were analyzed.

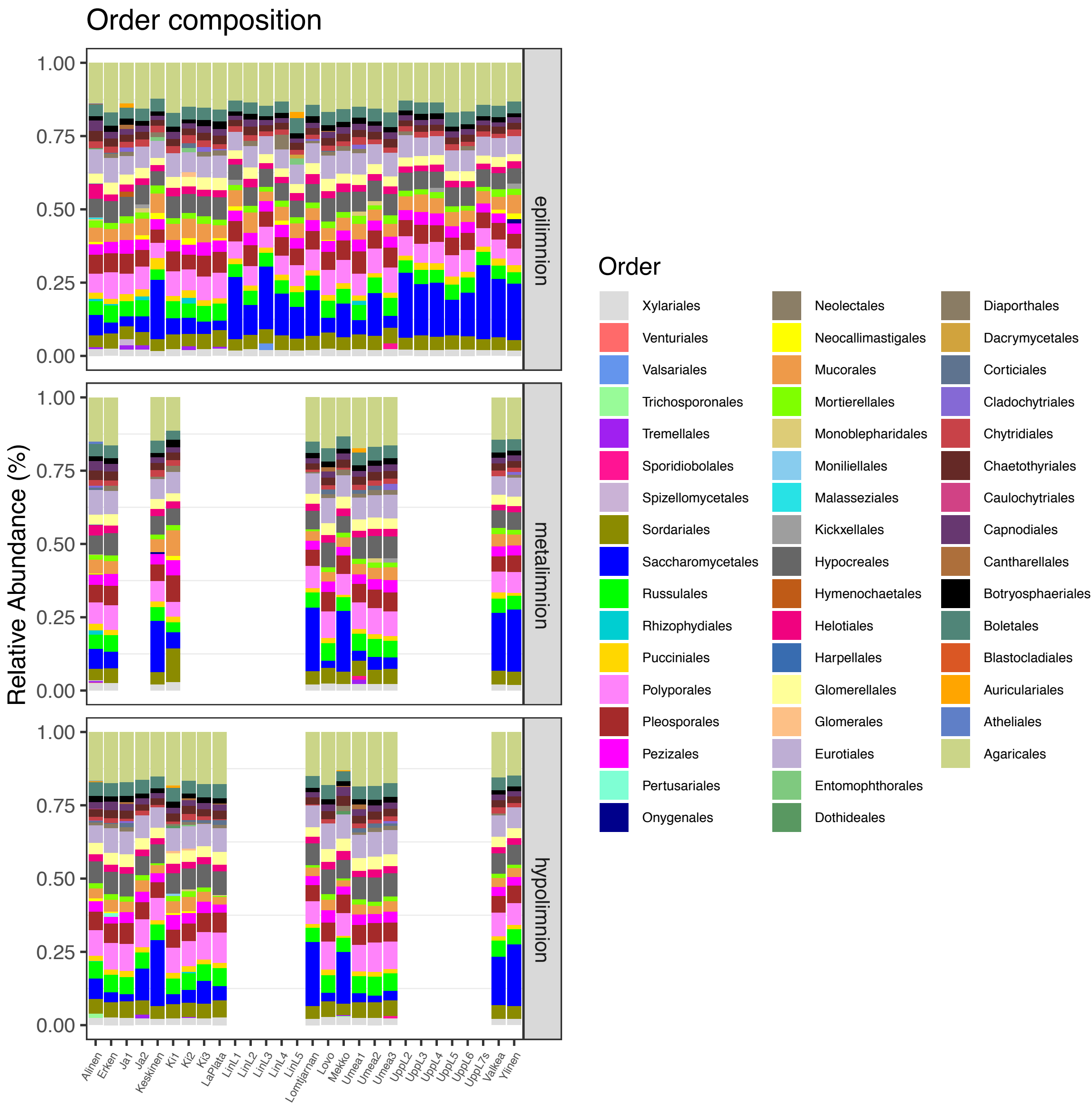


Figure 3. The fungal community composition for orders comprising > 0.01 % of the community across the lakes and at different depths.

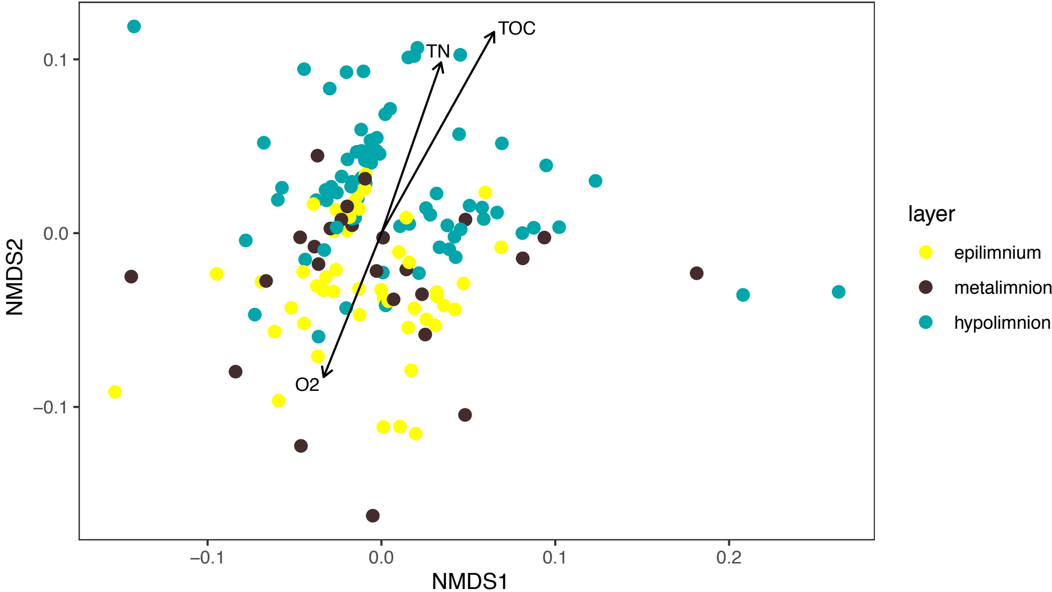


Figure 4. NMDS illustrating the composition of the fungal communities across different depth layers. Vectors of environmental variables that significantly correlated ($p < 0.05$) with the ordination were fitted onto the plot with envfit (TOC $r^2 = 0.28$, TN $r^2 = 0.17$, O_2 $r^2 = 0.13$). Stress = 0.14.

4. Impact of permafrost thaw on fungal diversity

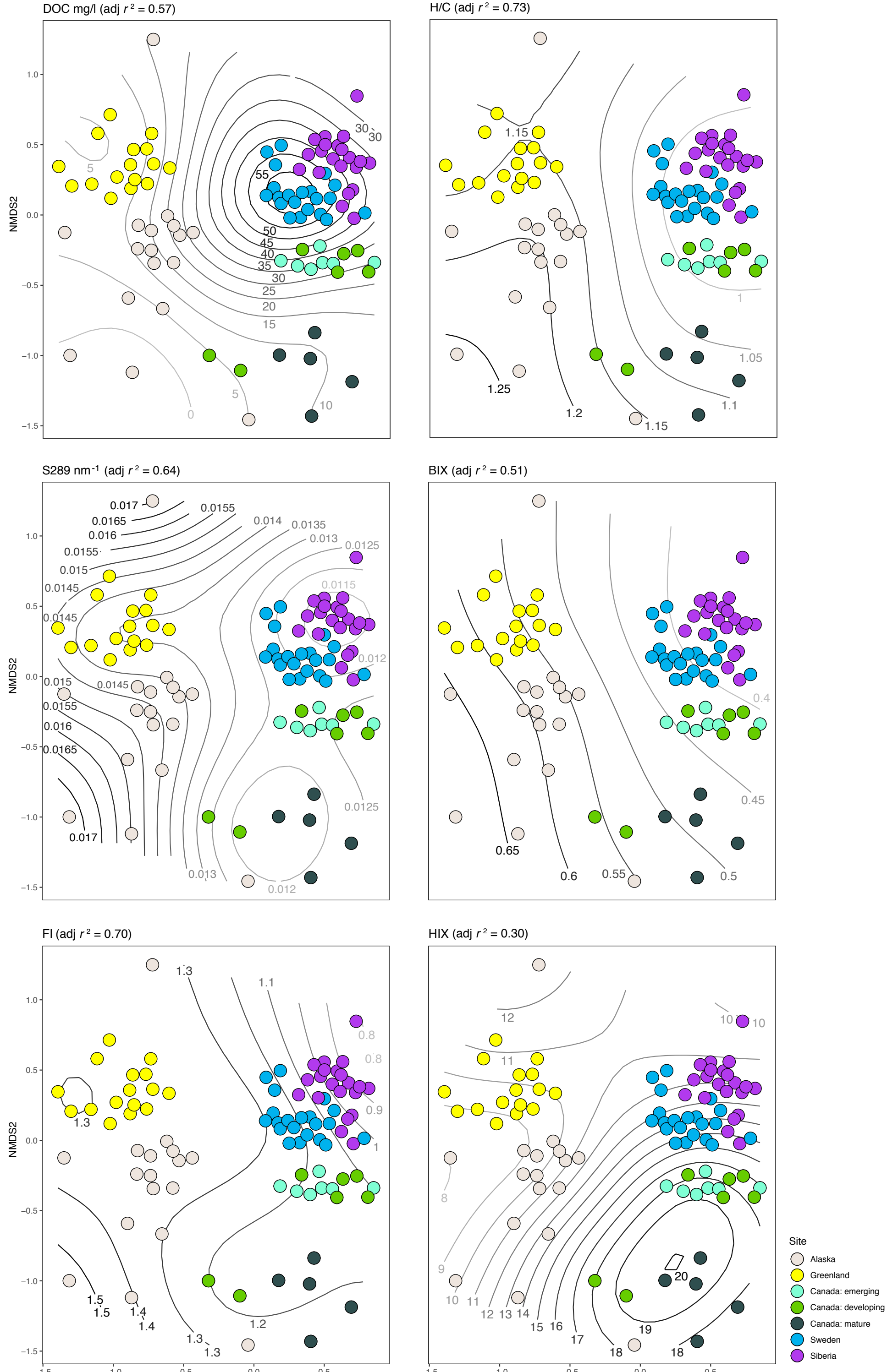
4.1 DOM quality linked with the composition of fungal communities in thermokarst ponds

In **paper III**, we analyze the fungal community composition in thaw ponds across a thawing permafrost gradient, and how organic matter quality and quantity correlate with these communities. Two sites, located in Alaska and Greenland, represented pristine sites, without visible effects on the landscape. The sites in Canada, Sweden and Russia represented degraded sites, and were going through active thermokarst. Also, for the Canadian site, the ponds represented different developmental stages, consisting of emerging, developing and mature ponds. This allowed us to explore the succession of the communities across the different developmental stages. For each site, we sampled water and sediment from 12 ponds and analyzed the ITS2 amplicons as described in Chapter 2. The water samples were analyzed for DOM quality and dissolved organic carbon (DOC) concentration.

As expected, for the degraded sites, the DOM proxies for quality pointed to a terrestrial source, with lower saturation, which can indicate higher aromaticity. The degraded sites also had higher DOC concentration. Opposite patterns were observed in the pristine sites, which showed fresher and autochthonous (i.e., microbial or algal derived) DOM, with higher saturation and, thus, likely lower aromaticity. At the Canadian site, results suggested a stronger influence of the autochthonous than allochthonous (i.e., terrestrial) DOM as the ponds develop and become more mature. These gradients of DOM quality and quantity were correlated with the fungal community composition (Figure 5). Sites with similar DOM quality shared

the highest number of OTUs. Also, among the dominant OTUs that were present at all sites, the abundances were either higher at pristine or degraded sites. This suggests that these OTUs had a preference for certain DOM qualities.

In fact, in statistical analyses the quality of DOM was one of the main factors that correlated with the community composition in the ponds. The water pH was also a significant factor, though highly correlated with DOM proxies related to allochthonous sources, and negatively correlated with DOC. This is not surprising as the thawing peat is one of main causes of acidification of thermokarst ponds (Pokrovsky *et al.* 2014). Therefore, it is challenging to separate the effects of pH and DOM quality as potential drivers of the community composition.



DOC mg/l (adj r^2 = 0.57)
H/C (adj r^2 = 0.73)
S289 nm^{-1} (adj r^2 = 0.64)
BIX (adj r^2 = 0.51)
FI (adj r^2 = 0.70)
HIX (adj r^2 = 0.30)
NMDS2
NMDS1
Site
Alaska
Greenland
Canada: emerging
Canada: developing
Canada: mature
Sweden
Siberia

Figure 5. NMDS of the water samples from the thermokarst ponds with contours of dissolved organic matter–related variables.

4.2 Declining beta diversity towards degraded sites

Beta diversity, in terms of variability in species composition among the ponds that we sampled at each site (12 ponds) or pond stage (4 ponds, in Canada) (Anderson *et al.* 2006), was estimated through the analyses of the multivariate homogeneity of group dispersions. The deviations of each sample from the group centroid was assessed by a permutational analysis of multivariate dispersions and differences between groups were verified and are shown in Figure 6.

Ponds from the degraded sites (Sweden and Siberia) showed significantly lower beta diversity than ponds from pristine sites. This means that the fungal communities among ponds from the degraded sites were more similar to each other; and pristine sites had the highest variation. This suggests a homogenization of the communities across ponds in regions of advanced permafrost thaw. Also, the higher diversity at the Canadian site can be explained by the variation of the developmental stages of the ponds.

It is difficult to predict the impacts of site-scale diversity loss. A decline in beta diversity could impact the multifunctionality of a meta-community, i.e. the landscape-level community (Mori *et al.* 2018). More studies are needed to elucidate how the functional potential of the fungal communities would be affected upon thaw.

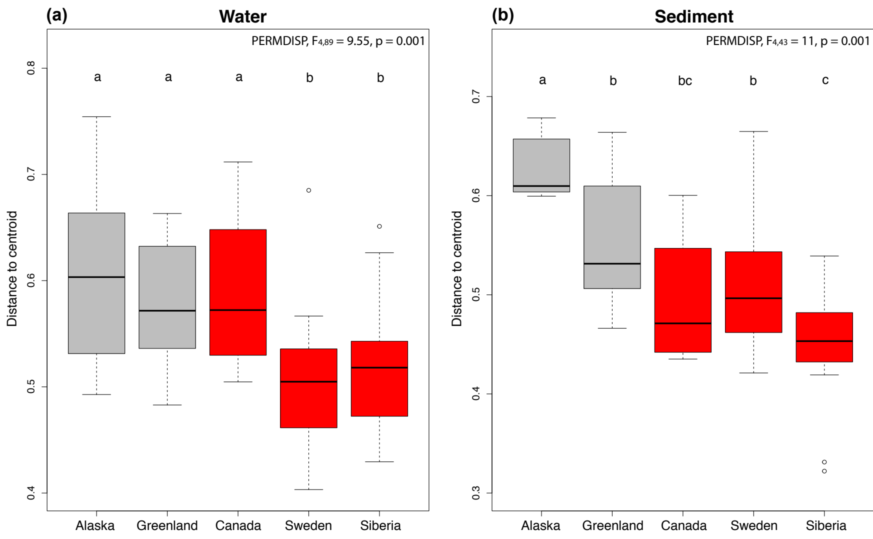


Figure 6. Boxplots showing, for each of the sites, the distances to the centroid for all the (a) water and (b) sediment samples within a site. Different letters indicate statistical differences between sites at $p < 0.05$, based on pairwise comparisons of group mean dispersions. Sites with same letters have similar dispersion. Gray boxes indicate pristine sites, whereas red boxes indicate degraded sites.

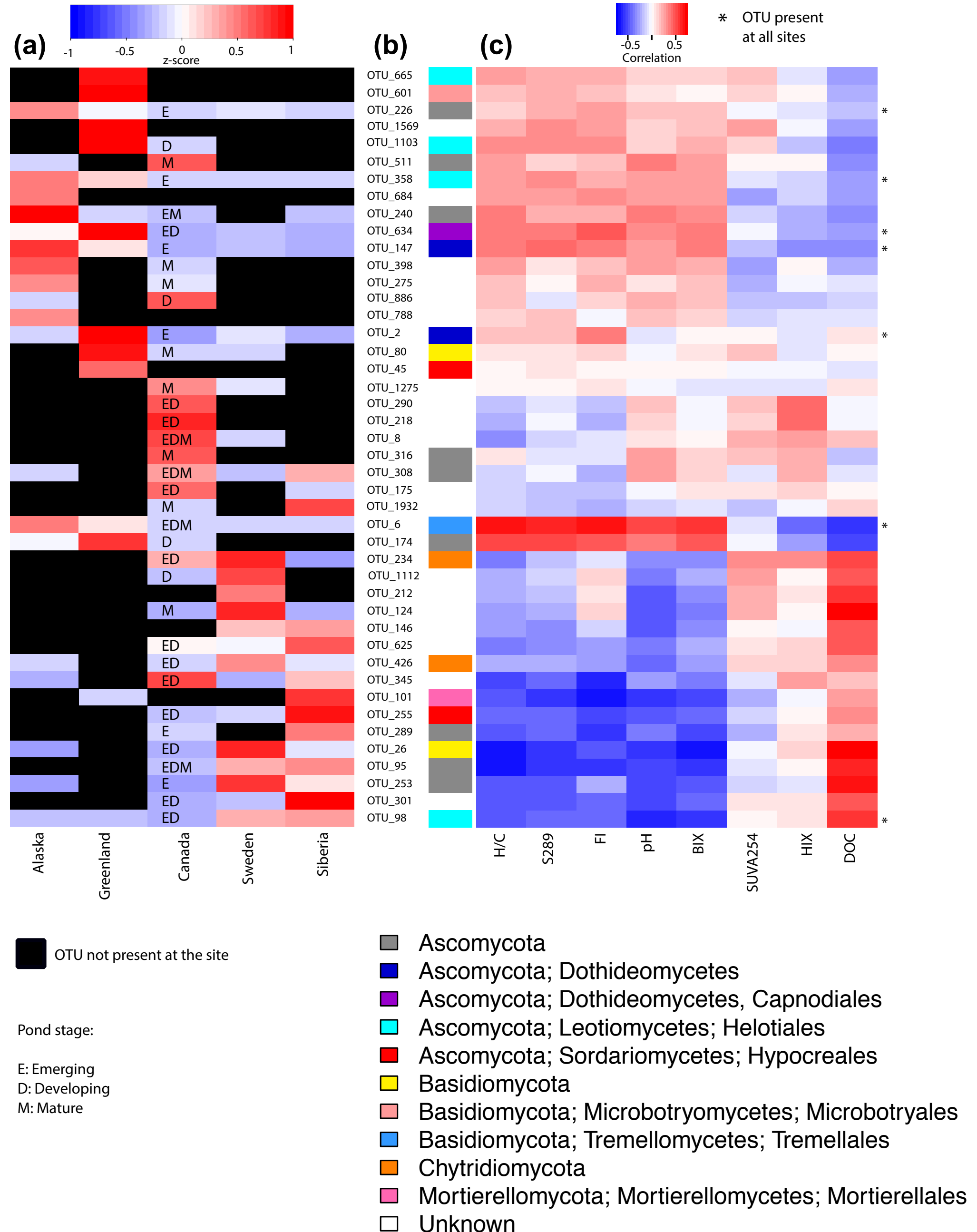
4.3 OTUs correlated with DOM quality

Some OTUs showed a clear correlation with proxies of DOM quality (Figure 7). OTUs that were abundant at pristine sites were negatively correlated with DOC concentration and positively correlated with variables indicative of autochthonous DOM (e.g., OTUs 6 and 174). Oppositely, OTUs that negatively correlated with these variables were more abundant in degraded sites, suggesting an affinity for more allochthonous DOM (e.g., OTUs 98, 289, and 345).

Different ecological strategies can be behind such dynamics. For instance, some species might be opportunistic ruderal/r-strategists, i.e., fast growers that can tolerate disturbances (Fontaine *et al.* 2003; Chagnon *et al.* 2013). Other species might be K-strategists: slow growers that can efficiently compete for limited resources (Andrews & Harris 1986). Upon permafrost thaw, a strong competition for the labile fraction of DOM is expected. This

would favor opportunistic ruderal r-strategists that can cope with the disturbances caused by the collapsing permafrost and have high affinity for the labile compounds. Studies in soil have shown that ascomycetes were able to rapidly grow in favorable conditions like at the initial stages of plant debris decay or after a disturbance (Lindahl *et al.* 2010; Vivelo & Bhatnagar 2019). This could be the case of some ascomycetes that were more abundant in emerging ponds and degraded sites. Alternatively, with the consumption of the labile DOM over time, more recalcitrant compounds remain. This could favor K-strategists that are able to thrive in more limiting conditions. This could be the case of basidiomycetes that are capable of breaking down recalcitrant carbon. These slow-growth fungi were found to increase in abundances at late stages of litter decomposition (Voriskova & Baldrian 2013), and could flourish at the most degraded sites which are dominated by allochthonous and potentially more recalcitrant DOM.

These findings contribute to the understanding on the dynamics between aquatic fungal communities and DOM of varying quality in thermokarst ecosystems. Elucidating such relationships is important to predict the impacts of climate change in permafrost areas, and to understand the ecosystem functioning.



(a)
-1
-0.5
0
0.5
1
z-score
(b)
(c)
-0.5
0.5
Correlation
* OTU present at all sites
OTU_665
OTU_601
OTU_226
OTU_1569
OTU_1103
OTU_511
OTU_358
OTU_684
OTU_240
OTU_634
OTU_147
OTU_398
OTU_275
OTU_886
OTU_788
OTU_2
OTU_80
OTU_45
OTU_1275
OTU_290
OTU_218
OTU_8
OTU_316
OTU_308
OTU_175
OTU_1932
OTU_6
OTU_174
OTU_234
OTU_1112
OTU_212
OTU_124
OTU_146
OTU_625
OTU_426
OTU_345
OTU_101
OTU_255
OTU_289
OTU_26
OTU_95
OTU_253
OTU_301
OTU_98
Alaska
Greenland
Canada
Sweden
Siberia
H/C
S289
FI
pH
BIX
SUVA254
HIX
DOC
OTU not present at the site
Pond stage:
E: Emerging
D: Developing
M: Mature
Ascomycota
Ascomycota; Dothideomycetes
Ascomycota; Dothideomycetes, Capnodiales
Ascomycota; Leotiomycetes; Helotiales
Ascomycota; Sordariomycetes; Hypocreales
Basidiomycota
Basidiomycota; Microbotryomycetes; Microbotryales
Basidiomycota; Tremellomycetes; Tremellales
Chytridiomycota
Mortierellomycota; Mortierellomycetes; Mortierellales
Unknown

Figure 7. Heatmap of the most abundant OTUs in the water samples for each of the sites and their correlation to variables related to carbon quality and pH. (a) Abundances of the OTUs per site, as given in Z-score standardized to total number of reads for each OTU. For the Canadian site, the presence/absence of each of the OTUs in each developmental stage is indicated by a letter. (b) The taxonomic assignment of the OTUs and (c) the Spearman rank correlation between the relative abundance of the OTUs and the carbon variables across all ponds.

