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Declining fungal diversity in Arctic freshwaters along a permafrost thaw gradient

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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 hydroecosystems, 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

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

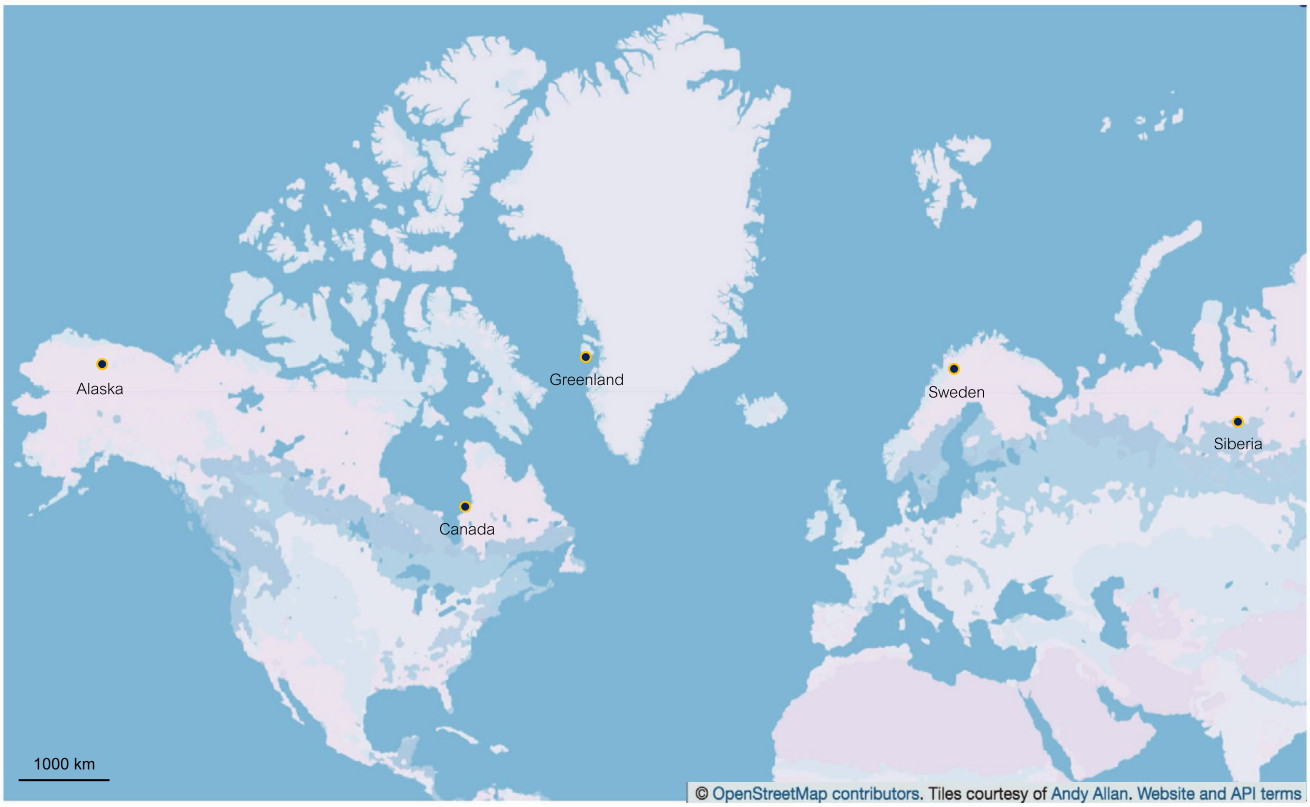

FIGURE 1 Map showing the sampled locations and an emerging pond at the Canadian site

