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Comparing Sediment Microbial Communities of Arctic Beaver Ponds to Tundra Lakes and Streams

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Key Points:

- Bacterial and archaeal communities of Alaskan tundra beaver ponds are unique from pristine Alaskan tundra lakes and streams
- Fungal communities of beaver ponds and tundra lakes and streams differ in overall composition but not dominant lineages
- The bacteria, archaea, and fungi of beaver ponds display greater similarity to tundra streams than to tundra lakes

Supporting Information:

Supporting Information may be found in the online version of this article.

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Abstract In recent decades the habitat of North American beaver (*Castor canadensis*) has expanded from boreal forests into Arctic tundra ecosystems. Beaver ponds in Arctic watersheds are known to alter stream biogeochemistry, which is likely coupled with changes in the activity and composition of microbial communities inhabiting beaver pond sediments. We investigated bacterial, archaeal, and fungal communities in beaver pond sediments along tundra streams in northwestern Alaska (AK), USA and compared them to those of tundra lakes and streams in north-central Alaska that are unimpacted by beavers. β-glucosidase activity assays indicated higher cellulose degradation potential in beaver ponds than in unimpacted streams and lakes within a watershed absent of beavers. Beta diversity analyses showed that dominant lineages of bacteria and archaea in beaver ponds differed from those in tundra lakes and streams, but dominant fungal lineages did not differ between these sample types. Beaver pond sediments displayed lower relative abundances of Crenarchaeota and Euryarchaeota archaea and of bacteria from typically anaerobic taxonomic groups, suggesting differences in rates of fermentative organic matter (OM) breakdown, syntrophy, and methane generation. Beaver ponds also displayed low relative abundances of Chytridiomycota (putative non-symbiotic) fungi and high relative abundances of ectomycorrhizal (plant symbionts) Basidiomycota fungi, suggesting differences in the occurrence of plant and fungi mutualistic interactions. Beaver ponds also featured microbes with taxonomic identities typically associated with the cycling of nitrogen and sulfur compounds in higher relative abundances than tundra lakes and streams. These findings help clarify the microbiological implications of beavers expanding into high latitude regions.

Plain Language Summary The North American beaver has moved into tundra regions of Alaska in recent decades as a result of more favorable habitat and population rebound from overtrapping in previous centuries. On the tundra, beavers create/engineer ponds, changing how water flows, carbon and nutrient cycling, and the rate of permafrost thawing. Our study compares the microbiology of Alaskan tundra beaver pond sediments to that of pristine tundra lakes and streams in an Arctic region still undisturbed by beavers. Our findings indicate that bacteria and archaea found in Arctic beaver ponds are different from those of tundra lakes and streams, yet dominant fungal lineages were not different between these settings. Based on the types of microbes found, beaver ponds may provide favorable conditions for plant-associated fungi, nitrogen- and sulfur-cycling bacteria, and aerobic bacteria, while tundra lakes and streams display particularly high relative abundances of fungal parasites and pathogens and anaerobic bacteria and archaea that are usually attributed with fermentative, methanogenic, and syntrophic or cooperative metabolisms. Our data provides a first look at the microbiology of far northern beaver ponds, an ecological setting that's unique to a drastically changing Arctic.

1. Introduction

As the Arctic has disproportionately warmed (Hinzman et al., 2005; Rantanen et al., 2022; Screen & Simmonds, 2010), the habitats of mammalian species, including the North American beaver (*Castor canadensis*), have spread into high latitude regions (Tape et al., 2018; Zhou et al., 2020) concurrent with the northern expansion of shrubby vegetation (Myers-Smith et al., 2011). Beaver populations are also rebounding from overtrapping

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in previous centuries (Bockstoce, 2009). This ongoing range shift of beavers into tundra regions of Alaska has occurred over several decades and has resulted in the doubling of beaver ponds in the Alaskan Arctic from 2003 to 2017 (Tape et al., 2022). Beavers are keystone species (Naiman et al., 1988), because when they dam streams in Arctic (and non-Arctic) watersheds it can lead to numerous consequences such as alterations to hydrological connectivity, wetland expansion, changes in riparian shrub composition, increases in floating ice regimes, and the thawing of permafrost (Jones et al., 2020; Tape et al., 2018, 2022).

It is unknown how ecosystem impacts of North American beaver will influence the sediment biogeochemistry, and the coincidental changes in microbial community structure, of aquatic settings in Arctic tundra ecosystems. Previous work showed that the damming of rivers by the Eurasian beaver (*Castor fiber*) in the Ob River watershed of Western Siberia drastically increased both the release of methane (CH_4) and the sequestration of carbon (C) (Cazzolla Gatti et al., 2018). Increased carbon sequestration was thought to have occurred through the formation of particulate organic matter, and its subsequent decomposition into dissolved organic and inorganic carbon (DOC and DIC) (Cazzolla Gatti et al., 2018). This DOC and DIC was then buried and sequestered in the sediment underlying beaver ponds (though some is released to water flow) (Cazzolla Gatti et al., 2018). Though long-term CO_2 release may be mitigated by beavers through carbon burial, pond formation may also trigger CH_4 release by causing abrupt thaw in ice-rich terrain (i.e., thermokarst) (Cazzolla Gatti et al., 2018; Tape et al., 2022). Abrupt thaw can mobilize deep and often labile permafrost carbon stores, which can be oxidized and transformed by soil and sediment microbial communities (Edward et al., 2022). The structure of beaver pond sediment microbial communities relates to how organic matter can be transformed and released from these changing Arctic systems.

Globally, permafrost soils store 1,400 to 1,600 Pg of C (Estop Aragonés et al., 2020), which is over twice the amount of C contained in the atmosphere (Schuur et al., 2008). The majority of permafrost C has been stored in a perennially frozen state for hundreds to thousands of years. Permafrost thaw can mobilize ancient C, releasing it to the atmosphere as greenhouse gas or transported to surface waters as dissolved or particulate OM. Given the large size of the permafrost C pool, release of thawed C to the atmosphere as greenhouse gases has the potential to enhance warming of Earth's climate (Edward et al., 2022; Schuur et al., 2008). The vulnerability of permafrost carbon to decomposition and subsequent greenhouse gas release can be dictated by the microbial community response to thaw (Feng et al., 2020). This alteration of bacterial and archaeal communities in thawing permafrost has generally been associated with an increase in greenhouse gas production (Graham et al., 2012). Bacteria and archaea in thawing permafrost accelerate carbon and nutrient cycling processes, including denitrification (Marushchak et al., 2021), ferrous iron reduction (Patzner et al., 2020), hydrogenotrophic and acetoclastic methanogenesis (McCalley et al., 2014), methanotrophy (Singleton et al., 2018), mercury methylation (Tarbier et al., 2021), sulfate reduction (Hultman et al., 2015; Mackelprang et al., 2016), and fermentation (Wu et al., 2022). The release of the potent greenhouse gas, nitrous oxide (N_2O) has also been attributed to the activities of nitrogen-cycling bacterial and archaeal communities in thawing ice-rich permafrost with high nitrogen (N) contents (Marushchak et al., 2021). However, it is unknown how the sediments of beaver-impacted watersheds will shift in the diversity of bacteria and archaea that carry out these metabolic processes.

Understanding the response of fungal communities to permafrost thaw is of additional importance, particularly given their complex roles in mediating C-cycle processes. Fungal communities in Canadian Arctic permafrost soils can degrade OM, and their community composition can change in response to thaw (Varsadiya et al., 2021). A shift of communities from those with high abundances of mycorrhizal taxa to communities dominated by fungal pathogens was observed when permafrost plateaus change to thermokarst bogs in boreal settings (Schütte et al., 2019). The abundance of saprotrophic fungi, the main decomposers of OM in soils (Grinhut et al., 2007), is an essential control on OM turnover rates in boreal soils (Kyaschenko et al., 2017). In contrast, symbiotic ectomycorrhizae (EM) can mitigate short-term litter decomposition by reducing N availability to saprotrophs, leading to decreased CO_2 release (Sterkenburg et al., 2018). However, the potential for EM-driven cellulose and lignin decomposition, through the production of extracellular enzymes, has also been documented (Moore et al., 2015). It is therefore relevant to assess the relative abundances of EM, pathogenic, and saprotrophic fungi in thawing permafrost soils to connect taxonomic identities with carbon and nutrient cycling processes. Fungi (and bacteria) degrade lignocellulosic material in soils by producing extracellular enzymes (Voříšková & Baldrian, 2013), and β-glucosidase activity levels capture this process. Fungi in beaver ponds therefore represent a key area of study for the assessment of complex OM breakdown.

Though several studies describe correlations between beaver activity and impacts on biogeochemical cycling (Cirmo & Driscoll, 1996; Johnston, 2017; Nummi et al., 2018; Čiuldienė et al., 2020), none to date have assessed impacts on the combined diversity of bacterial, archaeal, and fungal communities, which drive biogeochemical

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processes. Beaver ponds can contain microbial communities with a high potential for anaerobic metabolisms and higher microbial biomass and respiration rates, compared to streams without beaver activity (Roth et al., 2022; Songster-Alpin & Klotz, 1995). Anaerobic microbes, such as methanogens, can also release methylmercury (Wang et al., 2020), which is acutely toxic to animals and humans when it bioaccumulates in food webs (Farina et al., 2011) and can exist in high concentrations in beaver ponds (Levanoni et al., 2015; Roy et al., 2009; Čiuldienė et al., 2020). By studying the microbial diversity of Arctic beaver ponds, we can make inferences about how these organisms alter carbon, nutrient, and elemental cycling in watersheds influenced by beavers.

In this study, we characterized and compared communities of sediment bacteria, archaea, and fungi in two different Arctic settings: beaver ponds of northwest Alaska and tundra lakes and streams that feed into Toolik Lake, Alaska, an ecosystem unimpacted by beavers. Our aim was to determine whether the microbial communities of beaver ponds differ from those of the lakes and streams in an Arctic aquatic system that lacks beavers to assess how these mammals may alter the microbiology of Arctic watersheds. Though the two settings differ geographically and ecologically, the tundra lakes and streams are located in a well-studied reference watershed that exists in a semi-lentic (flowing water interspersed with standing water formations) state, similar to beaver ponds being interspersed throughout streams. Beavers also construct standing water formations in fluvial systems, therefore microbial community structures in beaver pond sediments could display high similarity to those of Arctic tundra streams. We hypothesized that the structure of sediment microbial communities of Alaskan beaver ponds differs from those of tundra lakes and streams lacking beavers because the buildup of vegetative OM and unique sediment physical characteristics of beaver ponds provide complex substrates for microbial metabolisms and unique sediment physical characteristics that influence microbial microhabitats, respectively. To address this hypothesis, we performed 16S small subunit (SSU) and 28S large subunit (LSU) ribosomal RNA (rRNA) gene sequencing to examine the structure of bacterial and archaeal and fungal communities in the beaver ponds and tundra lakes and streams. Our objective was to compare communities of bacteria, archaea, and fungi between the two Arctic settings by examining relative and absolute (16S only) abundances and phylogenetic and non-phylogenetic distance matrices paired with beta-diversity plots. Additionally, we sought to compare the breakdown potential of cellulosic OM (β-glucosidase activity) and sediment physical structure (texture) between the two ecological settings. These results provide insight into whether the community structure of microorganisms that drive complex biogeochemical cycling in beaver pond sediments will associate more closely to fluvial or lentic settings in tundra regions unimpacted by beavers.

2. Materials and Method

2.1. Sampling Method and Sample Site Description

All sample sites in this study were in the continuous permafrost zone, where >90% of the land area is underlain by permafrost. We collected samples from seven beaver ponds in the western Brooks Range in northwest Alaska (USA) that vary in elevation, watershed characteristics (permafrost, lithology), and sediment type. These ponds were formed from the damming of Rabbit Creek (RC) in Cape Krusenstern National Monument and Wrench Creek (WC) and Eli River (ER) in Noatak National Preserve. All ponds, except RC pond 1, were located on side channels of each riverine system. RC pond 1 was located on the main channel of a headwater stream (Figure S1 in Supporting Information S1). The only beaver ponds that displayed beaver activity were the Wrench Creek and Eli River ponds and Rabbit Creek pond 2. All other beaver ponds were abandoned. Sediment and surface water samples were collected from four ponds on RC, one pond on WC, and one pond on the ER (Figure 1; Figure S1 in Supporting Information S1). All beaver ponds exist in areas of low to moderate ground ice volumes (O'Donnell et al., 2016) and all sample sites were parts of lotic and fluvial systems. The Toolik Lake watershed was formed by glaciation >10,000 years ago (Hamilton, 2003), and its I-series lakes are characterized as ultra-oligotrophic, and they have high DOC (relative to the beaver ponds; most similar to RC pond 1) resulting from the drainage of tundra (Table 1).

All beaver pond sediment samples were obtained in August 2019 and all tundra lake and stream sediment samples were collected in August 2021. Beaver pond samples were taken by coring surface sediment from the periphery of the pond (depth: knee-to-waist level of the person sampling), along the dam at an approximate sediment depth of 2–5 cm with a PVC pipe and placing samples into Whirl Pak bags. These samples were kept on wet ice in the field until frozen at −80°C in Anchorage, AK, and then shipped on dry ice to Oregon State University (OSU) and stored at −80°C. Surface water samples for determining DOC concentration were collected from ponds in