5. Functional potential of aquatic fungal communities

The functional potential of the aquatic fungal communities in thermokarst ponds was investigated in **paper IV**. In this study, we have combined shotgun metagenomics, full genome sequencing and DOM characterization analyses to investigate the functional potential of water and sediment from thermokarst ponds. Additionally, we have looked at how genes involved in the carbon metabolism may interact with the quality of DOM.

We have analyzed the sediments and water of the 12 ponds from a palsa bog located in Abisko, Sweden – one of the locations of **papers II** and **III**. Here, I have successfully isolated 32 fungi from the sediments of the ponds, and six of these isolates were fully sequenced and functionally annotated (as described in Chapter 2). This allowed a complete analysis of the functional potential of these isolates and their abundances across the ponds. Additionally, fungal contigs obtained from the shotgun sequencing were used to look at the functional potential of the overall fungal community in the ponds.

5.1 Different functional potentials for water and sediment

The functional potential of our samples was analyzed in terms of relative abundances of CAZymes. Our analyses showed that sediment and water significantly differed in their composition of CAZymes. In the water, higher proportions of GTs were found (Figure 8). These enzymes participate in the assembly of carbohydrates, which suggests a higher potential for growth in the water.

In the sediment, a higher proportion of GHs and was observed (Figure 8). These hydrolases are involved in the breakdown of a variety of compounds

found in peat, such as cellulose and hemicellulose (Andlar *et al.* 2018). Moreover, the sediment samples had higher proportions of Helotiales isolates, whose genomes had the highest proportion of GHs among the isolates. This group of fungi are abundant in Arctic soils and are known for being efficient degraders of cellulose present in old OM (Newsham *et al.* 2018). The Helotiales were also more present in the sediment samples of degraded sites in **paper III**. It is possible that sediments of thermokarst ponds represent hotpots for the degradation of the OM coming from the thawing peat and that Helotiales play an important role.

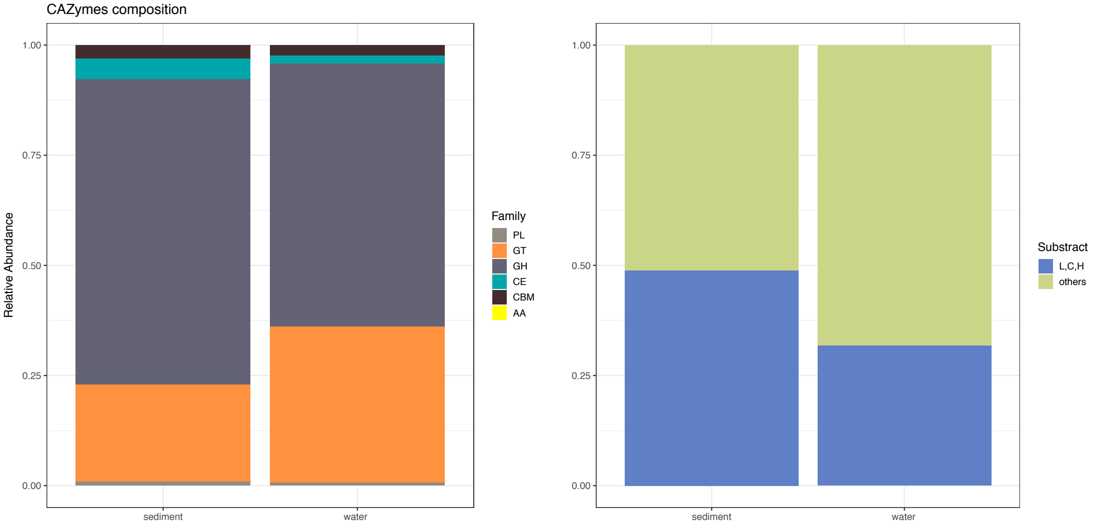


Figure 8. Relative abundances of CAZymes in the fungal communities, per sample type, showing: a) the proportion of CAZyme families; b) the proportion of CAZymes involved in lignin, cellulose or hemicellulose (L,C,H) degradation in relation to other CAZymes.

5.2 Correlations between CAZymes and DOM quality

The relationships between CAZymes and DOM quality in the water were investigated. We found that the overall composition of CAZymes, especially for the GTs, were positively correlated with DOM proxies for autochthonous

sources (S289, BIX, FI) and also of humic content (HIX, S289). Such correlations were also found when abundances of the CAZymes were correlated individually. For instance, certain GTs were correlated with high levels of BIX, FI and H/C, suggesting a connection with freshly synthesized OM of microbial sources. At the same time, some GHs correlated with low levels of these proxies, suggesting a link with OM of terrestrial origin. This likely reflects the ability of the aquatic fungal communities to use the carbon generated by the autochthonous production in the ponds and also to break down the carbon likely coming from the thawing permafrost.

Moreover, some of our isolates that were more relatively abundant in the water had the highest proportion of GTs in their genomes. This was the case of a *Umbelopsis angularis* isolate and a Microbotryomycetes. The relative abundance of the *Umbelopsis angularis* was especially high for pond P9, a pond with the lowest values for DOM humification and aromaticity, and the highest values of proxies for fresh and microbial-derived DOM. This also suggests that the DOM originated from the microbial communities in pond water can favor the growth of specific taxa.

Our findings suggest that the quality of the organic matter likely has an effect on the composition of fungal communities, given their ability to process different substrates. However, while the OM quality and availability can affect the microbial communities, microbes also affect the OM quality. Microorganisms transform and modify the OM and it is difficult to disentangle such relationships. Fungi, for instance, can degrade and produce HS (Rojas-Jimenez *et al.* 2017). Thus, further research is needed to investigate this complex ecosystem.

6. General conclusions and future perspectives

For a better estimation of the impacts of a warming climate in Northern landscapes to the aquatic ecosystems, it is crucial to understand the dynamics between microbial communities and organic matter. In this thesis, I have focused on aquatic fungal communities from freshwaters and explored their diversity, their relation to DOM and their functional potential towards the carbon cycle. We found that the quality of the organic matter is tightly linked with the aquatic fungal community composition, especially in permafrost areas affected by thawing. Our study in thermokarst ponds revealed a loss in beta diversity of the fungal communities in degraded sites. However, the impacts of this diversity loss are unknown and pertinent questions remain. How a decline in diversity affects the functional potential of these communities? Could such less diverse community be specialized in the degradation of more recalcitrant OM? Or, even further: how does it affect the activity of the communities? And how all of this can be translated in terms of ecosystem functioning? Further research is needed to address these questions.

The functional potential of fungal communities in the thermokarst ponds was explored in one of the sites affected by thawing. In this study, we observed different functional potentials for water and sediments. We found that the functional potential for the degradation of OM was greater in the sediment, whereas the water showed a greater potential for the biosynthesis of organic compounds required for growth. Also, there was a correlation of some CAZymes involved in cell growth to DOM derived from microbial production or algal biomass, as well as old terrestrial DOM. This suggests that the aquatic fungi could benefit from the carbon derived from multiple sources. However, these conclusions are limited to one site, which does not

capture all the variation in DOM quality found in permafrost sites that are differently affected by thawing. To address this, my following steps will be to extend the functional potential analysis to all sampled locations. In this way, I will be able to investigate the differences in functional potential across a thaw gradient, similarly to what was done with the community composition in **paper III**. Further, by evaluating all the sites, we would be able to investigate how the observed decline in taxonomic diversity relate to functional diversity. Would we observe an expansion or narrowing of the genetic functional potential?

Further research should include an investigation of the actual response of aquatic fungal communities upon thaw, and over time. This could be achieved with microcosms/mesocosms experiments that simulate the carbon inputs from the thawing peat under controlled conditions. This allows to observe the impacts of environmental change to fungal community diversity and environmental conditions, and would likely contribute to a more robust understanding on how the community structure is affected by thawing. Moreover, in this thesis, we have explored the functional potential of fungal communities, but not their actual activity - which would allow to answer which genes are active in which conditions. This could be achieved with metatranscriptomics studies or by targeting and quantifying specific genes of interest. I hope that my work would encourage the scientific community to take further steps on understanding the ecology of aquatic fungi.

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in ’t Zandt, M.H., Liebner, S. & Welte, C.U. (2020). Roles of Thermokarst Lakes in a Warming World. *Trends in Microbiology*, vol. 28 (9), pp. 769–779 Elsevier Current Trends.

Popular science summary

Fungi are found everywhere on Earth. This large and diverse group includes yeasts, mushrooms and molds. Several fungi are decomposers of the organic matter, but some can be parasites or live in association with plants. Despite their environmental and clinical relevance, there is still a lot to learn about them. It is estimated that we know only 10% of the fungal diversity. For some environments, this is particularly true. Most of what we know about fungi is based on studies of soil-dwelling fungi or fungi of clinical relevance. We still know very little about the fungi that live in water, which is the focus of my thesis. I have investigated fungal communities that are present in lakes and ponds in boreal and Arctic regions. I wanted to learn about who they are and what they can do, specially what their roles in the carbon cycling are. My research started with looking at how different fungal communities in different lakes in Scandinavia would be. This was a starting point to understand the factors that could be shaping the aquatic fungal communities in freshwater environments. For instance, I wanted to know if the availability of organic carbon and nutrients would favor the growth of certain groups of fungi and if this would have an impact on the fungal community composition. Our study of these lakes showed that each lake had a particular composition of fungal taxa and data suggested that carbon was an important variable driving this variation. This was a starting point for my biggest project. We know that climate change is threatening the Arctic ecosystems. One of the consequences of such warming climate is the thawing of the permafrost: soil that has been frozen for at least 2 consecutive years. When thawing occurs, there is a collapse of the landscape, which can result in the emergence of water bodies – the so called thermokarst ponds. While thawing is ongoing, these ponds receive a substantial amount of carbon that has been stored in the permafrost for millennia. Microorganisms play a key role in

recycling this carbon in such environments, and the biodegradation of the organic matter results in the release of greenhouse gasses (GHGs) to the atmosphere. This makes these ponds hotspots for GHG emissions. Fungi are notorious for their ability to degrade organic compounds in soil. Here, I am interested in the aquatic fungi from the thermokarst ponds. I want to know how their diversity is affected by the thawing permafrost, and how these fungal communities interact with the carbon coming from the thaw processes. To study this, water and sediments from ponds of five different regions across the Arctic were collected, representing a gradient of permafrost integrity – i.e., we have included sites that are not affected by the thaw (Alaska and Greenland – named pristine sites) and sites that were degraded (Canada, Sweden and Siberia). The goal was to investigate how the quality and quantity of carbon found in those sites would relate to the fungal community composition in the ponds. Our results show that the fungal composition was strongly correlated with the quality of the dissolved organic matter in the ponds. Sites that shared similar carbon quality shared the highest number of fungal species, showing a clear shift in the community composition along the thaw gradient. Another interesting finding was that the fungal beta-diversity decreased with permafrost thaw: ponds from the most degraded sites showed more homogeneous communities when compared to ponds in pristine sites. Also, at the Canadian site, we were able to look into developmental stages of the ponds, as the site had ponds that were emerging, developing and at a more mature stage. We observed that, as the ponds age, there is a change in the fungal community composition, which is also related to the transformation of the dissolved organic matter over time, converging to conditions more similar to the pristine ponds. In the Swedish site, I isolated fungi from the sediments of the ponds. Some of these isolates were subjected to full genome sequencing, to better evaluate their functional potential when it comes to the degradation of the organic matter. We also wanted to look at the functional potential of the entire fungal community from the ponds, which was achieved by metagenomic sequencing of the pond water and sediments. With this approach, we have assessed the entire genetic pool and looked for genes of interest that were involved in the carbon metabolism. We found that the communities from water had a different potential than the ones from the sediment. In the water, we observed a higher abundance of genes involved in growth, and some of these genes correlated with DOM derived from the microbial pool or algal biomass. Also, the

sediment had a greater potential for the degradation of the organic matter. We also observed that some of our isolates that had the greater potential for the OM breakdown were also more abundant in the sediment than in the water. It is possible that the pond sediments are important spots for the breakdown of the OM coming from the thawing peat. Our results contribute to understand the impacts of climate change in the Arctic and hopefully will encourage more research to explore the ecological role of aquatic fungi in the carbon cycling.

Populärvetenskaplig sammanfattning

Svampar finns överallt på jorden. Denna stora och mångsidiga grupp inehåller encelliga jästsvampar och flercelliga former som kan växa i stora sammanhängande mycel. Flera svampar är nedbrytare av det organiska materialet, men vissa kan vara parasiter eller leva tillsammans med växter och djur i symbios. Trots deras miljömässiga och kliniska relevans finns det fortfarande mycket att lära om dem. Det uppskattas att vi bara känner till 10% av svampdiversiteten. Det mesta vi vet om svampar är baserat på studier av jordlevande svampar eller svampar av klinisk relevans. Vi vet fortfarande väldigt lite om svamparna som lever i vatten, vilket är fokus för min avhandling. Jag har undersökt svampsamhällen som finns i sjöar och dammar i boreala och arktiska regioner. Jag ville lära mig mer om vilka de är och vad de kan göra, särskilt vad de har för roll i koldioxidcykeln. Min forskning började med att undersöka svampsamhällen i olika sjöar i Skandinavien. Detta var en utgångspunkt för att förstå de faktorer som formar samhällena i sötvattensmiljöer. Till exempel ville jag veta om tillgängligheten av organiskt kol och näringsämnen särskilt gynnar tillväxten av vissa grupper av svampar. Studierna visade att varje sjö hade en särskild sammansättning av svamparter och data tyder på att organiskt kol verkligen är en viktig variabel som reglerar denna variation.

Resultaten från kartläggningen av svampart i sjöar var sedan en utgångspunkt för mitt största projekt. Vi vet att klimatförändringarna hotar de arktiska ekosystemen och att en av konsekvenserna är upptining av permafrosten. När upptining sker leder det till en kollaps av landskapet, vilket kan resultera i uppkomsten av vattensamlingar - de så kallade termokarstdammarna. Medan upptining pågår får dessa dammar en betydande mängd kol som har lagrats i permafrosten i årtusenden. Mikroorganismer spelar en nyckelroll vid återvinning av detta kol i sådana

miljöer, och biologisk nedbrytning av det organiska materialet leder till utsläpp av växthusgaser till atmosfären. Detta gör dessa dammar till hotspots för utsläpp av växthusgaser. Svampar är välkända för sin förmåga att bryta ned organiska föreningar i jord. Frågan var vilken roll vattensvampar från termokarstdammarna spelar. Jag ville veta hur deras mångfald påverkas av den tinande permafrosten och hur dessa svampsamhällen interagerar med kolet som kommer från tiningsprocesserna. För att studera detta samlades vatten och sediment upp från dammar från fem olika regioner över Arktis, vilket representerar en gradient av nedbrytning av permafrosten - från platser som inte påverkats av tiningen (Alaska och Grönland) och platser som förändrats (Kanada, Sverige och Sibirien). Målet var att undersöka hur kvaliteten och kvantiteten av kol som finns på dessa platser förhåller sig till svampsamhällets sammansättning i dammarna. Våra resultat visar att sammansättningen av svampsamhället var starkt korrelerad med kvaliteten på det lösta organiska materialet i dammarna. Platser som hade en liknande kolkvalitet hade också det högsta antalet svamparter gemensamt, vilket visade ett tydligt skift i samhällssammansättningen längs tiningsgradienten. Ett annat intressant fynd var att svamparnas diversitet minskade med permafrost-tining: dammar från de mest nedbrutna platserna visade mer homogena samhällen jämfört med dammar på orörda platser. I Kanada kunde vi också undersöka utvecklingsstadierna av dammarna i en lokal gradient. Där såg vi också att när dammarna åldras sker det en förändring i svampsamhällets sammansättning, vilket är relaterat till omvandlingen av det upplösta organiska materialet över tid.

I Abisko i de svenska fjällen isolerade jag svampar från dammarnas sediment. Arvsmassan hos några av dessa isolat avlästes fullständig för att bättre kunna utvärdera deras funktionella kapacitet när det gäller nedbrytning av det organiska materialet. Vi ville också titta på den funktionella potentialen för hela svampgemenskapen från dammarna, vilket vi gjorde genom metagenomisk sekvensering av dammvattnet och sedimentet. Med denna metod kan man bedöma hela det genetiska materialet i ett prov, och vi letade särskilt efter gener som var inblandade i kolmetabolismen. Vi fann att samhällena från vatten hade en annan potential än de från sedimentet. I vatten observerade vi gener som är involverade i tillväxt, och de gener som korrelerade med löst organisk material, DOM, härrör från primärproduktion hos bakterier och alger. Våra resultat tyder på att de akvatiska svampsamhällena drar nytta av DOM som genereras av primära producenter,

framför allt organismer med fotosyntes. Svamparna i sedimentet hade en större genetisk potential för nedbrytning av det organiska materialet. Våra resultat bidrar till att förstå effekterna av klimatförändringar i Arktis och förhoppningsvis kommer att inspirera till mer forskning för att i detalj utforska vattensvampars ekologiska roll i koldioxidcykeln.

Acknowledgements

I would like to start thanking my main supervisor, Sari Peura, for giving me the opportunity to work in this exciting project. I could not be more grateful and appreciative of your trust. You were always there for me, knowing when to encourage me in the difficult moments or even telling me when it was time to stop (and that it would be ok to do so). Your enthusiasm while doing research is contagious, inspiring and motivating. We have done so many fun things together, and I truly could not have wished for a better supervisor.

Jan Stenlid, it was a real privilege to have you as my co-supervisor. Your wisdom and experience took our discussions to a much higher level. All of our meetings were so meaningful, you helped me a lot to organize my ideas and to think about the biology, the hypothesis and the questions. Your inputs helped me to know if I was on the right track and encourage me to become a better researcher.

Karina Clemmensen, I was very lucky to have you as my co-supervisor. I truly admire your ability of always coming up with questions that really make me think. The points you brought to discussion and your patience to explain and discuss concepts were crucial for my development. And I could not thank you enough for spending late hours reading the manuscripts, answering my questions and returning with valuable inputs – always with patience and kindness. I hope I wasn't too demanding, but I can say that working with you made a huge impact on my personal growth as a researcher.

Thanks to all the collaborators that contributed with the projects. Christian Wurzbacher, thank you for your participation in the design and all your help in this exciting project. Thanks to Anushree, Maxime, Domenico, Sarahi, Anna S., Renata…it has been a real pleasure to collaborate with you!

Thanks to my sister Anelise and Martin for carrying all the heavy stuff in the tundra and all the help in the field.

Katta and Eddy, I cannot imagine the lab without you. Thank you for all your guidance and patience and for showing me things around the lab. I cannot express how much I have enjoyed working next to you, our conversations, fikas. Rena and Maria J, you also were always there to help me with questions. The lab really felt like a second home to me and it would not have been the same without you all around.

Thanks to Sara Hallin, Maria Hellman, Chris Jones, Lea, Yvonne, Arpita, Aurélien, Jaanis, Gaëtan, Sari, Karina, Carles and Louis for all the nice discussions about Science during our meetings. Thank you for our discussions about statistics, bioinformatics, experimental design…I have learned a lot from all of you and your feedback has always been very important to me.

Thank you Anders Dahlberg and Petra for always helping me with the practical questions related with the PhD. Thank you Malin for all our nice fika discussions about Science and all sorts of things. Thank you Les for solving all the computer issues, always with a nice sense of humour. Thank you Jenny for always helping me with the administrative part. Thanks to all Mykopat family, which always made me feel so welcome at the department.

Thanks to all my PhD colleagues for always being a such supportive group, for the nice discussions, fikas and fun and relaxing moments outside work. Gaëtan, what can I say, you are a true friend. Thank you for the help in the field, for being supportive and for our discussions (even the philosophical ones that we have never finished). Thank you Deanne, I am so happy I met you, you are an amazing friend. Thank you for always listening to my concerns, dilemmas and also for sharing the happy moments.

À minha família, minha repleta gratidão. Minhas irmãs, Ane e Denise, são grandes exemplos para mim. Amo nossa sororidade, nossa rede de apoio, confiança...enfrentamos tudo. Meus pais, Ana e Willy, criaram três filhas de uma forma tão singular. Eu cresci em um lar sem preconceitos, onde éramos livres para viver do jeito que quiséssemos e estimulados a explorar o mundo. Mãe e pai, vocês nos apresentaram o mundo desde muito cedo, viajando, conhecendo lugares e pessoas. Fomos e somos tão privilegiados. Não há dúvidas de que isso despertou essa minha curiosidade pelas coisas, e me levou ao caminho da Ciência. Vocês abarrotaram as estantes de livros, me presentearam com enciclopédias (sim, sou dessa época) e nunca faltou

investimento e incentivo à educação. Muito, muito obrigada. A distância não é fácil, ainda mais nesse momento, mas nossa união transcende fronteiras.

Agradeço também aos meus professores que foram fundamentais para o meu aprendizado e desenvolvimento. Jeanine Marschner, eu muito provavelmente não teria seguido na área da microbiologia se não fosse por ti. A qualidade das tuas aulas é ímpar. Fernando Spilki, tu foste quem primeiro abriu as portas e sempre serei grata a todas as oportunidades incríveis que tive trabalhando no teu laboratório. Juliane Fleck, tu és um exemplo de humanidade e competência, aprendo tanto contigo. Ana Cláudia Franco, que sorte a minha ter sido tua orientanda, que projeto lindo que fizemos e que foi essencial na minha formação. Adriana Giongo, feliz o dia em que iniciamos nossa trajetória. Tu me acolheste quando eu mais precisava e sempre estiveste comigo.

Fernando, tu sabes da tua importância, e do meu apreço por nossa linda parceria. Obrigada por tua tranquilidade, amor e apoio. Ed Nozari, Lucas Matte, Ju Comerlato, Marcos, Carol, Patrícia, Vanessa, Felipe, Sandro, Cris, Nila, Nicole...mesmo longe vocês me acompanharam. Nossa amizade é inestimável.

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Community composition of aquatic fungi across the thawing Arctic

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Thermokarst activity at permafrost sites releases considerable amounts of ancient carbon to the atmosphere. A large part of this carbon is released via thermokarst ponds, and fungi could be an important organismal group enabling its recycling. However, our knowledge about aquatic fungi in thermokarstic systems is extremely limited. In this study, we collected samples from five permafrost sites distributed across circumpolar Arctic and representing different stages of permafrost integrity. Surface water samples were taken from the ponds and, additionally, for most of the ponds also the detritus and sediment samples were taken. All the samples were extracted for total DNA, which was then amplified for the fungal ITS2 region of the ribosomal genes. These amplicons were sequenced using PacBio technology. Water samples were also collected to analyze the chemical conditions in the ponds, including nutrient status and the quality and quantity of dissolved organic carbon. This dataset gives a unique overview of the impact of the thawing permafrost on fungal communities and their potential role on carbon recycling.

