Microbiological quality of drinking water in northern settlements: Influence of source water and treatment processes

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Highlights

- The microbiome of drinking water sources differs across northern settlements.
- Dissolved organic matter composition shapes bacterial community composition.
- Bacterial composition of treated water is set by the source and treatment process.
- Household storage tanks can promote bacterial regrowth in tap water.

Keywords: Climate change, aquatic microbiome, drinking water quality, drinking water supply, northern settlements, dissolved organic matter, bacterial community structure.

Abstract

Northern settlements face unique challenges regarding drinking water quality which may be exacerbated by rising pressures on freshwater ecosystems from climate change. Notably, browning, which results from the increasing addition of organic matter in surface water, could affect drinking water quality. In this study, we assessed the microbiological quality of water from source to tap, in settlements ─ including hamlets and northern villages ─ across Nunavut, Nunavik, and the Northwest Territories. We examined the bacterial composition of drinking water and its relationship with dissolved organic matter (DOM) and nutrients to identify the environmental drivers underlying changes in microbiological quality. Our study shows that the bacterial composition of drinking water sources, and consequently of water within the supply system, differs both spatially and temporally across settlements, and is associated with DOM and nutrient composition (dissolved organic carbon, total nitrogen, and proportions of fluorescent DOM components). Bacterial community composition (abundance and taxa) shifted along the drinking water systems, with patterns of reduction, stability, or increase from source to tap depending on the settlement. In some cases, bacterial abundance increased significantly in household taps, raising potential health concerns. Differences in treatment practices between settlements, particularly chlorination levels and the use (or absence) of filtration, influenced bacterial abundance, resulting in heterogeneous assemblages in treated waters. Overall, these findings highlight that drinking water monitoring must be adapted to local contexts and include regular temporal assessments to effectively identify risks related to microbiological water quality, particularly those arising from environmental changes that alter DOM composition.

1. Introduction

Climate-driven environmental changes, including altered precipitation regimes and permafrost thaw, are expected to increase pressures on freshwater resources, with consequences for ecosystem services such as drinking water supply. Arctic lakes are particularly vulnerable to shifts in precipitation, and changes in hydrological regimes under a warmer climate could further exacerbate the precariousness of drinking water sources in the North (Prowse et al., 2006), particularly in communities relying on a single source or a small shallow lake (Bakaic et al., 2017; 2018). In Nunavik, some northern villages have already reported changes in rivers flows, declining lake levels, and deteriorating water quality (Medeiros et al., 2017). Thawing permafrost also increases infiltration, leading to lake drainage and river drying, while mobilizing specific contaminants such as mercury and other metals stored in frozen soils (Prowse et al., 2006; Vonk et al., 2015; Loiko et al., 2017; Medeiros et al., 2017).

The browning of northern surface waters, driven by increases in organic matter inputs associated with changes in precipitation, terrestrial productivity, and permafrost thaw, can also affect water quality (Creed et al., 2018; Xenopoulos et al., 2021). It is well known that bacterial community composition is shaped by the nature of DOM (Logue et al., 2016; Comte et al., 2016; Tanentzap et al., 2019; Zhou et al., 2020). Hence, by modifying the DOM pool, browning is expected to alter freshwater ecosystem functioning, including a shift towards heterotrophic conditions and enhanced bacterial production (Roiha et al., 2016; Wauthy et al., 2017), which could impact drinking water quality due to an increase in bacterial biomass, if the treatment implemented does not have the capacity to adapt to source water quality variability. Consequently, as water treatment is generally limited to disinfection in northern communities (ITK 2020), browning is likely to alter the microbiological quality of drinking water sources. In addition, permafrost thaw and soil erosion can increase the transfer of soil microorganisms into freshwaters ecosystems, including potential pathogens in permafrost (Legendre et al., 2014;

Timofeev et al., 2019). Exposure to such microorganisms in drinking water may pose an emerging threat to public health. Despite this, the characterization of microbiomes in northern surface drinking water across different permafrost contexts remains limited and underexplored.

In northern settlements, drinking water supply typically involves pumping water from surface sources such as lakes or rivers, treating it at a local water treatment plant, delivering it to households via tank trucks, and storing it in household tanks for several days depending on consumption. Across Inuit Nunangat, including Inuvialuit Settlement Region, Nunavut, Nunavik, and Nunatsiavut (Northern Labrador), 41 of the 51 settlements rely exclusively on tank truck distribution systems for their water supply (ITK 2020). Surface water sources in northern settlements are vulnerable to chemical and microbiological contamination, posing health risks for residents (Medeiros et al., 2017). Potential hazards include the proximity of waste storage and contamination from animal migration, which can introduce harmful chemicals and pathogens leading to gastrointestinal illness that are frequently reported in these communities (Medeiros et al., 2017; Martin et al., 2007; Anaviapik-Soucie et al., 2015). Household storage tanks further increase vulnerability, as they are prone to *E. coli* contamination and bacterial regrowth (Farenhorst et al., 2017). Although high-level chlorination effectively inactivates pathogens, it presents additional challenges, as its reaction with dissolved organic matter (DOM) can produce disinfection by-products (Cortes & Marcos, 2018). Many residents collect raw water directly from lakes, rivers, snow, or lake ice. Raw water is preferred for its taste as it contains no chlorine (Cassivi et al., 2023) and because it avoids the truck or household tank storage conditions that can promote microbial growth (Martin et al., 2007; Wright et al., 2018 a-b; Ratelle et al., 2022). Water harvesting from the land also holds cultural importance as a traditional and intergenerational practice (Martin et al., 2007; Anaviapik-Soucie et al., 2015).

Monitoring the microbial composition of drinking water from source to tap is essential to evaluate microbiological risks associated with its consumption. While water quality is typically assessed using indicators such as coliforms and enteric pathogens (Health Canada 2020), profiling the taxonomic composition of bacterial communities offers a more comprehensive understanding of bacterial ecology, including potential pathogens that may affect water safety (Wang et al., 2018). However, few studies have investigated the microbiological quality of drinking water in the Arctic, while also accounting for regional differences in source water characteristics, particularly DOM composition, and treatment methods (Daley et al., 2018; Gora et al., 2020a-b).