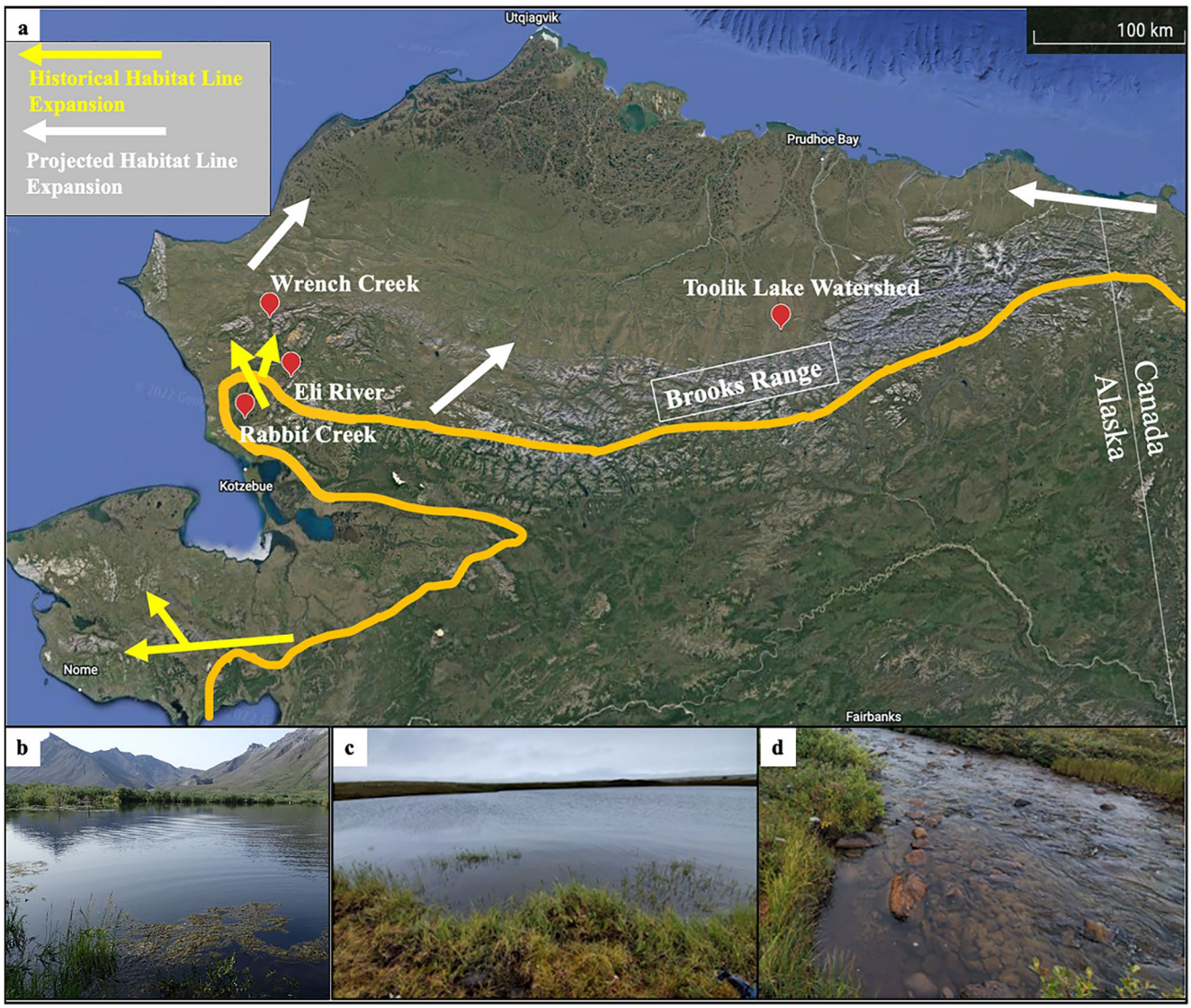


Figure 1. (a) Aerial view of sample site locations. Toolik Lake watershed represents all of the tundra lakes and streams, and Rabbit Creek represents each of the 5 Rabbit Creek sample sites. The orange line displays the approximate historical range limit for beavers, the yellow lines indicates known beaver habitat range expansion from the historical habitat line, and the white lines indicated projected future beavers spread; all adapted from Tape et al. (2018, 2022). (b) Image of Wrench Creek beaver pond. (c) Image of beaver unimpacted lake I-2. (d) Image of beaver unimpacted lake I-8 inlet stream. Wrench Creek picture taken by Michael Carey and Toolik Lake watershed images taken by Jason Dobkowski.

August 2019, field-filtered at 0.45 μm (GeoTech Versapor capsule filter), and stored in pre-cleaned amber glass bottles at 4°C. DOC concentration was determined by persulfate oxidation (Aiken, 1992) at the U.S. Geological Survey (Boulder, CO, USA). The maximum depth measurement of RC pond 2 (Table 1) was taken by towing a sonar (Deeper Smart Sonar Pro+ (Deepersonar, USA)) behind a boat throughout the lake and computationally finding the deepest point of the pond on the Deepersonar software. This technique was not available for the other beaver ponds, and the maximum depths of these ponds were qualitatively estimated by the person conducting the sampling by wading into the pond at the dam location (Table 1; sample sites with asterisks). DOC measurements of tundra lakes and streams are also from 2019.

Tundra lake and stream sample sites were located in a well-studied aquatic system, known as the I-series, that feeds into the southeastern portion of Toolik Lake, AK (Crump et al., 2007) at the Arctic Long Term Ecological Research site (ARC LTER), located at Toolik Field Station. These sites were composed of five lakes: I-2, I-3, I-4, I-5, and I-swamp and two streams: a headwater stream that flows into lake I-8 (I-8 inlet) and the connecting

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Table 1
Sample Site Geographic and Physical Characteristics

Sample site	Latitude	Longitude	Elevation (m)	Sample type	Surface area (m^2)	Approximate age (Years old)	Maximum water feature depth (m)
RC1	67.566574	−163.463202	107	Beaver Pond	2,825	5–9	NA
RC1 Outlet	67.566574	−163.463202	107	Beaver Pond Outlet Stream	NA	5–9	NA
RC2	67.496707	−163.615501	54	Beaver Pond	24,651	23–43	1.83
RC4	67.482256	−163.675693	42	Beaver Pond	20,819	14	3–4*
RC4 Side	67.482256	−163.675693	42	Beaver Pond	NA	14	NA
ER	67.805323	−161.864649	294	Beaver Pond	29,818	19	1–2*
WC	68.311408	−162.691287	312	Beaver Pond	15,633	19	1–2*
I-2	68.571037	−149.565214	785	Tundra Lake	94,532	>10,000	18
I-3	68.574673	−149.583618	774	Tundra Lake	34,536	>10,000	4.9
I-4	68.578931	−149.579989	770	Tundra Lake	83,688	>10,000	8
I-5	68.587573	−149.590297	767	Tundra Lake	210,238	>10,000	8.4
I-Swamp	68.610781	−149.600742	736	Tundra Lake	19,124	>10,000	5.9
I8 Inlet	68.6085333	−149.587633	744	Tundra Stream	NA	>10,000	NA
I-8 to I-9	68.6183833	−149.5965	728	Tundra Stream	NA	>10,000	NA

Note. Elevation measurements taken from Crump et al., 2007. Asterisks (*) indicate maximum beaver pond water depths estimated by wading.

stream between lakes I-8 and I-9 (I-8 to I-9) (Figure S2 in Supporting Information S1). Lakes I-2, I-3, I-4, and I-5 directly connect to each other (Crump et al., 2007) (I-swamp further down gradient) (Figure S2 in Supporting Information S1). Of importance, the stream sites are located within a separate tributary into Toolik Lake than the lake sites (Figure S2 in Supporting Information S1). Samples from tundra lakes and streams were collected as for the beaver ponds by scooping or coring sediments from the periphery of lakes at similar depths to the beaver ponds and in areas where sediment had settled out within streambeds. Samples were placed in Whirl-Pak bags and stored on wet ice in the field before transfer to a −80°C freezer at Toolik Field Station. They were then shipped on dry ice to Oregon State University where they were stored at −80°C. DOC measurements were performed on surface water samples from each tundra lake and stream site in 2019. The surface water samples were acidified with 100 µL trace-metal-grade HCl per ∼60 mL sample volume and stored at 4°C until being shipped to the University of Michigan on dry ice, and DOC was measured on a Shimadzu TOC-5000. These samples were not filtered due to the water being oligotrophic. DOC values for these samples were converted from micromolar concentrations to mass per volume stoichiometrically using the molar mass of carbon. Maximum depth measurements of tundra lakes (Table 1) were taken using a surface-calibrated Hydrolab DS5 sonde.

The composition of vegetation surrounding water features (beaver ponds and tundra lakes and streams) varied little by sampling location. Vegetation classifications included erect dwarf shrub tundra (Rabbit Creek) and tussock sedge, dwarf shrub, moss tundra (Wrench Creek, Eli River, and tundra lakes and streams) (Jorgenson et al., 2009). For the beaver ponds, dwarf shrubs are dominated by *Betula nana*, moss cover includes a mix of feather mosses (e.g., *Hylocomium spendens*) and *Sphagnum spp*., and tussock sedges are dominated by *Eriophorum vaginatum* (Jorgenson et al., 2009). The tundra lakes and streams are further characterized as being moist, acidic tundra dominated by graminoids (Walker & Maier, 2008). Abundant plant species in these sites include *Eriophorum vaginatum-Sphagnum* and *Carex bigelowii-Sphagnum* (Walker & Maier, 2008). The approximate age ranges of beaver ponds (Table 1) were estimated from time series by calculating tasseled cap wetness (TCW) derived from annual cloud-free mosaics of Landsat images of each beaver pond polygon. Statistical tests were then performed on the TCW time series to identify breakpoint years (points in time of significant changes to TCW) where structural changes occurred (Zeileis et al., 2003).

2.2. β-Glucosidase and Sediment Texture Analyses

Measurements of β-glucosidase activity and texture were conducted by the Oregon State University Soil Health Lab, based on existing methods (Allison & Vitousek, 2005; Ashworth et al., 2001; German et al., 2011). Briefly,

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β-glucosidase activity was measured fluorometrically by preparing sediment slurries of 1 g dried sediment mixed with 100 mL of 50 mM sodium acetate buffer, adjusted to pH 5. The slurries were homogenized and pipetted into a 96-well plate and enzyme activity was measured using a BioTek Synergy H1 Hybrid Multi-Mode plate reader at an excitation of 365 nm and emission of 445 nm. Measurements of enzyme activity were given as nmol β-glucosidase per g sediment per h. Sand, silt, and clay percentages of samples were measured using a hydrometer in 1 L of water and dried sediment, following the removal of cementing and flocculating agents and particles greater than 2 mm. The Eli River beaver pond and lakes I-3 and I-swamp all had insufficient amounts of dried sediment for texture analysis.

2.3. DNA Extractions and High Throughput Sequencing Preparation

Microbial DNA was extracted from 0.25 g of frozen sediment from each sample with Qiagen DNeasy Powersoil DNA isolation kits (Qiagen, [Hilden, Germany]) following the manufacturer's protocol. No water samples were taken for microbial DNA extractions. To obtain absolute abundance measurements of bacteria and archaea, internal standards of *Thermus thermophilus* strain hb8 genomes (purchased from the American Type Culture Collection as purified DNA) were added to bead-beating tubes after the addition of the sediment, following established protocols (Leight et al., 2018; Smets et al., 2016). Additions of the internal standard were performed in duplicate so that *T. thermophilus* represented 0.5% (first replicate) and 1.0% (second replicate) of total microbial DNA per sample, based on previously measured Qubit values of total extracted DNA from each sample. For bacteria and archaea, the v4 hypervariable region of the 16S SSU rRNA gene was PCR-amplified with universal, indexed 515F-806R 16S primers (Apprill et al., 2015; Parada et al., 2016). PCR was performed in 50 µL reactions containing 1 µL forward and reverse primers (0.2 nM final concentration each), 25 µL of Platinum™ II Hot-Start PCR Master Mix (2X) (Invitrogen™[Waltham, Massachusetts]), 2–10 µL of DNA template, and 13–21 µL of PCR water. Thermocycler conditions were performed following the PCR master mix manufacturer's procedure, and consisted of the following steps: initial denaturation for 2 min at 94°C, 32–35 cycles of denaturation at 94°C for 15 s, annealing at 60°C for 15 s, and extension at 72°C, followed by a 10 min hold at 72°C.

Fungal PCR was performed by amplifying the D2 variable region of the 28S LSU rRNA gene using universal, non-indexed LR22R/LR3 primers (Mueller et al., 2016). The D2 gene sequence was chosen because it is a suitable target for phylogenetic tree building (Reich & Labes, 2017) and because the commonly-used ITS gene region can have low taxonomic resolution with environmental samples (Banos et al., 2018). All fungal PCR was performed on 50 µL reactions containing 1 µL forward and reverse primers (0.2 nM final concentration each), 25 µL of Platinum™ II Hot-Start PCR Master Mix (2X), 10 µL of DNA template, and 13 µL of PCR water. Thermocycler conditions were the same as those for 16S PCR with the following exceptions: 28–30 cycles of the denaturation, annealing, and extension steps were performed, with the extension step at 68°C, and no final hold at 72°C. All PCR products were purified with Qiagen PCR Purification Kits, following the manufacturer's protocol, and gel electrophoresis was performed on all purified amplicons to ensure proper amplicon sizing. All 16S purified amplicons were pooled to equimolar concentrations and sent, along with unpooled 28S amplicons, to the Oregon State University Center for Quantitative Life Sciences (CQLS) for index PCR (for 28S amplicons) and multiplexing. All bacterial, archaeal, and fungal samples were then sequenced with 2 × 250 paired-end Illumina MiSeq high throughput sequencing by the CQLS.

2.4. Microbial Community Data Analysis and Statistical Methods

All raw MiSeq reads were demultiplexed by the CQLS. Illumina adapter trimming and initial quality filtering, dropping reads with Phred scores of <20, was performed with Trim Galore (v0.6.7), following the trimming of most adapter sequences by the CQLS. Read quality reports were generated with FastQC (v0.11.9) and visualized with MultiQC (Ewels et al., 2016). DADA2 (Callahan et al., 2016) was used in base R (v4.1.1) for further read filtering, generating contigs, and assigning filtered reads to bacterial, archaeal, and fungal ASVs, following default parameters, except for pseudo-pooling being conducted at the denoising step. Pseudo-pooling allowed for better recognition of ASVs in each sample based on prior information (Nalley et al., 2022). All 16S sample technical replicates (0.5% (1) and 1.0% (2) *T. thermophilus* added) were averaged into single samples with the "merge_samples" command from the phyloseq package (McMurdie & Holmes, 2013), following denoising. Technical replicates were combined by mean, rather than sum, to avoid bias due to several samples (I8-I9 stream, lake I3, and RC1 outlet stream) that lacked technical replicates. Bacterial and archaeal ASVs were assigned

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taxonomy with the Silva (v138.1) 16S reference database (Quast et al., 2013) and fungal ASVs were assigned taxonomy with the Ribosomal Database Project (RDP) (Cole et al., 2014) fungal 28S LSU training set 11. The Decontam package (Davis et al., 2018) was used to remove any ASVs identified as contaminants, based on their existence in negative control samples, with default parameters. All chloroplast, mitochondria, and *T. thermophilus* 16S ASVs and all fungal ASVs not classified to the kingdom fungi were removed from further community analysis. Due to the large number of fungi that were unclassified at the phylum level, these ASV sequences were all exported as fasta files and were re-classified with the RDP web-based classifier (Wang et al., 2007). All non-fungal ASVs, identified by the RDP classifier, were then removed from further analysis in RStudio (R Core Team, 2022). 16S gene absolute abundance quantifications were performed following the protocol of Leight et al. (2018). Briefly, 16S rRNA gene absolute abundance values were calculated for non-*T. thermophilus* 16S ASVs by comparing sequence read relative abundance values of all non-*T. thermophilus* reads to the ratio of (*T. thermophilus* genomes added)/(*T. thermophilus* sequences reads present) (where *T. thermophilus* reads were corrected for *T. thermophilus* copy number). This approach was not used to calculate fungal 28S rRNA gene absolute abundance because fungal internal controls were not added to the samples. Absolute abundance values were not corrected for 16S gene copy number variation due to the high variability of copy numbers across bacterial and archaeal phyla (Větrovský & Baldrian, 2013).