Background & Summary

Frozen tundra soils hold one of the Earth's largest pools of organic carbon. With ongoing climate change, permafrost is thawing rapidly, especially in the Arctic and Subarctic regions, causing the release of a large fraction of this carbon[1,2]. The thawing of permafrost creates small and shallow waterbodies, hereafter referred to as thermokarst ponds[3]. The vast amount of organic matter released from the degrading permafrost ends up in these ponds[4], where it can sink and be stored in the sediment, or be recycled in the microbial loop, generating greenhouse gases (GHG) as end products[5,6]. Most of the research on the microbial activity in the thermokarst ponds concentrates on prokaryotes[7–10] and, despite the central role of fungi as decomposers of the organic matter in terrestrial ecosystems[11–13], very little is known about the fungal communities in aquatic environments, especially in the Arctic. To our knowledge, only one earlier study has specifically targeted the fungi in thermokarst ponds, highlighting that a major part of the aquatic fungal community in the thermokarst ponds belongs to unknown phyla[14].

In this dataset, we collected surface water, detritus and sediment from thermokarst ponds in five different permafrost areas in the Arctic. These areas represent different stages of permafrost degradation from unaffected permafrost sites (represented by Alaska and Greenland, pristine sites) to sites affected by increasing severity of thermokarst activity (represented by Canada, Sweden and Russia, degraded sites) (Fig. 1). For each site, 12 ponds were sampled. Moreover, at the Canadian site, the ponds represented three different stages of permafrost thaw, including emerging, developing and mature thermokarst ponds; four ponds were sampled for each of these three stages[8,14]. This allowed us to investigate whether there is a succession of the community over pond development, when the quality and availability of carbon sources gradually changes. All the samples were extracted for the total metagenomic DNA, which was then amplified for fungal ITS2 region of the ribosomal genes and sequenced using PacBio (Fig. 2). We also collected water samples for chemical and optical analyses, in order to investigate nutrients and GHG concentrations as well as the quantity and quality of the dissolved organic matter (DOM). This included nutrients (dissolved nitrogen (DN), nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), sulfate (SO_4^{2-}), total phosphorous (total P)), total iron (Fe), GHG (carbon dioxide (CO_2), methane (CH_4)) and dissolved organic

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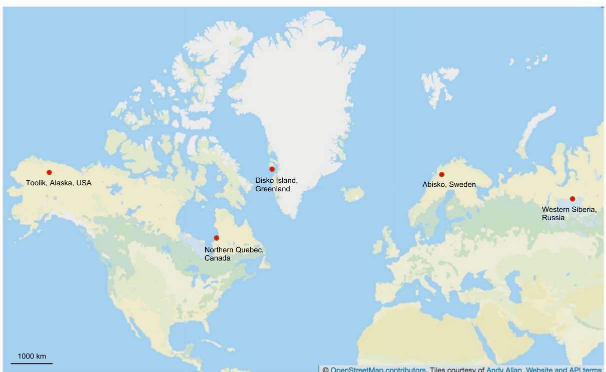

Fig. 1 Map showing the five locations of the sampling sites: Alaska, Canada, Greenland, Sweden and Siberia.

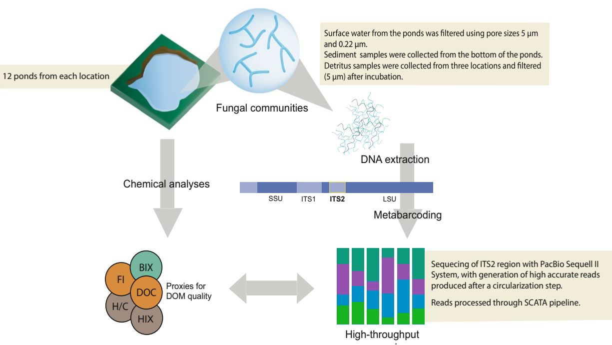

Fig. 2 Workflow illustrating the experimental design for sampling and the generation of the amplicon and chemical data.

carbon (DOC) concentrations, as well as various proxies of DOM such as fluorescence index (FI), freshness index (BIX), humification index (HIX), specific ultraviolet absorbance ($SUVA_{254}$), spectral slope for the intervals 279–299 nm (S_{289}) and average H/C and O/C.

The aim of this data collection was to study how the fungal diversity is affected by permafrost thaw and the resulting inputs of organic matter to the thermokarst ponds. Further, the impact of general chemical conditions in the ponds and their relationship to fungal community composition was addressed. Generally, this dataset gives unique insights into the composition of fungal communities in aquatic habitats in the Arctic. Thus, the data can be used to study the general composition of arctic fungal communities and how the community changes together with their environment, such as the availability of the carbon substrates. Importantly, it also expands the database for fungal ITS sequences with a large number of previously unencountered sequences, widening the knowledge and database available for studying fungal diversity in undersampled biomes. Additionally, this dataset can be useful for studies that explore the Arctic fungal taxonomy and their geographic distribution.

Methods

Study sites. We sampled ponds in the following five sites representing different regional-scale permafrost integrity: Toolik, Alaska, USA; Qeqertarsuaq, Disko Island, Greenland, Denmark; Whapmagoostui-Kuujjuarapik, Nunavik, Quebec, Canada; Abisko, Sweden and Khanymey, Western Siberia, Russia (Online-only Table 1). The aim was to include representatives of different stages of permafrost thaw in order to understand whether responses can be generalized across different geographic and environmental conditions.

The sampling site in Alaska is located in a continuous permafrost area, mostly dominated by moss-tundra characterized by tussock-sedge *Eriophorum vaginatum* and *Carex bigelowii*, and dwarf-shrub *Betula nana* and

Salix pulchra[15]. The average depth of the active layer in 2017 was ~50 cm[16]. Records of surface air temperature from 1989 to 2014 showed no significant warming trend, and there was no significant increase in the mean maximum thickness of the active layer or maximum thaw depth[17].

The sampling site in Greenland is located in the Blæsedalen Valley, south of Disko Island, and is characterized as a discontinuous permafrost area. From 1991 to 2011, Hollensen *et al.*[18] observed an increase of the mean annual air temperatures of 0.2 °C per year in the area, while Hansen *et al.*[19] highlighted that sea ice cover reduced 50% from 1991 to 2004. Soil temperatures recorded by the Arctic Station from the active layer of the coarse marine stratified sediments also showed an increase over the years[18]. The sampling site is comprised of wet sedge tundra, and the dominating species are *Carex rariflora*, *Carex aquatilis*, *Eriophorum angustifolium*, *Equisetum arvense*, *Salix arctophila*, *Tomentypnum nitens* and *Aulacomnium turgidum*[20].

The Canadian site is located within a sporadic permafrost zone, in a palsa bog, in the valley of Great Whale river, close to the river mouth to Hudson Bay. The vegetation consists of a coastal forest tundra, dominated by the species *Carex* sp. and *Sphagnum* sp.[21] Since the mid-1990s, there has been a significant increase in the surface air temperature of the region for spring and fall, which has been correlated to a decline of sea ice coverage in Hudson Bay[22]. This area has experienced an accelerated thawing of the permafrost over the past decades, resulting in the collapse of palsas and the emergence of thermokarst ponds as well as significant peat accumulation[21,23]. In this specific site, thermokarst ponds at different development stage can be found, from recently emerging to older, mature thermokarstic waterbodies. The stage of the ponds was estimated based on the distance between the pond and the edge of the closest palsa, as well as based on satellite images[14]. The edges of the emerging ponds reached a maximum of 1 m from the closest palsa and were less than 0.5 m deep, whereas the edges of the developing ponds had a maximum distance of 2–3 m to the closest palsa and were ~1 m deep. Mature ponds were identified based on satellite images and were up to 60 years old.

The Swedish site is located in a discontinuous permafrost zone at the Stordalen palsa mire, on an area of collapsed peatland affected by active thermokarst. The region has experienced an increase in mean annual air temperature and active layer thickness since the 1980s, which has been followed by a shift to wetter conditions[24]. The vegetation found on the surface of the palsa depressions of Stordalen mire is dominated by sedges (*Eriophorum vaginatum*, *Carex* sp.) and mosses (*Sphagnum* sp.)[24,25].

The Russian site is located in a discontinuous permafrost area in Western Siberia Lowland, near Khanymey village. The sampling site is a flat frozen palsa bog with a peat depth no more than 2 m, and is affected by active thermokarst, resulting in the emergence of thermokarst ponds[26,27]. The vegetation is dominated by lichens (*Cladonia* sp.), schrubs (*Ledum palustre*, *Betula nana*, *Vaccinium vitis-idaea*, *Andromeda polifolia*, *Rubus chamaemorus*) and mosses (*Sphagnum* sp.)[28].

Sample collection. At all sites, water from the depth of 10 cm was collected from 12 ponds, totaling 60 ponds for the full dataset. Unfiltered water samples were collected for total P analysis. For analyzing Fe, various dissolved anions and cations, DOC concentrations, and perform optical and mass spectrometry analyses on DOM, water was filtered through GF/F glass fiber filters (0.7 µm, 47 mm, Whatman plc, Maidstone, United Kingdom). Moreover, water samples were collected in order to measure GHG (CO_2 and CH_4) concentrations. Water, detritus and sediment samples were also collected from ponds for fungal community analyses. Water samples were collected and filtered sequentially first through 5 µm Durapore membrane filter (Millipore, Burlington, Massachusetts, USA) and then through a 0.22 µm Sterivex filter (Millipore) to capture fungal cells of different sizes. The samples were filtered until clogging or up to a maximum of 3.5 liters (filtered volume ranging from 0.1 l to 3.5 l). Surface sediments were sampled from each of the ponds, with the exception of the Canadian site, where only one emerging and three developing ponds were sampled for sediments. From the sites in Alaska, Greenland, and Sweden, also detritus samples (dead plant material) were collected. The detritus was washed in the lab using tap water, followed by overnight incubation in 50 ml tap water to induce sporulation. The use of tap water may have added fungal spores to the samples, which should be kept in mind when using the detritus data. After the incubation, the water was filtered through a 5 µm pore size filter and the filter was stored at −20 °C.

All the samples for DNA extraction were transported to the laboratory frozen, with the exception of the Alaskan samples, which were freeze dried prior to transportation. The samples transported frozen were freeze dried prior to DNA extraction to ensure similar treatment of all samples. The samples for nutrient and carbon measurements were transported frozen with the exception of samples for DOC and fluorescence analyses, which were transported cooled.

Chemical analyses. All chemical, optical and mass spectrometry results are provided in OSF[29]. DOC quantification was carried out using a carbon analyzer (TOC-L + TNM-L, Shimadzu, Kyoto, Japan). Accuracy was assessed using EDTA at 11.6 mg C/l as a quality control (results were within + − 5%) and the standard calibration range was of 2–50 mg C/l. Fe(II) and Fe(III) were determined by using the ferrozine method[30], but instead of reducing Fe(III) with hydroxylamine hydrochloride, ascorbic acid was used[31]. Absorbance was measured at 562 nm on a spectrophotometer (UV/Vis Spectrometer Lambda 40, Perkin Elmer, Waltham, Massachusetts, USA). The samples were diluted with milli-Q water if needed. The concentration of total P was determined using persulfate digestion[32]. The anion NO_3^- was measured on a Metrohm IC system (883 Basic IC Plus and 919 Autosampler Plus; Riverview, Florida, USA). NO_3^- were separated with a Metrosep A Supp 5 analytical column (250 × 4.0 mm) which was fit with a Metrosep A Supp 4/5 guard column at a flow rate of 0.7 ml/min, using a carbonate eluent (3.2 mM Na_2CO_3 + 1.0 mM $NaHCO_3$). SO_4 was analyzed using Metrohm IC system (883 Basic IC Plus and 919 Autosampler Plus, Riverview), NH_4^+ spectrophotometrically as described by Solórzano[33], and NO_2^- and DN as in Greenberg *et al.*[34].

For the gas analyses, samples from Alaska and Canada were taken as previously described in Kankaala *et al.*[35], except that room air was used instead of N_2 for extracting the gas from the water. Shortly, 30 ml of water was taken

into 50 ml syringes, which were warmed to room temperature prior to extraction of the gas. To each syringes 0.5 ml of HNO_3 and 10 ml of room air was added and the syringes were shaken for 1 min. Finally, the volumes of liquid and gas phases were recorded and the gas was transferred into glass vials that had been flushed with N_2 and vacuumed. For Greenland, Sweden and Russia 5 ml of water was taken for the gas samples with a syringe and immediately transferred to 20 ml glass vials filled with N and with 150 µL H_2PO_4 to preserve the sample. All gas samples were measured using gas chromatography (Clarus 500, Perkin Elmer, Polyimide Uncoated capillary column 5 m x 0.32 mm, TCD and FID detector respectively).

Optical analyses. In order to characterize DOM, we recorded the absorbance of DOM using a UV-visible Cary 100 (Agilent Technologies, Santa Clara, California, USA) or a LAMBDA 40 UV/VIS (PerkinElmer) spectrophotometer, depending on sample origin. $SUVA_{254}$ is a proxy of aromaticity and the relative proportion of terrestrial versus algal carbon sources in DOM[36] and was determined from DOC normalized absorbance at 254 nm after applying a corrective factor based on iron concentration[37]. S_{289} enlights the importance of fulvic and humic acids related to algal production[38] and were determined for the intervals 279–299 nm by performing regression calculations using SciLab v 5.5.2.[39]

We also recorded fluorescence intensity on a Cary Eclipse spectrofluorometer (Agilent Technologies), across the excitation waveband from 250–450 nm (10 nm increments) and emission waveband of 300–560 nm (2 nm increments), or on a SPEX FluoroMax-2 spectrofluorometer (HORIBA, Kyoto, Japan), across the excitation waveband from 250–445 nm (5 nm increments) and emission waveband of 300–600 nm (4 nm increments), depending on sample origin. Based on the fluorometric scans, we constructed excitation-emission matrices (EEMs) after correction for Raman and Raleigh scattering and inner filter effect[40]. We calculated the FI as the ratio of fluorescence emission intensities at 450 nm and 500 nm at the excitation wavelength of 370 nm to investigate the origin of fulvic acids[41]. Higher values (~1.8) indicate microbial derived DOM (autochthonous), whereas lower values (~1.2) indicate terrestrial derived DOM (allochthonous), from plant or soil[42]. HIX is a proxy of the humic content of DOM and was calculated as the sum of intensity under the emission spectra 435–480 nm divided by the peak intensity under the emission spectra 300–445 nm, at an excitation of 250 nm. Higher values of HIX indicate more complex, higher molecular weight, condensed aromatic compounds[43,44]. BIX emphasizes the relative freshness of the bulk DOM and was calculated as the ratio of emission at 380 nm divided by the emission intensity maximum observed between 420 and 436 nm at an excitation wavelength of 310 nm[45]. High values (>1) are related to higher proportion of more recently derived DOM, predominantly originated from autochthonous production, while lower values (0.6–0.7) indicate lower production and older DOM[42,44].

High resolution mass spectrometry. 50 ml water samples were collected from each of the ponds and were filtered with a Whatman GF/F filter for mass spectrometry analyses. For each sample, 1.5 ml of water was dried completely with a vacuum drier, and was then re-dissolved in 100 µL 20% acetonitrile, 80% water with three added compounds as internal standards (Hippuric acid, glycyrrhizic acid and capsaicin, all at 400 ppb v/v). Samples were filtered to an autosampler vials and injected at 50 µL onto the column. In order not to overload the detectors, some of the higher concentration samples were injected at a lower volume, to give a maximum of 20 µg carbon loaded.

High-performance liquid chromatography – high resolution mass spectrometry (ESI-HRMS) was conducted as described in Patriarca *et al.*[46] using a C18-Evo column (100 × 2.1 mm, 2.6 µm; Phenomenex, Torrance, California, USA). The ESI-HRMS data was averaged from 2–17 min to allow formula assignment to a single mass list. Formulas considered had masses 150–800 m/z, 4–50 carbon (C) atoms, 4–100 hydrogen (H) atoms, 1–40 oxygen (O) atoms, 0–1 nitrogen (N) atoms and 0–1 13 C atoms. Formulas were only considered if they had an even number of electrons, H/C 0.3–2.2 and $O/C \leq 1$. The data are presented as a number of assigned formulas and weighted average O/C ratio, H/C ratio and m/z.

The analysis was run in two batches (36 and 24 samples per run, respectively) and to the latter run, three samples of Suwannee River fulvic acid (SRFA, reference material) were added. At the moment of the run, the DOC concentration of these samples was unknown, so 50 µL was injected. From high resolution mass spectrometry, average H/C and a number of assigned formulas were obtained. The H/C can be used as a proxy of DOM aliphatic content; higher H/C values (>1) indicate more saturated (aliphatic) compounds, whereas values lower than 1 indicate more unsaturated, aromatic molecules[47].

DNA extraction, ITS2 amplification and sequencing. All samples for molecular analyses (water and detritus filters and sediments) were extracted using DNeasy PowerSoil® kit (Qiagen, Hilden, Germany), following the manufacturer's recommendations for low input DNA. Extracts were eluted in 100 µl of Milli-Q water and DNA concentrations were measured with Qubit dsDNA HS kit. The fungal ribosomal internal transcribed spacer 2 (ITS2) sequences were amplified using a modified ITS3 Mix2 forward primer from Tedersoo[48], named ITS3-mkmix2 CAWCGATGAAGAACGCAG, and a reverse primer ITS4 (equimolar mix of cwmix1 TCCTCCGCTTAyTgATAtGc and cwmix2 TCCTCCGCTTAtTrATAtGc)[14]. Each sample received a unique combination of primers containing identification tags generated by Barcrawl[49]. All tags had a minimum base difference of 3 and a length of 8 nucleotides. Both forward and reverse primer tags were extended by two terminal bases (CA) at the ligation site to avoid bias during ligation of sequencing adaptors, and the forward primer tag also had a linker base (T) added to it[50]. The list of primers and tags is found in Supplementary Table S1. PCR reactions were performed on a final volume of 50 µl, with an input amount of DNA ranging from 0.07 ng to 10 ng, 0.25 µM of each primer, 200 µM of dNTPs, 1U of Phusion™ High-Fidelity DNA Polymerase (Thermo Fisher Scientific, Waltham, Massachusetts, USA), 1X Phusion™ HF Buffer (1X buffer provides 1.5 mM $MgCl_2$, Thermo Fisher Scientifics) and 0.015 mg of BSA. PCR conditions consisted of an initial denaturation cycle at 95 °C for 3 min, followed by 21–35 cycles for amplification (95 °C for 30 sec, 57 °C for 30 sec and 72 °C for 30 sec), and final extension

at 72 °C for 10 min. In order to reduce PCR bias, all samples (in duplicates) were first submitted to 21 amplification cycles. In case of insufficient yield, the number of cycles was increased up to 35 cycles (see the records on the number of cycles for each of the samples in Supplementary Table S2).

The PCR products were purified with Sera-Mag™ beads (GE Healthcare Life Sciences, Marlborough, Massachusetts, USA), visualized on a 1.5% agarose gel and quantified using Qubit dsDNA HS kit. The purified PCR products were randomly allocated into three DNA pools (20 ng of each sample), which were purified with E.Z.N.A.® Cycle-Pure kit (Omega Bio-Tek, Norcross, Georgia, USA). Nine of the samples (4 water, 1 sediment and 4 detritus) were left out of the pools because of too little PCR product, giving a total of 203 samples for sequencing (Online-only Table 1). Negative PCR controls were added to each pool, as well as a mock community sample containing 10 different fragment sizes from the ITS2 region of a chimera of *Heterobasidium irregular* and *Lophium mytilinum*, ranging from 142 to 591 bases, as described by Castaño *et al.*[51]. The size distribution and quality of all the pools were verified with BioAnalyzer DNA 7500 (Agilent Technologies), and purity was assessed by spectrophotometry (OD 260:280 and 260:230 ratios) using NanoDrop (Thermo Fisher Scientific). The libraries were sequenced at Science for Life Laboratory (Uppsala University, Sweden), on a Pacific Biosciences Sequel instrument II, using 1 SMRT cell per pool. This PacBio technology allows the generation of highly accurate reads (>99% accuracy) which are produced based on a consensus sequence after a circularization step.

Quality filtering of reads, clustering and taxonomy identification of clusters. The sequencing resulted in a total of 1071489 sequences, ranging from 397 to 9184 sequences per sample (average on 2551 sequences per sample). The raw sequences were filtered for quality and clustered using the SCATA pipeline (https://scata.mykopat.slu.se/, accessed on May 19th, 2020). For quality filtering, sequences from each pool were screened for the primers and tags, requiring a minimum of 90% match for the primers and a 100% match for the tags. Reads shorter than 100 bp were removed, as well as reads with a mean quality lower than 20, or containing any bases with a quality lower than 7. After this filtering, 582234 sequences were retained in the data. The sequences were clustered at the species level by single-linkage clustering at a clustering distance of 1.5%, with penalties of 1 for mismatch, 0 for gap open, 1 for gap extension, and 0 for end gaps. Homopolymers were collapsed to 3 and unique genotypes across all pools were removed. For a preliminary taxonomy affiliation of the clusters, hereafter called OTUs (Operational Taxonomic Units), sequences from the UNITE + INSD dataset for Fungi[52] database were included in the clustering process. After the clustering, the data included 518128 sequences, divided among 8218 OTUs. For taxonomical annotation, all OTUs with a minimum of ten total reads in the full dataset were included, retaining 3108 OTUs and 498414 sequences in the taxonomical analysis.

Data Records

The raw sequences are deposited in the NCBI SRA database under accession number PRJNA701021 (Biosample accession numbers SAMN17843604-SAMN17843806)[53]. All the raw mass spectrometry data is available at the Mass Spectrometry Interactive Virtual Environment (MassIVE) under the accession number MSV000086952[54]. The fungal OTU sequences and their taxonomic classification, as well as all the environmental data related to the samples are deposited in Open Science Framework (OSF)[29].

Technical Validation

The DNA extractions were done in a laminar flow hood with a UV-C lamp and handled separately for each of the locations to avoid any possible cross-contamination between the samples or sites. For the PCRs, the samples were first randomized into three groups including samples from all locations to minimize the risk of batch effect at the sequencing step. Negative controls were included in the PCR step and added to the pools. The negative controls created 4, 12 and 4 sequences for pools 1, 2 and 3, respectively. For each pool, 100 ng of a positive control containing mock communities (as described in the methods section) was added. The mock communities captured all different fragment sizes. The sequences were controlled rigorously by removing singletons and rare reads, as described in the methods section, to remove any sequences that might be chimeric reads or a result of erroneous amplification. Finally, the sequences were compared against multiple databases and their taxonomy was verified manually to ensure that only fungal sequences were included to the tree shown in Fig. 2. For the resulting data, correlations between the number of OTUs and filtered water volume were checked to verify that the differential filtering volumes did not introduce a bias to the richness of the communities (Supplementary Figure S1).