In this study, we investigated changes in the microbial composition of drinking water throughout the entire supply chain (from source to tap) in settlements of Nunavut, Nunavik, and the Northwest Territories, and examined their relationship with the composition of DOM (i.e., its chromophoric and fluorescent components, and dissolved organic carbon concentrations; Fellman et al., 2010) and nutrient levels. We aimed to identify environmental conditions that may contribute to microbiological quality issues, including the presence of pathogens. We hypothesised that: 1) the abundance and composition of bacterial communities in source waters would vary with local environmental contexts and DOM composition; and (2) bacterial communities would shift markedly along the supply chain, with bacterial abundance decreasing in treated waters.

2. Materials and Methods

2.1.Study sites

The study involved eight northern settlements across northern Canada: Salluit (SAL), Kangirsuk (KGS), and Kangiqsualujjuaq (KAN) in Nunavik (northern Quebec); Cambridge Bay (CB), Taloyoak (TAL), Mittimatalik (MIT), and Qamani'tuaq (QAM) in Nunavut; and Fort Good Hope (FGH) in the Northwest Territories (Figure S1). Most settlements are in regions of continuous permafrost, with the exceptions of

Fort Good Hope, Kangirsuk, and Kangiqsualujjuaq, which lie in areas of discontinuous permafrost. In all settlements, drinking water is sourced from surface water and distributed by truck, except for SAL where it is sourced from a combination of surface and groundwater. Treatment generally consists of chlorination at the treatment station prior to distribution, with additional processes applied in certain settlements (Table S2). Ultraviolet disinfection is relatively common (implemented in 5 of the 8 settlements), whereas coagulation and filtration are rarely used (applied at FGH and CB).

2.2.Sample collection and processing

Between 2019 and 2021, water samples were collected at multiple points along the drinking water systems (Table S1): the supply sources (SW), raw water reservoir of the treatment plants when present (RW), chlorination point (CW), distribution truck (TW), household taps (HO), and public taps (PT). In SAL and KAN, the truck water (TW) consists of water sampled from the loading arm at the treatment station which is used to fill the distribution truck. Most settlements rely on lakes for their drinking water sources (6 of 8 hamlets), with the exceptions of FGH which draw from the Mackenzie River and SAL which draw from the Kuuguluk River and from groundwater within a Talik (an unfrozen zone within permafrost) beneath the river (Lemieux et al., 2016). Additional sources (AW) were also sampled, including Jackfish Lake in FGH (a potential alternate source), an unidentified source in TAL, Kuuguluk River in SAL, and a mountain spring used by part of the community in KAN. Because the study period overlapped with the COVID-19 pandemic and associated restrictions on access to the North, sampling campaigns were carried out in close collaboration with northern research partners and local settlements. This made the project a rare example of large-scale community-based research implemented with standardised sampling procedures. Settlements and partners received detailed protocols, and where possible, experienced operators trained new local members. Training sessions were organized as part of this study and related initiatives (e.g., ArctiConnexion: https://arcticonnexion.ca/) to help build local

141 capacity. Temporal variation in microbiological water quality was assessed at two scales: over two years
142 (2019 and 2021) in FGH, MIT, and KAN, and seasonally in 2021 in MIT, KAN, and CB. Samples were
143 processed upon receipt at the Institut national de la recherche scientifique (Québec).

144 **2.3.Water chemistry analyses**

145 Water temperature and pH were recorded *in situ* using an Oakton pHTestr 5 probe, and free and total
146 chlorine concentrations were measured with a Hach DR300 pocket colorimeter. These parameters were
147 measured onsite at the time of sampling. However, some measurements may have been taken with a
148 delay, which could have affected the temperatures recorded. Free chlorine and total chlorine at the
149 treatment point (TW or CW) were used as proxies for effective chlorination and overall chlorination
150 level, respectively. The difference between these two measures was calculated as consumed chlorine,
151 representing chlorine lost to reactions (e.g., with DOM, ammonia, or biofilm) or by decay. Total
152 phosphorus (TP) was measured using an Astoria2 analyzer (Astoria-Pacific), and total nitrogen (TN)
153 with a Lachat Quikchem 8500 (Hach Company).

154 Dissolved organic carbon (DOC, mg L^{-1}) was quantified by UV oxidation with ammonium persulfate
155 using a total organic carbon analyzer (Sivers M9, Veolia). Dissolved organic matter (DOM) was
156 characterized through its chromophoric (CDOM) and fluorescent (FDOM) fractions. Absorbance spectra
157 (200-800 nm) were acquired with a Cary300 dual beam spectrophotometer (Varian, 1 cm cuvette) to
158 calculate the absorption coefficient at 320 nm (a_{320}, m^{-1}; proxy of CDOM quantity), the specific UV
159 absorbance index at 254 nm (SUVA, , L mgC^{-1} m^{-1}; proxy of aromaticity), and the spectral slope
160 between 275 and 295 nm (S_{285}, nm^{-1}; proxy of DOM molecular size). Fluorescence excitation/emission
161 matrices (EEMs; excitation/emission range: 250-450 nm/300-600 nm) were obtained using a Cary
162 Eclipse spectrofluorimeter (Varian). Fluorescence matrices from 196 northern samples were processed
163 to build a Parallel Factor Analysis (PARAFAC) model. Six distinct fluorophore groups or components

were identified (detailed description provided in Herrera et al., 2025): four humic-like terrestrial components (HT1-4), one humic-like microbial component (HM1), and one protein-like (tryptophan-like) component (Try). Component assignments were confirmed using the *Openfluor* database (Murphy et al., 2014) with a similarity index above 0.95. FDOM composition was described by the abundance of these components (in Raman units, RU) and their relative proportion. The sum of the six components (F_{tot}, RU) was used as a proxy for total FDOM quantity.