To generate phylogenetic trees, sequences of filtered ASVs were imported into Mothur (Schloss et al., 2009) to generate alignment files against the Silva and RDP databases for bacteria and archaea and fungi, respectively, with the "align.seqs" command. The alignment files were then used to construct 16 and 28S phylogenetic trees with FastTree (Price et al., 2009). Each phylogenetic tree was then exported into iTOL (Letunic & Bork, 2021) where all branches with bootstrap values below 0.50 were trimmed and trees were rooted at their midpoints. These phylogenetic trees were then exported into R and combined with 16 and 28S taxonomy and count tables to create Phyloseq objects (McMurdie & Holmes, 2013). The Phyloseq objects were rarefied to minimum depths of 14,806 reads and 5,686 reads for 16S and 28S ASVs, respectively. Chao1, Shannon, and Faith's phylogenetic diversity alpha diversity measurements (Figures S3 and S4 in Supporting Information S1) were conducted with the packages, btools and MicrobiotaProcess (Xu et al., 2023). The Chao1 index examines taxonomic richness with a particular emphasis on rare taxa (Kim et al., 2017). The Shannon index estimates diversity by accounting for both taxonomic richness (the number of taxa present) and evenness (how evenly distributed the relative abundances of taxa are). Faith's phylogenetic diversity (PD) index measures the sum of the lengths of branches in a phylogenetic tree that lead to nodes found in each sample (Faith, 1992). All box plots were made with ggplot2 (Wickham, 2016).

Phyloseq (McMurdie & Holmes, 2013) was used to generate distance matrices, of weighted-UniFrac, unweighted-Unifrac, and Bray distances, and to plot NMDS (non-metric multidimensional scaling) ordinations, along with ggplot2 (Wickham, 2016). When UniFrac (both weighted and unweighted) was used as a distance method, differences between sample sites correspond to the phylogenetic relatedness between sites when calculating the distance matrix (Lozupone & Knight, 2005). The vegan (Oksanen et al., 2020) package was used for PERMANOVA (permutational multivariate analysis of variance) using distance matrices and the "adonis2" command for two group comparisons between beaver ponds and tundra lakes and streams and the "betadisper," and "permutest" commands to compare variances of these two groups, with default parameters. Family-level taxonomic vectors were also constructed using vegan (Oksanen et al., 2020). The FungalTraits (Põlme et al., 2020) database was used to assign functional information to fungal taxonomic units (taxa). This was performed by importing the database into R and using the "intersect" command from the dplyr (Wickham et al., 2023) package (reexported from base R), to align common genera, and associated functional information, between the taxonomy table of the fungal Phyloseq object and the FungalTraits database.

Statistical tests for differences in means of β-glucosidase, DOC, bacterial and archaeal absolute abundance, and abundance of fungal functional guilds between beaver ponds and tundra lakes and streams were performed using Welch's unequal variance *t*-tests to correct for non-constant variance in RStudio with the stats (R Core Team, 2022) package. All statistical tests were performed in RStudio (R Core Team, 2022). Significance tests for differences in alpha diversity measurements between beaver and non-beaver (tundra lakes and streams) water features were performed with Wilcoxon signed-rank tests. Results were deemed to be significant if *p*-values fell below 0.05. Shapiro tests for normality of β-glucosidase and bacterial and archaeal absolute abundance values were conducted using the stats package (R Core Team, 2022) and gave *p*-values > 0.05, showing that the data was approximately normally distributed (fail to reject Null hypothesis that data is approximately normal). A residual

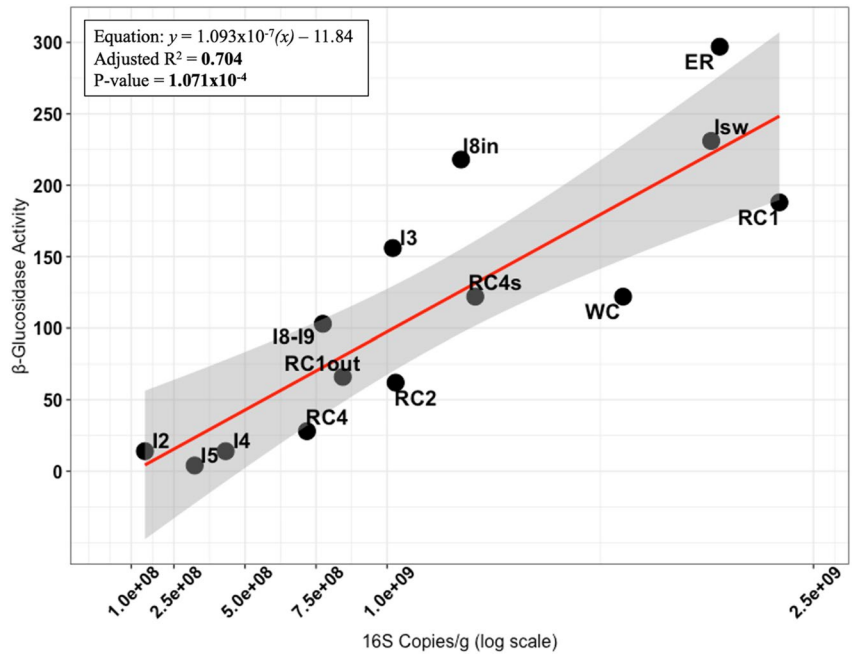


Figure 2. Scatterplot of β-glucosidase activity levels (nmol enzyme/g sediment/h) plotted against bacterial and archaeal absolute abundance. Line of best fit calculated from linear regression (response = β-glucosidase activity; explanatory = absolute abundance). The gray shading displays standard error. Isw = I-Swamp; RC1out = RC1 Outlet.

v. fitted values plot was utilized using the "plot" function in the base R (R Core Team, 2022) package to confirm linearity and the lack of strong outliers. Finally, a Breusch-Pagan test was performed on the linear regression model in using the lmteset package (Zeileis & Hothorn, 2002) and displayed a p-value > 0.05, which signified a lack of heteroskedasticity (fail to reject Null hypothesis that data is homoscedastic) of the model.

3. Results

3.1. Sediment Enzyme and Physical Analyses and Sample Site Characteristics

β-glucosidase enzyme activity, which serves as a proxy for microbial degradation of plant organic matter, varied greatly between beaver pond and tundra lakes and streams (Figure S5 in Supporting Information S1), ranging from 4 to 297 nmol enzyme/g sediment/h. Average β-glucosidase activity levels (mean ± standard deviation) for beaver ponds was 126.43 ± 91.67 nmol enzyme/g sediment/h and the average for tundra lakes and streams was 105.71 ± 98.33 nmol enzyme/g sediment/h. These water features did not significantly differ (p-value > 0.05) in β-glucosidase activity. Relatively high β-glucosidase activity values were seen in the ER beaver pond, lake I-swamp, and the I-8 inlet stream, which appeared to coincide with high 16S gene absolute abundance values (Figure 2). Absolute abundance values varied across sites from 1.40×10^8 to 2.36×10^9 16S copies/g sediment, and were on average (mean ± standard deviation) higher for beaver ponds than tundra lakes and streams (beaver ponds = $1.47 \times 10^9 \pm 6.61 \times 10^8$; tundra lakes and streams = $8.71 \times 10^8 \pm 6.84 \times 10^8$), though this difference was not significant (p-value > 0.05). To further examine the correlation between absolute abundance and β-glucosidase activity linear regression was performed with β-glucosidase activity as the response and absolute abundance as the explanatory variable, which displayed a significant relationship between the two variables (p-value of linear model <0.05) (Figure 2). This provides evidence that the enzyme was produced by bacteria (in addition to fungi), because the abundance of 16S gene copies is an indicator for bacterial biomass (Smets et al., 2016). All Rabbit Creek beaver pond sites, except for RC pond 1, displayed similar texture classifications to the tundra streams (silty/sandy clay loam) (Table 2). All other sample sites were mainly composed of fine-grained material, ranging from clay to clay loam sediments. DOC concentrations were on average (mean ± standard deviation) lower for beaver ponds (2.67 ± 2.40 mgC/L) than tundra lakes and streams (6.57 ± 0.32 mgC/L) (Table 2). Beaver ponds differed significantly in DOC concentrations than those of tundra lakes and streams (p-value = 0.0050) and RC1 and RC1 outlet were much higher in DOC concentrations than all other beaver ponds (Table 2).

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Table 2
Sediment Enzyme Rates, Texture, DOC, and Internal Standard Results

Sample site	β-Glucosidase (nmol/g sed/hr)	Sand %	Silt %	Clay %	Texture classification	DOC (mg/L C)	Bacterial and archaeal biomass (16S gene copies/g sed)
RC1	188	25	34	41	Clay	6.1	2.38×10^9
RC1 Outlet	66	57	12	31	Sandy Clay Loam	6.2	8.44×10^8
RC2	62	67	5	27	Sandy Clay Loam	1.6	1.03×10^9
RC4	28	11	56	33	Silty Clay Loam	1.6	7.18×10^8
RC4 Side	122	2	64	35	Silty Clay Loam	1.6	1.31×10^9
ER	297	NES	NES	NES	NES	0.7	2.17×10^9
WC	122	14	31	55	Clay	0.9	1.83×10^9
I-2	14	30	32	37	Clay Loam	6.6	1.48×10^8
I-3	156	NES	NES	NES	NES	6.9	1.02×10^9
I-4	14	20	34	46	Clay	7.1	4.32×10^8
I-5	4	43	21	36	Clay Loam	6.4	3.23×10^8
I-Swamp	231	NES	NES	NES	NES	6.3	2.14×10^9
I8 Inlet	218	7	62	31	Silty Clay Loam	6.3	1.26×20^9
I-8 to I-9	103	67	11	32	Sandy Clay Loam	6.4	7.74×10^8

Note. Texture classifications were calculated with the USDA soil texture calculator (Soil Survey Staff, 2019). NES = not enough sediment.

3.2. Bacterial and Archaeal Alpha Diversity and Taxonomic Abundances

Three different alpha diversity indices (Chao1, Faith's PD, and Shannon indices) were used to examine the diversity and richness of bacterial, archaeal, and fungal taxa. Interestingly, the tundra lakes and streams were on average higher than beaver ponds in every alpha diversity measurement (Figure S3 in Supporting Information S1) for bacteria and archaea, especially Faith's PD, though these results were not significant. RC pond 2 also displayed the highest diversity of any sample sites, in all three indices (Figure S3 in Supporting Information S1). Bacteria represented the vast majority of taxonomic alignments of 16S ASVs in both sites, but were notably more dominant in the beaver ponds (~97% of ASVs) than the tundra lakes and streams (~82% of ASVs). The five most abundant bacterial phyla across sample sites, from high to low abundance, were the Proteobacteria (22.3%), Acidobacteriota (14.6%), Bacteroidota (11.4%), Verrucomicrobiota (11.1%), and Chloroflexi (9.3%). Bacterial orders of high relative abundance in beaver ponds included Chthoniobacterales (7.7%), Vicinamibacterales (6.8%), and Pyrinomonadales (5.0%), while Bacteroidales (5.8%), SBR1031 (Anaerolinea Class) (3.0%), and Anaerolineales (4.1%) displayed high relative abundances in tundra lakes and streams (Figure 3). Burkholderiales (9.9%), Rhizobiales (4.5%), Planctomycetales (2.0%), Pirellulales (2.3%), Pedosphaerales (2.9%), and Verrucomicrobiales (2.0%) all displayed high relative abundances across all sample sites (Figure 3). Taxa related to sulfur cycling (Frank et al., 2016; Kjeldsen et al., 2019; Ward et al., 2021) existed across sample sites, especially in the tundra lakes and the ER and WC beaver ponds. Desulfocapsaceae (0.23%) genera were found in the ER, WC, and RC pond 2 beaver ponds and lakes I-3 and I-4. Desulfobulbaceae (0.03%) taxa were found in limited relative abundances (highest in ER and WC beaver ponds) and Thermodesulfovibrionia (0.31%) was also found in lakes I-3, I-4, and I-5 and the ER and WC ponds.

Several archaea related to methanogenic taxa showed higher relative abundances in tundra lakes and streams than beaver ponds and included Methanobacteriales (0.62%), Methanosarcinales (0.10%), and Methanomicrobiales (0.007%) (Figure 3). Bathyarchaeia (0.48%) (among the unclassified Archaea) also displayed higher relative abundances in tundra lakes and streams than beaver ponds (Figure 3). Woesearcheales (0.22%) archaea, which are thought to interact syntrophically with methanogens and other anaerobes through hydrogen and acetate exchange (Liu et al., 2018), were found in all sites except for the tundra streams and RC1. Prominent uncultured families in the Bacteroidales were only found in tundra lakes and streams and included Bacteroidetes vadinHA17 (4.1%) and BD2-2 (0.09%). Genera in the Burkholderiales were found in high relative abundance across sample sites and included *Ellin6067* (1.2%) and *Rhodoferax* (0.62%). *Geobacter* (0.67%) was also a prominent genus across all

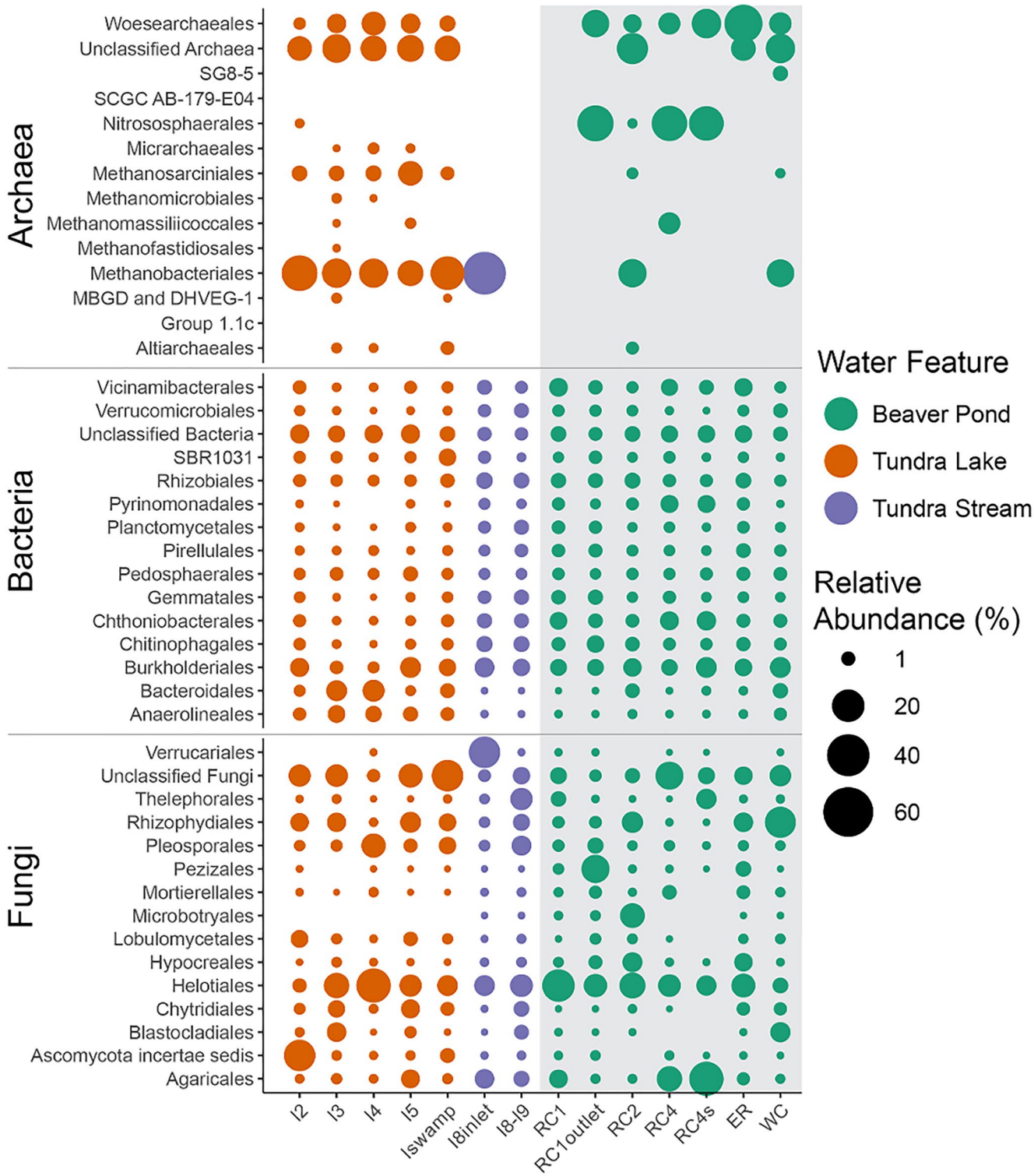


Figure 3. Within-kingdom relative abundance of the top 15 (14 for archaea) most abundant taxonomic orders across all sample sites. Relative abundance was calculated on the basis of per-sample total reads within each kingdom. Beaver pond sample sites are indicated by gray shading.