Usage Notes

To verify that the acquired OTUs were of fungal origin, we scrutinized all the OTUs with a minimum of ten total reads for their phylogenetic origin, retaining 3108 OTUs and 498414 sequences in the analysis. For taxonomic annotation, the Protax-Fungi[55] and massBLASTer analyses available through the Pluto-F platform of the UNITE database (https://plutof.ut.ee/, accessed on May 23rd 2020) were used. An OTU was assigned to a taxonomic level if the Protax identification probability was at least 95% and matched the taxonomy based on the massBLAST against the UNITE species hypothesis (SH) database. To support the taxonomic identification and to discard all non-fungal OTUs, a phylogenetic tree was built, which included all the OTUs with at least 50 reads in total. For building the tree, the ITS2 region was extracted with ITSx[56], aligned with MUSCLE[57] and a Neighbor-Joining phylogenic tree was constructed using MEGA7[58], with a p-distance, bootstrap of 1000 replicates, gamma distributed rates and gaps treated as pairwise deletions. As reference sequences, 29 different eukaryotes were selected from the ITS2 database[59] (http://its2.bioapps.biozentrum.uni-wuerzburg.de/, accessed on August 18th 2020 – accession numbers: AB084092, AY752993, HQ219352, GQ402831, AF228083, AF053158, AF163102, JN113133, AF353997, GU001158, JQ340345, AM396560, FJ946912, EU812490, KJ925151, KF772413, JX988759, AF315074, AJ400496, AJ566147, AY458037, AY479922, EF060369, FN397599, JF750409, KF524372, U65485, Z48468, AY499004). Eight references were further derived from GenBank (https://www.ncbi.nlm.nih.gov/genbank/, accessed on

September 29th 2020 - accession numbers: KC357673, AF508774, AY676020, MN158348, HM161704, AY264773, AB906385, AJ296818). These sequences cover different eukaryote lineages: Centroheliozoa, Choanoflagellida, Ichthyosporea, Oomycetes, Streptophyta, Chlorophyta, Rhodophyta, Cercozoa, Amoebozoa, Apusozoa, Cryptophyta, Haptophyceae, Heterolobosea, Katablepharidophyta, Arthropoda, Picozoa, Alveolata, Cnidaria, Stramenopiles, Protostomia and Porifera. Additionally, 530 fungal sequences (SHs from UNITE database) were selected to represent different fungal lineages and included the closest SH matches to each of the OTUs. Finally, to further establish higher level taxonomy, BLASTn searches (E-value cutoff of 1e-3, against the NCBI nucleotide database (https://www.uppmax.uu.se/resources/databases/blast-databases/) were run on September-October 2020, excluding uncultured organisms and environmental samples), for all the OTUs. The (at least) 10 best hits from the BLASTn search were evaluated as follows: an OTU was classified as a "likely fungi" if all resulting hits would match fungal sequences. The OTUs that had also matched other eukaryotic sequences were checked manually, and classified as a "likely fungi" if the best hits were fungal sequences, and also based on the quality of the alignments (higher query coverage and identity %). Any OTU that could not be classified as a "likely fungi" from the BLASTn searches and/or would cluster with any other eukaryote reference in the phylogenetic tree was discarded. The final taxonomic assignment of all fungal OTUs was based on matches to UNITE SHs and Protax probabilities. OTUs with undetermined phylum, class or order were assigned to higher taxonomic levels whenever supported by the phylogenetic tree (considering a minimal bootstrap value of 70%) or BLASTn results (min of 90% query coverage and 90% identity for class, and 100% query coverage and min 97% identity for order). The final data set with 1334 OTUs (178531 sequences) identified as fungi is presented in Supplementary Figure S2.

Code availability

The code used for the extraction of ITS2 regions using ITSx and for the Blastn searches are provided in Supplementary Note 1.

Received: 9 March 2021; Accepted: 29 July 2021;
Published online: 19 August 2021

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Acknowledgements

We thank the staff at the Toolik Field Station, Arctic Station, CEN W-K Station, Abisko Research Station and Khanymey Station, as well as Anelise Kluge, Gaëtan Martin and Martin Petersson for their assistance with the samplings. We offer our gratitude to the Cree and Inuit communities in Whapmagoostui-Kuujjuarapik for giving us access to their ancestral lands. Katarina Ihrmark, Maria Jonsson, Christoffer Bergvall and Anna Ferguson are acknowledged for their assistance with the laboratory analyses. We are grateful to the INTERACT and Science for Life Laboratory for funding. The computations were performed on resources provided by SNIC through Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX) under Project SNIC 2020-5-196.

Author contributions

M.K. planned the samplings together with C.W. and S.P., did the molecular laboratory work and sequence analyses and wrote the first draft of the manuscript together with S.P. C.W. planned the study together with S.P., planned the sampling together with M.K. and S.P., and participated in the collection of the samples. M.W. participated in the collection of the samples, analyzed carbon samples and data. K.E.C. instructed the amplicon processing. J.H. analyzed carbon samples and data. K.E. analyzed carbon samples and data. J.S. helped with the study design and data analysis. S.P. planned the study together with C.W., planned the samplings together with C.W. and M.K., participated in the collection of the samples and wrote the first draft of the manuscript together with M.K. All authors participated in the revision of the manuscript.

Funding
Open access funding provided by Swedish University of Agricultural Sciences.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41597-021-01005-7.

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Online-only Table 1 Description of the sampled sites and the number of samples with successful amplification of the ITS 2 region for each site and sample type. .

From: Community composition of aquatic fungi across the thawing Arctic

Research station	Month and year of sampling	Number of amplified samples	Permafrost type	Active thermo-karst	Climate zone	Annual precipi-tation	Annual mean temperature/July mean temperature	Altitude (a.s.l.)	Coordinates
Toolik Field Station, **Alaska**, USA	July, 2017	11/12 water 12 sediment 11 detritus	Continuous	No	Low Arctic	318 mm	−8.7 °C / 10.8 °C	720 m	68°37′ N 149°35′ W
Arctic Station, **Greenland**, Denmark	July, 2019	10/12 water 12 sediment 8 detritus	Discontinuous	No	Low/high Arctic	436 mm	−3.2 °C / 7.6 °C	20 m	69°15′ N 53°34′ W
CEN WK Station, Quebec, **Canada**	July, 2017	12/12 water 4 sediment	Sporadic Ponds represent emerging, developing and mature stages of permafrost thaw	Yes	Subarctic	648 mm	−4 °C / 12.7 °C	50 m	55°16′ N 77°45′ W
Abisko Research Station, **Sweden**	July, 2018	12/12 water 12 sediment 12 detritus	Discontinuous	Yes	Subarctic	310 mm	−0.6 °C / 11 °C	385 m	68°21′ N 18°49′ E
Khanymey Research Station, **Russia**	July, 2019	11/12 water 11 sediment	Discontinuous	Yes	Subarctic	436 mm	−6 to −3.5 °C / 15.5 to 17 °C	70 m	63°43′ N 75°57′ E

1. The stations are ordered according to increasing level of thermokarst activity. The coordinates are for the stations, the sample coordinates can be found at OSF[29]. The number of samples for water is showing the number of successfully amplified water samples for 0.2 and 5 μm filters, respectively. WK = Whapmagoostui-Kuujjuarapik Research Complex.

Back to article page ›

Scientific Data (*Sci Data*) ISSN 2052-4463 (online)

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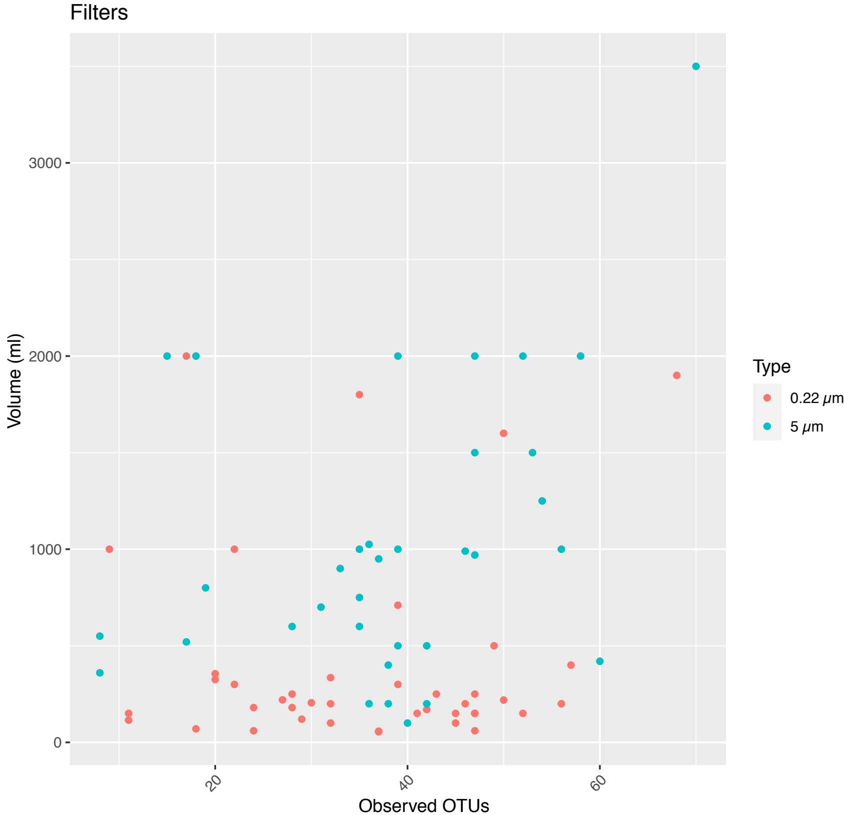
Supplementary Information



Supplementary Figure S1 – Plots of sampled volume (ml) x number of observed OTUs, per sample. Red dots are samples filtered with 0.22 µm filters, and blue dots represent 5 µm filters. Spearman correlations between sample volume and number of observed OTUs was 0.30 (p = 0.08) for 5 µm filtered samples, and 0.014 (p = 0.93) for 0.22 µl filters.

Supplementary Table S1 – The barcodes used for creating the sequencing libraries.

Primer_ID with tag name	Tag **(bold)** and primer sequences
Forward tagged primers:	
ITS3-mkmix2-A	**CATACCAGCAT**CAWCGATGAAGAACGCAG
ITS3-mkmix2-B	**CATCACGACAT**CAWCGATGAAGAACGCAG
ITS3-mkmix2-C	**CAAAGACCGAT**CAWCGATGAAGAACGCAG
ITS3-mkmix2-D	**CACAACACCAT**CAWCGATGAAGAACGCAG
ITS3-mkmix2-E	**CACTAGCACAT**CAWCGATGAAGAACGCAG
ITS3-mkmix2-F	**CACATGTCCAT**CAWCGATGAAGAACGCAG
ITS3-mkmix2-G	**CAGGATCACAT**CAWCGATGAAGAACGCAG
ITS3-mkmix2-H	**CAGGCAAGTAT**CAWCGATGAAGAACGCAG
Reverse tagged primers:	
ITS4-cwmix1-01	**CAAACCTCCA**TCCTCCGCTTAY*TG*ATAT*GC
ITS4-cwmix2-01	**CAAACCTCCA**TCCTCCGCTTAT*TR*ATAT*GC
ITS4-cwmix1-02	**CATGAACGAC**TCCTCCGCTTAY*TG*ATAT*GC
ITS4-cwmix2-02	**CATGAACGAC**TCCTCCGCTTAT*TR*ATAT*GC
ITS4-cwmix1-03	**CATCTCAGAC**TCCTCCGCTTAY*TG*ATAT*GC
ITS4-cwmix2-03	**CATCTCAGAC**TCCTCCGCTTAT*TR*ATAT*GC
ITS4-cwmix1-04	**CAATCTAGCC**TCCTCCGCTTAY*TG*ATAT*GC
ITS4-cwmix2-04	**CAATCTAGCC**TCCTCCGCTTAT*TR*ATAT*GC
ITS4-cwmix1-05	**CAACTAGGAC**TCCTCCGCTTAY*TG*ATAT*GC
ITS4-cwmix2-05	**CAACTAGGAC**TCCTCCGCTTAT*TR*ATAT*GC
ITS4-cwmix1-06	**CAAGCAGTGA**TCCTCCGCTTAY*TG*ATAT*GC
ITS4-cwmix2-06	**CAAGCAGTGA**TCCTCCGCTTAT*TR*ATAT*GC
ITS4-cwmix1-07	**CACTGAGGAA**TCCTCCGCTTAY*TG*ATAT*GC
ITS4-cwmix2-07	**CACTGAGGAA**TCCTCCGCTTAT*TR*ATAT*GC
ITS4-cwmix1-08	**CACAATGGTG**TCCTCCGCTTAY*TG*ATAT*GC
ITS4-cwmix2-08	**CACAATGGTG**TCCTCCGCTTAT*TR*ATAT*GC
ITS4-cwmix1-09	**CACGATGAGA**TCCTCCGCTTAY*TG*ATAT*GC
ITS4-cwmix2-09	**CACGATGAGA**TCCTCCGCTTAT*TR*ATAT*GC
ITS4-cwmix1-10	**CACGAACAAG**TCCTCCGCTTAY*TG*ATAT*GC
ITS4-cwmix2-10	**CACGAACAAG**TCCTCCGCTTAT*TR*ATAT*GC
ITS4-cwmix1-11	**CACGCACTAA**TCCTCCGCTTAY*TG*ATAT*GC
ITS4-cwmix2-11	**CACGCACTAA**TCCTCCGCTTAT*TR*ATAT*GC
ITS4-cwmix1-12	**CAGCTATGAG**TCCTCCGCTTAY*TG*ATAT*GC
ITS4-cwmix2-12	**CAGCTATGAG**TCCTCCGCTTAT*TR*ATAT*GC

Bases followed by * are PTO-protected nucleotides that prevent mismatch corrections by proof-reading polymerases.

Supplementary Note 1 – Code for extracting ITS2 regions with ITSx and Blastn searches.

ITSx command line:

```
ITSx -i 3108_OTUs_scata4743.fasta -o ITSx_3108_OTUs_scata4743 --save_regions all
```

Blastn command line:

```
blastn -db nt -query 3108_OTUs_scata4743.fasta -negative_gilist sequence.gi -evalue 1e-3 -max_target_seqs 10 -max_hsps 10 -out Result_blastn_OTUs_scata4743 -outfmt "6 qseqid sseqid pident length mismatch gapopen qstart qend sstart send evalue bitscore staxids sscinames scomnames sblastnames sskingdoms stitle qcovs"
```

Supplementary Table S2. Scheme of the three pools of samples and the number of PCR cycles used to create the sequencing libraries.

Pool 1

A - 1	T1 0.2 31c	A - 2	SAS2C 5 28c	A - 3	R10 0.2 28c	A - 4	GR3 5 28c	A - 5	P1 sed 21c	A - 6		A - 7	T2 d 28c	A - 8	T9 sed 22c	A - 9	GR3 sed 21c	A - 10	P10 5 20c	A - 11	P2 d 22c	A - 12	
B - 1	T3 0.2 28c	B - 2	B4 5 28c	B - 3	R3 5 28c	B - 4	GR6 5 28c	B - 5	P2 sed 20c	B - 6		B - 7	T4 d 28c	B - 8	T10 sed 22c	B - 9	GR4 sed 21c	B - 10	P11 5 21c	B - 11	P4 d 25c	B - 12	
C - 1	T4 0.2 28c	C - 2	P9 0.2 28c	C - 3	R7 5 23c	C - 4	GR8 5 28c	C - 5	P3 sed 23c	C - 6		C - 7	T7 d 28c	C - 8	T11 sed 25c	C - 9	GR5 sed 23c	C - 10	6 sed 21c	C - 11	P5 d 23c	C - 12	
D - 1	T9 0.2 35c	D - 2	P9 5 29c	D - 3	GR1 0.2 28c	D - 4	P1 0.2 24c	D - 5	P9 sed 23c	D - 6		D - 7	T8 d 28c	D - 8	T12 sed 25c	D - 9	GR6 sed 23c	D - 10	9 pond C2 sed 22c	D - 11	P6 d 25c	D - 12	
E - 1	T1 5 31c	E - 2	P12 5 28c	E - 3	GR2 0.2 28c	E - 4	P4 0.2 27c	E - 5	R3 sed 20c	E - 6		E - 7	GR1 d 28c	E - 8	B4 0.2 29c	E - 9	I1 5 25c	E - 10	10 pond B1 sed 22c	E - 11		E - 12	
F - 1	T2 5 28c	F - 2	R8 0.2 8c	F - 3	GR3 0.2 28c	F - 4	P8 0.2 27c	F - 5	R5 sed 20c	F - 6		F - 7	GR2 d 28c	F - 8	C2 0.2 27c	F - 9	I3 5 25c	F - 10	11 sed 22c	F - 11		F - 12	
G - 1	T3 5 27c	G - 2	R4 0.2 28c	G - 3	GR4 0.2 28c	G - 4	R4 5 25c	G - 5	R6 sed 23c	G - 6		G - 7	GR5 d 28c	G - 8	C5 0.2 29c	G - 9		G - 10	neg	G - 11		G - 12	
H - 1	T5 5 24c	H - 2	R7 0.2 28c	H - 3	GR2 5 31c	H - 4	R2 5 24c	H - 5	R7 sed 23c	H - 6		H - 7	GR6 d 28c	H - 8	I1 0.2 25c	H - 9		H - 10		H - 11		H - 12	

Pool 2

A - 1	T2 0.2 28c	A - 2	T4 sed 27c	A - 3	SAS2A 5 26c	A - 4	P11 0.2 28c	A - 5	R1 5 26c	A - 6	12 pond G1 sed 20c	A - 7	GR9 5 30c	A - 8	P7 5 24c	A - 9		A - 10	T3 d 31c	A - 11	T1 d 31c	A - 12	
B - 1	T5 0.2 28c	B - 2	T5 sed 25c	B - 3	SAS2B 5 25c	B - 4	P1 5 25c	B - 5	R6 5 21c	B - 6	14 sed 21c	B - 7	GR10 5 30c	B - 8	R10 sed 25c	B - 9		B - 10	T5 d 28c	B - 11	GR3 d 31c	B - 12	
C - 1	T6 0.2 28c	C - 2	T7 sed 27c	C - 3	SAS2D 5 25c	C - 4	P3 5 25c	C - 5	R8 5 22c	C - 6	19 sed 22c	C - 7	GR11 5 31c	C - 8		C - 9	neg	C - 10	T6 d 25c	C - 11		C - 12	
D - 1	T7 0.2 31c	D - 2	T8 sed 27c	D - 3	G1 5 28c	D - 4	P8 5 27c	D - 5	R11 5 25c	D - 6	P10 sed 21c	D - 7	GR12 5 32c	D - 8		D - 9		D - 10	P1 d 25c	D - 11		D - 12	
E - 1	T4 5 25c	E - 2	SAS2A 0.2 31c	E - 3	1 sed 28c	E - 4	R1 0.2 28c	E - 5	GR11 0.2 Failed*	E - 6	P11 sed 22c	E - 7	GR12 sed 21c	E - 8		E - 9		E - 10	P3 d 25c	E - 11		E - 12	
F - 1	T7 5 29c	F - 2	SAS2B 0.2 28c	F - 3	P2 0.2 27c	F - 4	R3 0.2 25c	F - 5	GR9 0.2 31c	F - 6	R8 sed 21c	F - 7	GR7 sed 22c	F - 8		F - 9		F - 10	P7 d 26c	F - 11		F - 12	
G - 1	T8 5 28c	G - 2	SAS2C 0.2 31c	G - 3	P3 0.2 29c	G - 4	R9 0.2 25c	G - 5	GR10 0.2 28c	G - 6	R11 sed Failed	G - 7	GR8 sed 22c	G - 8		G - 9	P12 d 25c	G - 10	GR4 d Failed	G - 11		G - 12	
H - 1	P4 sed 28c	H - 2	SAS2D 0.2 31c	H - 3	P5 0.2 28c	H - 4	R2 0.2 29c	H - 5	GR12 0.2 Failed	H - 6	R12 sed 21c	H - 7	GR9 sed 22c	H - 8		H - 9	P12 sed 25c	H - 10	GR10 d Failed	H - 11	GR12 d 30c	H - 12	

Pool 3

A - 1	T6 5 22c	A - 2	C5 5 23c	A - 3	P5 5 26c	A - 4	R5 5 22c	A - 5	GR11 sed 22c	A - 6	T10 0.2 28c	A - 7	P2 5 20c	A - 8		A - 9	P12 0.2 31c	A - 10		A - 11	T9 d 28c	A - 12	GR7 d 30c
B - 1	T1 sed 25c	B - 2	H1 5 232c	B - 3	P6 5 23c	B - 4	R12 5 22c	B - 5	GR5 0.2 28c	B - 6	T11 0.2 28c	B - 7	P4 5 22c	B - 8		B - 9	T8 0.2 Failed	B - 10		B - 11	T10 d 28c	B - 12	GR8 d Failed
C - 1	T2 sed 22c	C - 2	8 pond C5 sed 23c	C - 3	P5 sed 25c	C - 4	R1 sed 25c	C - 5	GR6 0.2 28c	C - 6	T12 0.2 31c	C - 7	P10 0.2 22c	C - 8	G1 0.2 27c	C - 9	neg	C - 10		C - 11	T11 d 28c	C - 12	GR9 d 28c
D - 1	T3 sed 25c	D - 2	16 sed 25c	D - 3	P6 sed 28c	D - 4	R2 sed 25c	D - 5	GR7 0.2 25c	D - 6	T9 5 28c	D - 7	GR10 sed 20c	D - 8	H1 0.2 25c	D - 9		D - 10		D - 11		D - 12	GR11 d Failed
E - 1	T6 sed 26c	E - 2	17 sed 25c	E - 3	P7 sed 25c	E - 4	R4 sed 25c	E - 5	GR8 0.2 25c	E - 6	T10 5 28c	E - 7	GR1 5 31c	E - 8		E - 9		E - 10		E - 11	P8 d 22c	E - 12	
F - 1	B1 0.2 26c	F - 2	21 "tiny" sed 25c	F - 3	P8 sed 25c	F - 4	R9 sed 25c	F - 5	R5 0.2 25c	F - 6	T11 5 25c	F - 7	GR4 5 32c	F - 8		F - 9		F - 10		F - 11	P9 d 24c	F - 12	
G - 1	B1 5 23c	G - 2	P6 0.2 27c	G - 3	R9 5 29c	G - 4	GR1 sed 25c	G - 5	R6 0.2 28c	G - 6	R11 0.2 Failed	G - 7	GR5 5 31c	G - 8		G - 9		G - 10		G - 11	P10 d 24c	G - 12	
H - 1	C2 5 23c	H - 2	P7 0.2 27c	H - 3	R10 5 22c	H - 4	GR2 sed 25c	H - 5	I3 0.2 27c	H - 6	R12 0.2 29c	H - 7	GR7 5 32c	H - 8		H - 9		H - 10		H - 11	P11 d 24c	H - 12	

*samples that did not amplify and were left out of the pools for sequencing

III

Received: 27 April 2021 | Accepted: 3 August 2021
DOI: 10.1111/gcb.15852

WILEY

PRIMARY RESEARCH ARTICLE

Declining fungal diversity in Arctic freshwaters along a permafrost thaw gradient

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Funding information
Science for Life Laboratory; INTERACT

Abstract

Climate change–driven permafrost thaw has a strong influence on pan-Arctic regions, via, for example, the formation of thermokarst ponds. These ponds are hotspots of microbial carbon cycling and greenhouse gas production, and efforts have been put on disentangling the role of bacteria and archaea in recycling the increasing amounts of carbon arriving to the ponds from degrading watersheds. However, despite the well-established role of fungi in carbon cycling in the terrestrial environments, the interactions between permafrost thaw and fungal communities in Arctic freshwaters have remained unknown. We integrated data from 60 ponds in Arctic hydro-ecosystems, representing a gradient of permafrost integrity and spanning over five regions, namely Alaska, Greenland, Canada, Sweden, and Western Siberia. The results revealed that differences in pH and organic matter quality and availability were linked to distinct fungal community compositions and that a large fraction of the community represented unknown fungal phyla. Results display a 16%–19% decrease in fungal diversity, assessed by beta diversity, across ponds in landscapes with more degraded permafrost. At the same time, sites with similar carbon quality shared more species, aligning a shift in species composition with the quality and availability of terrestrial dissolved organic matter. We demonstrate that the degradation of permafrost has a strong negative impact on aquatic fungal diversity, likely via interactions with the carbon pool released from ancient deposits. This is expected to have implications for carbon cycling and climate feedback loops in the rapidly warming Arctic.