2.4.Bacterial composition analyses

For bacterial abundance analysis, 5 mL water subsamples were fixed with glutaraldehyde (2.5% final concentration) and stored at -80°C until analysis. Abundance was determined by flow cytometry (Accuri C6 Plus, Becton Dickinson Biosciences) using SYBR-Green to stain DNA, following Mazoyer et al. (2022). For bacterial community composition, triplicate 500 mL subsamples were filtered onto 0.2 µm PES filters (Milipore) and stored at -80°C. DNA was extracted with the PowerWater DNA extraction kit (Qiagen), quantified using a Qubit fluorometer with the high-sensitivity assay (Invitrogen; concentration range: 1-50 ng μl^{-1}), and sequenced at the Integrated Microbiome Resource (Dalhousie University). Sequencing targeted the V4-V5 regions of the 16S rRNA gene, amplified with primers 515f-926r (Walters et al., 2015), using the Illumina MiSeq platform. Some treated water samples could not be processed due to insufficient DNA yield, likely reflecting low microbial biomass after treatment, which prevented successful sequencing. In total, 131 samples were successfully analyzed (60 natural water, 71 treated water).

Bioinformatics were conducted on Alliance Canada servers using DADA2 (Callahan et al., 2016) implemented in R (R Core Team, 2025). Processing steps included primer removal, denoising, quality filtering of forward and reverse reads (*filterAndTrim* function at positions 250 and 225, respectively), merging paired-end reads (*mergePairs* function), and chimera removal (*removeBimeraDenovo*

function). Non-bacterial sequences (Archaea, Eukaryotes) were excluded, and taxonomic assignments of bacterial 16S sequences were performed with the SILVA database (v138) using the *assignTaxonom* function in DADA2.

2.5.Statistical analyses

All statistical analyses were performed using Rstudio (version 4.5.0). Bacterial composition was analyzed with the *phyloseq* package (McMurdie & Holmes 2013). To account for differences in sequencing depth, data were normalized using rarefaction (*rarefy_even_depth* function, depth = 6102 reads per sample, selected from rarefaction curve analysis) for alpha diversity analyses, and by relative abundances for beta diversity analyses. Alpha diversity was estimated using the Shannon index (*diversity* function, *vegan* package), and differences between water types and sampling points were assessed with Kruskal-Wallis rank-sum tests due to the non-normal distribution of data (determined by the Shapiro-Wilk's test). Beta diversity was examined by testing for homogeneity of group dispersions (*betadisper* function, *vegan* package; Oksanen et al., 2025).

Permutational multivariate analysis of variance (PERMANOVA; *adonis2*, *vegan* package) was used to test effects of spatial variation (settlement), temporal variation (interannual, intra-annual), water type (natural vs treated), sampling point, and treatment procedure (filtration, UV disinfection, initial chlorination level) on bacterial and DOM composition (including FDOM components, DOC, TP, TN, F_{tot}, a_{320}, SUVA, and S_{285}). Interannual effects were tested in settlements sampled across years (FGH, KAN, and MIT), while intra-annual (monthly) variation was tested in settlements sampled across months in 2021 (CB, MIT, and KAN). The interaction between settlement and the temporal variable (year or month) was included in the models.

Pairwise comparisons of bacterial and DOM composition among settlements were assessed using the *pairwise.adonis* function (*PairwiseAdonis* package), with Benjamini-Hochberg correction to control for

multiple comparisons. Mantel tests (*mantel*, *vegan* package) were conducted to assess Spearman correlations between bacterial composition and both DOM-nutrient composition and water chemistry variables (pH, temperature, and free chlorine in treated waters). The resulting p-values were adjusted to account for multiple comparisons using the Benjamini-Hochberg method. Relationships were visualized through non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity, using the *ordinate* and *plot_ordination* functions (*phyloseq* package). Environmental variables were fitted to NMDS ordinations using the *envfit* function (*vegan* package). To assess the effects of settlement, water type, and sampling point on individual components of DOM and nutrients, ANOVA or Kruskal-Wallis tests were performed, depending on whether ANOVA assumptions were met.

We also tested differences in bacterial abundance between natural and treated waters, as well as across sampling points, using the Kruskal-Wallis test with permutations (*kruskal_test* function, *rstatix* package) for each settlement. Settlements with only one observation per sampling point (QAM, TAL, KGS, and SAL) were excluded from this analysis. To identify the best predictors of bacterial abundance among the DOM and nutrient composition parameters described above, we performed a linear regression using stepwise model selection (*stepAIC* function, *MASS* package). Bacterial abundance data were square root transformed to improve the normality and homogeneity of residuals. Moreover, a separate linear regression model was used to assess the effect of treatment procedures on bacterial abundance in treated waters. Explanatory variables included settlement, sampling point, total chlorine, free chlorine, consumed chlorine, chlorination level (total chlorine at the treatment step), effective chlorination (free chlorine at the treatment step), and presence-absence of filtration and UV disinfection steps.

Finally, in addition to overall community analysis, we assessed the presence of ASVs (amplicon sequence variants) assigned to genera associated with potential disinfection resistance and environmental opportunistic pathogenicity based on the literature.

2.6.Quantitative Polymerase Chain Reaction and Microbial Source Tracking

Real-time quantitative polymerase chain reaction (qPCR) was used to detect and quantify *Enterococci* in drinking water sources and raw water (reservoirs) from KAN, KGS, FGH, CB and TAL, using the USEPA 1609.1 method (USEPA, 2015; Saleem et al., 2023). *Enterococci* are widely used as fecal bacteria indicators for assessing drinking water quality, as their presence is associated with an increased risk of gastrointestinal illness (Health Canada, 2020). Microbial Source Tracking (MST) was also performed using digital PCR targeting the human-specific *Bacteroides* DNA marker HF183. This marker is well characterized in terms of its prevalence and concentration in wastewater and has been widely used in MST studies (e.g., Ahmed et al., 2016; Mayer et al., 2018). Real-time PCR detection of *Bacteroides* markers (including HF183) has been shown to correlate with the presence of pathogens such as *Escherichia coli* O-157, *Salmonella*, and enterotoxigenic *E. coli*, making it a reliable indicator of human fecal contamination in water (Savichtcheva et al., 2007). In addition, the seagull-specific *Catellicoccus* DNA marker (Gull4), typically found in gull feces (Koskey et al., 2016), was used to identify avian fecal contamination. Assays for dPCR used previously published primer and probe sets for the human HF183 marker (Green et al., 2014) and the seagull Gull4 marker (Ryu et al., 2012) and are described in Edge et al., 2021). Reactions were loaded onto a 20,000 micro-well chip (QuantStudio™ 3D Digital PCR 20K Chip v2, and PCR assays were carried out using the ProFlex PCR System (ThermoFisher). Chips were read using a QuantStudio™ 3D Digital PCR Instrument (ThermoFisher), and results were analyzed using the QuantStudio™ AnalysisSuite™. All qPCR and digital PCR assays included appropriate negative and positive controls.