sample sites. Taxa related to methanotrophic bacteria (Guerrero-Cruz et al., 2021; Yang et al., 2022) were found in high relative abundances in tundra lakes and the WC beaver pond and included Crenothrix and Methylobacter, and Methylacidiphilaceae, which was found nearly exclusively in lake I-2. Taxa related to aerobic heterotrophic bacteria (Brewer et al., 2016; Kant et al., 2011) were found in high relative abundances in all sample sites,

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especially the RC beaver ponds, and included Chthoniobacter and Candidatus Udaeobacter, of the Chthoniobacterales. The WC beaver pond was the only sample site that contained *Sulfuricurvum* (Campylobacterota), in a high relative abundance (3.7%).

3.3. Fungal Alpha Diversity and Functional Guilds

Trends in fungal diversity, based on the Faith's PD, Chao1, and Shannon indices, were markedly different from bacterial and archaeal diversity (Figure S4 in Supporting Information S1). The tundra lakes and streams were higher in phylogenetic diversity but lower, though only slightly, than the beaver ponds in the Chao1 and Shannon indices. RC1 and the I-8 to I-9 stream showed the highest diversity index values for the beaver ponds and tundra streams and lakes, respectively. All fungal ASVs that were identified to the phylum level were represented, from high to low abundance, by the six phyla: Ascomycota (51.9%; Verrucariales, Pleosporales, Pezizales, Hypocreales, Helotiales, and Ascomycota *incertae sedis*, in Figure 3), Basidiomycota (22.5%; Thelephorales, Microbotryales, and Agaricales in Figure 3), Chytridiomycota (16.79%; Rhizophydiales, Lobulomycetales, Chytridiales in Figure 3), Blastocladiomycota (2.7%; Blastocladiales in Figure 3), Fungi *incertae sedis* (majority Zygomycota class) (1.8%; Mortierellales in Figure 3) and few ASVs of Glomeromycota (0.02%). Lakes I-2, I-4, I-swamp, and the I-8 inlet stream were especially dominated by Ascomycota. All tundra lake and stream sites, except for lake I-4 and the I-8 inlet stream, showed high relative abundances of Chytridiomycota. Notably, lake I-swamp showed a high relative abundance of unclassified taxa in class Sordariomycetes, of which many species are saprotrophic and participate in nutrient cycling (Lee et al., 2019). For the beaver ponds, RC ponds 1 and 2 and outlet pond 1 were dominated by Ascomycota, while RC pond 4 and pond 4 side were dominated by Basidiomycota. The WC pond differed greatly from other beaver ponds and displayed high relative abundances of Chytridiomycota and Blastocladiomycota and low relative abundances of Basidiomycota and Ascomycota.

Hebeloma (4.3%) (Agaricales in Figure 3) was the most abundant genus across all sample sites, especially in the RC pond 4 sites. Taxa related to ericoid mycorrhizal fungi of the Heliotales Order (Figure 3) (Walker et al., 2011), were abundant across all sites, especially in lake I-4 and RC1. The Fungi *incertae sedis* were represented mainly by Zygomycota *incertae sedis*. Of significance within this class is *Mortierella* (1.1%), which occurred in high relative abundances in beaver ponds. Taxa in the Rhizophydiales were found in high relative abundances in all sample sites, especially the WC pond (Figure 3). This group of fungi was represented mainly by *Betamyces* (4.1%), species of which have been attributed with the colonization of microplastics in Arctic lakes (González-Pleiter et al., 2021), potentially providing evidence for the breakdown of material with anthropogenic origins.

FungalTraits results displayed clear differences in functional potentials of fungi between beaver ponds and tundra lakes and streams. Fungal functional guilds were distilled into three trophic modes: pathotrophs, symbiotrophs, and saprotrophs. Pathotrophs were mainly composed of plant pathogens, but also included animal pathogens, parasites, endophytic parasites, and lichen parasites. Symbiotrophs were almost entirely composed of ectomycorrhizae, with the addition of orchid-root-associated ectomycorrhizae, lichens, endophytes, and one plant pathogen. Saprotroph functional guilds were diverse and mainly composed of unidentified saprotrophic fungi, but also included wood, dung, litter, leaf, and soil saprotrophs. Based on genera identified by the database, beaver ponds were 42.3% saprotrophic, 7.5% pathotrophic, and 50.1% symbiotrophic and tundra lakes and streams were 40.1% saprotrophic, 23.5% pathotrophic, and 36.6% symbiotrophic. Though the relative abundance of each trophic mode did not significantly differ (p-value > 0.05) between beaver ponds and tundra lakes and streams, a majority of beaver pond functional guilds were represented by high relative abundances of ectomycorrhizae and low relative abundances of pathogenic fungi, compared to tundra lakes and streams.

Nearly all ectomycorrhizal taxa belonged to the class Agaricomycetes (relative abundances out of all fungal taxa; 11.8% in beaver ponds; 5.45% in tundra lakes and streams) and were most abundant in the RC beaver ponds. Prominent genera included *Hebeloma*, *Inocybe* (0.53%), *Sebacina* (0.12%), and *Cortinarius* (0.17%). *Ascobolus* (3.0%) was an abundant saprotroph in the ER beaver pond and RC pond 1. Other abundant saprotrophs across the sample sites included *Lulworthia* (0.26%), *Entoloma* (0.11%), *Mortierella*, and *Galerina* (0.18%). Finally, fungi related to pathogenic taxa (Giotis et al., 2009; Shoemaker & Babcock, 1989) were found across sample sites and included *Phaeosphaeria* (0.29%; highest in RC ponds and lake I-5) and *Pyrenochaeta* (0.02%; highest in I8 inlet stream).

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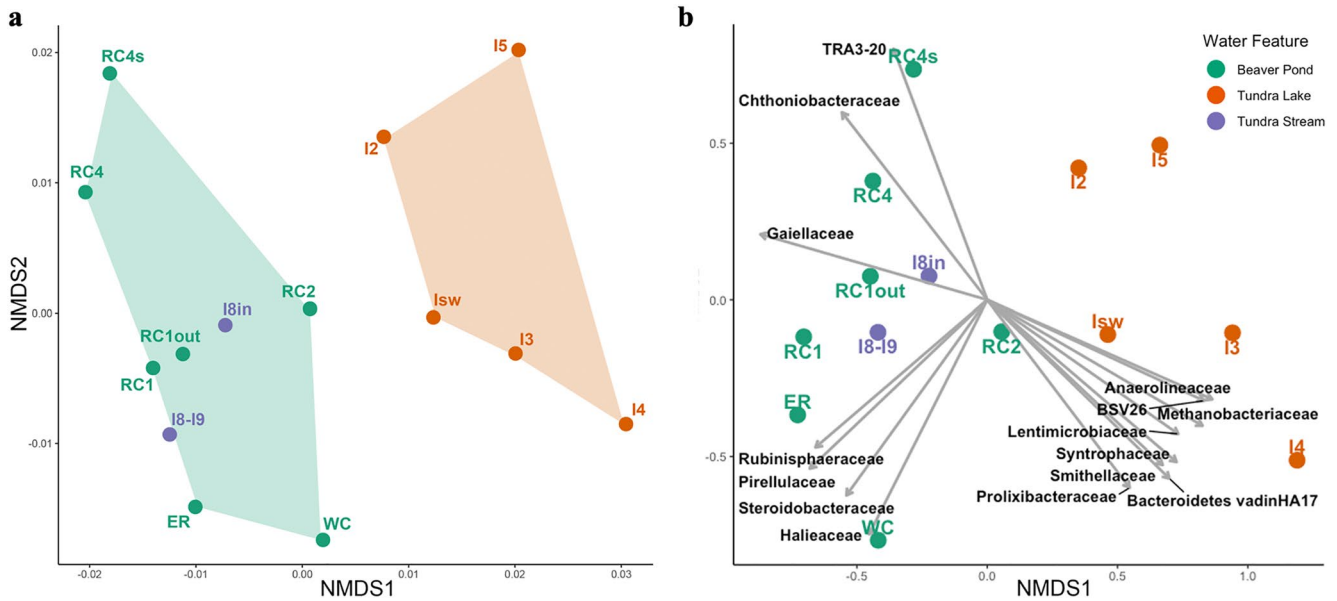


Figure 4. NMDS plots, based on (a) weighted UniFrac distance, displaying differences in bacterial and archaeal lineages between beaver ponds and tundra lakes and streams, with dominant taxa weighted and (b) Bray-Curtis dissimilarity, displaying bacterial and archaeal ASV compositional differences between sample sites. (a) Green shading in corresponds to the area of the ordination covered by beaver pond water features and orange shading corresponds to the area of the ordination covered by tundra lakes. Stress = 0.089. (b) Taxonomic vectors of the top 15 most significant families (all p-values < 0.05) partially explain the distribution of the points. Stress = 0.082.

3.4. Bacterial and Archaeal Community Composition

Unweighted and weighted UniFrac were used to examine phylogenetic differences in the diversity of communities, based on the presence and absence of lineages, and differences between dominant lineages of sites, based on the relative abundance of ASVs (nodes on the tree), respectively. Both UniFrac (weighted and unweighted) and Bray-Curtis dissimilarity were used to separate NDMS plots to display differences in the presence/absence of phylogenies (or lineages; unweighted), dominant lineages (weighted), and ASV relative abundance-related compositional differences (Bray-Curtis) between beaver ponds and tundra lakes and streams. The bacterial and archaeal communities of each tundra stream appeared to be more similar to beaver ponds than to tundra lakes (Figure 4a). Communities were significantly different between the two groups (beaver ponds and tundra lakes and streams) regardless of relative abundance weighting (weighted UniFrac: PERMANOVA F-statistic = 2.15 and p-value = 0.019; unweighted UniFrac: PERMANOVA F-statistic = 1.65 and p-value = 0.016). Both weighted (Figure 4a) and unweighted (Figure S6a in Supporting Information S1) UniFrac results displayed similar ordination patterns on NMDS plots, and tundra streams clustered more closely to beaver ponds than tundra lakes. Variance was not significantly different between the two groups (weighted and unweighted UniFrac permutest p-values > 0.05). Both RC pond sites displayed the highest similarity in community abundance to the tundra streams (Figure 4a; Figure S6a in Supporting Information S1).

Apparent differences between the abundance of ASVs in beaver ponds and lakes were observed when Bray-Curtis dissimilarity was used (Figure 4b). The Bray-Curtis dissimilarity plot indicates that differences between beaver ponds and tundra lakes and streams were caused by ASV compositional differences in addition to differences in the compositions of phylogenetic lineages (PERMANOVA F-statistic = 1.97 and p-value = 0.017; permutest F-statistic = 1.00 and p-value = 0.329). Methanobacteriaceae, Syntrophaceae, Anaerolinaceae, BSV26, Smithellaceae, Prolixibacteraceae, Bacteroidetes vadinHA17, and Lentimicrobiaceae separated lakes I-swamp, I-3, and I-4, and to a lesser extent RC pond 2 (Figure 4b). The WC and ER beaver ponds were structured by similar taxa, such as Steroidobacteraceae, Pirellulaceae, Rubinisphaeraceae.