KEYWORDS
aquatic fungi, Arctic, dissolved organic matter, fungal diversity, permafrost thaw, thermokarst ponds

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1 | INTRODUCTION

Climate change–induced increase in annual mean temperature is particularly affecting the Arctic and Subarctic landscapes, as the temperature in the North is increasing 2–3 times faster than the global mean temperature (Post et al., 2019). In the Arctic regions, this increase in temperature is causing permafrost thaw, which in return is inducing a mobilization of a large fraction of the vast carbon storage in the permafrost (Schuur et al., 2015). In fact, the frozen surface tundra soils store about 1035 gigatons (Gt) of carbon, which is twice as much as the carbon present in the atmosphere (Hugelius et al., 2014), and 120 Gt of this stored carbon is expected to be released as greenhouse gases (GHGs) by 2100 (Schaefer et al., 2014). These GHGs are further accelerating the global warming and causing a vicious circle that is threatening the existence of the Arctic environments. One result of the erosion and collapse of the permafrost is the emergence of the so-called thermokarst ponds and lakes (Vonk et al., 2015), which receive large inputs of recently thawed organic matter and nutrients as the degradation of the landscape progresses (In 't Zandt et al., 2020; Serikova et al., 2019). This change in the environment has a large impact on the biota of the region, including the microorganisms that experience a drastic change in the quality and quantity of the substrates available for them to metabolize.

The dissolved organic matter (DOM) found in the thermokarst lakes and ponds is dominated by allochthonous organic carbon (Wauthy et al., 2018). Although part of the DOM may sink and be stored in the sediment, a large part of it is recycled in the microbial loop, generating GHGs such as carbon dioxide (CO_2) and methane (CH_4) as end products (Negandhi et al., 2013). Thus, microbial metabolism in thermokarst ponds has a key role in venting out the GHGs and determining the fate of the carbon released from thawing permafrost. This makes the thermokarst pond hotspots in carbon cycling, and, consequently, key environments for the climate change. Although most of the research carried out on thermokarst ponds has focused on prokaryotes (Peura et al., 2020; Vigneron et al., 2019), little is known about the role of microeukaryotes, such as fungi, which may have a central role in degrading especially the allochthonous fractions of DOM (Grossart & Rojas-Jimenez, 2016) released from the thawing watershed. Although our knowledge about the fungi in aquatic environments is sparse, global estimates suggest that the amount of carbon cycled via fungi is as large as the amount cycled through the bacteria (Bar-On et al., 2018), highlighting the urgency to study the fungal communities and their interactions with the environment.

Generally, both quality and quantity of the substrates have a significant impact on the composition of the microbial community (Peura et al., 2020). In aquatic environments, prokaryotes are the main decomposers of labile and/or low-molecular-weight DOM (Berggren et al., 2010; Mostofa et al., 2013). However, bacteria are not efficient in degrading semilabile terrigenous DOM (Herlemann et al., 2014), whereas fungi are known for their key role in the decomposition of plant debris, including particulate organic matter (Canhoto et al., 2016), thereby providing carbon substrates for the microbial loop (Fabian et al., 2017; Roberts et al., 2020). In addition, by the production of oxidative enzymes, such as laccases and peroxidases, some freshwater fungi can degrade large and complex polymers, including humic substances, that dominate permafrost carbon (Wauthy et al., 2018), and even transform these into more aromatic compounds (Masigol et al., 2019). Considering the variation in the metabolic capacity of fungi, it can be expected that the quality of the carbon has a pronounced impact on the fungal community composition. Despite the known importance of fungi in the initial decomposition of organic matter, the composition of fungal communities in aquatic environments is poorly understood, and their functional and ecological roles are drastically understudied (Grossart et al., 2019). Furthermore, there are no studies addressing how the change in the quality and quantity of the carbon compounds available for the aquatic fungi following the permafrost thaw affects these key degraders.

The few studies that have investigated fungal communities in circumpolar landscapes are mostly focused on soil, and members of the Helotiales order have been found to be dominant, both in Arctic and Antarctic soils (Deslippe et al., 2012; Geml et al., 2015). Helotiales is one of the most species-rich orders of Ascomycota and includes various life strategies, such as saprotrophs, pathogens, endophytes, mycorrhizal fungi, and aquatic hyphomycetes (Grossart et al., 2019). In Antarctic permafrost soils, members of Helotiales possess cellulases capable of degrading ancient carbon more efficiently than other taxa (Newsham et al., 2018). Considering the prevalence and decomposer role of Helotiales in permafrost soils, it can be hypothesized that they may be important players also in thermokarst ponds and their sediments. To our knowledge, no studies on Helotiales in such environments exist, but one of the few studies addressing fungal communities in thaw ponds found Ascomycota to be the dominant phylum followed by a large number of unclassified fungi, but the finer taxonomic levels were not evaluated (Wurzbacher et al., 2017). Thus, given the importance of thermokarst ponds for recycling terrigenous organic matter in Arctic regions (Roiha et al., 2016), more studies are needed to dissect fungal communities in aquatic habitats before we can understand how fungal communities and their decomposer activities interact with nutrient and carbon cycling in this dynamic environment.

In this study, we investigated how fungal diversity and community composition in Arctic ponds are impacted by permafrost thaw and the related changes in carbon quality, and carbon and nutrient availability. We also explored whether dominant fungal taxa have a pan-Arctic distribution or show habitat specificity. We collected samples from a total of 60 ponds from five hydro-ecosystems representing different stages of permafrost integrity and located in five different regions across the Arctic, encompassing Alaska, Greenland, Canada, Sweden, and Western Siberia. Sites in Alaska and Greenland had relatively intact permafrost, without thermokarst, and are hereafter referred to as pristine sites. The other three sites, located in Canada, Sweden, and Western Siberia, represented a gradient toward more degraded permafrost. Twelve ponds were sampled at each site. In addition, at the Canadian site, the sampled ponds

displayed a developmental stage gradient, including emerging, developing, and mature ponds, representing pond development up to 60 years after permafrost collapse (Wurzbacher et al., 2017). This allowed us to investigate the community succession over pond development, as the quality and availability of carbon sources change under the same climatic and permafrost regimes. Pond water and sediments were sampled for fungal community analyses using ITS amplicon sequencing. Environmental variables, including nutrient concentrations and organic carbon quantity and quality, were further analyzed from the water. As carbon quality has been shown to be an important determinant of bacterial community composition (Peura et al., 2020), we hypothesized that also the fungal community composition and diversity have clear connections to DOM quality, which, in turn, is directly linked with the degradation status of the permafrost and the pond developmental stage. Such links have important implications on carbon cycling and climate feedback loops in warming Arctic regions.

2 | MATERIALS AND METHODS

2.1 | Study sites

We sampled a gradient of ponds representing various stages of permafrost degradation in the following five sites: Toolik, Alaska, USA; Qeqertarsuaq, Disko Island, Greenland, Denmark; Whapmagoostui-Kuujjuarapik, Nunavik, Quebec, Canada; Abisko, Sweden and Khanymei, Western Siberia, Russia (Table 1, Figure 1, Figure S1). The aim was to include representatives of different stages of permafrost integrity to understand whether responses can be generalized across different geographic and environmental conditions. A detailed description of the study sites can be found in Kluge et al. (2021).

Shortly, the sampling site in Alaska is located in a continuous permafrost area, mostly dominated by moss tundra. The average active layer depth for the sampling year (2017) was ~50 cm (Shaver & Rastetter, 2019), and there were no signs of a warming trend or increase in the thickness of the active layer in the area (Hobbie et al., 2017). The site in Greenland is located in the Blæsedalen Valley, south of Disko Island, and is characterized as a discontinuous permafrost area mostly dominated by wet sedge tundra (Christiansen et al., 2017). An increase in mean air annual temperature of 0.2°C per year has been observed from 1991 to 2001 in the region (Hollesen et al., 2015).

The Canadian site is a palsa bog located in a river valley in Hudson Bay, within a sporadic permafrost zone and surrounded by coastal forest tundra (Bhiry et al., 2011). Since the mid-1990s, there has been a significant increase in the surface air temperature of the region (0.5–0.9°C per decade) (Hochheim & Barber, 2014), and the area has experienced an accelerated thawing of the permafrost over the past decades, including collapsing of palsa and emergence of thermokarst ponds (Bhiry et al., 2011) (Figure 1). The thermokarst ponds at this site represent different developmental stages of

TABLE 1 Description of the sampling sites

Research station	Month and year of sampling	Permafrost type	Active thermokarst	Climate zone	Annual precipitation (mm)	Annual air mean temperature/ July air mean temperature (°C)	Altitude (m, a.s.l.)	Coordinates	Mean distance and range among the ponds (m)
Toolik Field Station, Alaska, USA	July, 2017	Continuous	No	Low Arctic	318	−8.7/10.8	720	68°37′N 149°35′W	8791 53.8–19,963
Arctic Station, Disko Island, Greenland, Denmark	July, 2019	Discontinuous	No	Low/high Arctic	436	−3.2/7.6	20	69°15′N 53°34′W	261.5 12.7 – 571.2
CEN WK Station, Nunavik, Quebec, Canada	July, 2017	Sporadic	Yes	Subarctic	648	−4/12.7	50	55°16′N 77°45′W	98.1 3.1–249.2
Abisko Research Station, Abisko, Sweden	July, 2018	Discontinuous	Yes	Subarctic	310	−0.6/11	385	68°21′N 18°49′E	80.5 6–231.1
Khanymey Research Station, Western Siberia, Russia	July, 2019	Discontinuous	Yes	Subarctic	436	−6 to −3.5/15.5 to 17	70	63°43′N 75°57′E	415.5 16–1796.7

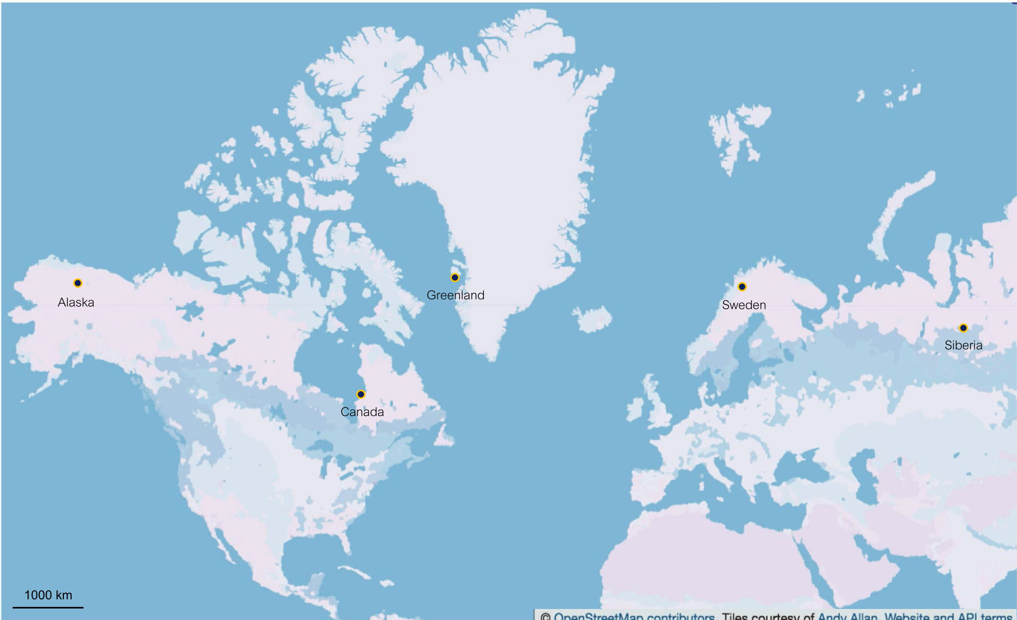

FIGURE 1 Map showing the sampled locations and an emerging pond at the Canadian site

permafrost thaw, including recently emerged, developing and mature thermokarstic waterbodies. To estimate the stage of the pond, we used the distance between the pond and the edge of the palsa (for emerging and developing ponds) or satellite pictures (mature ponds) (Wurzbacher et al., 2017). The emerging ponds had a maximum distance of 1 m from the palsa, were less than 0.5 m deep, and were estimated to be 2–5 years old; whereas the developing ponds were 2–3 m away from the palsa, were approximately 1 m deep, and were estimated to be a maximum of 10 years old. Mature ponds at the site were up to 60 years old based on satellite imaging and about 2 m deep. For each of the stage classes, four ponds were sampled.

The Swedish site is located at the Stordalen palsa mire dominated by sedges (*Eriophorum vaginatum*, *Carex* sp.) and mosses (*Sphagnum* sp.) (Olefeldt et al., 2012) and located on a discontinuous permafrost zone. The sampling location is a collapsed peatland affected by active thermokarst development, where active layer thickness has been increasing since the 1980s, following an increase of ~1.5°C in annual air mean temperature since 1974 (Swindles et al., 2015). The

Siberian site is a flat, frozen palsa bog affected by active thermokarst development, resulting in the emergence of thermokarst ponds (Pokrovsky et al., 2014) and located in a discontinuous permafrost area in the Western Siberia Lowland. The vegetation is dominated by lichens and *Sphagnum* mosses (Loiko et al., 2019). In this study, the Canadian, Swedish, and Siberian sites with active thermokarst development are referred as "degraded." The different pond developmental stages were only observed at the Canadian site. For the other sites, ponds were very similar in size and were no deeper than 0.5 m, and, thus, are expected to represent the same stage of permafrost degradation within each site.

2.2 | Experimental design and sampling

A total of 12 ponds were sampled for each site, and all samplings were conducted in July (Table 1). Surface-water samples were collected in duplicates from the middle of each pond for molecular analyses. The water was filtered sequentially through 5 and 0.22-µm pore size Durapore and Sterivex filters (Millipore), respectively, until clogging or up to a maximum of 3.5 L. The two pore sizes were used for maximal capture of fungal cells of different sizes. After the collection of the water samples, surface sediments from the bottom of each pond were collected for molecular analyses using gloved hands, at a water depth of approximately 20 cm. For the Canadian site, however, only one emerging and three developing ponds were sampled for sediments. The filters and sediments were transported frozen, except the Alaskan samples that were freeze-dried at the site. All other filters and sediments were freeze-dried immediately on arrival to the laboratory in Sweden. Freeze-dried samples were stored at room temperature in a silica desiccator for a maximum of 6 months until DNA extraction. The extractions were performed in batches, per sample site, in a laminar flow hood with an UV-C lamp, and the extracted DNA was stored at −80°C until amplification. We also collected water samples to analyze the chemical conditions in the ponds, including pH, nitrate (NO_3^-), total phosphorus (TP), total dissolved iron (Fe), and dissolved organic carbon (DOC) concentrations; and to carry out optical and mass spectrometry analyses to investigate carbon quality. Samples for chemical analyses were transported frozen to the laboratory, with the exception of samples collected for DOC and fluorescence analyses, which were refrigerated.

2.3 | Molecular and chemical analyses

The details of DNA extraction and PCR amplification are described in Kluge et al. (2021). Briefly, the water and sediment samples were eluted in 100 µl of Milli-Q water and extracted with DNeasy PowerSoil® kit (Qiagen), following the manufacturer's recommendations for low-input DNA. For the PCR amplification and sequencing, samples were first randomized into three groups, which included samples from all sites to minimize the risk of batch effect. The fungal ribosomal internal transcribed spacer 2 (ITS2) sequences were amplified using a modified ITS3 Mix2 forward primer from Tedersoo et al. (2015), named ITS3-mkmix2 CAWCGATGAAGAACGCAG, and a reverse primer ITS4 (equimolar mix of cwmix1 TCCTCCGCTTAyTgATAtGc, and cwmix2 TCCTCCGCTTAtTrATAtGc) from Wurzbacher et al. (2017), each sample receiving a unique combination of primers. PCR reactions were performed on a final volume of 50 µl, with an input amount of DNA ranging from 0.07 to 10 ng, using Phusion™ High-Fidelity DNA Polymerase (Thermo Fisher Scientific). The PCRs consisted of an initial denaturation cycle at 95°C for 3 min, followed by 21–35 cycles for amplification (95°C for 30 s, 57°C for 30 s, and 72°C for 30 s), and final extension at 72°C for 10 min. All samples were run in duplicates, first using 21 amplification cycles, and, in case of insufficient yield, the number of cycles was increased up to 35 cycles (for details, see Kluge et al., 2021). The products were purified with Sera-Mag™ beads (Merck), quantified using Qubit dsDNA HS kit and then randomly allocated into three equimolar DNA pools (20 ng of each sample), which were purified with E.Z.N.A.® Cycle-Pure kit (Omega Bio-Tek). For five of the samples, we were unable to produce a PCR product, and these samples could not be included to the pools. In addition, negative controls from the PCR and a positive control containing a mock community were included (for details of the mock community, see Kluge et al., 2021). The libraries were sequenced at Science for Life Laboratory (Uppsala University, Sweden), on a Pacific Biosciences Sequel instrument, using 1 SMRT cell per pool.

The quantification of NO_3^-, TP, Fe, and DOC concentrations were carried out as described in Kluge et al. (2021). In addition, several proxies of carbon quality and composition were analysed using optical analyses and mass spectrometry, including fluorescence index (FI), humification index (HIX), freshness index (BIX), spectral slope for the intervals 279–299 nm (S289), specific ultraviolet absorbance (SUVA254) and hydrogen-to-carbon ratio (H/C). FI investigates the origin of fulvic acids (McKnight et al., 2001), while HIX and BIX are proxies of the humic content and the relative freshness of the DOM, respectively (Huguet et al., 2009). SUVA254 is a proxy of aromaticity and the relative proportion of terrestrial versus algal carbon sources in DOM (Weishaar et al., 2003), and S289 indicates the share of fulvic and humic acid compounds related to algal production (Loiselle et al., 2009). H/C can be used as a proxy of DOM aliphatic content (Riedel et al., 2016). More details about these proxies and their respective calculations are available in Kluge et al. (2021). The pH of surface water was measured on site using a ProDSS probe (YSI—Swedish and Siberian samples), a RBRconcerto probe (RBR—Alaskan samples), or colorimetric strips (Greenland). The pH was not measured at the Canadian site, and the pH values used in this study are from a previous sampling campaign from 2014, when the same ponds were sampled following the same methods as the Alaskan samples (see Wurzbacher et al., 2017).

2.4 | Processing of the reads, clustering, and taxonomy identification

Sequences were filtered for quality and clustered using the SCATA pipeline (https://scata.mykopat.slu.se/, accessed on May 19, 2020).

The filtering, clustering, and taxonomical assignment of the reads are described in detail in Kluge et al. (2021). Briefly, a minimum of 90% match for the primers and a 100% match for the tags were required. Reads shorter than 100 bp or a mean quality lower than 20 were removed, as well as reads containing bases with a quality lower than 7. The parameters used for clustering were clustering distance of 0.015, minimum alignment to consider of 0.85, maximum penalty of 1, gap open penalty of 0, gap extension penalty of 1, end gap weight of 0.0, collapse homopolymers 3, and removing singletons. Only operational taxonomic units (OTUs) with a minimum of 10 total reads were kept for the taxonomic identification. Taxonomy assignments were done with the Protax-Fungi server (Abarenkov et al., 2018) using 95% identification probability cutoff combined with massBLASTer analyses from the Pluto-F platform using best matches to species hypothesis (SH) in the UNITE database (https://plutof.ut.ee/, accessed on May 23, 2020). An OTU was assigned to a taxonomic level if the Protax-Fungi identification and massBLASTer results were in agreement. In addition, a BLASTn search was done with all the OTUs, and at least the 10 best hits were evaluated. All OTUs matching only with fungi as well as OTUs matching also to other eukaryotic sequences but displaying their best hits with fungi were classified as "likely fungi." Additionally, the phylogenetic placement of some of the abundant OTUs was verified using a Neighbor-Joining tree (for detail see Kluge et al., 2021). OTUs with undetermined phyla, class, or order based on Protax-Fungi and SH searches were assigned to those higher taxonomic levels if supported by the phylogeny results and BLASTn results. After OTU filtering, any sample that resulted in less than a total of 100 reads was excluded from all the analyses.

2.5 | Statistical analyses

All statistical analyses were performed using R v 4.0.2. (*R: The R Project for Statistical Computing*, n.d.) using functions from the *vegan* (Dixon, 2003) and *phyloseq* packages (McMurdie & Holmes, 2013). Two subsets of samples from all sites were analysed based on sample type: one containing all surface-water samples, from both filters (0.22 and 5 μm pore sizes), and one with only sediment samples. Another subset encompassing only water samples from Canada (from both filters) was also created to investigate the community succession over pond development.

Following rarefaction to even sequencing depth, Venn diagrams were used to visualize the number of shared OTUs across sites and sample types using the *MicEco* package (Russel, 2020). In addition, for rarefied subsets, alpha diversity was estimated with Shannon, Simpson's, and Pielou's evenness, number of observed OTUs and Chao1 richness estimates. Differences in alpha diversity indices between sites and pond stages (for the Canadian water samples only) were analyzed by Kruskal–Wallis tests, with pairwise comparisons using Dunn's post hoc test. Spearman correlations were carried out to evaluate whether the number of samples per site or the filtered volume would correlate to diversity indices (as described in more detail in Kluge et al., 2021).