3. Results

3.1. Bacterial composition of water sources

Bacterial assemblages of drinking water sources (including SW, RW, and AW) differed among settlements (PERMANOVA, $p < 0.01$; Figure 1a; Figure S2; Table S3). More specifically, the bacterial composition of Kangiqsualujjuaq (KAN) source differed significantly from that of Fort Good Hope (FGH) and Mittimatalik (MIT) (pairwise-adonis, $p < 0.05$). For instance, the relative abundances of the bacterial classes Acidobacteriae and Planctomycetes were higher in KAN, while Acidimicrobia was more abundant in FGH (Figure 1b). However, overall bacterial abundance in source waters did not differ significantly between settlements (Kruskal-Wallis $p > 0.05$). The bacterial community composition also varied over time (monthly) in 2021 (PERMANOVA, $p < 0.05$; Figure 1c).

Significant associations were observed between bacterial composition and the DOM and nutrient characteristics of the source waters. Notably, bacterial composition correlated with the proportions of FDOM components, absolute abundance of component HM1 and DOC concentration, the spectral index S_{285} (a proxy for DOM molecular size), and total nitrogen (TN) (Mantel test, $p < 0.05$; Table S4; Figure 1b). The DOM and nutrient composition of source waters also differed significantly between settlements (PERMANOVA, $p < 0.05$) and across sampling months. Higher concentrations of TN and DOC, as well as greater proportions of humic-like terrestrial FDOM components (HT1-HT3), were observed in FGH, MIT, and CB compared to the Nunavik northern villages KAN and KGS (see Herrera et al., 2025). Additionally, higher values of a_{320} (a proxy for CDOM quantity) and increased concentrations of HT1-HT3 components were observed in April and August compared to July and September 2021, particularly in MIT, CB, and KAN.

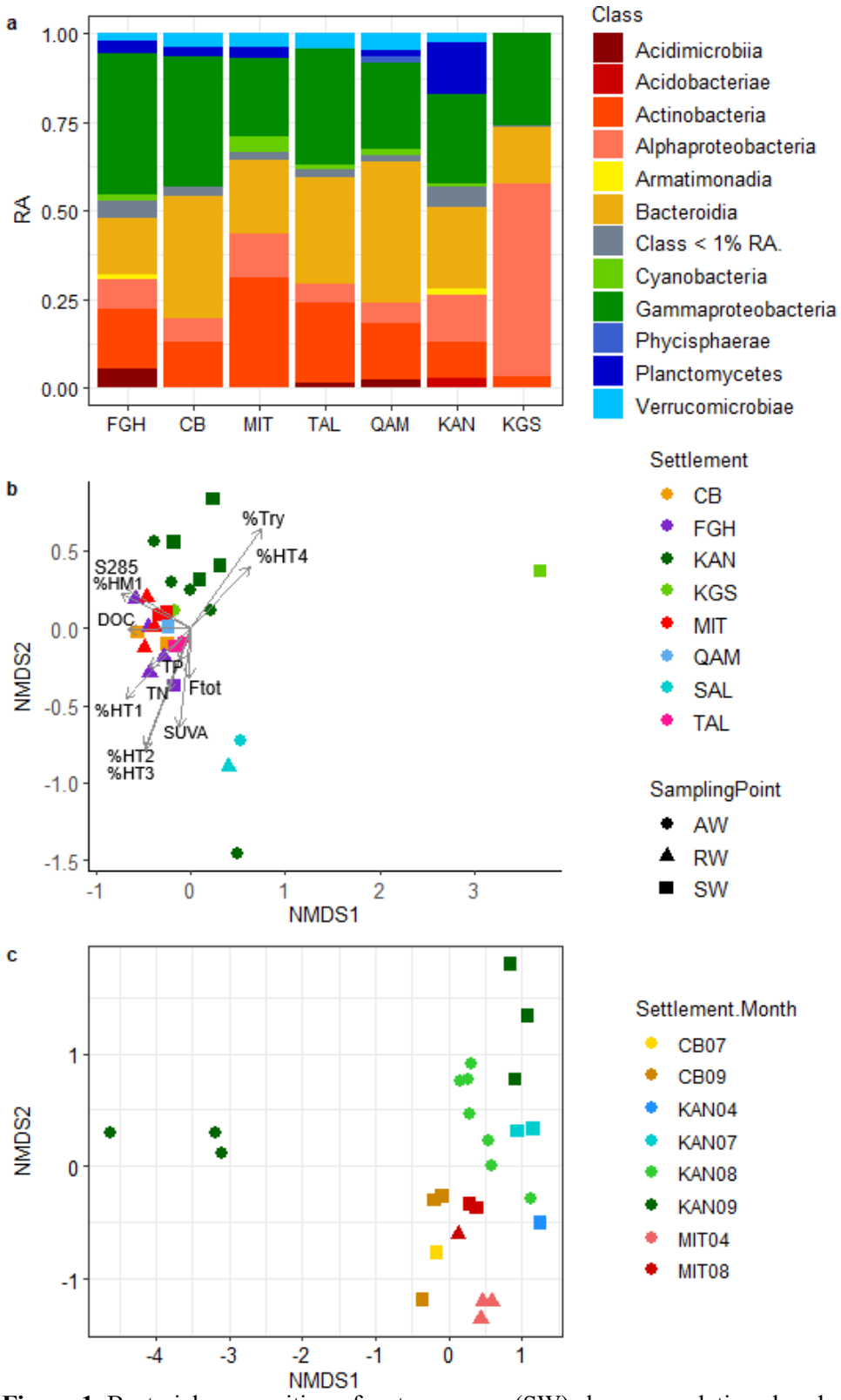

Figure 1. Bacterial composition of water sources (SW) shown as relative abundance (RA) of bacterial classes (a), and NMDS ordination (Bray-Curtis) illustrating variability in bacterial communities of natural waters (SW, RW, AW) across settlements, and their relationships with DOM and nutrient composition (b), and among months in 2021 (c).