3.5. Fungal Community Composition

The results of fungal community weighted and unweighted UniFrac ordinations differed noticeably from those of bacteria and archaea (Figure 5a). Based on the statistical results, similar taxa of dominant fungi appeared to

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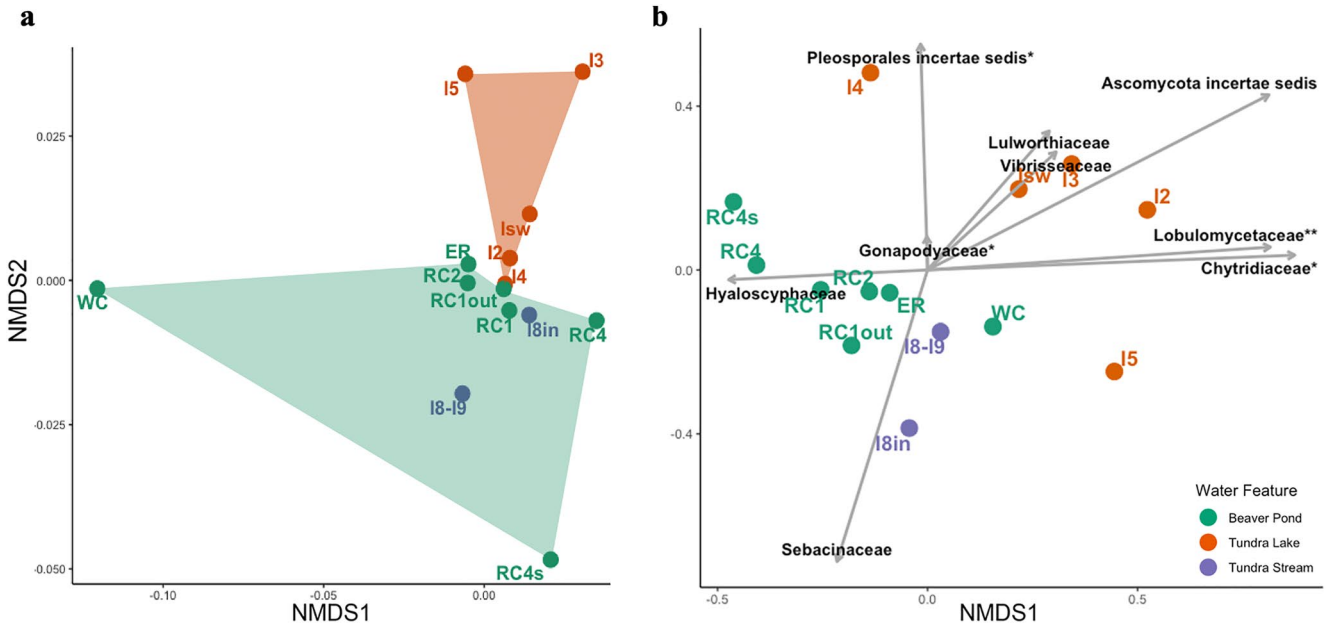


Figure 5. NMDS plots based on (a) weighted UniFrac distance, displaying differences in fungal communities between beaver ponds and tundra lakes and streams, with dominant taxa weighted and (b) based on Bray-Curtis dissimilarity, displaying fungal ASV compositional differences among sample sites. (a) Stress = 0.108. (b) Taxonomic vectors of significant families (p-value < 0.10) partially explain the distribution of points. Asterisks indicate significant family vectors (p-value < 0.05). Stress = 0.158.

exist between the two groups, beaver ponds and tundra streams and lakes (weighted UniFrac: PERMANOVA F-statistic = 1.20 and p-value = 0.106), while the presence and absence of fungal taxa was significantly different between the two groups (unweighted UniFrac: PERMANOVA F-statistic = 1.65 and p-value = 0.016). Ordination results for weighted (Figure 5a) and unweighted (Figure S6b in Supporting Information S1) UniFrac differed greatly. Separation between the clustering of water feature sample sites was discernible with unweighted UniFrac, though lake I-4 clustered more closely to beaver ponds than the other tundra lakes (Figure S6b in Supporting Information S1). Conversely, on the weighted UniFrac ordination tundra lakes, I-4, I-2, and I-swamp overlapped with the beaver ponds (Figure 5a). Sample site variance of fungal communities did not significantly differ between the two groups (weighted and unweighted UniFrac permutest p-values > 0.05). In addition to the tundra streams, lakes I-2, I-4, and I-swamp all clustered closely to beaver ponds, except the WC pond and RC pond 4 side pond (Figure 5a), which displayed similarities between these two sample sites in dominant fungal taxa. Differences between the WC beaver pond and the other ponds may be driven by the high relative abundances of Chytridiomycota and Blastocladiomycota and low abundances of Basidiomycota. Interestingly, the RC4 pond differed from the RC4 side pond (Figure 5a) even though these sites are adjacent to each other. Similar results to unweighted UniFrac were obtained when Bray-Curtis dissimilarity was used to structure the ordination (Figure 5b). Fungal ASV compositions were significantly different between beaver ponds and tundra lakes and streams, while variance between these two groups did not differ (PERMANOVA F-statistic = 1.39 and p-value = 0.002; permutest F-statistic = 1.00 and p-value = 0.326).

The influence of fungal families on the placement of sample sites on the ordination were plotted as vectors where significant (p-value < 0.05) families are indicated with an asterisk and nearly significant (0.05 < p-value < 0.10) families lack asterisks (Figure 5b). Significant families included the Pleosporales *incertae sedis*, (*Massariosphaeria* and *Lophiotrema*), Gonapodyceae (*Gonapodya*), many Chytridaceae genera, and the Lobulomycetaceae (*Maunachytrium*, *Lobulomyces*, and *Clydaea*) (Figure 5b). Each of these families belong to the Chytridiomycota phylum and appeared to have impacts mainly on the distribution of the tundra lakes in the ordination. *Massariosphaeria*, typically an endophytic genus (Li et al., 2020) in the Pleosporales *incertae sedis* (Figure 5b), influenced the divergence of lake I-4 from the rest of the tundra lakes. The tundra streams again clustered more closely to beaver ponds than tundra lakes, which is consistent with the relative abundance of fungi of the Sebacinaceae family (Phylum, Basidiomycota). Though not statistically significant, fungi in the Hyaloscyphaceae (*Hyaloscypha*, *Lachnum*, and *Hydrochina*) influenced the placement of RC beaver ponds, especially ponds 1 and 4, in the ordination.

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4. Discussion

4.1. Beaver Ponds and Tundra Lakes and Streams Differ in Extracellular Enzyme Activity and Physical Structure

The spread of woody vegetation, the decrease in extended cold temperature in winter, and the increase in winter stream discharge in northern latitudes have stimulated an expansion of previously-known habitats of the North American beaver (Jones et al., 2020; Tape et al., 2018, 2022). These ecosystem engineers have profound ecological impacts on the watersheds that they dam and likely change the microbiology of Arctic rivers and streams. The semi-lentic nature of both the beaver ponds and the tundra lakes and streams (Figures S1 and S2 in Supporting Information S1) provides an ideal comparison between beaver-impacted and beaver-unimpacted environmental systems. Examining β-glucosidase activity levels allows for the assessment of one of the many microbially-produced enzymes used to degrade vegetative OM. The cleavage of cellobiose to glucose by β-glucosidase is the rate-limiting step of cellulose decomposition (Zhang et al., 2020). This enzyme is produced primarily by heterotrophic microbes (Turner et al., 2002), including many bacterial phyla, in particular Firmicutes, Actinobacteria, Proteobacteria, and Acidobacteria, members of which were distributed across sample sites, and also by Ascomycota and Basidiomycota fungi (Pathan et al., 2017). Basidiomycota are prominent decomposers of cellulose due to the growth of these fungi on dead wood or litter (Baldrian & Valášková, 2008). In addition, saprotrophs can breakdown plant material, and the saprotrophic genera (identified by FungalTraits), *Lulworthia*, *Entoloma*, *Mortierella*, and *Galerina* were distributed across sample sites of all three water features and contain species capable of decomposing wood (Enjalbert et al., 2004) and cellulose (Meyers & Scott, 1968).

Cellulose is the most common carbohydrate found in plant biomass (Zhang et al., 2020) and the activity of β-glucosidase is therefore an indicator for the degree of decomposition of plant material in environmental settings. Though each of the tundra lakes and streams were all found within the same watershed, the variation of β-glucosidase activity levels was higher for these sample sites than beaver ponds (Figure S4 in Supporting Information S1), which could be driven by gradients within the I-series catchment (downgradient higher activity and upgradient lower activity). β-glucosidase activity in the ER pond was much higher than all other sample sites, which suggests a high degree of heterotrophic activity in this beaver pond. Beaver ponds had higher average β-glucosidase activity levels (see Section 3.1), relative abundances of Basidiomycota fungi, and bacterial and archaeal absolute abundances (significantly associated with β-glucosidase activity levels) than the tundra lakes and streams, which provides evidence for elevated cellulose breakdown in beaver ponds. The extracellular decomposition of this refractory polysaccharide would also provide accessible glucose as a carbon and energy source for microbes in the local environment.

In contrast to β-glucosidase activity levels, sediment texture did not appear to differ consistently between beaver and tundra lakes and streams. Previous studies have shown that textural heterogeneity (the evenness between sand, silt, and clay percentages) is a major factor in the structuring of soil fungal and bacterial communities (Bach et al., 2010; Seaton et al., 2020), and that increases in heterogeneity positively influence microbial biomass (Hu et al., 2014). Though similarities were observed between sediment texture classifications (Table 1), individual sand, silt, and clay percentages differed greatly between sample sites. Sample sites with relatively even sand, silt, and clay percentages included RC pond 1 and lakes I-2, I-4, and I-5, however no discernible relationship existed between textural heterogeneity and bacterial and archaeal absolute abundances. A larger sample size is likely needed to identify relationships between sediment texture and bacterial and archaeal biomass, based on absolute abundance of the 16S rRNA gene.

4.2. Bacteria and Archaea Associated With CH_4- and Nitrogen-Cycling Differ Between Beaver Ponds and Tundra Lakes and Streams

Understanding the diversity of microbes involved in CH_4 and N_2O cycling in beaver ponds is a key step in understanding the climatic impacts of these beaver-impacted environmental settings because of the global warming potential of these two greenhouse gases (Ali et al., 2013). Past research has shown that global beaver habitat proliferation leads to net increases in CH_4 emissions resulting from pond formation caused by stream damming (Whitfield et al., 2015). However, beaver ponds in this study showed lower relative abundances of putatively methanogenic archaea (i.e., methanogens) than tundra lakes and streams. RC pond 2 and the WC pond were the only beaver ponds that contained somewhat high relative abundances of methanogens, which suggests that

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beavers may stimulate CH_4 release in some systems, particularly older ponds as they become more similar to lakes, though potentially at lower levels than Arctic lakes unimpacted by beavers. Further evidence for this claim comes from RC pond 1 and its outlet stream, the youngest beaver pond sites (Table 1), which lacked methanogenic archaea in our study. Of additional importance is discerning which methanogenesis pathways are occurring, based on dominant archaeal taxa, as different forms of methanogenesis in Arctic sediments can influence CH_4 production under scenarios where temperature is increasing (Blake et al., 2015). Based on the relative abundance of methanogen genera present, hydrogenotrophic methanogenesis could be the dominant pathway (*Methanobacterium*), though acetate and formate could also be used as substrates (*Methanosarcina*) (Liebner et al., 2015; Schauer & Ferry, 1980).

It is possible that a large portion of CH_4 produced in beaver ponds and tundra lakes is oxidized by bacteria, because samples that contained high relative abundances of methanogenic archaea also contained high relative abundance of bacteria closely related to methanotrophic organisms (i.e., methanotrophs). The absolute abundance of methanotrophs was higher than that of methanogens in nearly all samples (Figure S7 in Supporting Information S1), indicating methanogens may have been under-sampled and could inhabit deeper sediment layers. The absolute abundances of methanogens in RC pond 2 and the WC pond were higher than lakes I-2 and I-5, indicating that methanogen biomass may be high in these beaver ponds, though this was not reflected by relative abundance, likely due to the overshadowing of bacterial relative abundance over archaeal relative abundance. The WC pond also displayed high absolute abundances of methanotrophs, implicating this beaver pond as a potential hotspot for CH_4 cycling, with atmospheric release possibly mitigated by oxidation. However, lakes I-3, I-4, and I-swamp had much higher absolute abundances of methanogens than all beaver ponds and the tundra lakes and streams contained an order of magnitude higher average absolute abundance of methanogens (1.86×10^7) than beaver ponds (3.79×10^6). More research is needed to measure CH_4 concentrations in water and fluxes from ponds and stream to evaluate the impact of beaver impoundments on CH_4 releases in Alaska.

Beaver pond microbes showed a relatively high potential for nitrogen cycling compared to tundra lakes and stream based on the taxa that were detected. The capacity for N fixation may be present at all sample sites due to the high relative abundance Rhizobiales (Figure 3), an order of Alphaproteobacteria that includes many N-fixing organisms (Altshuler et al., 2019). In addition, a high relative abundance of *Bradyrhizobium* (Rhizobiales Order; Figure 3), a genus that includes N-fixing species (Altshuler et al., 2019), occurred in all beaver ponds and tundra streams, but not in tundra lakes. N_2O is released by bacteria due to incomplete denitrification (Palta et al., 2013), an anaerobic process that is primarily reliant on the generation of nitrate from nitrification by ammonia-oxidizing bacteria (AOB) and/or archaea (AOA) (Beeckman et al., 2018). High relative abundances of Nitrosomonadaceae (Burkholderiales Order; Figure 3), a family of Burkholderiales containing species capable of ammonia oxidation (Podlesnaya et al., 2020), were found across sample sites, especially the beaver ponds. Though weakly based on 16S rRNA gene identity, this could indicate a higher prevalence of ammonia oxidation in beaver ponds than in other sample sites, which could provide substrate for denitrification.

The nitrite-oxidizing genus *Nitrospira*, and the potentially denitrifying genera *Thiobacillus* and *Pseudomonas* were all nearly exclusive to the beaver ponds and tundra streams, which provides limited evidence for coupled nitrification and denitrification in these sites (Altshuler et al., 2019; Daims et al., 2015). Members of the Rhodobacterales, which were positively correlated with N_2O release in Arctic cryosols (Altshuler et al., 2019), also displayed highest relative abundances in beaver ponds and tundra streams. Finally, unclassified members of the Rokubacteriales, which were positively correlated with total nitrogen content in northern peatlands (Ivanova et al., 2021), were in high relative abundances in beaver ponds and tundra streams, especially the RC ponds. These combined results display a potential for N-cycling, and possible N_2O release if denitrification is occurring and incomplete, in both beaver ponds and tundra streams, but to a much lesser degree in tundra lakes.

4.3. Putative Anaerobic Bacteria and Archaea Differ Between Beaver Ponds and Tundra Lakes and Streams

Evidence for anaerobic microbial metabolisms existed in all sample sites, however, the tundra lakes appear to harbor the most extensive diversity of bacteria and archaea taxa related to organisms with anaerobic metabolisms. Methanobacteriaceae, Syntrophaceae, Anaerolinaceae, BSV26, Smithellaceae, Prolixibacteraceae, Bacteroidetes vadinHA17, and Lentimicrobiaceae all contain species that undergo anaerobic metabolisms (Cai et al., 2022; Galushko & Kuever, 2021; Kuever, 2014; Liang et al., 2015; Schauer & Ferry, 1980; Sun et al., 2016; Zhou

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et al., 2019) and influenced the placement of tundra lakes (Figure 4b). Taxa in the Clostridiales, Anaerolineales, and Bacteroidales were also present in high relative abundances across all sample sites, but especially tundra lakes (Figure 3). This combined evidence suggests that the anaerobic breakdown of OM via fermentation may be a dominant pathway in Arctic lakes and, to a lesser extent, beaver ponds. It is also possible that syntrophic coupling between bacteria undergoing fermentation, which produce the substrates for methanogenesis (H_2, CO_2, and acetate), and methanogenic archaea (Buan, 2018) could favor anaerobic OM decomposition and CH_4 release in Arctic lakes unimpacted by beavers. This theoretical syntrophy may, however, be absent or less influential in the RC beaver ponds, other than RC pond 2, due to the lack of observed methanogens in these sample sites. All tundra lakes and streams had higher DOC concentrations than beaver ponds, which would provide a greater substrate for OM decomposition and the production of macromolecules to fuel methanogenesis. Tundra streams contained far lower relative abundances of taxa related to organisms with anaerobic metabolisms than tundra lakes, which could partially drive bacterial and archaeal community similarity between tundra streams and beaver ponds.