Beta diversity, in terms of the variability in species composition among ponds for each site or pond stage (Anderson et al., 2006), was assessed through the analyses of the multivariate homogeneity of group dispersions (i.e., deviations of each sample from the group centroid) by a permutational analysis of multivariate dispersions (PERMDISP, betadisper function of *vegan* package), and differences between groups were verified by the permutest function of *vegan* package. Beta diversity was also visualized with nonmetric multidimensional scaling (NMDS). All beta diversity analyses were based on Bray–Curtis dissimilarities calculated after Hellinger transformation of the nonrarefied subsets. For all subsets of water samples, chemical variables were fitted into the NMDS plots (ordisurf function from the *vegan* package) to vizualize their variation across the permafrost integrity gradient. To assess the contribution of categorical (e.g., site and pond age) and chemical variables to fungal community variations, a permutational multivariate analysis of variance (PERMANOVA; Anderson, 2017) was conducted on the nonrarefied subsets of water samples using Bray–Curtis distance as dissimilarity index, and the number of permutations was fixed at 1000. Collinearity between variables was tested using Spearman's rank correlation and highly correlated variables (Spearman's $\rho^2 > .70$) were excluded from the model. Mantel tests with 9999 permutations were conducted to evaluate the correlation (Spearman) between fungal community composition (Bray–Curtis distance matrix) of pond water samples and environmental variables (Euclidean distance matrices). The BIOENV function (*vegan* package) was used to examine the highest Spearman rank correlation between these variables and the community matrix. Because the geographical distance within ponds can affect the beta diversity of a site, Mantel correlograms were used to test spatial autocorrelation for each site. To compare DOM-related variables and pH between the sites and pond stages, nonparametric Kruskal–Wallis (with Dunn's post hoc test) or parametric one-way ANOVA (with Tukey's honestly significant difference post hoc test) tests were performed, depending on normality and homogeneity of variance assumptions for each of the variables.

Additionally, the 50 most abundant OTUs (based on total relative abundance, nonrarefied data sets) for each site (water and sediment subsets, containing all sites) were considered to be dominant OTUs. From those dominant OTUs, the 10 most abundant OTUs in the water samples for each site were selected to build a heatmap with the heatmap.2 function of the *gplots* package (Warnes et al., 2020). Here, abundance of OTUs per site, given in *Z*-score standardized to total number of reads for each OTU (based on rarefied subset), was correlated (Spearman rank correlation) with DOM-related variables (H/C, S289, BIX, FI, SUVA254, HIX and DOC). A summary of all used subsets, including rarefaction, is found in Table S1.

3 | RESULTS

After quality control and filtering, the data set included a total of 1280 OTUs, represented by 142778 reads across 142 samples. In the rarefied data, each site had its own characteristic community, and

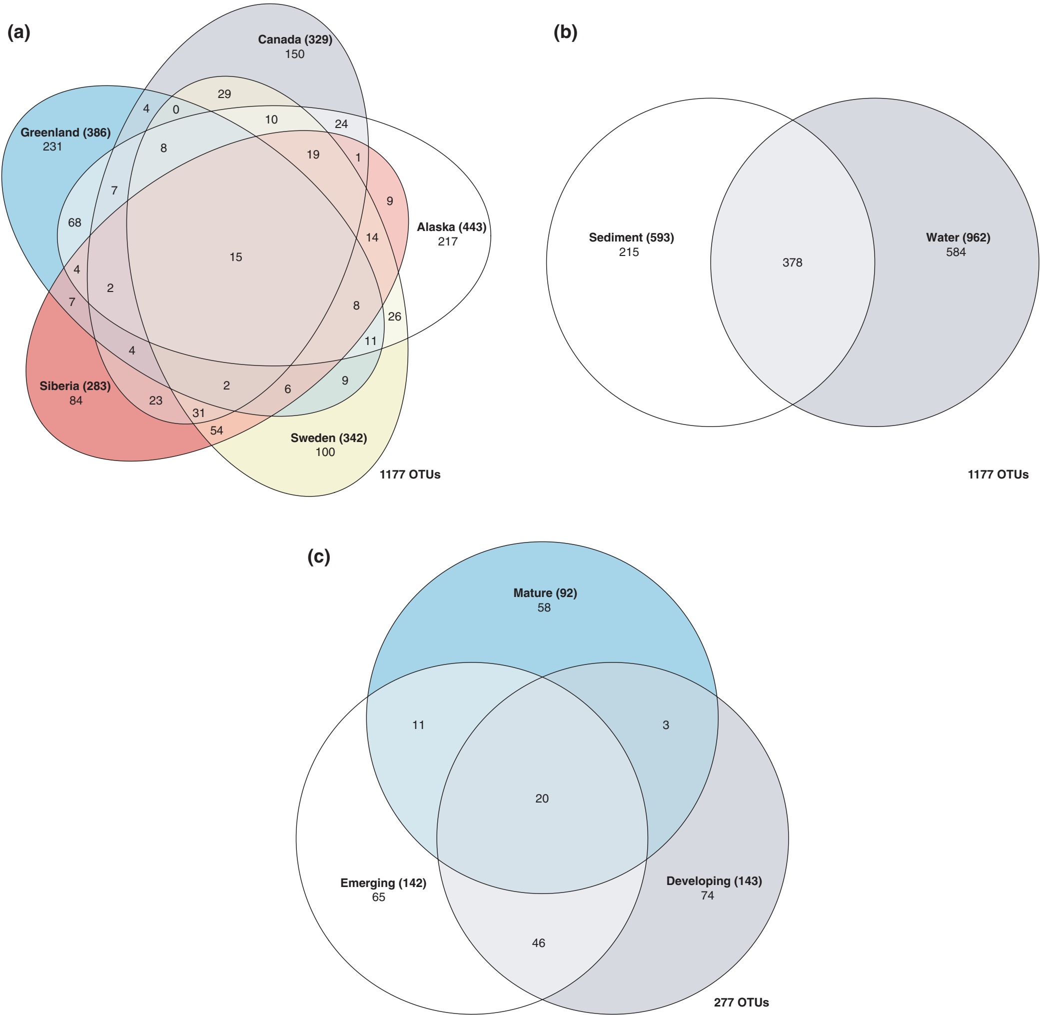

FIGURE 2 Venn diagrams of the shared OTUs across sites (a), by sample type (b), and by pond developmental stage for the Canadian water samples (c), based on rarefied datasets. Numbers in parenthesis indicate the total number of OTUs for each group

only 15 OTUs (1.3% of the total number of OTUs; 11.65% of total sequences) were present at all sites (Figure 2a). Greenland had the highest proportion of site-specific OTUs (OTUs present only at one site) with 60% of OTUs being site-specific (Figure 2a). The lowest number of site-specific OTUs were found at Swedish and Siberian sites (29% and 30%, respectively). Alaska and Greenland had the highest number of OTUs exclusively shared between two sites, followed by Sweden and Siberia (Figure 2a). The water and sediment samples shared 30% of the OTUs (Figure 2b). When Canadian water samples were analyzed alone (Figure 2c), only 7.2% of the OTUs were shared across all ponds, with mature ponds having the highest proportion of stage-specific OTUs (63% of all OTUs in mature ponds only present in mature ponds).

The alpha diversity of fungal communities was similar in both the water and sediments across all sites (Figure S2). The most degraded site (Siberia) had somewhat lower values in the sediments, but this difference was not significant. Regarding the developmental stage, the ponds from the Canadian site suggested a decreasing number of species as the ponds aged, but, again, the difference was not significant (Figure S3).

Beta diversity was illustrated in an NMDS plot based on Bray–Curtis distances of all samples (Figure 3). The samples clustered

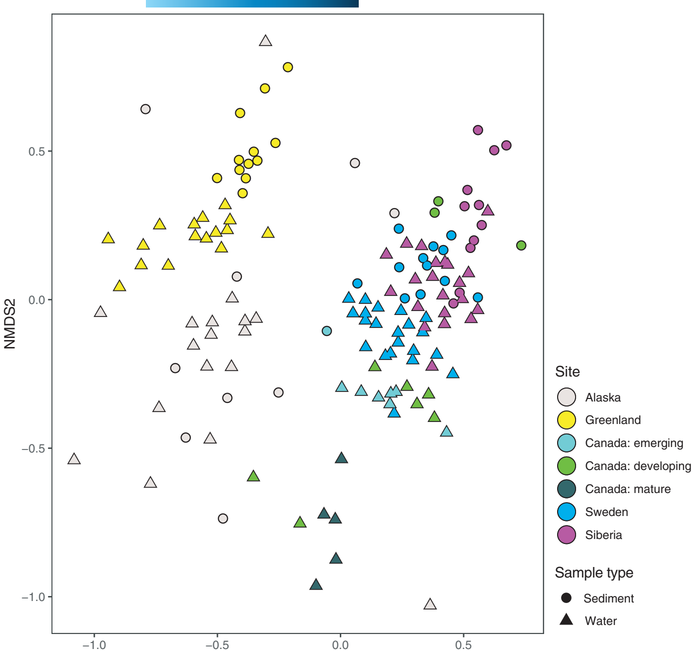

FIGURE 3 Nonmetric multidimensional scaling plot displaying beta diversity by Bray–Curtis dissimilarities (based on Hellinger-transformed abundances) for fungal communities of water and sediment samples (stress = 0.198)

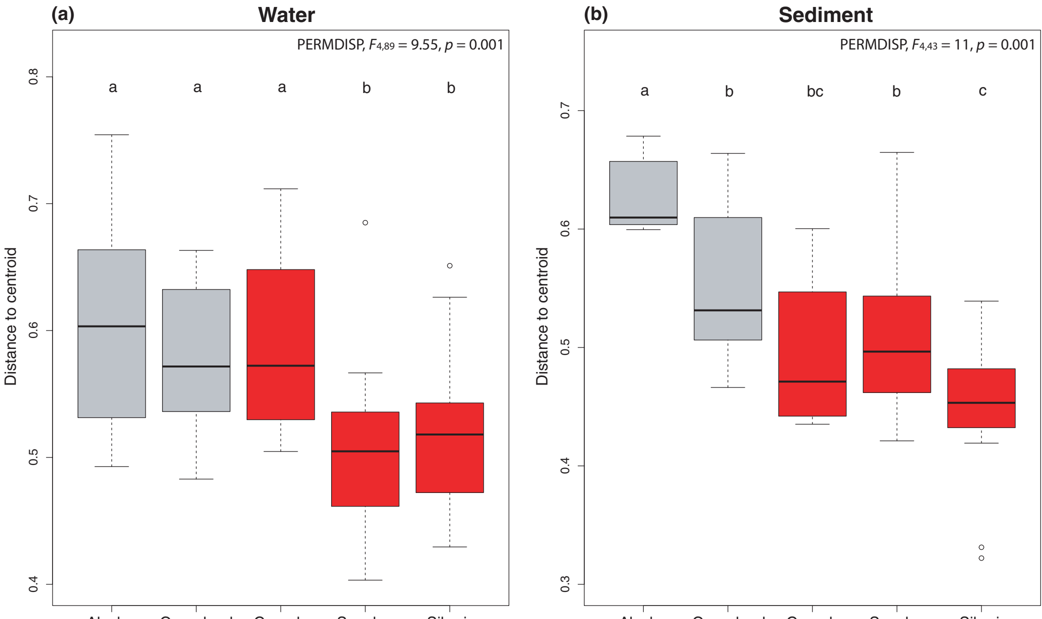

FIGURE 4 Boxplots showing, for each of the sites, the distances to the centroid for all the (a) water and (b) sediment samples within a site. The distances are based on Bray–Curtis dissimilarity matrices, with permutational analysis of multivariate dispersions (PERMDISP) test results. Different letters indicate statistical differences between sites at $p < .05$, based on pairwise comparisons of group mean dispersions. Sites with same letters have similar dispersion. Gray boxes indicate pristine sites, whereas red boxes indicate degraded sites

according to the sites and reflected the gradient of permafrost integrity along NMDS1 axis, with more degraded Canadian, Swedish, and Siberian samples clustering together in association with positive NMDS1 scores. The NMDS also confirmed that Canadian water samples separated by pond developmental stage, with the mature ponds clustering away from the emerging and most of the developing ponds, which, in turn, were more similar to Swedish and Siberian ponds (Figure 3). Samples from the pristine sites in Alaska and Greenland, were clearly separated along the second NMDS axis. Overall, beta diversity among the ponds within each site decreased as community composition became less variable with progressing permafrost degradation, as illustrated by a shorter distance to the centroid for the degraded sites (Figure 4). The community dispersion was, thus, especially low in Sweden and Siberia compared with the more pristine sites, for both water and sediment (Figure 4). This decline in beta diversity was in line with a lower site-level richness at the sites with more advanced stage of permafrost degradation (Figure 2a). The differences in group mean dispersions of water samples were highly significant, and pairwise comparisons revealed two statistically different groups: One consisting of the pristine sites (Alaska, Greenland) and Canada, and the second covering the most degraded sites (Sweden and Siberia) (Figure 4, Table S2). For sediment samples, the differences in dispersion were also highly significant among sites, displaying a similar pattern (Figure 4, Table S2). For the Canadian water samples analyzed by pond stage, there was a trend with emerging ponds seemingly having the lowest dispersion, whereas the developing ponds had the highest, but there were no significant differences between the groups (Figure S4). Spatial autocorrelation of the fungal communities was significant for Greenland ($p = .0054$, $r = .31$), Canada ($p = .0001$, $r = .54$), and Sweden ($p = .0003$, $r = .41$). However, for Alaska and Siberia, the sites with the highest and lowest beta diversity, respectively, there was no spatial autocorrelation.

The DOC concentrations were significantly higher in more degraded sites, as well as in emerging and developing Canadian ponds, compared with pristine sites and mature Canadian ponds (Figures S5 and S6). For DOM quality proxies, the ponds from the pristine sites, Alaska and Greenland, had higher FI, S289, BIX, and lower HIX, all indicative of more freshly derived DOM from autochthonous production (Figure 5, Figure S5). The opposite patterns for these proxies were seen in the more degraded systems in Canada, Sweden, and Siberia, indicating older, more degraded, and allochthonous DOM (Figure 5, Figure S5). The SUVA254 was lower at Alaskan ponds, and higher in Greenland, Canada, and Sweden (Figures S5 and S7). In addition, for the Canadian site, mature ponds had significantly higher FI than emerging ponds, which suggests more autochthonous, that is, microbial or algal-derived, DOM compared with earlier stages of pond development (Figure S6). The H/C displayed a significant gradient from more pristine to more degraded sites (Figure 5, Figure S5). Ponds from Sweden, Siberia, and emerging and developing ponds from Canada had H/C values that suggest unsaturated, more aromatic/phenolic compounds, whereas ponds in Alaska, Greenland, and mature Canadian ponds had values that point to more saturated, aliphatic compounds. The pH was the lowest in the most degraded sites; however, the difference between Sweden and Greenland was not significant (Figure S5). Mature ponds had pH values significantly higher than emerging ponds (Figure S6). NO_3^- and TP also followed a gradient, with the most degraded sites displaying the highest concentrations (Figure S7). Fe, however, displayed higher concentrations in Alaska (Figure S7).

TABLE 2 The results of permutational multivariate analysis of variance (PERMANOVA) and Mantel tests for the influence of pH, DOM proxies, and nutrients on the fungal community structure (Hellinger-transformed OTU abundance data) for water samples from all of the sites (upper panel) and for Canadian samples only (lower panel), based on Bray–Curtis dissimilarity matrices with 1000 permutations. *p*-values were adjusted with the Benjamini and Hochberg method. *df* = 1 for all PERMANOVA results

	PERMANOVA			Mantel test	
	F	R^2	*p* adjusted	*R*	*p*
Water (all sites)					
pH	7.8768	.0723	.001	.5132	.001
H/C	4.9424	.0454	.001	.6539	.001
S289	1.7115	.0157	.008	.5371	.001
HIX	2.8762	.0264	.001	.1244	.005
SUVA254	3.0439	.0280	.001	.1548	.001
NO_3	2.0140	.0185	.001	−.0303	.707
TP	2.1357	.0196	.002	.0300	.248
Fe	2.2912	.0210	.001	.1527	.002
Water (Canada)					
pH	5.1796	.1813	.004	.6105	.0001
H/C	2.1653	.0758	.010	.6193	.0001
S289	2.4860	.0803	.007	.2195	.0236
HIX	2.3894	.0837	.005	.2636	.0133
NO_3	1.1540	.0404	.246	−.0230	.5282
TP	2.2481	.0787	.004	.4298	.0001
Fe	1.9437	.0680	.013	.3547	.0054

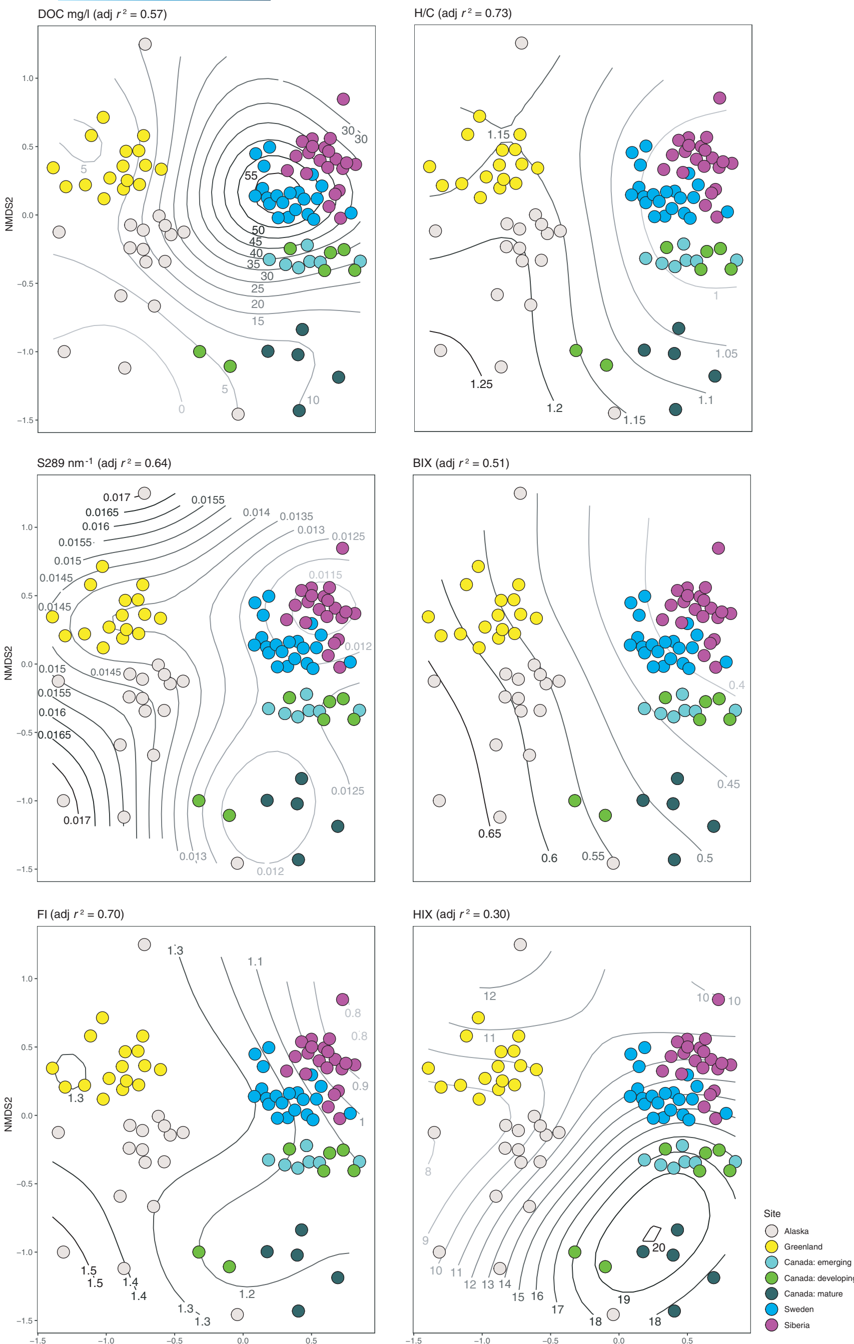

FIGURE 5 Nonmetric multidimensional scaling of all water samples with contours of dissolved organic matter–related variables placed by the ordisurf function ($p < .0001$)

OTU not present at the site

Pond stage:

E: Emerging
D: Developing
M: Mature

Ascomycota
Ascomycota; Dothideomycetes
Ascomycota; Dothideomycetes, Capnodiales
Ascomycota; Leotiomycetes; Helotiales
Ascomycota; Sordariomycetes; Hypocreales
Basidiomycota
Basidiomycota; Microbotryomycetes; Microbotryales
Basidiomycota; Tremellomycetes; Tremellales
Chytridiomycota
Mortierellomycota; Mortierellomycetes; Mortierellales
Unknown

FIGURE 6 Heatmap of the 10 most abundant OTUs in the water samples for each of the sites (44 OTUs in total, across all sites) and their correlation to variables related to carbon quality and pH. (a) Abundances of the OTUs per site, as given in *Z*-score standardized to total number of reads for each OTU (rarefied dataset). For the Canadian site, the presence/absence of each of the OTUs in each developmental stage is indicated by a letter. (b) The taxonomic assignment of the OTUs, down to the lowest classified level and (c) the Spearman rank correlation between the relative abundance of the OTUs and the carbon variables across all ponds

The overall community composition in the pond water was mainly driven by the pond stage and site (Table S3). When continuous variables were tested as explanatory variables in the PERMANOVA model (Table 2), all variables were significant across all sites ($p < .05$ for all). H/C was the variable that correlated the most with the community composition (Mantel test, Table 2), followed by S289 and pH. The same three variables also showed the highest correlation with the community composition according to BIOENV (Spearman $\rho = .67$). For the analysis on Canadian samples alone (Table 2), all variables, except NO_3^-, were significant ($p < .05$) (PERMANOVA, Table 2). H/C and pH, followed by TP, were the variables that correlated the most with community composition (Mantel test, Table 2). In addition, pH, H/C, S289, HIX, and TP were the variables with the highest correlation with the community composition in the Canadian ponds according to BIOENV (Spearman $\rho = .80$).

On a taxonomic level, a high proportion of OTUs remained unknown, even at the phylum level (47.42%, representing 26.61% of the reads) (Figure S8). The most abundant phylum at all sites was Ascomycota, followed by Basidiomycota. In addition, Mortierellomycota and Chytridiomycota composed a significant fraction of the community at some sites (Figure S8a). At the class level, Leotiomycetes was the most abundant group and found especially in the sediment samples (Figure S8a). Mature ponds showed a higher proportion of Ascomycota compared with the emerging and developing ponds (Figure S8b). Among the 50 most abundant OTUs from each site, 215 and 207 dominant OTUs (representing 67%–98% of the reads per site) were enumerated from the water and sediment samples, respectively, across all sites. Among the Ascomycota, the most frequent classes for both sample types were Leotiomycetes and Dothideomycetes, with low abundance of Sordariomycetes, Eurotiomycetes, and Lecanoromycetes (Table S4). Pezizomycetes were present only in the sediment samples. Among the Basidiomycota, the most frequent classes for both sample types were Agaricomycetes and Tremellomycetes, followed by Malasseziomycetes and Ustilaginomycetes. Overall, the most frequent classified order was Helotiales, followed by Agaricales. However, a large fraction of the dominant OTUs remained unknown (48.8% and 31.4% for water and sediment samples, respectively; Table S4).