Most drinking water sources and reservoir samples tested positive for *Enterococci*, with levels varying across settlements and sampling dates (Table S5). The human-specific Bacteroides marker HF183, used to detect fecal contamination, was not detected in any of the samples. However, the seagull-associated *Catellicoccus* marker (Gull4) was detected in both the source water and reservoir of MIT in July 2019. Notably, this same sampling period and location exhibited the highest levels of *Enterococci*.

3.2. Bacterial community composition along the drinking water supply systems

Bacterial composition along the drinking water supply systems varied significantly across settlements and over time, both between years and among months (PERMANOVA, $p < 0.01$) (Figure 2b-c; Table S3). In particular, the bacterial community composition in KAN's distribution system differed significantly from those in MIT, CB, and FGH (Pairwise PERMANOVA, $p < 0.05$), while the composition in MIT also differed from SAL ($p < 0.05$). For instance, a notable separation in the bacterial composition of house tap water was observed across the settlements (Figure 2a). Changes observed between years include an increase in the relative abundance of bacterial class Planctomycetes and a decrease in the relative abundance of bacterial class Bacteroidia in the drinking water systems of MIT and KAN in 2021 compared to 2019. A decrease in the relative abundance of Alphaproteobacteria was also observed for FGH (2020) and KAN (2021). Among the months, we observed a lower relative abundance of Bacteroidia and a higher relative abundance of Oligoflexia in September (at CB and KAN), as well as an increased relative abundance of Cyanobacteria in August (at MIT and KAN) (Figure S3). Bacterial abundance along the supply systems also varied significantly between settlements and across months (Kruskal-Wallis, $p < 0.05$; Table S6). Higher abundances were observed in MIT and TAL compared to FGH, KAN, KGS, and SAL, with peak levels occurring in July (post-hoc Dunn' test, $p < 0.05$; Figure 3).

The composition of DOM and nutrients, which also differed across settlements and over time (PERMANOVA, $p < 0.05$), was significantly associated with bacterial community composition along the drinking water systems. Specifically, bacterial composition correlated with DOC concentration, humic-like terrestrial compound HT4, humic-like microbially processed compound HM1, TN, and the proportions of the different FDOM components (Mantel, $p < 0.05$; Table S4). An additional correlation was observed with water temperature. Moreover, bacterial abundance along the drinking water systems was explained by DOM composition, settlement, and sampling point (linear regression model LM1: $R^2 = 0.58$, $p < 0.01$; Table S7). More precisely, bacterial abundance was positively correlated with DOC and component HT2 (a humic-like terrestrial compound related to fulvic acids) and negatively correlated with component HM1 (a humic-like, microbially processed autochthonous compounds) and with CDOM concentration (a_{320}) (Figure S4).