The WC and ER beaver ponds showed the highest potential for microbial sulfur cycling among beaver ponds, and WC was the only site that contained *Sulfuricurvum* abundance. Sulfide oxidation, potentially coupled with denitrification, could therefore be uniquely driven by *Sulfuricurvum* in this beaver pond (Cron et al., 2019; Kodama & Watanabe, 2004). Anaerobic taxa, including those that cycle sulfur and iron, methanogens, and syntrophic and fermenting taxa, have the ability to methylate mercury (Bravo et al., 2018; Peterson et al., 2020). ASVs of the Geobacteraceae family, species of which have been identified as the main mercury methylators in iron-rich freshwater sediments (Bravo et al., 2018), were in high relative abundance across sample sites, especially lake I-2, RC ponds 1 and 2, and the WC beaver pond. *Geobacter*, a taxon attributed with mercury methylation (Lu et al., 2016), displayed the highest relative abundance in lake I-3, the I-8 inlet stream, RC ponds 1 and 2, and the WC pond. Also, ASVs of the *Syntrophus* genus, species of which are capable of anaerobic metabolisms and mercury methylation (Gilmour et al., 2013), were observed in high relative abundances in lakes I-2, I-3, and I-4 and, to a lesser extent, the WC pond. Though the mercury methylation genes, *hgcAB* are harbored by a broad phylogeny of bacteria and archaea and exist in an array of environmental settings (Podar et al., 2015), enhanced methylmercury production would likely occur in sample sites with increased relative abundances of putatively anaerobic taxa such as the WC and RC2 beaver ponds and lakes I-swamp, I-3, and I-4.

4.4. Fungi Differ by Functional Guilds but Not Dominant Lineages Between Beaver Ponds and Tundra Lakes and Streams

Results from weighted UniFrac showed that dominant fungal communities did not significantly differ between the two sample groups (Figure 4b), however the FungalTraits results portrayed beaver ponds and tundra lakes and streams as harboring fungi with distinct dominant functional traits. This suggests that functional guilds may not be restricted to certain taxonomic annotations of dominant fungi. Tundra lakes and streams appeared to harbor high relative abundances of fungi that can degrade dead OM and parasitize plants and animals whereas the beaver ponds were dominated by ASVs typically assigned as ectomycorrhizae. Beaver ponds overall contained similar levels of saprotrophic fungi to tundra lakes and streams, though the saprotrophic genera *Mortierella* and *Ascobolus* displayed heightened relative abundances in beaver ponds. *Mortierella* have been shown to be carry out plant growth promotion and cellulose decomposition (Ozimek & Hanaka, 2021) and *Ascobolus* abundance was correlated with OM, N, and P availability in bauxite residues (Dong et al., 2021). Unlike other water-inundated permafrost settings (Schütte et al., 2019), the damming of rivers by beavers may favor plant:fungi mutualistic interactions, based on the high relative abundance of ectomycorrhizae in beaver ponds. Ectomycorrhizae, particularly species of *Hebeloma*, also influence the cycling of nitrogen (Tibbett et al., 1999), therefore the prevalence of ectomycorrhizal fungi in beaver ponds complements the heightened relative abundance of putative N-cycling bacteria in these same sample sites. The fungal functional guilds of tundra lakes resembled previously described boreal thermokarst settings where fungal communities shift away from a dominance of plant mutualists (Schütte et al., 2019).

The relative contribution of pathotrophic fungi in our analysis was likely underestimated in our study because parasitic fungi of the Chytridiomycota phylum (Rojas-Jimenez et al., 2017) are absent from the FungalTraits database, but had high relative abundances in some samples. For example, the lack of ectomycorrhizae and high relative abundance of Chytridiomycota in the WC beaver pond suggests a closer fungal functional and taxonomic

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relationship between this sample site and tundra lakes and streams, which could be influenced by its high latitude location. However, a caveat to this conclusion is that the WC pond did not display high relative abundances of saprotrophic fungi. CO_2 release from dead OM decomposition could be magnified by saprotrophs in tundra lakes and streams, while ectomycorrhizae could stimulate photosynthesis and concurrent CO_2 drawdown through plant:fungal interactions in the RC and ER beaver ponds. However, CO_2 release by ectomycorrhizal OM decomposition could also occur in beaver ponds. This could partially explain why beaver ponds displayed higher average β-glucosidase activity levels than tundra lakes and streams, but percent compositions of saprotrophic fungal taxa, which would likely produce the enzyme, were largely uniform between beaver ponds and tundra lakes and streams.

4.5. Microbial Communities in Beaver Ponds Are More Similar to Those in Tundra Streams Than in Tundra Lakes

We anticipated that microbial communities of Alaskan beaver ponds would differ from those of tundra lakes and streams, and would feature elevated alpha diversity because woody vegetation in beaver ponds may stimulate sediment microbial multifunctionality, driven by high diversity (Delgado-Baquerizo et al., 2016). We found evidence to support the hypothesis that beaver ponds host different sediment communities than tundra lakes and streams, but did not find evidence for elevated alpha diversity. While dominant bacterial and archaeal communities differed between beaver ponds and tundra lakes and streams, dominant fungal taxa were similar in these two ecological settings. However, tundra streams clustered more closely to beaver ponds than tundra lakes on each NMDS plot, meaning that dominant beaver pond fungal communities could be more similar to streams in composition. The combined results of bacterial, archaeal, and fungal community sequencing also showed tundra streams to be more microbiologically similar to beaver ponds than tundra lakes. This finding was unexpected because the tundra streams are located the same catchment as the tundra lakes and so would seem likely to resemble each other due to proximity.

Additionally, differences in geographic distance and elevation between the streams and beaver ponds would suggest that dissimilarities between these settings might be evident. The presence of flowing water in beaver ponds (beavers dam free-flowing streams and rivers) may influence microbial community similarities through the deposition of larger sized sediment particles (sand and silt) carried by moving water. Variations in sand, silt, and clay percentages influence bacterial diversity (Yao et al., 2019). The similarities of texture classifications (Table 2) between the tundra streams and the RC beaver ponds (all but pond 1) therefore could have partially caused taxonomic overlaps. This is displayed by the bacterial and archaeal community ordinations (Figures 4 and 5), where the I-8 to I-9 and I-8 inlet streams are close to RC beaver ponds. The similarity between RC pond 1 and outlet and the tundra streams in bacterial and archaeal beta diversity may have been partially due to the high DOC concentrations of these beaver pond sample sites, compared to the rest of the beaver ponds (Table 2), which in turn could favor bacterial heterotrophs. Further, the RC sites are located in headwaters and contain free-flowing water from mainstem Rabbit Creek, and are therefore ecologically similar to the tundra streams. Younger beaver ponds may feature heightened DOC and resemble streams unimpacted by beavers, yet could become more similar to tundra lakes with age.

5. Conclusion

The spread of the North American beaver to northern latitudes will likely have important impacts on the ecology and biogeochemistry of watersheds where they build dams. This study characterized the structure of microbial communities of Alaskan beaver ponds and differentiated them from established Arctic lakes and streams where beavers are absent. Our research suggests that unique bacterial, archaeal, and fungal communities inhabit beaver ponds and could drive a diverse array of potential biogeochemical cycles (carbon, nitrogen, sulfur, mercury, and iron). Though similar assemblages of dominant fungal lineages occurred between beaver ponds and tundra lakes and streams, fungal phylogenetic diversity (unweighted UniFrac) and ASV compositions differed significantly between these two settings. It could be that beaver ponds become more similar to Arctic lakes with age and older beaver ponds harbor more methanogenic archaea, methanotrophic bacteria, and anaerobic taxa. However, more research is necessary to support this conclusion. Based on taxonomic identities, beaver ponds appeared to harbor high relative abundances of N-cycling bacteria and fungi, with implications for microbially-driven N_2O release from beaver ponds. The release of CO_2 from the breakdown of vegetative OM may be slightly higher in beaver

ponds than tundra lakes and streams, stemming from bacterial and fungal heterotrophic activity. However, this release may be mitigated by increased beaver pond plant photosynthesis, stimulated by ectomycorrhizae and carbon burial in sediment. Future functional and activity-based analyses of microbial communities in these types of systems would provide greater insight into the specific processes occurring in high latitude beaver ponds. Larger sample sizes and sediment cores (with depth profile maintenance and replicates) should also be taken to avoid under-sampling methanogens and other anaerobic taxa. Measurements of carbon burial rates should be made to assess the release of carbon from beaver ponds and into streams and rivers. Finally, seasonal data would help to assess how beaver pond communities change when ponds freeze over. Further research into the microbiology of beaver ponds will help to discern how this keystone species impacts Arctic aquatic ecosystems as it continues to spread north.

Data Availability Statement

The datasets presented in this study can be found in the NCBI sequence read archive (Leinonen et al., 2011); PRJNA924313: https://www.ncbi.nlm.nih.gov/sra/PRJNA924313.

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References

Aiken, G. R. (1992). Chloride interference in the analysis of dissolved organic carbon by the wet oxidation method. *Environmental Science and Technology*, *26*(12), 2435–2439. https://doi.org/10.1021/es00036a015

Ali, M. A., Hoque, M. A., & Kim, P. J. (2013). Mitigating global warming potentials of methane and nitrous oxide gases from rice paddies under different irrigation regimes. *Ambio*, *42*(3), 357–368. https://doi.org/10.1007/s13280-012-0349-3

Allison, S. D., & Vitousek, P. M. (2005). Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biology and Biochemistry*, *37*(5), 937–944. https://doi.org/10.1016/j.soilbio.2004.09.014

Altshuler, I., Ronholm, J., Layton, A., Onstott, T. C., Greer C, W., & Whyte, L. G. (2019). Denitrifiers, nitrogen-fixing bacteria and N_2O soil gas flux in high Arctic ice-wedge polygon cryosols. *FEMS Microbiology Ecology*, *95*(5), fiz049. https://doi.org/10.1093/femsec/fiz049

Apprill, A., McNally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic Microbial Ecology*, *75*(2), 129–137. https://doi.org/10.3354/ame01753

Ashworth, J., Keyes, D., Kirk, R., & Lessard, R. (2001). Standard procedure in the hydrometer method for particle size analysis. *Communications in Soil Science and Plant Analysis*, *32*(5–6), 633–642. https://doi.org/10.1081/css-100103897

Bach, E. M., Baer, S. G., Meyer, C. K., & Six, J. (2010). Soil texture affects soil microbial and structural recovery during grassland restoration. *Soil Biology and Biochemistry*, *42*(12), 2182–2191. https://doi.org/10.1016/j.soilbio.2010.08.014

Baldrian, P., & Valášková, V. (2008). Degradation of cellulose by basidiomycetous fungi. *FEMS Microbiology Reviews*, *32*(3), 501–521. https://doi.org/10.1111/j.1574-6976.2008.00106.x

Banos, S., Lentendu, G., Kopf, A., Wubet, T., Glöckner, F. O., & Reich, M. (2018). A comprehensive fungi-specific 18S rRNA gene sequence primer toolkit suited for diverse research issues and sequencing platforms. *BMC Microbiology*, *18*(1), 190. https://doi.org/10.1186/s12866-018-1331-4

Beeckman, F., Motte, H., & Beeckman, T. (2018). Nitrification in agricultural soils: Impact, actors and mitigation. *Current Opinion in Biotechnology*, *50*, 166–173. https://doi.org/10.1016/j.copbio.2018.01.014

Blake, L. I., Tveit, A., Øvreås, L., Head, I. M., & Gray, N. D. (2015). Response of methanogens in Arctic sediments to temperature and methanogenic substrate availability. *PLoS One*, *10*(6), e0129733. https://doi.org/10.1371/journal.pone.0129733

Bockstoce, J. R. (2009). *Furs and frontiers in the far North: The contest among Native and foreign nations for the Bering Strait Fur trade*. Yale University Press.

Bravo, A. G., Zopfi, J., Buck, M., Xu, J., Bertilsson, S., Schaefer, J. K., et al. (2018). Geobacteraceae are important members of mercury-methylating microbial communities of sediments impacted by waste water releases. *ISME Journal*, *12*, 802–812. https://doi.org/10.1038/s41396-017-0007-7

Brewer, T. E., Handley, K. M., Carini, P., Gilbert, J. A., & Fierer, N. (2016). Genome reduction in an abundant and ubiquitous soil bacterium 'Candidatus Udaeobacter copiosus.'. *Nature Microbiology*, *2*, 1–7.

Buan, N. R. (2018). Methanogens: Pushing the boundaries of biology. *Emerging Topics in Life Sciences*, *2*(4), 629–646. https://doi.org/10.1042/etls20180031

Cai, Y.-J., Liu, Z.-A., Zhang, S., Liu, H., Nicol, G. W., & Chen, Z. (2022). Microbial community structure is stratified at the millimeter-scale across the soil–water interface. *ISME COMMUN*, *2*(1), 53. https://doi.org/10.1038/s43705-022-00138-z

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Cazzolla Gatti, R., Callaghan, T. V., Rozhkova-Timina, I., Dudko, A., Lim, A., Vorobyev, S. N., et al. (2018). The role of Eurasian beaver (Castor fiber) in the storage, emission and deposition of carbon in lakes and rivers of the River Ob flood plain, western Siberia. *Science of the Total Environment*, *644*, 1371–1379. https://doi.org/10.1016/j.scitotenv.2018.07.042

Cirmo, C. P., & Driscoll, C. T. (1996). The impacts of a watershed $CaCO_3$ treatment on stream and wetland biogeochemistry in the Adirondack Mountains. *Biogeochemistry*, *32*(3), 265–297. https://doi.org/10.1007/bf02187142

Čiuldienė, D., Vigricas, E., Belova, O., Aleinikovas, M., & Armolaitis, K. (2020). The effect of beaver dams on organic carbon, nutrients and methyl mercury distribution in impounded waterbodies. wbio 2020.