The 10 most abundant OTUs in water from each site (a total of 44 OTUs across all sites) were further correlated with the proxies of carbon quality and quantity (Figure 6). Only seven of the abundant OTUs were present at all sites (OTUs 226, 634, 147, 358, 2, 6, and 98), with the first six of these cosmopolitan OTUs being more abundant at the pristine sites, and OTU 98, a member of the Helotiales, increasing in frequency at more degraded sites (Figure 6, cosmopolitan taxa marked with asterisks). Instead, most of the abundant OTUs in the degraded sites were not found at the pristine sites. With the exception of one OTU (OTU 6, Basidiomycota), all cosmopolitan OTUs belonged to Ascomycota. Although at most sites only a limited number of dominant OTUs from other sites were detected, at the Canadian site only nine dominant OTUs were missing and, overall, mature ponds shared a limited number of OTUs with emerging or developing ponds. As a reflection of the overall community patterns, the OTUs that were abundant at the pristine sites were negatively correlated with DOC concentration and positively correlated with variables indicative of autochthonous (i.e., S289, BIX and FI) and more aliphatic (H/C) DOM (e.g., OTUs 6 and 174). The OTUs that negatively correlated with these DOM quality variables, suggesting a positive interaction with more allochthonous DOM (e.g., OTUs 98, 289, and 345), were more abundant at the degraded sites.

4 | DISCUSSION

Our data set, representing a pan-Arctic gradient of thermokarst activity, clearly showed that the beta diversity of the fungal community decreased and the community composition changed along permafrost degradation, suggesting a homogenization of communities as the permafrost thaws. This is in line with a recent study at the Tibetan Plateau, which suggested decreasing alpha diversity of the bacterial and fungal community when permafrost soils were incubated in the laboratory to induce thaw, mainly attributed to the loss of rare species (Chen et al., 2020). Interestingly, the same study showed that, despite the loss in taxonomic diversity, the abundance of genes related to carbon degradation increased upon permafrost thaw, suggesting that fungal community members could be activated as resources became available, which could potentially increase the GHG emissions. The community composition in our ponds supported the structuring impact of permafrost thaw; the highest number of shared OTUs was observed in the sites with a similar degradation status, while, overall, the sites did not share many OTUs. For bacterial and archaeal communities, the structuring effect of permafrost thaw has been shown in multiple studies (Deng et al., 2015; Mondav et al., 2017), but for aquatic environments critically affected by permafrost thaw, and especially for fungi, the research is lacking behind.

Although in the total data set the beta diversity decreased with increasing permafrost thaw, the local-stage gradient in Canada did not show any decrease in beta diversity with pond development. However, the mature ponds did have lower richness compared with emerging and developing ponds, suggesting impacts to alpha diversity. In addition, ponds from stages with more similar DOM quality (emerging and developing ponds) shared more OTUs. The Canadian site also showed the highest number of abundant OTUs (Figure 6) as well as the highest number of dominant OTUs from other sites, which could be a reflection of larger variety of different ecological niches represented by the gradient in pond development included in our sampling design. Most of the dominant OTUs that displayed a pan-Arctic distribution were more abundant at pristine sites (e.g., OTUs 6 and 147), with the exception of one Helotiales species (OTU 98) that displayed an increasing abundance with higher permafrost degradation (Figure 6). Thus, permafrost thaw would appear to induce a change in site-level beta diversity as well as in community composition. At the same time, the results suggest that only a limited number of species had the capacity to outcompete the other species, or alternatively, some species were able to grow under more limited conditions (e.g., more recalcitrant OM), reflecting ecological theories

on ruderal/r-strategy and K-strategy species (Fontaine et al., 2003). Both these explanations are plausible and could also reflect different stages in the degradation process. Moreover, emerging Canadian ponds included the highest number of the dominant OTUs present at the Canadian site (Figure 6), and the lowest total richness was observed in mature ponds, which could be due to some of the community members blooming as the ponds start to emerge and possible filtering effect as ponds develop. To our knowledge, there are no other studies specifically evaluating the beta diversity of aquatic fungal communities in thaw ponds, although a study in Siberia has looked into the diversity of micro-eukaryotes in thermokarst ponds and polygonal ponds only filled by precipitation (Moigne et al., 2020). This study showed that thermokarst ponds presented more similar communities than the polygonal ponds and also presented a niche-specific community composition, similar to our findings.

The distances among the ponds sampled at each site was taken into consideration as it could have affected the perceived beta diversity, as dispersal limitation and a higher variation of environmental variables across the landscape are expected in larger areas (Comte et al., 2016). Accordingly, spatial autocorrelation was observed at sites where some of the ponds were very close to each other (Greenland, Canada, and Sweden). That is, in a small-scale landscape, there is likely no dispersal limitation among the ponds that are adjacent (i.e., a few meters) to each other, while communities become different among more distant ponds. At the same time, the beta diversity differed significantly between these sites that showed spatial autocorrelation. Greenland and Sweden, for instance, displayed significantly different beta diversity values despite the fact that both had spatial autocorrelation. Contrastingly, Alaska and Siberia, which were the sites with the highest distances between the ponds, showed no spatial autocorrelation. Thus, although autocorrelation may have had some impact on the diversity at the sites with short distances between the ponds, it is not likely to have influenced the overall decrease in diversity from pristine to degraded sites. Furthermore, the high diversity observed among the ponds in Canada is likely caused by the different developmental pond stages, rather than spatial factors.

Overall, across all sites, the carbon quality and quantity clearly followed the status of permafrost integrity, with pristine sites displaying more autochthonous DOM, whereas degraded sites were characterized by more allochthonous DOM. As for the gradient of pond developmental stages at the Canadian site, the proxies for DOM quality in the emerging and developing ponds indicated more allochthonous DOM, as well as higher DOC levels, compared with mature ponds. However, it is important to notice that, for more mature ponds, the distance from the sampling point to the sediment was significantly larger (2–3 m) than in the emerging and developing ponds (0–1 m). Folhas et al. (2020) analyzed the surface and bottom water of one of the mature ponds included in our study. They found that surface DOM was more aromatic and potentially more recalcitrant than the bottom DOM, suggesting that the labile DOM in surface water may be degraded rapidly, either by microbial activity or photodegradation. Besides, as recently suggested (Peura et al., 2020), the impact of permafrost thaw may be more diluted in the mature ponds, which become more similar with the pristine ponds, having stronger influence of the autochthonous than allochthonous carbon. Hence, the processes affecting the fungal community composition in the mature ponds might not be directly related to permafrost thaw.

Generally, permafrost thaw has been linked to a release of labile carbon compounds (Abbott et al., 2014) and nutrients (Reyes & Lougheed, 2015), which could induce a bloom of fungal taxa with high growth rates at the earlier phase of the thaw process, outcompeting the slower growing fungi that might inhabit the peat at the pristine stage. In line with this expectation, nutrient levels at the more degraded sites were high, which could have boosted microbial growth. However, labile DOM can be rapidly bio- and photodegraded (Drake et al., 2015; Panneer Selvam et al., 2017) and part of the labile DOM coming from thawing permafrost can be processed or filtered during its transportation through soil before reaching the waterbodies (Vonk et al., 2015). Thus, there is likely a strong competition for the labile DOM fraction in the microbial community, which can favor fast-growing species with high affinity for these substrates, likely coupled with a ruderal/r-strategy (Chagnon et al., 2013; Fontaine et al., 2003). Given their ability to cope with disturbances, these opportunistic ruderal/r-strategists can benefit from the collapsing permafrost, especially in the beginning of the thawing process when there are ample inputs of carbon and nutrient resources. In studies evaluating the processing of soil organic matter, some members of the Ascomycota have been shown to rapidly increase in abundance whenever conditions are favorable, such as at the early stages of plant litter decay and after a disturbance (Lindahl et al., 2010; Vivelo & Bhatnagar, 2019). Such dynamics could underlie the higher abundance of some Ascomycota on degraded sites and in emerging ponds (OTUs 95, 98 and 253).

At the same time, as resources become scarce and labile organic matter is being degraded, ponds can be left with more recalcitrant compounds. The lower H/C ratios found in degraded sites indicate less aliphatic compounds, which may refer to less labile DOM, as studies on the bioavailability of DOM in Arctic rivers have reported a rapid consumption of aliphatic compounds of permafrost DOC by the microbial community (Spencer et al., 2015; Textor et al., 2019). Consequently, along the permafrost degradation process, only a limited number of species might have the capacity to grow and persist in such C-limited conditions, benefiting K-strategists, which are slow growers adapted to use minimal resources (Andrews & Harris, 1986). An example of slow growers are Basidiomycetes that are able to break down recalcitrant carbon (Osono, 2007), and an increase in their abundances has been observed at late stages of litter decomposition (Voriskova & Baldrian, 2013). This mechanism could be behind the dynamics of, for example, Basidiomycota OTU 26, with higher abundance at the degraded Swedish site, and the lowest abundance at the pristine sites (Figure 6). Moreover, a recent study highlighted that constant and small carbon inputs to soil could shift the fungal community to *K*-strategists, increasing the decomposition rate (Zhou et al., 2021). Thus, it may be that the frequent carbon inputs coming

from the thawing permafrost at degraded sites favor the *K*-strategists in the ponds. This so-called priming effect of fresh carbon on the degradation of the existing carbon has been studied in freshwaters with conflicting results; some studies discard this phenomenon in freshwaters (Catalán et al., 2015) while other studies suggest that priming is an important process in lakes (Bianchi et al., 2015). Thus, in the light of literature, we can not dismiss nor verify that priming would be affecting the community composition at the degraded sites.

Overall, regardless of the strategy, the OTUs that were present in at least four sites, were either more abundant at the degraded or at the pristine sites, and many tended to correlate with either more autochthonous or more allochthonous DOM (Figure 6). This further suggests affinity for certain DOM compounds. At the same time, humic substances are resistant to bio- and photodegradation (Shirokova et al., 2019), and some freshwater fungi, for example, ascomycetes, are capable of synthesizing aromatic compounds, such as humic acids (Rojas-Jimenez et al., 2017). The combination of slow degradation and simultaneous production of humic substances could explain why the DOM humification proxy (HIX), with the exception of the Canadian site, has not varied significantly across all sites, as part of the available carbon may be converted to humic substances. In fact, the DOM quality covaried with the community composition, supporting our hypothesis that the quality of organic matter strongly influences the species composition, similarly to what has been shown for soil fungal communities (Huang et al., 2021), while the fungal community also has an impact on the transformation of carbon compounds.

A factor closely related to carbon concentration in the water is pH. As soil acidity is a strong predictor of fungal communities (Tedersoo et al., 2014), pH was included as a factor in our analyses. The pond water pH had a significant and strong effect on the community composition. Water pH also correlated negatively with DOC and positively with H/C, which was reflected in a tendency for pristine and mature ponds to be less acidic. This is in accordance to studies across different permafrost zones in Siberia, where peat leaching in more active thermokarst ponds is a main source of DOC and cause of acidification (Pokrovsky et al., 2014). However, vegetation, such as a high prevalence of mosses in the landscape, may also contribute to decreasing pH (Pokrovsky et al., 2014). Therefore, it is challenging to determine to which extent pH is an initial driver of the fungal diversity or whether a reflection of the permafrost thawing process. Nevertheless, all our results combined show a clear correlation and influence of DOM variables to the community composition and diversity. This strongly suggests carbon being a main driver of the fungal community composition. However, it needs to be kept in mind that the carbon was strongly correlated with pH, and our data do not allow disentangling this relationship.

Another finding in our study is the high proportion of unknown OTUs, even among the dominant OTUs, which points toward the general lack of coverage of fungal sequences from similar environments in taxonomical databases. Thus, it is not uncommon to find studies on freshwater environments, including thermokarst ponds (Heeger et al., 2018; Wurzbacher et al., 2017), as well as permafrost soil (Hu et al., 2014), showing that a significant part of the fungal community cannot be taxonomically assigned. This highlights the gap in the knowledge on aquatic fungal diversity and function, and toward understudied groups such as Chytridiomycota, which have been shown to be dominant in ponds and lakes in Greenland (Bomberg et al., 2019). This lack of representability of aquatic fungi in currently available databases makes it difficult to relate taxonomic composition to functional roles. Nevertheless, among the classified OTUs for the order level, it was possible to see a predominance of the order Helotiales among the dominant OTUs for both water and sediment samples (Table S4). In addition, in water samples, among the 10 most dominant OTUs for each site (Figure 6), five out of the seven OTUs shared across all sites were ascomycetes with two of them belonging to Helotiales. Helotiales was also the most prevalent classified order among the OTUs that were shared between at least two sites. This dominance of Ascomycota has been shown for Canadian thaw ponds (Wurzbacher et al., 2017) as well as the dominance of Helotiales for Arctic and Antarctic soils (Deslippe et al., 2012; Newsham et al., 2018). Compared with other fungal taxa, members of the order Helotiales have been shown to be efficient in degrading ancient cellulose in Antarctic soil (Newsham et al., 2018). In our analysis, OTUs from this order were associated with either pristine-like or degraded DOM profiles, pointing toward a wide range of environmental preferences among the species with various abilities to flourish during the different stages of permafrost thaw. Interestingly, members of Agaricomycetes were among the classified Basidiomycota in water and sediment, and most of these genera were ectomycorrhizal (e.g. *Cortinarius*, *Tomentella*, *Inocybea*, and *Lactarius*). Given their dependence on plant hosts, the DNA from these species found in water were likely from spores which persisted after initial pond development. In fact, we observed fruiting bodies surrounding shallow ponds in Greenland. Nevertheless, a few aquatic Agaricomycetes have been described and were found in deep groundwater in Greenland (Bomberg et al., 2019; Frank et al., 2010), suggesting that at least some of the Agaricomycetes in our samples could be true aquatic fungi.

In conclusion, our study sheds light on how the fungal communities may interact with DOM of varying quality that is found at the different stages of permafrost degradation and how this may affect the fungal diversity. This is particularly important considering the changing Arctic climate, where GHG emissions are tightly related to microbial decomposition rates. A loss in beta diversity could result in a loss of multifunctionality (Mori et al., 2018), and further research is needed to evaluate such effects on the functional potential of the fungal communities and how this is impacting the venting of GHGs in these rapidly changing environments. An understanding of such relationships is a key to predicting the long-term impacts of global warming on the Northern landscapes and on the ecology of the aquatic habitats. Our findings should encourage further studies to investigate the functional roles of aquatic fungi and how fungal communities respond to the thawing of the permafrost in thermokarst ecosystems.

ACKNOWLEDGMENTS

We offer our gratitude to the Cree and Inuit communities in Whapmagoostui-Kuujjuarapik (W-K) for giving us access to their

ancestral lands. We thank the staff at the Toolik Field Station, Arctic Station, Centre for Northern Studies (CEN) W-K Station, Abisko Research Station, and Khanymey Station, as well as Anelise Kluge, Gaëtan Martin, and Martin Petersson for their assistance in the field sampling. We are also grateful to the CEN W-K Station former manager, Maxime Saunier, for providing us transportation and lab facilities. Katarina Ihrmark, Maria Jonsson, Christoffer Bergvall, and Anna Ferguson are acknowledged for their assistance with the laboratory analyses. We also thank the two anonymous reviewers of this manuscript for their valuable inputs. The funding was provided by the Canada Research Chairs Program, the Canada Foundation for Innovation, and the CEN. We are grateful to the INTERACT (FunCaST, and AquaFun) and Science for Life Laboratory for funding. The computations were performed on resources provided by the Swedish National Infrastructure for Computing (SNIC), partially funded by the Swedish Research Council through grant agreement no. 2018-05973, through Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX) under Project SNIC 2020-5-196.

CONFLICT OF INTERESTS

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

SP planned the study together with CW. Sampling was planned by SP, CW, and MK. SP, MK, CW, and MW participated in the collection of the samples. MK performed the molecular laboratory work and sequence and data analyses. KEC instructed the amplicon processing and analyses. JS helped with the study design and data analysis. MW, JAH, and KE analyzed carbon samples and data, and MR helped interpreting the data. MK took lead in writing the manuscript, with the help of SP. All authors participated in the revision of the manuscript.

DATA AVAILABILITY STATEMENT

The raw sequences are deposited in the NCBI SRA database, at https://www.ncbi.nlm.nih.gov/sra, under accession number PRJNA701021. The full description of the data processing and all raw data used in this study are also presented in data descriptor (Kluge et al., 2021).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Kluge, M., Wauthy, M., Clemmensen, K. E., Wurzbacher, C., Hawkes, J. A., Einarsdottir, K., Rautio, M., Stenlid, J., & Peura, S. (2021). Declining fungal diversity in Arctic freshwaters along a permafrost thaw gradient. *Global Change Biology*, 00, 1–18. https://doi.org/10.1111/gcb.15852

Supplementary Information

Table S1 - Subsets of data used in the analyses

Sample type	Site	Number of samples	Number of unique OTUs	Number of samples after rarefying	Number of unique OTUs after rarefying	Reads per OTU after rarefying
Water and sediment	All sites	142	1280	111	1177	304
Water	All sites	94	1113	82	950	199
Sediment	All sites	48	776	43	621	405
Water	Canada	19	362	18	277	152

Table S2 – Pairwise comparisons of the groups from permutational analysis of multivariate dispersions (PERMDISP) tests, based on 999 permutations. Observed *p*-values are below diagonal and permuted *p*-values are above diagonal.

Water

Site	Alaska	Greenland	Canada	Sweden	Siberia
Alaska	-	0.375	0.747	0.001	0.001
Greenland	0.380	-	0.541	0.002	0.002
Canada	0.721	0.544	-	0.001	0.002
Sweden	0.0002	0.0003	0.00009	-	0.407
Siberia	0.0006	0.001	0.0004	0.423	-

Sediment

Site	Alaska	Greenland	Canada	Sweden	Siberia
Alaska	-	0.009	0.003	0.001	0.001
Greenland	0.007	-	0.166	0.100	0.003
Canada	0.0007	0.152	-	0.750	0.280
Sweden	0.0001	0.101	0.750	-	0.044
Siberia	0.0000013	0.001	0.279	0.049	-

Table S3 – Permutational multivariate analysis of variance (PERMANOVA) testing the influence of site or pond stage, filter type, pH, DOM proxies and nutrients on the fungal community structure (Hellinger-transformed OTU abundance data) for water samples from all sites and for Canadian water samples, based on Bray–Curtis dissimilarity matrices with 1000 permutations. *P*-values were adjusted with the Benjamini & Hochberg method.

All sites: water	**DF**	***F***	**R^2**	***p* adjusted**
Site	4	8.5562	0.2649	0.002
Filter type	1	3.0945	0.0240	0.002
pH	1	2.7823	0.0215	0.002
H/C	1	1.8361	0.01421	0.002
S289	1	1.8734	0.01450	0.003
HIX	1	1.5683	0.0121	0.019
SUVA254	1	1.3099	0.0101	0.08
NO_3	1	2.4221	0.0188	0.002
TP	1	1.6857	0.0107	0.021
Fe	1	1.3811	0.0107	0.065

Canada: water	**DF**	***F***	**R^2**	***p* adjusted**
Pond stage	2	4.8011	0.2532	0.005
Filter type	1	2.6978	0.0711	0.007
pH	1	4.7822	0.1261	0.005
H/C	1	2.9676	0.0782	0.006
S289	1	2.2718	0.0599	0.007
HIX	1	1.9933	0.0526	0.014
NO_3	1	1.5343	0.0405	0.056
TP	1	1.7769	0.0469	0.040
Fe	1	2.3019	0.0607	0.010

Table S4 – Proportions of each taxonomic level of dominant OTUs (water and sediment samples)

Water (215 OTUs)	Proportion
Ascomycota	**31,16%**
c_unknown	**15,35%**
o_unknown	15,35%
Dothideomycetes	**6,51%**
Capnodiales	1,40%
Dothideales	0,93%
Mycosphaerellales	0,47%
Pleosporales	2,33%
o_unknown	1,40%
Eurotiomycetes	**0,47%**
Eurotiales	0,47%
Lecanoromycetes	**0,47%**
Lecanorales	0,47%
Leotiomycetes	**6,51%**
Helotiales	6,05%
o_unknown	0,47%
Saccharomycetes	**0,47%**
Saccharomycetales	0,47%
Sordariomycetes	**1,40%**
Diaporthales	0,47%
Hypocreales	0,93%
Basidiomycota	**14,42%**
Agaricomycetes	**3,72%**
Agaricales	2,79%
Russulales	0,47%
Thelephorales	0,47%
c_unknown	**5,12%**
o_unknown	5,12%
Malasseziomycetes	**0,93%**
Malasseziales	0,93%
Microbotryomycetes	**0,47%**
Microbotryales	0,47%
Pucciniomycetes	**0,47%**
Pucciniales	0,47%
Tremellomycetes	**3,26%**
Cystofilobasidiales	0,93%
Filobasidiales	0,47%
Tremellales	1,86%
Ustilaginomycetes	**0,47%**
Urocystidales	0,47%
Chytridiomycota	**3,72%**
c_unknown	**3,72%**
o_unknown	3,72%
Mortierellomycota	**1,86%**
Mortierellomycetes	**1,86%**
Mortierellales	1,86%
p_unknown	**48,84%**
c_unknown	**48,84%**
o_unknown	48,84%
Total	**100,00%**

Sediment (207 OTUs)	Proportion
Ascomycota	**50,24%**
c_unknown	**26,57%**
o_unknown	26,57%
Dothideomycetes	**5,31%**
Capnodiales	0,48%
Pleosporales	2,42%
o_unknown	2,42%
Eurotiomycetes	**0,97%**
Chaetothyriales	0,97%
Lecanoromycetes	**0,48%**
Lecanorales	0,48%
Leotiomycetes	**14,01%**
Helotiales	9,66%
o_unknown	4,35%
Pezizomycetes	**0,48%**
Pezizales	0,48%
Sordariomycetes	**2,42%**
Coniochaetales	0,48%
Hypocreales	1,45%
o_unknown	0,48%
Basidiomycota	**13,53%**
Agaricomycetes	**9,18%**
Agaricales	3,86%
Boletales	0,48%
Sebacinales	0,48%
Thelephorales	1,45%
o_unknown	2,90%
c_unknown	**4,35%**
o_unknown	4,35%
Chytridiomycota	**0,97%**
c_unknown	**0,97%**
o_unknown	0,97%
Mortierellomycota	**3,86%**
Mortierellomycetes	**3,86%**
Mortierellales	2,90%
o_unknown	0,97%
p_unknown	**31,40%**
c_unknown	**31,40%**
o_unknown	31,40%
Total	**100,00%**

Figure S1 – Pictures of the sites showing the ponds.