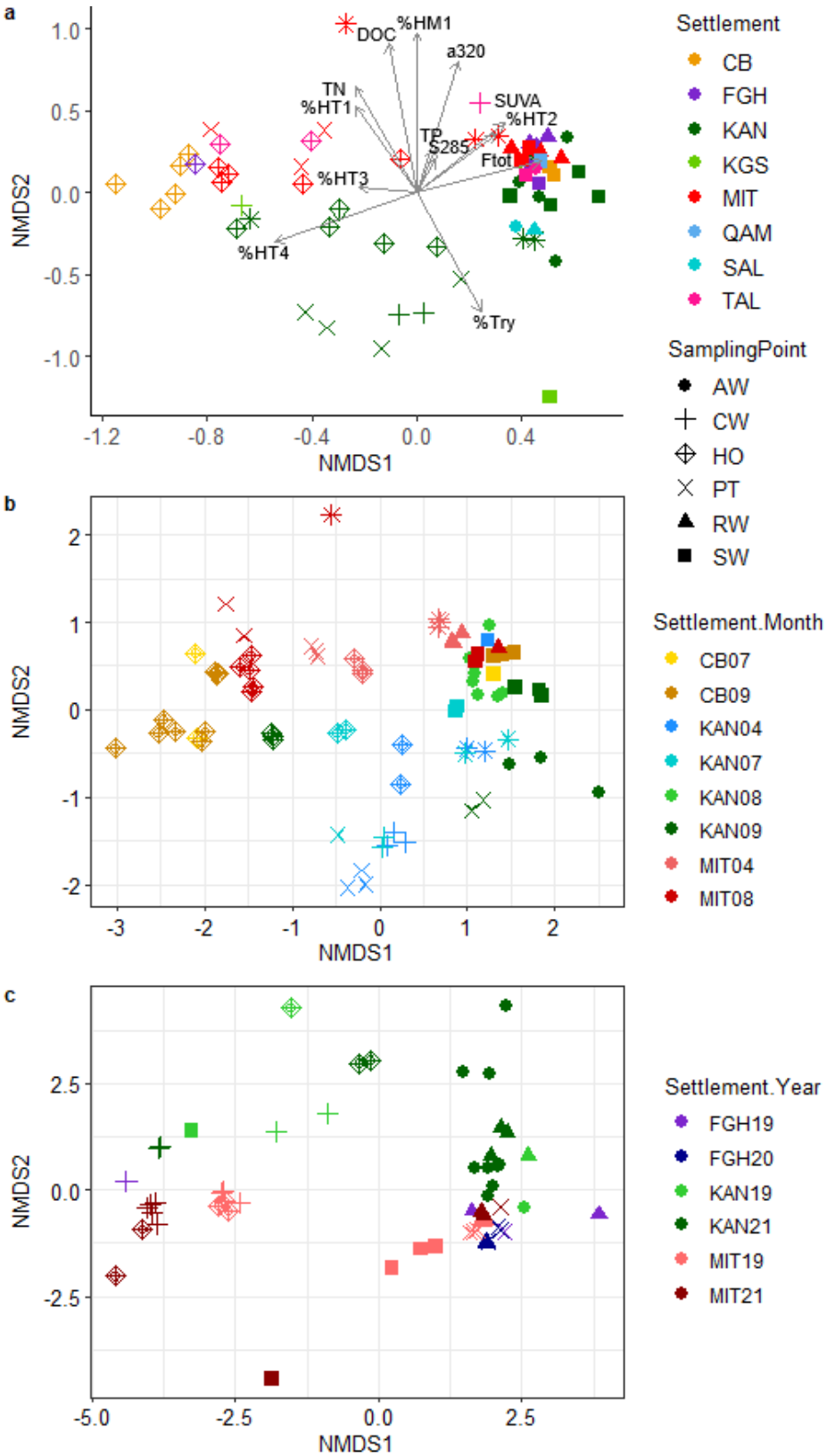

Figure 2. NMDS ordination (Bray-Curtis) presenting the spatial variability and relationships with the DOM and nutrient composition (a), and the temporal variability of bacterial community composition along the drinking water supply systems across months (b) and years (c).

3.3. Bacterial abundance and composition of treated water

We then examined how water treatment influenced bacterial community composition and abundance along the drinking water supply systems using PERMANOVA, Kruskal-Wallis tests, and linear regression models. Patterns of change in bacterial abundance across the supply system varied across settlements (Figure 3). In some cases, treatment appeared effective in reducing bacterial abundance. For example, significant reductions were observed in FGH (by 97%; Kruskal-Wallis, $p < 0.01$; Table S6). Notable but untested reductions were also observed in KGS (by 90%) and QAM (by 55%), though statistical significance could not be assessed due to limited sample sizes (only one observation per group). In contrast, other settlements showed no significant changes in bacterial abundance along the supply system (at MIT, TAL, and KAN, Kruskal-Wallis, $p > 0.05$), and, in some cases, an increase in bacterial abundance at the tap. Specifically, higher abundances at the household tap (HO) were noted in CB, QAM, and SAL compared to treated water at the chlorination point (CW) or in the distribution truck (TW). However, these differences were not statistically significant, likely due to low sample sizes at individual sampling points, limiting the power of the Kruskal-Wallis tests.

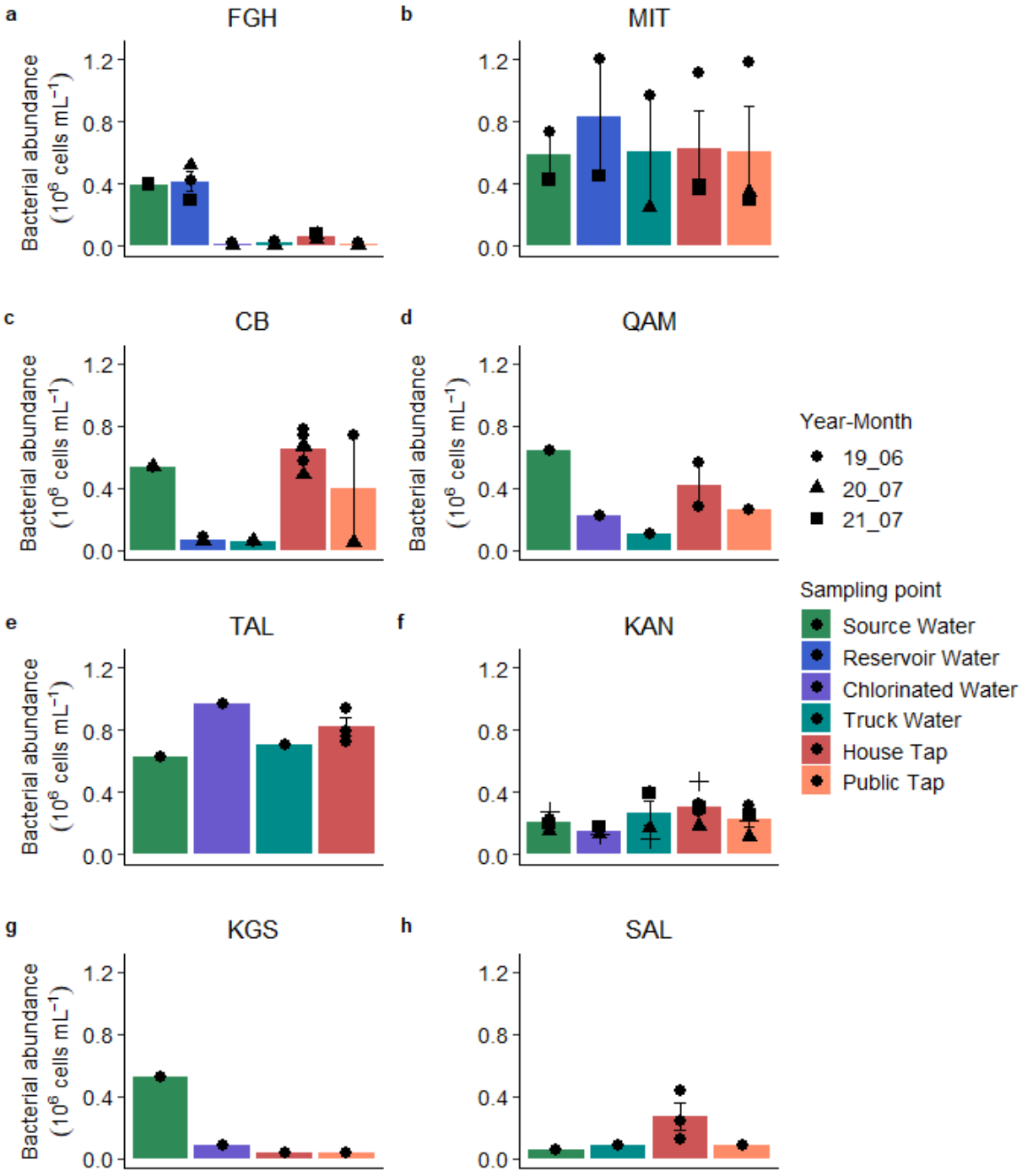

Figure 3. Bacterial abundance (10^6 cells mL^{-1}) measured along the drinking water supply systems across eight northern settlements (FGH: Fort Good Hope, MIT: Mittimatalik, CB: Cambridge Bay, QAM: Qammani'tuaq, TAL: Taloyoak, KAN: Kangiqsualujjuaq, KGS: Kangirsuk, and SAL: Salluit).