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

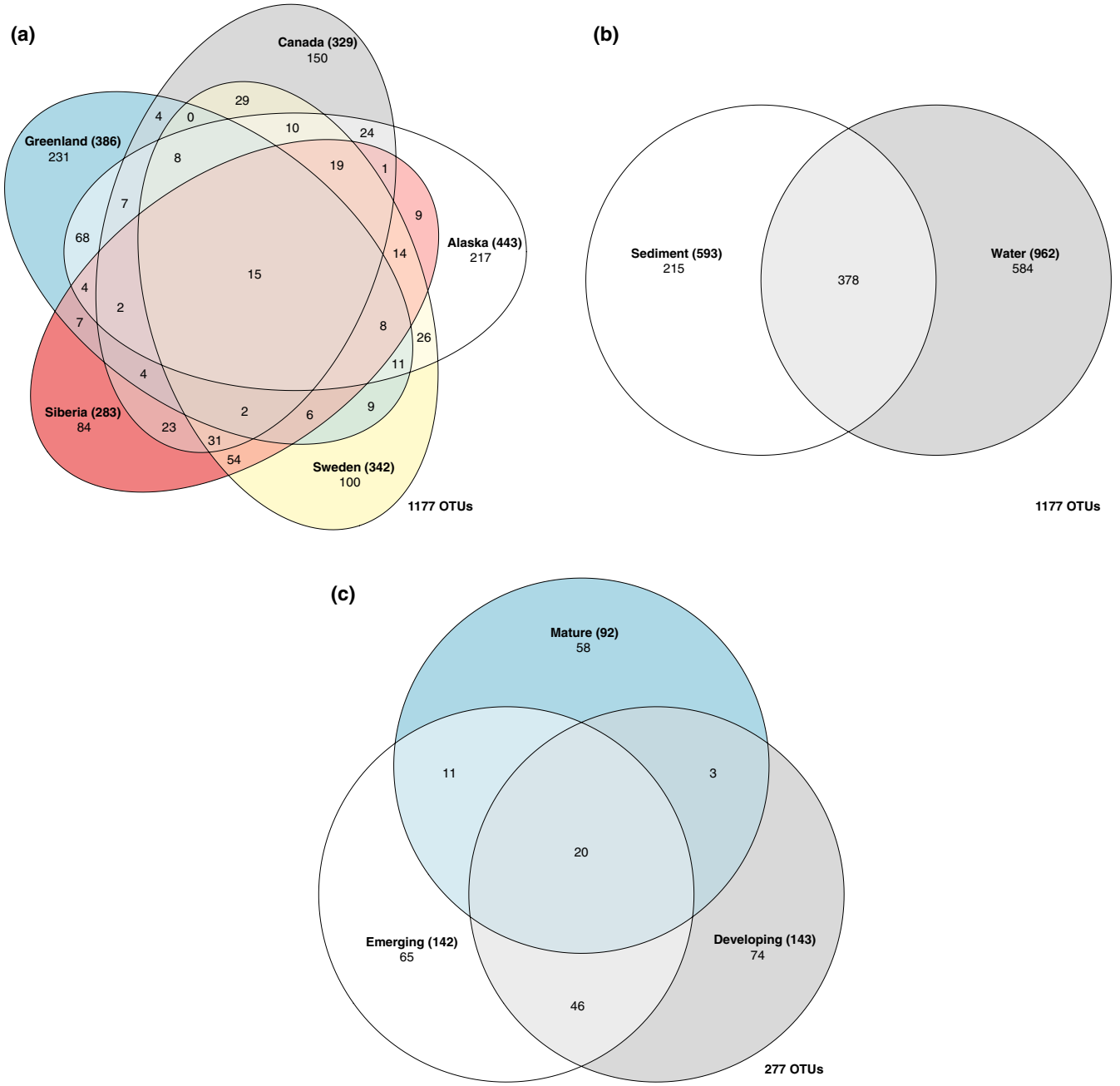

FIGURE 2 Venn diagrams of the shared OTUs across sites (a), by sample type (b), and by pond developmental stage for the Canadian water samples (c), based on rarefied datasets. Numbers in parenthesis indicate the total number of OTUs for each group