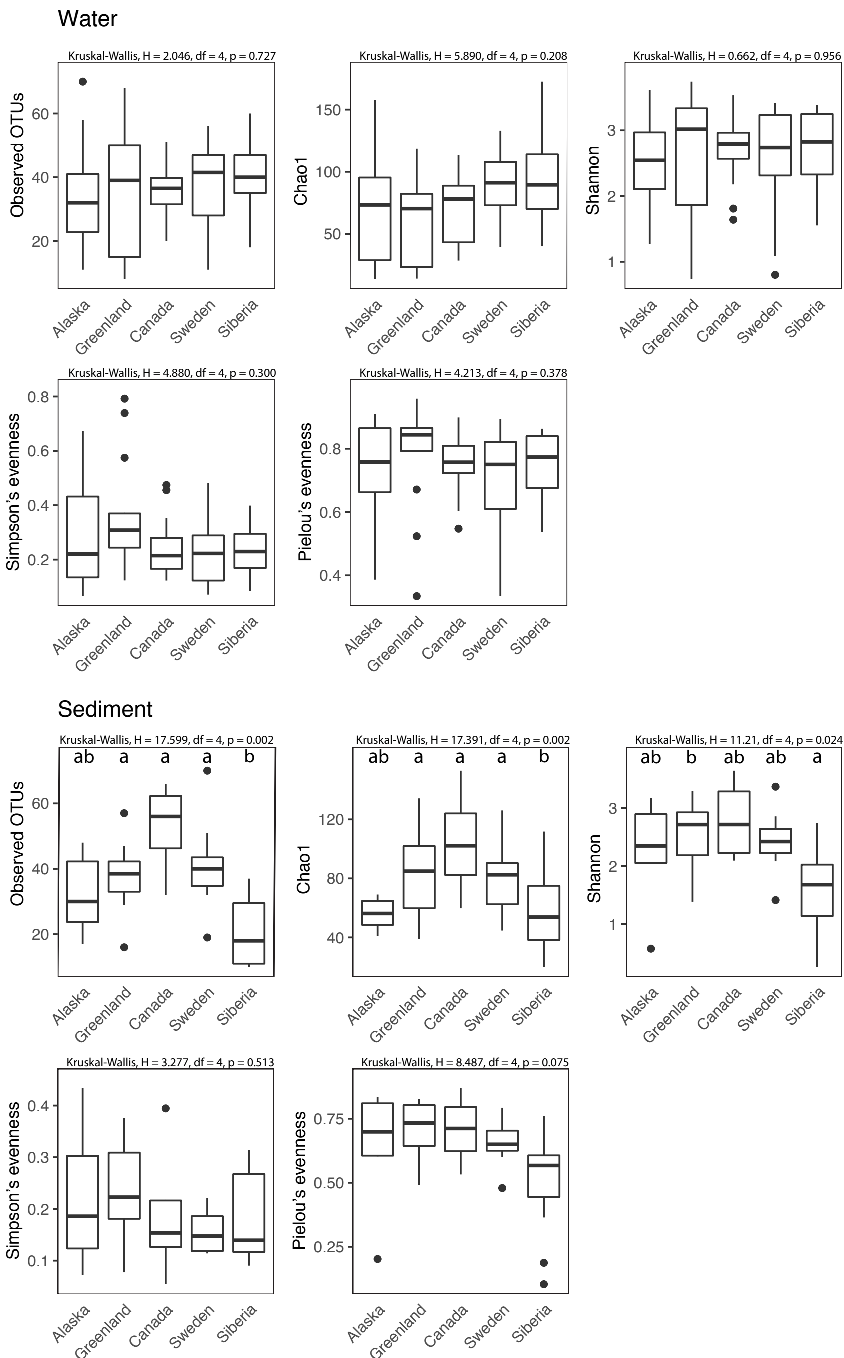

Figure S2 – Alpha diversity of the ponds per site (pond water and sediment), with results from Kruskal-Wallis tests. Different letters indicate statistical differences between sites at $p < 0.05$, based on pairwise comparisons (Dunn's test) of group means. Sites sharing the same letters are not significantly different from each other.

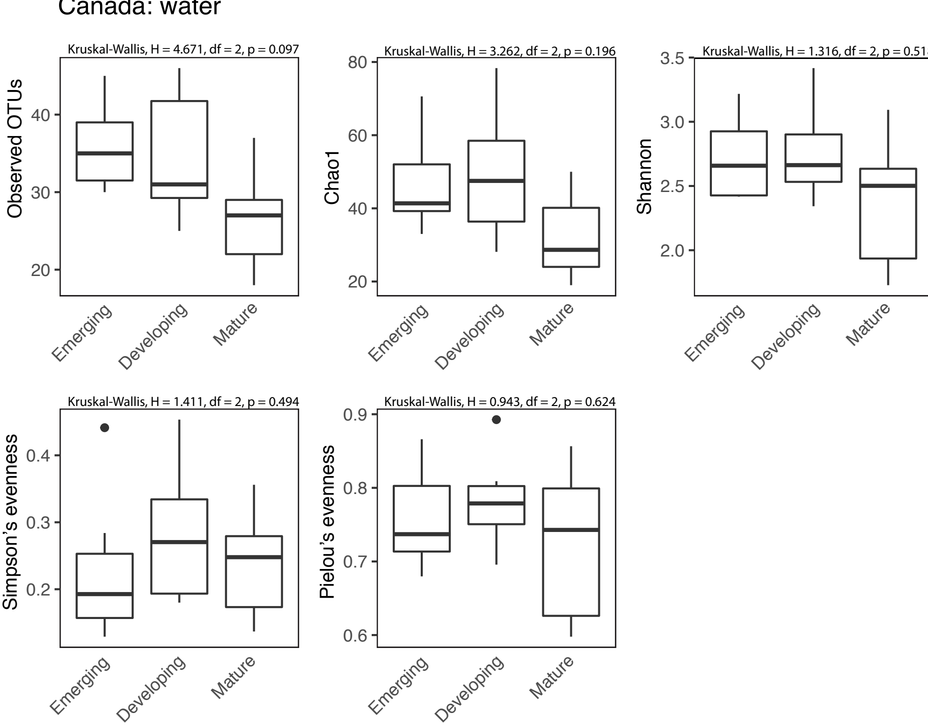

Figure S3 – Alpha diversity of the ponds per pond developmental stage in the Canadian site, with results from Kruskal-Wallis tests.

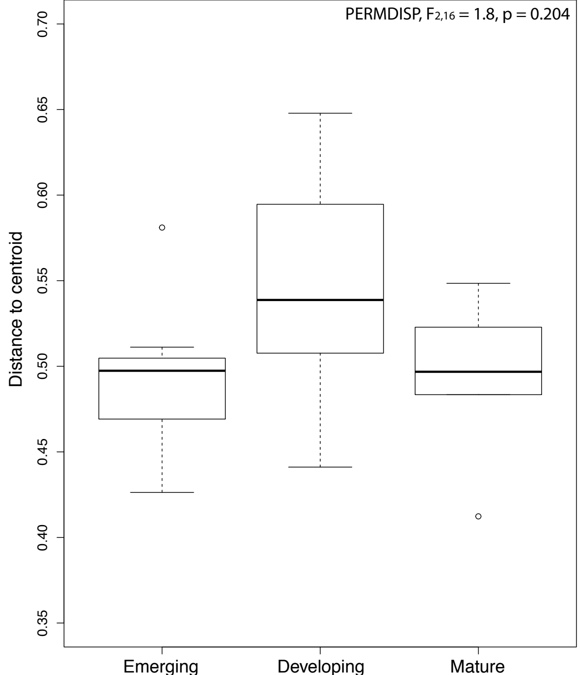

Figure S4 - Boxplot showing the distances of each of the samples within a pond stage to the centroid of the same stage, based on Bray–Curtis dissimilarity matrices, for each pond stage on Canadian water samples.

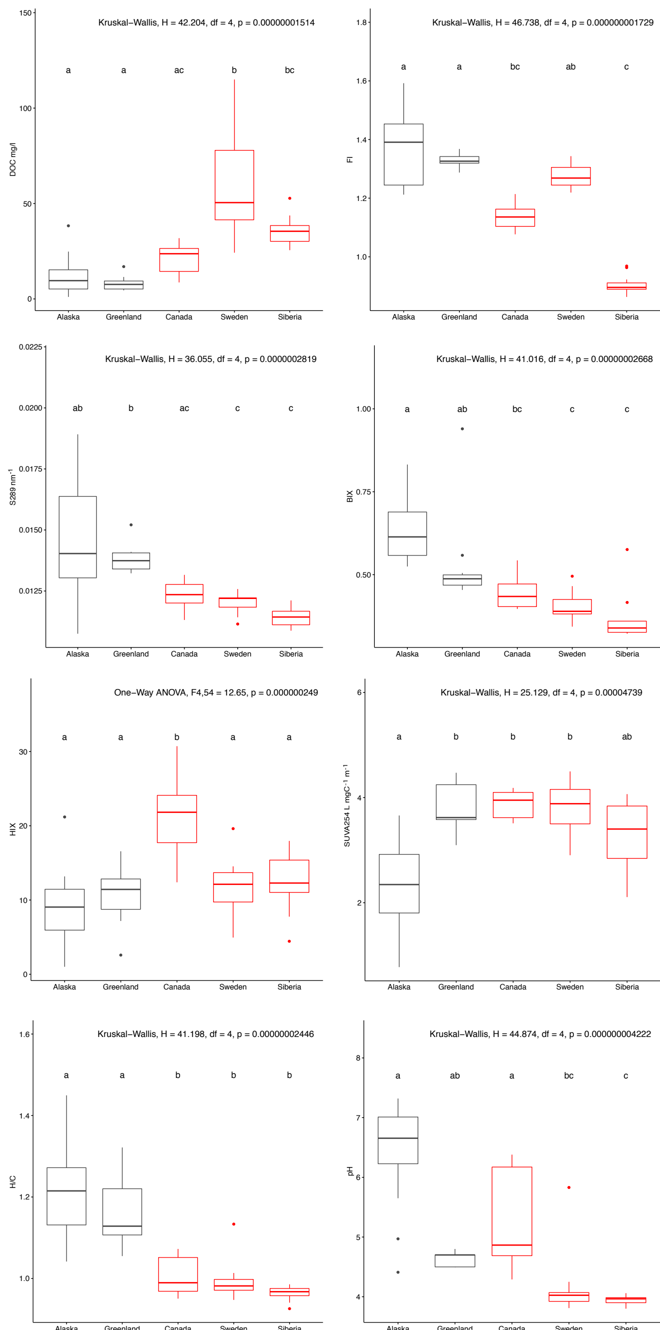

Figure S5 - Boxplots showing the DOM related variables and pH of all ponds for each site (n=12 per site). Grey boxes indicate pristine sites, whereas red boxes indicate degraded sites. Different letters indicate statistical differences between sites at $p < 0.05$, based on Tukey's HSD (One-Way ANOVA) or Dunn's (Kruskal-Wallis) post hoc tests. Sites sharing the same letters are not significantly different from each other.

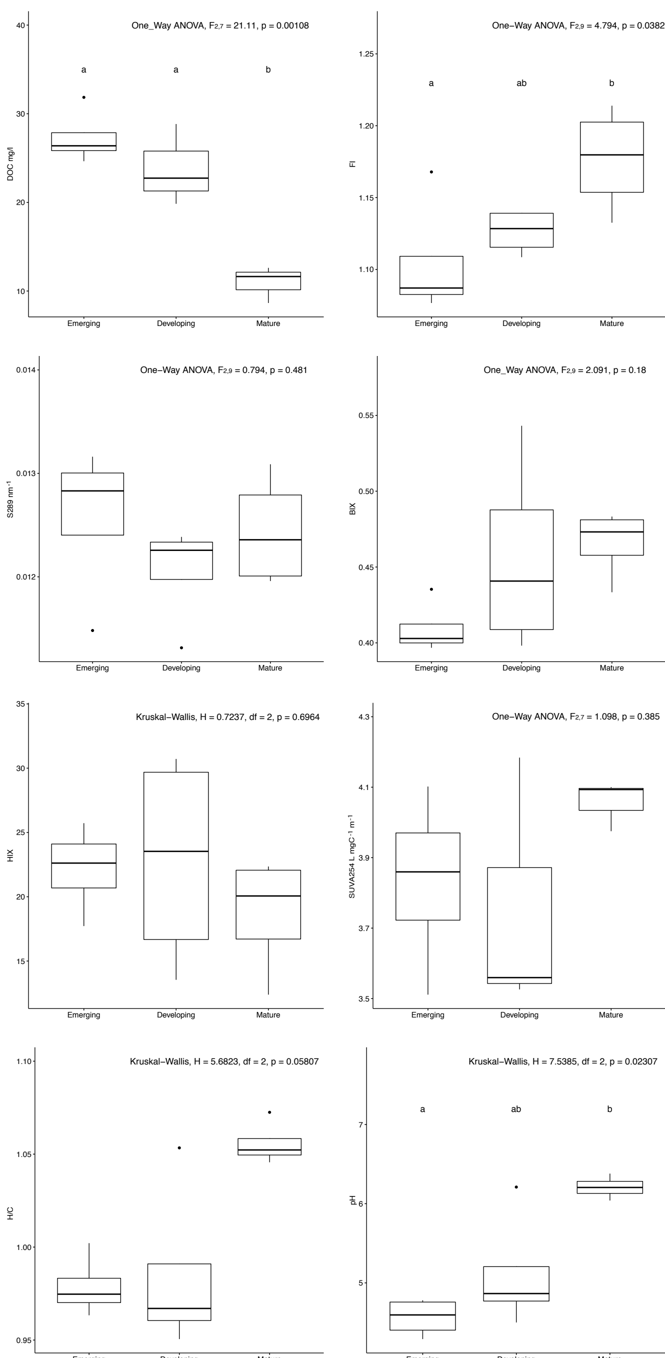

Figure S6 - Boxplots showing the DOM related variables and pH, of all Canadian ponds for each pond stage (n=4 per stage). Different letters indicate statistical differences between sites at $p < 0.05$, based on Tukey's HSD (One-Way ANOVA) or Dunn's (Kruskal-Wallis) post hoc tests. Pond stages sharing the same letters are not significantly different from each other.

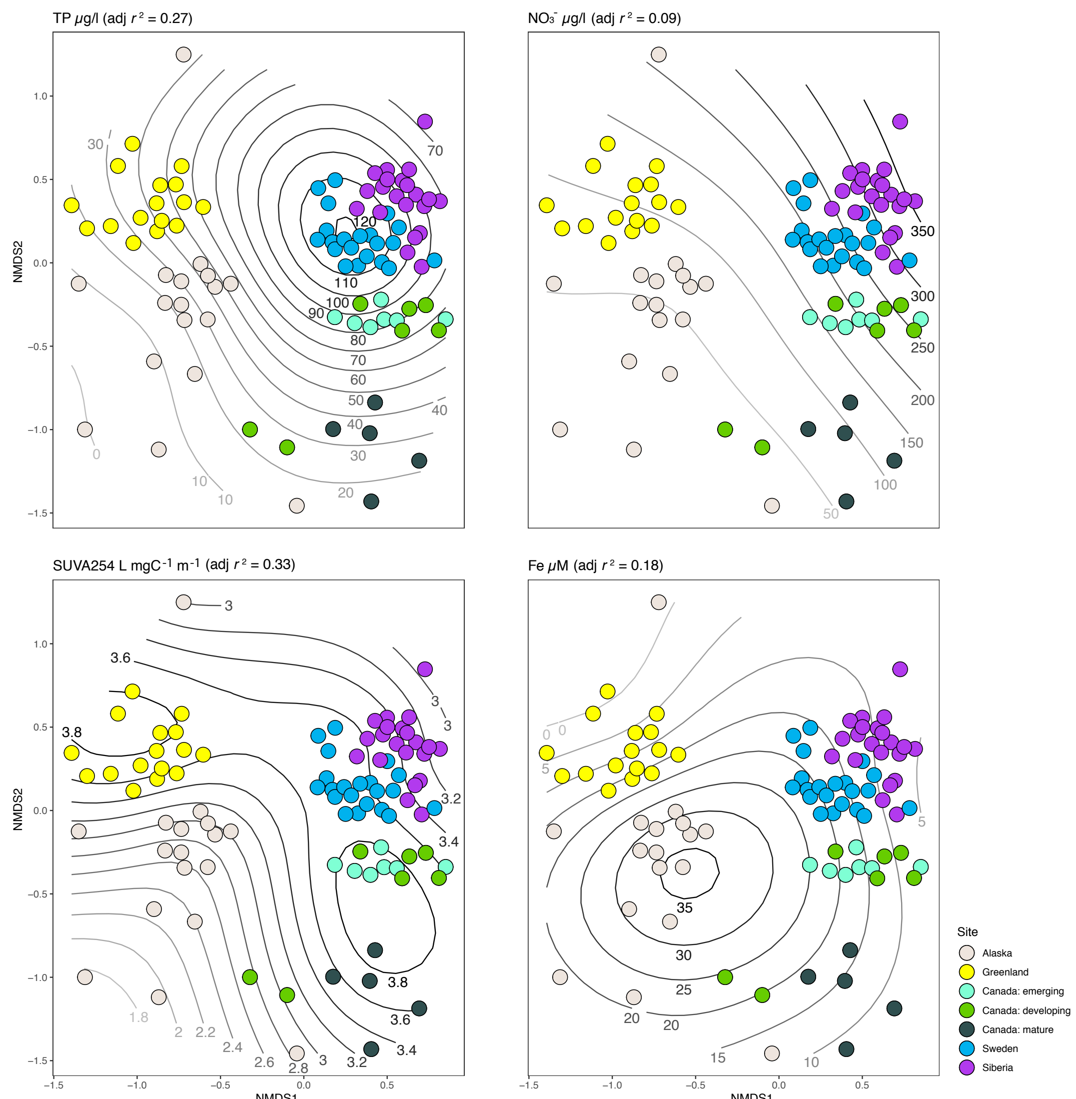

Figure S7 - Non-metric multidimensional scaling (NMDS) of water samples from all sites, with variables related to nutrients, SUVA254 and iron, placed by ordisurf function ($p < 0.01$).

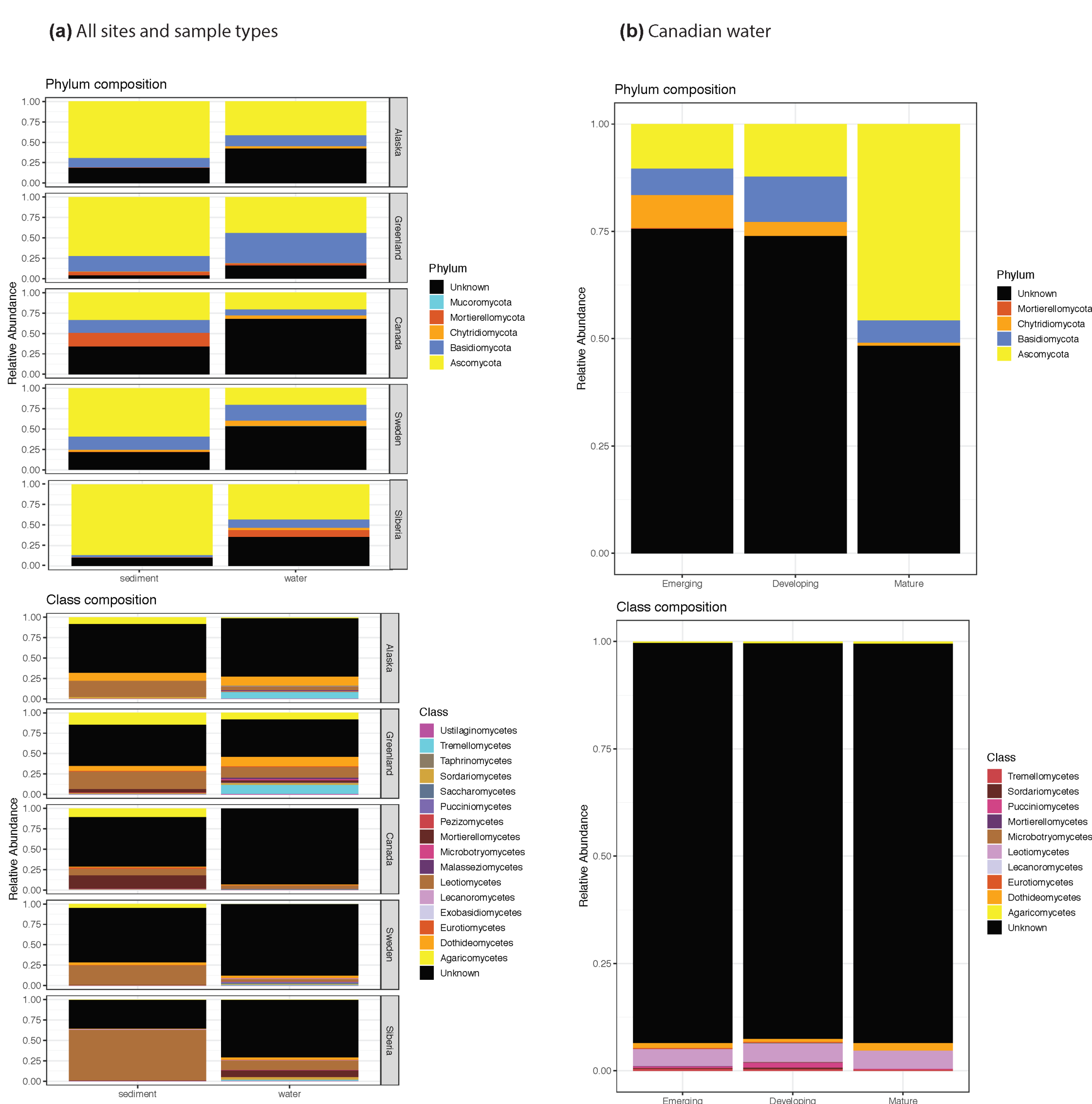

Figure S8 – The taxonomic composition of fungal OTUs of **(a)** all sites and sample types and **(b)** Canadian water samples; at the phylum and class levels.

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Doctoral Thesis No. 2021:71

Fungi are important decomposers of the organic matter in soils but little is known about their ecological role in freshwater ecosystems. Here, aquatic fungal communities were explored for their diversity and functional potential towards the carbon metabolism. The composition of these communities was tightly linked with the quality of the organic matter. This was more evident in communities found in Arctic ponds, across a gradient of permafrost integrity, which showed potential for the degradation of organic matter from different sources.

Mariana Kluge received her graduate education at the Department of Forest Mycology and Plant Pathology, SLU, Uppsala. She received her M.Sc. in Agricultural and Environmental Microbiology from the Federal University of Rio Grande do Sul, RS, Brazil.

Acta Universitatis Agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

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Online publication of thesis summary: http://pub.epsilon.slu.se/

ISSN 1652-6880

ISBN (print version) 978-91-7760-819-6

ISBN (electronic version) 978-91-7760-820-2