We also assessed the effect of treatment steps on bacterial removal in treated water using a linear regression model. Bacterial abundance was predicted by settlement, chlorination level at the treatment

step, consumed chlorine, and water temperature (LM2, $R^2 = 0.86$, $p < 0.01$; Table S7). Specifically, bacterial abundance was negatively correlated with chlorination level, and positively correlated with both water temperature and consumed chlorine levels (Figure 4). Among treatment procedures, the presence of a filtration step (implemented in FGH and CB) was associated with significantly lower bacterial abundance. Mean abundance was 0.25 x 10^6 cells mL^{-1} when filtration was included, compared to 0.36 x 10^6 cells mL^{-1} in its absence (Kruskal-Wallis, $p < 0.05$). However, filtration was not retained as a significant predictor in the final regression model.

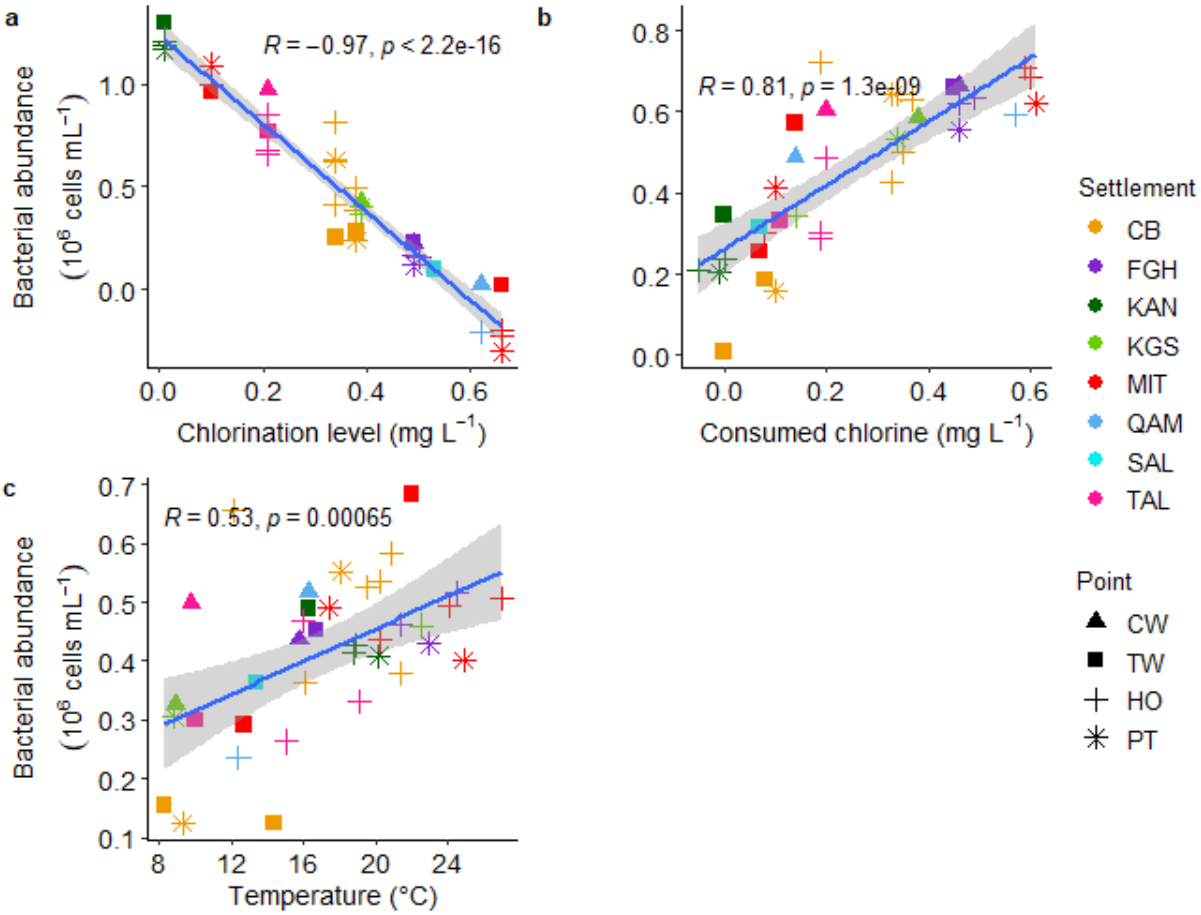

Figure 4. Conditional relationships between bacterial abundance in treated water (10^6 cells mL^{-1}) and (a) chlorination level (total chlorine at the treatment step), (b) consumed chlorine concentration (x-axis shown on a square root scale), and (c) temperature. Relationships are derived from the linear regression model LM2, with corresponding Pearson correlation coefficients (R) indicated.

Bacterial community composition changed along the drinking water supply systems, with assemblages of natural waters differing significantly from those in treated waters (PERMANOVA, $p < 0.05$; Figure

352 5a-b). Specifically, bacterial classes with higher relative abundance in natural waters, such as
353 Bacteroidia and Actinobacteria, were replaced in treated waters by other classes, notably
354 Alphaproteobacteria and Planctomycetes (Figures 5b). In several tap water samples, the relative
355 abundance of Alphaproteobacteria increased significantly (in FGH, CB, TAL, MIT, and KAN; Figure
356 S5). Gammaproteobacteria also showed increased relative abundance in KAN and TAL or was sustained
357 through to the tap in MIT. ASVs affiliated with genera linked to disinfection resistance and
358 environmental opportunism (*e.g.*, *Hyphomicrobium*, *Methylobacterium*, *Mycobacterium*, *Legionella*,
359 *Pseudomonas*) were detected in certain treated water samples.