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

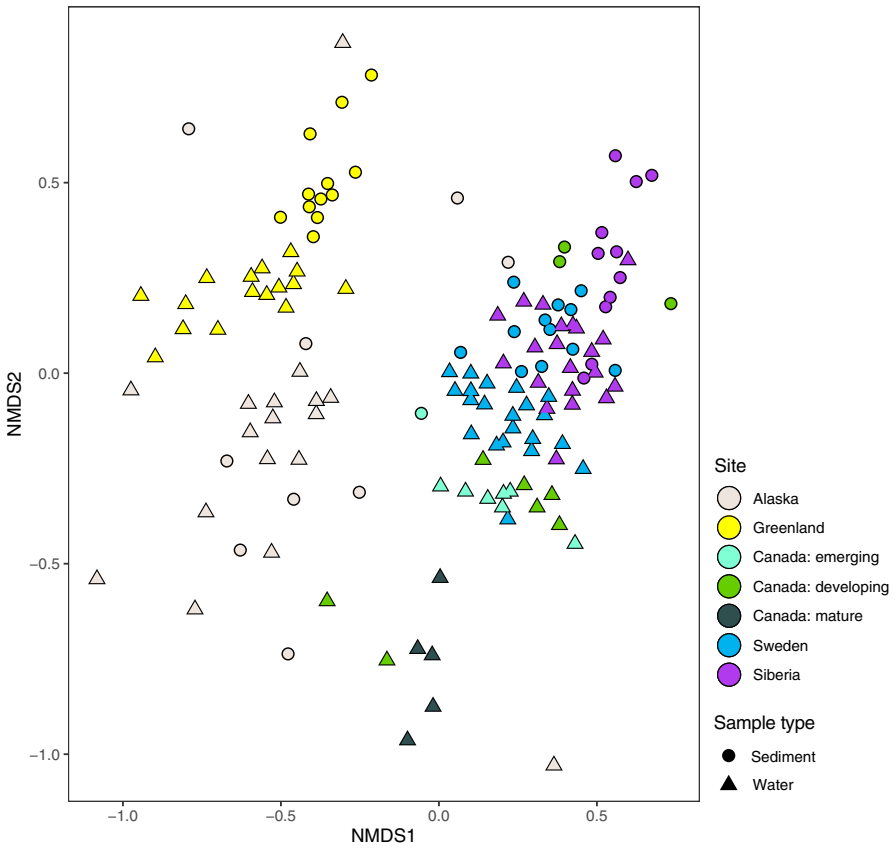

FIGURE 3 Nonmetric multidimensional scaling plot displaying beta diversity by Bray–Curtis dissimilarities (based on Hellinger-transformed abundances) for fungal communities of water and sediment samples (stress = 0.198)