Cole, J. R., Wang, Q., Fish, J. A., Chai, B., McGarrell, D. M., Sun, Y., et al. (2014). Ribosomal database project: Data and tools for high throughput rRNA analysis. *Nucleic Acids Research*, *42*(D1), D633–D642. https://doi.org/10.1093/nar/gkt1244

Cron, B., Henri, P., Chan, C. S., Macalady, J. L., & Cosmidis, J. (2019). Elemental sulfur formation by Sulfuricurvum kujiense is mediated by extracellular organic compounds. *Frontiers in Microbiology*, *10*, 2710. https://doi.org/10.3389/fmicb.2019.02710

Crump, B., Adams, H., Hobbie, J., & Kling, G. (2007). Crump BC, Adams HE, Hobbie JE, Kling GW. Biogeography of bacterioplankton in lakes and streams of an Arctic tundra catchment. *Ecology*, *88*(6), 1365–1378. https://doi.org/10.1890/06-0387

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Daims, H., Lebedeva, E. V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., et al. (2015). Complete nitrification by Nitrospira bacteria. *Nature*, *528*(7583), 504–509. https://doi.org/10.1038/nature16461

Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*, *6*(1), 226. https://doi.org/10.1186/s40168-018-0605-2

Delgado-Baquerizo, M., Maestre, F. T., Reich, P. B., Jeffries, T. C., Gaitan, J. J., Encinar, D., et al. (2016). Microbial diversity drives multifunctionality in terrestrial ecosystems. 1. *Nature Communications*, *7*, 10541. https://doi.org/10.1038/ncomms10541

Dong, M., Shao, Y., Xu, Z., Liu, X., Xu, Y., Hu, X., et al. (2021). Resilience of fungal flora in bauxite residues amended with organic matter and vermiculite/fly ash. *Journal of Environmental Management*, *284*, 112052. https://doi.org/10.1016/j.jenvman.2021.112052

Edward, A. G. S., Abbott, B. W., Commane, R., Ernakovich, J., Euskirchen, E., Hugelius, G., et al. (2022). Permafrost and climate change: Carbon cycle feedbacks from the warming Arctic. *Annual Review of Environment and Resources*, *47*(1), 343–371. https://doi.org/10.1146/annurev-environ-012220-011847

Enjalbert, F., Cassanas, G., Rapior, S., Renault, C., & Chaumont, J.-P. (2004). Amatoxins in wood-rotting Galerina marginata. *Mycologia*, *96*(4), 720–729. https://doi.org/10.2307/3762106

Estop Aragonés, C., Olefeldt, D., Abbott, B. W., Chanton, J. P., Czimczik, C. I., Dean, J. F., et al. (2020). Assessing the potential for mobilization of old soil carbon after permafrost thaw: A synthesis of 14C measurements from the northern permafrost region. *Global Biogeochemical Cycles*, *34*, e2020GB006672. https://doi.org/10.1029/2020GB006672

Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: Summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, *32*(19), 3047–3048. https://doi.org/10.1093/bioinformatics/btw354

Faith, D. P. (1992). Conservation evaluation and phylogenetic diversity. *Biological Conservation*, *61*, 1–10. https://doi.org/10.1016/0006-3207(92)91201-3

Farina, M., Rocha, J. B. T., & Aschner, M. (2011). Mechanisms of methylmercury-induced neurotoxicity: Evidence from experimental studies. *Life Sciences*, *89*(15–16), 555–563. https://doi.org/10.1016/j.lfs.2011.05.019

Feng, J., Wang, C., Lei, J., Yang, Y., Yan, Q., Zhou, X., et al. (2020). Warming-induced permafrost thaw exacerbates tundra soil carbon decomposition mediated by microbial community. *Microbiome*, *8*(1), 3. https://doi.org/10.1186/s40168-019-0778-3

Frank, Y. A., Kadnikov, V. V., Lukina, A. P., Banks, D., Beletsky, A. V., Mardanov, A. V., et al. (2016). Characterization and genome analysis of the first facultatively alkaliphilic thermodesulfovibrio isolated from the deep terrestrial subsurface. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.02000

Galushko, A., & Kuever, J. (2021). Smithellaceae. In *Bergey's manual of systematics of archaea and bacteria* (pp. 1–3). John Wiley & Sons, Ltd.

German, D. P., Weintraub, M. N., Grandy, A. S., Lauber, C. L., Rinkes, Z. L., & Allison, S. D. (2011). Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biology and Biochemistry*, *43*(7), 1387–1397. https://doi.org/10.1016/j.soilbio.2011.03.017

Gilmour, C. C., Podar, M., Bullock, A. L., Graham, A. M., Brown, S. D., Somenahally, A. C., et al. (2013). Mercury methylation by novel microorganisms from new environments. *Environmental Science and Technology*, *47*(20), 11810–11820. https://doi.org/10.1021/es403075t

Giotis, C., Markelou, E., Theodoropoulou, A., Toufexi, E., Hodson, R., Shotton, P., et al. (2009). Effect of soil amendments and biological control agents (BCAs) on soil-borne root diseases caused by Pyrenochaeta lycopersici and Verticillium albo-atrum in organic greenhouse tomato production systems. *European Journal of Plant Pathology*, *123*(4), 387–400. https://doi.org/10.1007/s10658-008-9376-0

González-Pleiter, M., Velázquez, D., Casero, M. C., Tytgat, B., Verleyen, E., Leganés, F., et al. (2021). Microbial colonizers of microplastics in an Arctic freshwater lake. *The Science of the Total Environment*, *795*, 148640. https://doi.org/10.1016/j.scitotenv.2021.148640

Graham, D. E., Wallenstein, M. D., Vishnivetskaya, T. A., Waldrop, M. P., Phelps, T. J., Pfiffner, S. M., et al. (2012). Microbes in thawing permafrost: The unknown variable in the climate change equation. 4. *The ISME Journal*, *6*, 709–712. https://doi.org/10.1038/ismej.2011.163

Grinhut, T., Hadar, Y., & Chen, Y. (2007). Degradation and transformation of humic substances by saprotrophic fungi: Processes and mechanisms. *Fungal Biology Reviews*, *21*(4), 179–189. https://doi.org/10.1016/j.fbr.2007.09.003

Guerrero-Cruz, S., Vaksmaa, A., Horn, M. A., Niemann, H., Pijuan, M., & Ho, A. (2021). Methanotrophs: Discoveries, environmental relevance, and a perspective on current and future applications. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.678057

Hamilton, T. D. (2003). *Glacial geology of the Toolik lake and upper Kuparuk river regions Technical Report*. University of Alaska. Institute of Arctic Biology.

Hinzman, L. D., Bettez, N. D., Bolton, W. R., Chapin, F. S., Dyurgerov, M. B., Fastie, C. L., et al. (2005). Evidence and implications of recent climate change in Northern Alaska and other Arctic regions. *Climatic Change*, *72*(3), 251–298. https://doi.org/10.1007/s10584-005-5352-2

Hu, Y., Xiang, D., Veresoglou, S. D., Chen, F., Chen, Y., Hao, Z., et al. (2014). Soil organic carbon and soil structure are driving microbial abundance and community composition across the arid and semi-arid grasslands in northern China. *Soil Biology and Biochemistry*, *77*, 51–57. https://doi.org/10.1016/j.soilbio.2014.06.014

Hultman, J., Waldrop, M. P., Mackelprang, R., David, M. M., McFarland, J., Blazewicz, S. J., et al. (2015). Multi-omics of permafrost, active layer and thermokarst bog soil microbiomes. 7551. *Nature*, *521*, 208–212. https://doi.org/10.1038/nature14238

Ivanova, A. A., Oshkin, I. Y., Danilova, O. V., Philippov, D. A., Ravin, N. V., & Dedysh, S. N. (2021). Rokubacteria in northern Peatlands: Habitat preferences and diversity patterns. *Microorganisms*, *10*(1), 11. https://doi.org/10.3390/microorganisms10010011

Johnston, C. A. (2017). The biogeochemistry of boreal beaver ponds. In C. A. Johnston (Ed.), *Beavers: Boreal ecosystem engineers* (pp. 177–200). Springer International Publishing.

Jones, B. M., Tape, K. D., Clark, J. A., Nitze, I., Grosse, G., & Disbrow, J. (2020). Increase in beaver dams controls surface water and thermokarst dynamics in an Arctic tundra region, Baldwin Peninsula, northwestern Alaska. *Environmental Research Letters*, *15*(7), 075005. https://doi.org/10.1088/1748-9326/ab80f1

Jorgenson, M. T., Roth, J. E., Miller, P. F., Macander, M. J., Duffy, M. S., Wells, A. F., et al. (2009). An ecological land survey and landcover map of the Arctic Network 321.

Kant, R., van Passel, M. W. J., Palva, A., Lucas, S., Lapidus, A., Glavina del Rio, T., et al. (2011). Genome sequence of Chthoniobacter flavus Ellin428, an aerobic heterotrophic soil bacterium. *Journal of Bacteriology*, *193*(11), 2902–2903. https://doi.org/10.1128/jb.00295-11

Kim, B.-R., Shin, J., Guevarra, R. B., Lee, J. H., Kim, D. W., Seol, K.-H., et al. (2017). Deciphering diversity indices for a better understanding of microbial communities. *Journal of Microbiology and Biotechnology*, *27*(12), 2089–2093. https://doi.org/10.4014/jmb.1709.09027

Kjeldsen, K. U., Schreiber, L., Thorup, C. A., Boesen, T., Bjerg, J. T., Yang, T., et al. (2019). On the evolution and physiology of cable bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(38), 19116–19125. https://doi.org/10.1073/pnas.1903514116

Kodama, Y., & Watanabe, K. (2004). Sulfuricurvum kujiense gen. nov., sp. nov., a facultatively anaerobic, chemolithoautotrophic, sulfur-oxidizing bacterium isolated from an underground crude-oil storage cavity. *International Journal of Systematic and Evolutionary Microbiology*, *54*(6), 2297–2300. https://doi.org/10.1099/ijs.0.63243-0

21698961, 2023, 8, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2023JG007408 by Oregon State University, Wiley Online Library on [13/08/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Kuever, J. (2014). The family syntrophaceae. In E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, & F. Thompson (Eds.), *The prokaryotes: Deltaproteobacteria and epsilonproteobacteria* (pp. 281–288). Springer.
Kyaschenko, J., Clemmensen, K. E., Karltun, E., & Lindahl, B. D. (2017). Below-ground organic matter accumulation along a boreal forest fertility gradient relates to guild interaction within fungal communities. *Ecology Letters*, *20*(12), 1546–1555. https://doi.org/10.1111/ele.12862
Lee, S. H., Park, H. S., Nguyen, T. T. T., & Lee, H. B. (2019). Characterization of three species of Sordariomycetes isolated from freshwater and soil samples in Korea. *Mycobiology*, *47*(1), 20–30. https://doi.org/10.1080/12298093.2019.1574372
Leight, A. K., Crump, B. C., & Hood, R. R. (2018). Assessment of fecal indicator bacteria and potential pathogen co-occurrence at a shellfish growing area. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.00384
Leinonen, R., Sugawara, H., & Shumway, M. (2011). The sequence read archive [Software]. Nucleic Acids Research, *39*, D19–D21. https://doi.org/10.1093/nar/gkq1019
Letunic, I., & Bork, P. (2021). Interactive tree of Life (iTOL) v5: An online tool for phylogenetic tree display and annotation. *Nucleic Acids Research*, *49*(W1), W293–W296. https://doi.org/10.1093/nar/gkab301
Levanoni, O., Bishop, K., Mckie, B. G., Hartman, G., Eklöf, K., & Ecke, F. (2015). Impact of beaver pond colonization history on methylmercury concentrations in surface water. *Environmental Science and Technology*, *49*(21), 12679–12687. https://doi.org/10.1021/acs.est.5b03146
Li, X., Wang, H., Li, X., Li, X., & Zhang, H. (2020). Distribution characteristics of fungal communities with depth in paddy fields of three soil types in China. *Journal of Microbiology*, *58*(4), 279–287. https://doi.org/10.1007/s12275-020-9409-8
Liang, B., Wang, L.-Y., Mbadinga, S. M., Liu, J.-F., Yang, S.-Z., Gu, J.-D., & Mu, B.-Z. (2015). Anaerolineaceae and Methanosaeta turned to be the dominant microorganisms in alkanes-dependent methanogenic culture after long-term of incubation. *AMB Express*, *5*(1), 37. https://doi.org/10.1186/s13568-015-0117-4
Liebner, S., Ganzert, L., Kiss, A., Yang, S., Wagner, D., & Svenning, M. M. (2015). Shifts in methanogenic community composition and methane fluxes along the degradation of discontinuous permafrost. *Frontiers in Microbiology*, *6*. https://doi.org/10.3389/fmicb.2015.00356
Liu, X., Li, M., Castelle, C. J., Probst, A. J., Zhou, Z., Pan, J., et al. (2018). Insights into the ecology, evolution, and metabolism of the widespread Woesearchaeotal lineages. *Microbiome*, *6*(1), 102. https://doi.org/10.1186/s40168-018-0488-2
Lozupone, C., & Knight, R. (2005). UniFrac: A new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, *71*(12), 8228–8235. https://doi.org/10.1128/aem.71.12.8228-8235.2005
Lu, X., Liu, Y., Johs, A., Zhao, L., Wang, T., Yang, Z., et al. (2016). Anaerobic mercury methylation and demethylation by Geobacter bemidjiensis Bem. *Environmental Science and Technology*, *50*(8), 4366–4373. https://doi.org/10.1021/acs.est.6b00401
Mackelprang, R., Saleska, S. R., Jacobsen, C. S., Jansson, J. K., & Taş, N. (2016). Permafrost meta-omics and climate change. *Annual Review of Earth and Planetary Sciences*, *44*(1), 439–462. https://doi.org/10.1146/annurev-earth-060614-105126
Marushchak, M. E., Kerttula, J., Diáková, K., Faguet, A., Gil, J., Grosse, G., et al. (2021). Thawing Yedoma permafrost is a neglected nitrous oxide source. *Nature Communications*, *12*(1), 7107. https://doi.org/10.1038/s41467-021-27386-2
McCalley, C. K., Woodcroft, B. J., Hodgkins, S. B., Wehr, R. A., Kim, E.-H., Mondav, R., et al. (2014). Methane dynamics regulated by microbial community response to permafrost thaw. 7523. *Nature*, *514*, 478–481. https://doi.org/10.1038/nature13798
McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217
Meyers, S. P., & Scott, E. (1968). Cellulose degradation by Lulworthia floridana and other lignicolous marine fungi. *Marine Biology (Berlin)*, *2*(1), 41–46. https://doi.org/10.1007/bf00351636
Moore, J. A. M., Jiang, J., Post, W. M., & Classen, A. T. (2015). Decomposition by ectomycorrhizal fungi alters soil carbon storage in a simulation model. *Ecosphere*, *6*(3), 29. https://doi.org/10.1890/es14-00301.1
Mueller, R. C., Gallegos-Graves, L. V., & Kuske, C. R. (2016). A new fungal large subunit ribosomal RNA primer for high-throughput sequencing surveys. *FEMS Microbiology Ecology*, *92*.
Myers-Smith, I. H., Forbes, B. C., Wilmking, M., Hallinger, M., Lantz, T., Blok, D., et al. (2011). Shrub expansion in tundra ecosystems: Dynamics, impacts and research priorities. *Environmental Research Letters*, *6*(4), 045509. https://doi.org/10.1088/1748-9326/6/4/045509
Naiman, R., Johnston, C., & Kelley, J. (1988). Alteration of North American streams by beaver. *BioScience*, *38*(11), 753–762. https://doi.org/10.2307/1310784
Nalley, E. M., Donahue, M. J., & Toonen, R. J. (2022). Metabarcoding as a tool to examine cryptic algae in the diets of two common grazing surgeonfishes, Acanthurus triostegus and A. nigrofuscus. *Environmental DNA*, *4*(1), 135–146. https://doi.org/10.1002/edn3.206
Nummi, P., Vehkaoja, M., Pumpanen, J., & Ojala, A. (2018). Beavers affect carbon biogeochemistry: Both short-term and long-term processes are involved. *Mammal Review*, *48*(4), 298–311. https://doi.org/10.1111/mam.12134
O'Donnell, J. A., Aiken, G. R., Swanson, D. K., Panda, S., Butler, K. D., & Baltensperger, A. P. (2016). Dissolved organic matter composition of Arctic rivers: Linking permafrost and parent material to riverine carbon. *Global Biogeochemical Cycles*, *30*(12), 1811–1826. https://doi.org/10.1002/2016gb005482
Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2020). Vegan community ecology package version 2.5-7.
Ozimek, E., & Hanaka, A. (2021). Mortierella species as the plant growth-promoting fungi present in the agricultural soils. *Agriculture*, *11*(7), 7. https://doi.org/10.3390/agriculture11010007
Palta, M. M., Ehrenfeld, J. G., & Groffman, P. M. (2013). Denitrification and potential nitrous oxide and carbon dioxide production in brownfield wetland soils. *Journal of Environmental Quality*, *42*(5), 1507–1517. https://doi.org/10.2134/jeq2012.0392
Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology*, *18*(5), 1403–1414. https://doi.org/10.1111/1462-2920.13023
Pathan, S. I., Žifčáková, L., Ceccherini, M. T., Pantani, O. L., Větrovský, T., & Baldrian, P. (2017). Seasonal variation and distribution of total and active microbial community of *β*-glucosidase encoding genes in coniferous forest soil. *Soil Biology and Biochemistry*, *105*, 71–80. https://doi.org/10.1016/j.soilbio.2016.11.003
Patzner, M. S., Mueller, C. W., Malusova, M., Baur, M., Nikeleit, V., Scholten, T., et al. (2020). Iron mineral dissolution releases iron and associated organic carbon during permafrost thaw. 1. *Nature Communications*, *11*, 6329. https://doi.org/10.1038/s41467-020-20102-6
Peterson, B. D., McDaniel, E. A., Schmidt, A. G., Lepak, R. F., Janssen, S. E., Tran, P. Q., et al. (2020). Mercury methylation genes identified across diverse anaerobic microbial guilds in a eutrophic sulfate-enriched lake. *Environmental Science and Technology*, *54*(24), 15840–15851. https://doi.org/10.1021/acs.est.0c05435
Podar, M., Gilmour, C. C., Brandt, C. C., Soren, A., Brown, S. D., Crable, B. R., et al. (2015). Global prevalence and distribution of genes and microorganisms involved in mercury methylation. *Science Advances*, *1*(9), e1500675. https://doi.org/10.1126/sciadv.1500675