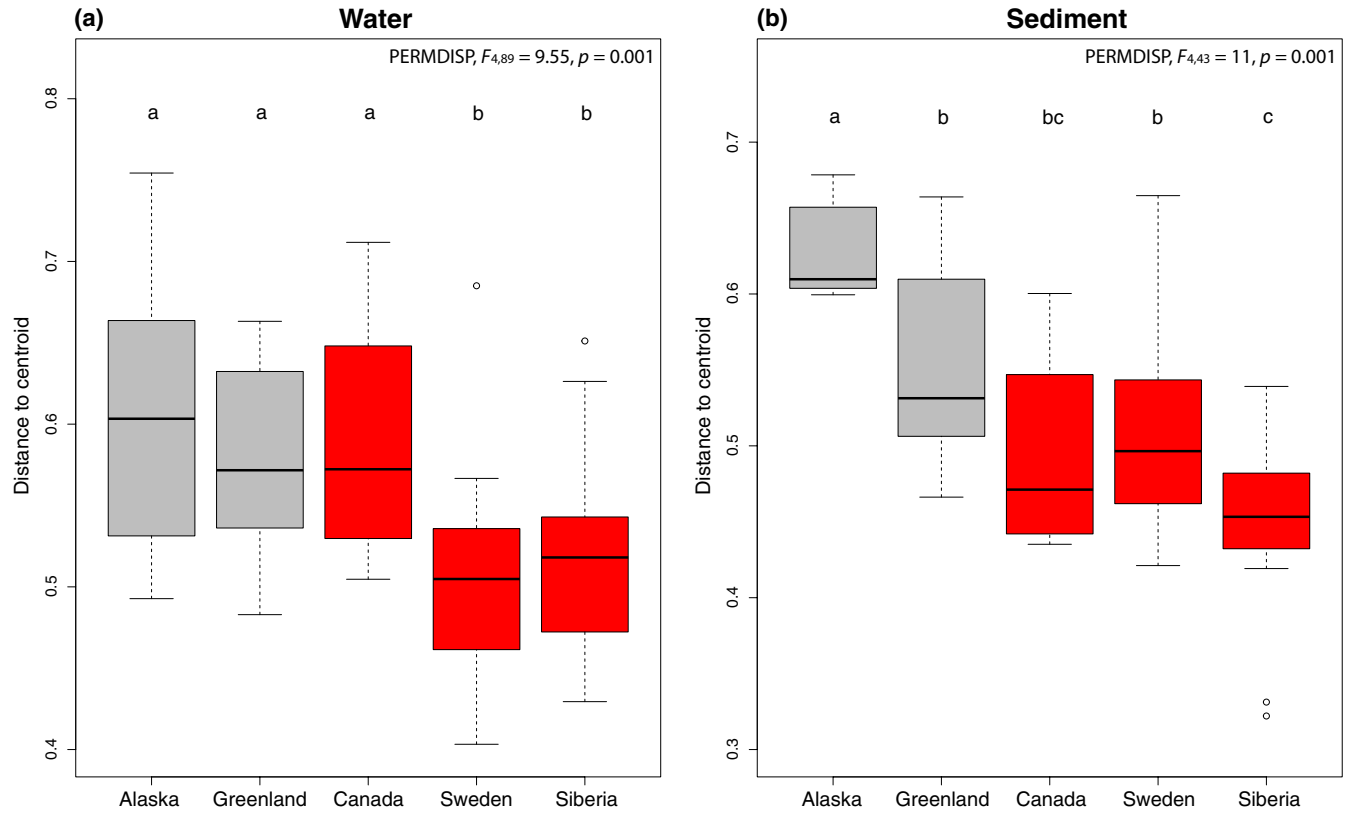

FIGURE 4 Boxplots showing, for each of the sites, the distances to the centroid for all the (a) water and (b) sediment samples within a site. The distances are based on Bray–Curtis dissimilarity matrices, with permutational analysis of multivariate dispersions (PERMDISP) test results. Different letters 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

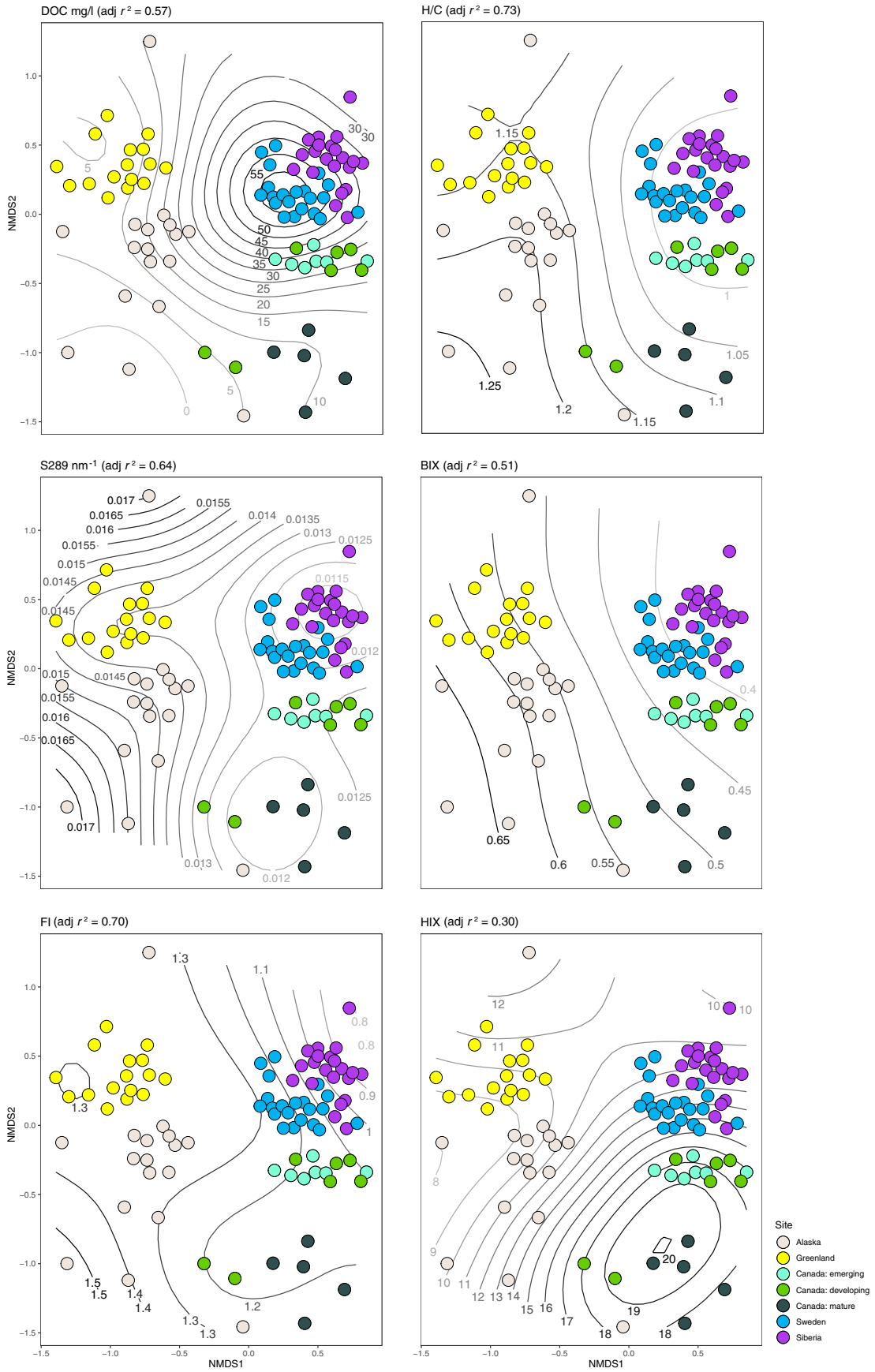

FIGURE 5 Nonmetric multidimensional scaling of all water samples with contours of dissolved organic matter–related variables placed by the ordisurf function ($p < .0001$)

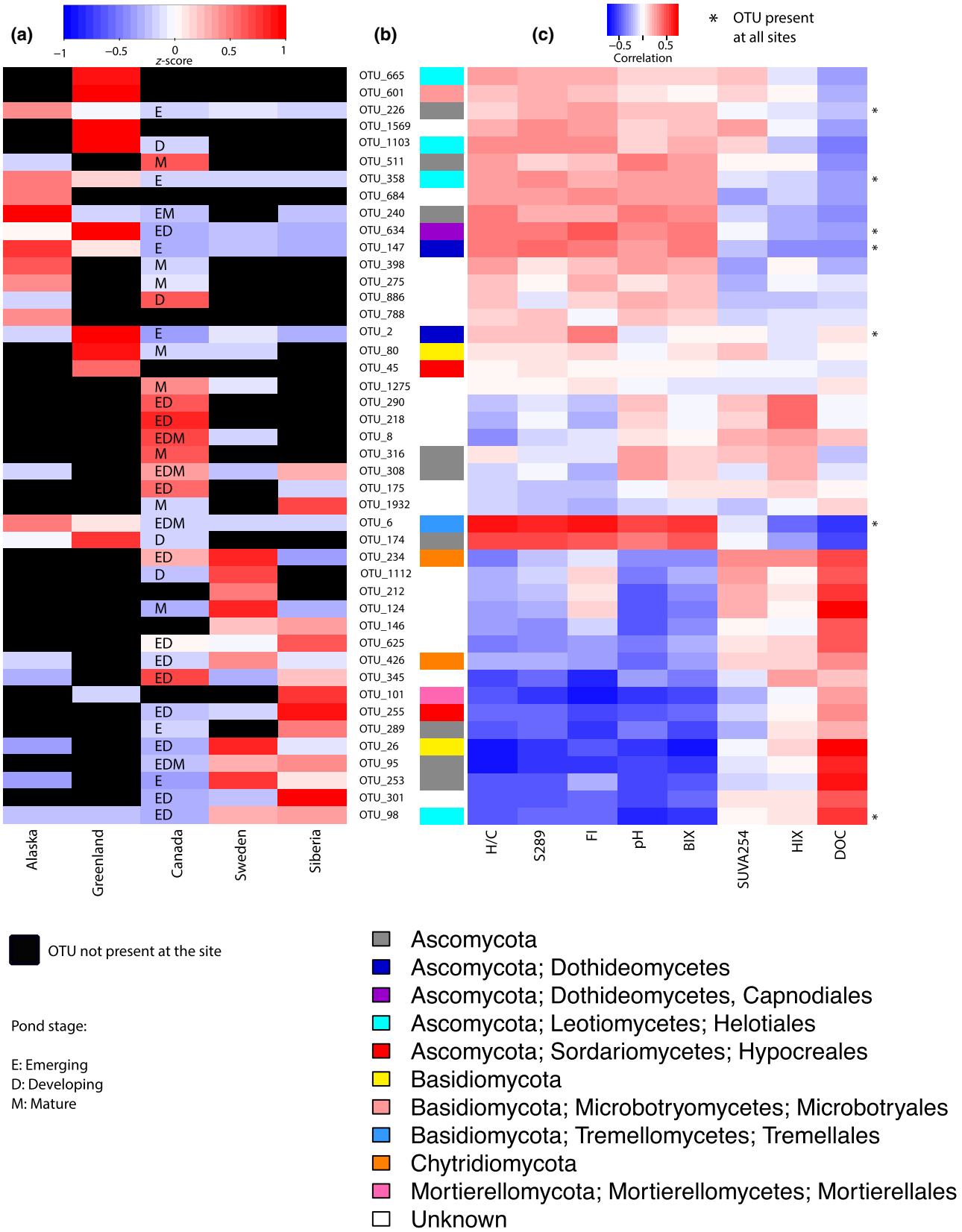

FIGURE 6 Heatmap of the 10 most abundant OTUs in the water samples for each of the sites (44 OTUs in total, across all sites) and their correlation to variables related to carbon quality and pH. (a) Abundances of the OTUs per site, as given in *Z*-score standardized to total number of reads for each 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.

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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

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