21698961, 2023, 8, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2023JG007408 by Oregon State University, Wiley Online Library on [13/08/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Podlesnaya, G., Krasnopeev, A., Potapov, S., Tikhonova, I., Shtykova, Y., Suslova, M., et al. (2020). Diversity of nitrifying bacteria in microbial communities from water and epilithic biofilms of the Lake Baikal littoral zone. *Limnology and Freshwater Biology*, *4*, 1008–1010. https://doi.org/10.31951/2658-3518-2020-a-4-1008

Põlme, S., Abarenkov, K., Henrik Nilsson, R., Lindahl, B. D., Clemmensen, K. E., Kauserud, H., et al. (2020). FungalTraits: A user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Diversity*, *105*, 1–16. https://doi.org/10.1007/s13225-020-00466-2

Price, M. N., Dehal, P. S., & Arkin, A. P. (2009). FastTree: Computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution*, *26*(7), 1641–1650. https://doi.org/10.1093/molbev/msp077

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), D590–D596. https://doi.org/10.1093/nar/gks1219

Rantanen, M., Karpechko, A. Y., Lipponen, A., Nordling, K., Hyvärinen, O., Ruosteenoja, K., et al. (2022). The Arctic has warmed nearly four times faster than the globe since 1979. 1. *Communications Earth & Environment*, *3*, 1–10.

R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Reich, M., & Labes, A. (2017). How to boost marine fungal research: A first step towards a multidisciplinary approach by combining molecular fungal ecology and natural products chemistry. *Marine Genomics*, *36*, 57–75. https://doi.org/10.1016/j.margen.2017.09.007

Rojas-Jimenez, K., Wurzbacher, C., Bourne, E. C., Chiuchiolo, A., Priscu, J. C., & Grossart, H.-P. (2017). Early diverging lineages within Cryptomycota and Chytridiomycota dominate the fungal communities in ice-covered lakes of the McMurdo Dry Valleys, Antarctica. 1. *Scientific Reports*, *7*, 15348. https://doi.org/10.1038/s41598-017-15598-w

Roth, H. K., Nelson, A. R., McKenna, A. M., Fegel, T. S., Young, R. B., Rhoades, C. C., et al. (2022). Impact of beaver ponds on biogeochemistry of organic carbon and nitrogen along a fire-impacted stream. *Environmental Sciences: Processes & Impacts*, *24*(10), 1661–1677. https://doi.org/10.1039/D2EM00184E

Roy, V., Amyot, M., & Carignan, R. (2009). Beaver ponds increase methylmercury concentrations in canadian shield streams along vegetation and pond-age gradients. *Environmental Science and Technology*, *43*(15), 5605–5611. https://doi.org/10.1021/es901193x

Schauer, N. L., & Ferry, J. G. (1980). Metabolism of formate in Methanobacterium formicicum. *Journal of Bacteriology*, *142*(3), 800–807. https://doi.org/10.1128/jb.142.3.800-807.1980

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, *75*(23), 7537–7541. https://doi.org/10.1128/aem.01541-09

Schütte, U. M. E., Henning, J. A., Ye, Y., Bowling, A., Ford, J., Genet, H., et al. (2019). Effect of permafrost thaw on plant and soil fungal community in a boreal forest: Does fungal community change mediate plant productivity response? *Journal of Ecology*, *107*(4), 1737–1752. https://doi.org/10.1111/1365-2745.13139

Schuur, E. A. G., Bockheim, J., Canadell, J. G., Euskirchen, E., Field, C. B., Goryachkin, S. V., et al. (2008). Vulnerability of permafrost carbon to climate change: Implications for the global carbon cycle. *BioScience*, *58*(8), 701–714. https://doi.org/10.1641/b580807

Screen, J. A., & Simmonds, I. (2010). The central role of diminishing sea ice in recent Arctic temperature amplification. 7293. *Nature*, *464*, 1334–1337. https://doi.org/10.1038/nature09051

Seaton, F. M., George, P. B. L., Lebron, I., Jones, D. L., Creer, S., & Robinson, D. A. (2020). Soil textural heterogeneity impacts bacterial but not fungal diversity. *Soil Biology and Biochemistry*, *144*, 107766. https://doi.org/10.1016/j.soilbio.2020.107766

Shoemaker, R., & Babcock, C. (1989). Phaeosphaeria. *Canadian Journal of Botany*, *67*(5), 1500–1599. https://doi.org/10.1139/b89-199

Singleton, C. M., McCalley, C. K., Woodcroft, B. J., Boyd, J. A., Evans, P. N., Hodgkins, S. B., et al. (2018). Methanotrophy across a natural permafrost thaw environment. *ISME Journal*, *12*(10), 2544–2558. https://doi.org/10.1038/s41396-018-0065-5

Smets, W., Leff, J. W., Bradford, M. A., McCulley, R. L., Lebeer, S., & Fierer, N. (2016). A method for simultaneous measurement of soil bacterial abundances and community composition via 16S rRNA gene sequencing. *Soil Biology and Biochemistry*, *96*, 145–151. https://doi.org/10.1016/j.soilbio.2016.02.003

Soil Survey Staff. (2019). *Soil texture calculator*. USDA Natural Resources Conservation Service. Retrieved from https://data.nal.usda.gov/dataset/soil-texture-calculator

Songster-Alpin, M., & Klotz, R. (1995). A comparison of electron transport system activity in stream and beaver pond sediments. *Canadian Journal of Fisheries and Aquatic Sciences*, *52*(6), 1318–1326. https://doi.org/10.1139/f95-128

Sterkenburg, E., Clemmensen, K. E., Ekblad, A., Finlay, R. D., & Lindahl, B. D. (2018). Contrasting effects of ectomycorrhizal fungi on early and late stage decomposition in a boreal forest. *ISME Journal*, *12*(9), 2187–2197. https://doi.org/10.1038/s41396-018-0181-2

Sun, L., Toyonaga, M., Ohashi, A., Tourlousse, D. M., Matsuura, N., Meng, X.-Y., et al. (2016). Lentimicrobium saccharophilum gen. nov., sp. nov., a strictly anaerobic bacterium representing a new family in the phylum Bacteroidetes, and proposal of Lentimicrobiaceae fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, *66*(7), 2635–2642. https://doi.org/10.1099/ijsem.0.001103

Tape, K. D., Clark, J. A., Jones, B. M., Kantner, S., Gaglioti, B. V., Grosse, G., & Nitze, I. (2022). Expanding beaver pond distribution in Arctic Alaska, 1949 to 2019. *Scientific Reports*, *12*(1), 7123. https://doi.org/10.1038/s41598-022-09330-6

Tape, K. D., Jones, B. M., Arp, C. D., Nitze, I., & Grosse, G. (2018). Tundra be dammed: Beaver colonization of the Arctic. *Global Change Biology*, *24*(10), 4478–4488. https://doi.org/10.1111/gcb.14332

Tarbier, B., Hugelius, G., Kristina Sannel, A. B., Baptista-Salazar, C., & Jonsson, S. (2021). Permafrost thaw increases methylmercury formation in subarctic Fennoscandia. *Environmental Science and Technology*, *55*(10), 6710–6717. https://doi.org/10.1021/acs.est.0c04108

Tibbett, M., Sanders, F. E., Cairney, J. W. G., & Leake, J. R. (1999). Temperature regulation of extracellular proteases in ectomycorrhizal fungi (Hebeloma spp.) grown in axenic culture. *Mycological Research*, *103*(6), 707–714. https://doi.org/10.1017/s0953756298007813

Turner, B. L., Hopkins, D. W., Haygarth, P. M., & Ostle, N. (2002). *β*-Glucosidase activity in pasture soils. *Applied Soil Ecology*, *20*(2), 157–162. https://doi.org/10.1016/s0929-1393(02)00020-3

Varsadiya, M., Urich, T., Hugelius, G., & Bárta, J. (2021). Fungi in permafrost-affected soils of the Canadian Arctic: Horizon- and Site-specific keystone taxa revealed by co-occurrence network. *Microorganisms*, *9*, 1943. https://doi.org/10.3390/microorganisms9091943

Větrovský, T., & Baldrian, P. (2013). The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses. *PLoS One*, *8*(2), e57923. https://doi.org/10.1371/journal.pone.0057923

Voříšková, J., & Baldrian, P. (2013). Fungal community on decomposing leaf litter undergoes rapid successional changes. *ISME Journal*, *7*(3), 477–486. https://doi.org/10.1038/ismej.2012.116

Walker, D. A., & Maier, H. A. (2008). *Vegetation in the vicinity of the Toolik field station, Alaska technical report*. University of Alaska. Institute of Arctic Biology.

21698961, 2023, 8, Downloaded from https://agupubs.onlinelibrary.wiley.com/doi/10.1029/2023JG007408 by Oregon State University, Wiley Online Library on [13/08/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Walker, J. F., Aldrich-Wolfe, L., Riffel, A., Barbare, H., Simpson, N. B., Trowbridge, J., & Jumpponen, A. (2011). Diverse Helotiales associated with the roots of three species of Arctic Ericaceae provide no evidence for host specificity. *New Phytologist*, *191*(2), 515–527. https://doi.org/10.1111/j.1469-8137.2011.03703.x

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, *73*(16), 5261–5267. https://doi.org/10.1128/aem.00062-07

Wang, Y., Roth, S., Schaefer, J. K., Reinfelder, J. R., & Yee, N. (2020). Production of methylmercury by methanogens in mercury contaminated estuarine sediments. *FEMS Microbiology Letters*, *367*(23), fnaa196. https://doi.org/10.1093/femsle/fnaa196

Ward, L. M., Bertran, E., & Johnston, D. T. (2021). Expanded genomic sampling refines current understanding of the distribution and evolution of sulfur metabolisms in the Desulfobulbales. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.666052

Whitfield, C. J., Baulch, H. M., Chun, K. P., & Westbrook, C. J. (2015). Beaver-mediated methane emission: The effects of population growth in Eurasia and the Americas. *Ambio*, *44*(1), 7–15. https://doi.org/10.1007/s13280-014-0575-y

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag. Retrieved from https://ggplot2.tidyverse.org

Wickham, H., François, R., Henry, L., Müller, K., & Vaughan, D. (2023). dplyr: A grammar of data manipulation. Retrieved from https://dplyr.tidyverse.org, Retrieved from https://github.com/tidyverse/dplyr

Wu, L., Yang, F., Feng, J., Tao, X., Qi, Q., Wang, C., et al. (2022). Permafrost thaw with warming reduces microbial metabolic capacities in subsurface soils. *Molecular Ecology*, *31*(5), 1403–1415. https://doi.org/10.1111/mec.16319

Xu, S., Zhan, L., Tang, W., Wang, Q., Dai, Z., Zhou, L., et al. (2023). MicrobiotaProcess: A comprehensive R package for deep mining microbiome. *Innovation*, *4*(2), 100388. https://doi.org/10.1016/j.xinn.2023.100388. https://www.sciencedirect.com/science/article/pii/S2666675823000164

Yang, Y., Chen, J., Pratscher, J., & Xie, S. (2022). DNA-SIP reveals an overlooked methanotroph, Crenothrix sp., involved in methane consumption in shallow lake sediments. *Science of the Total Environment*, *814*, 152742. https://doi.org/10.1016/j.scitotenv.2021.152742

Yao, Z., Du, S., Liang, C., Zhao, Y., Dini-Andreote, F., Wang, K., & Zhang, D. (2019). Bacterial community assembly in a typical estuarine marsh with multiple environmental gradients. *Applied and Environmental Microbiology*, *85*(6), e02602. https://doi.org/10.1128/aem.02602-18

Zeileis, A., & Hothorn, T. (2002). Diagnostic checking in regression relationships. *R News*, *2*(3), 7–10. https://CRAN.R-project.org/doc/Rnews/

Zeileis, A., Kleiber, C., Krämer, W., & Hornik, K. (2003). Testing and dating of structural changes in practice. *Computational Statistics & Data Analysis*, *44*(1–2), 109–123. https://doi.org/10.1016/s0167-9473(03)00030-6

Zhang, X., Ma, B., Liu, J., Chen, X., Li, S., Su, E., et al. (2020). *β*-Glucosidase genes differentially expressed during composting. *Biotechnology for Biofuels*, *13*(1), 174. https://doi.org/10.1186/s13068-020-01813-w

Zhou, J., Tape, K. D., Prugh, L., Kofinas, G., Carroll, G., & Kielland, K. (2020). Enhanced shrub growth in the Arctic increases habitat connectivity for browsing herbivores. *Global Change Biology*, *26*(7), 3809–3820. https://doi.org/10.1111/gcb.15104

Zhou, L.-Y., Yu, Z.-L., Xu, W., Mu, D.-S., & Du, Z.-J. (2019). Maribellus luteus gen. nov., sp. nov., a marine bacterium in the family Prolixibacteraceae isolated from coastal seawater. *International Journal of Systematic and Evolutionary Microbiology*, *69*(8), 2388–2394. https://doi.org/10.1099/ijsem.0.003495

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