360 Bacterial composition also changed significantly along the distribution systems after chlorination. House
361 tap water (HO) differed from chlorinated water (CW), truck-distributed water (TW), and public taps
362 (PT), with significant differences also observed between PT and TW (Pairwise-PERMANOVA $p <$
363 0.05). There was a significant effect of filtration, chlorination level, and free chlorine on the bacterial
364 composition of treated waters (PERMANOVA $p < 0.01$; Figure 5c; Table S3).

365 Beta diversity was significantly higher in treated waters compared to source waters (mean Bray-Curtis
366 dissimilarity = 0.67 vs 0.60; Betadisper, $p < 0.01$), indicating greater variability in bacterial composition
367 post-treatment. In contrast, alpha diversity was significantly lower in treated waters (mean Shannon
368 index = 6.48 vs 7.13 in raw waters; Kruskal-Wallis, $p < 0.05$; Figure S6).

369 The composition of DOM and nutrients also differed between raw and treated waters within settlements,
370 as evidenced by a significant interaction between hamlet and water type (PERMANOVA, $p < 0.05$;
371 Table S2). Across all settlements, raw waters were consistently enriched in humic-like microbial FDOM
372 component HM1 and the protein-like component Try (Kruskal-Wallis $p < 0.05$). However, no significant
373 differences were found in overall DOM quantitative proxies (DOC, a_{320}, F_{tot}) or nutrient levels (TP, TN)
374 between raw and treated waters except in FGH (see Herrera et al., 2025 for settlement-level details).

Relationships between bacterial composition and DOM-nutrient composition were also observed for treated waters. Bacterial assemblages were significantly correlated with DOC, the six FDOM, TN, a_{320} (a proxy for CDOM quantity), F_{tot} (FDOM quantity), and the S_{285} index (DOM molecular size) (Mantel test, $p < 0.05$; Table S4, Figure 5c). A significant positive correlation with water pH was also observed ($p < 0.05$).

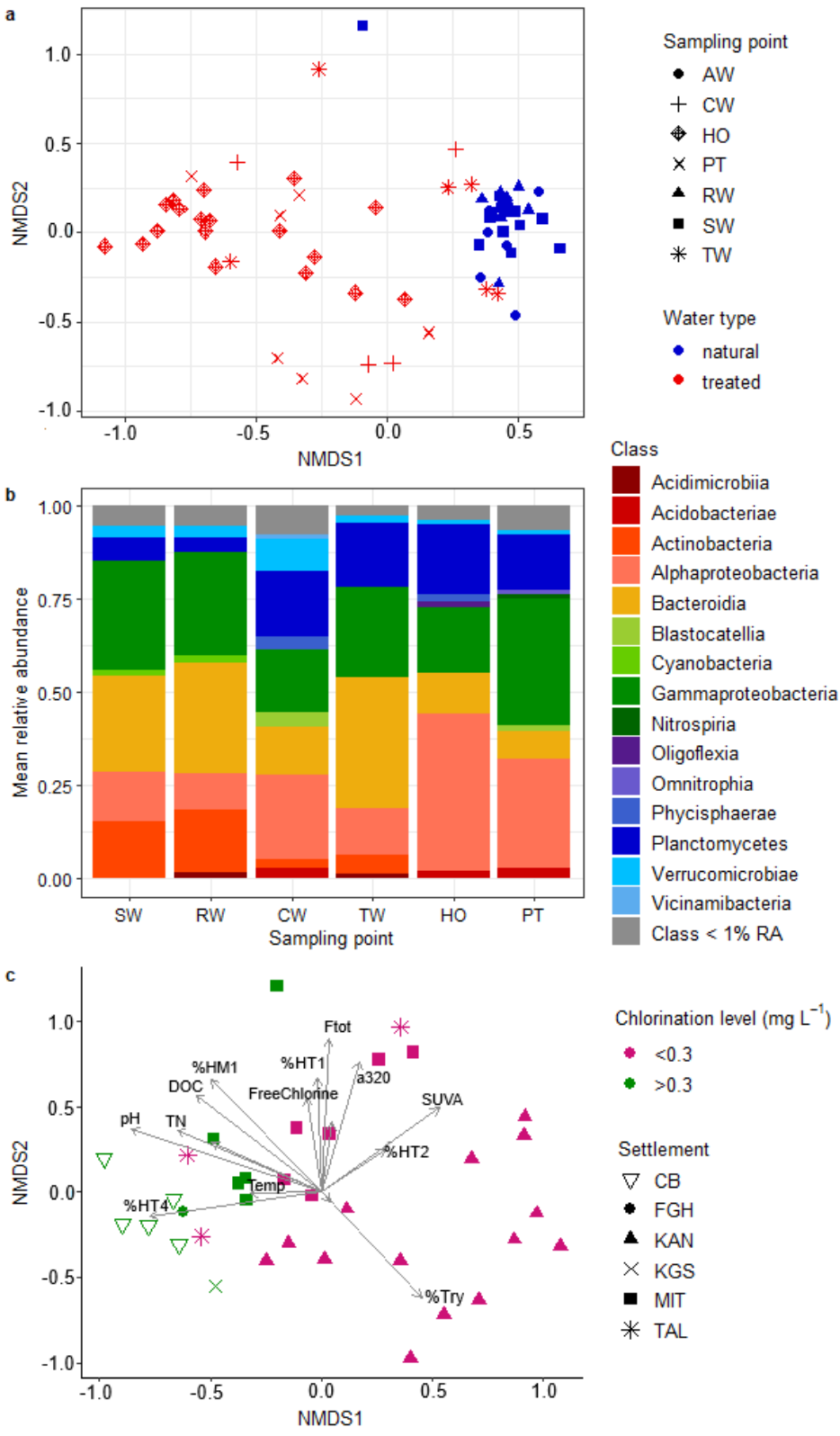

Figure 5. (a) NMDS ordination presenting dissimilarity in bacterial community composition between natural and treated waters. (b) Relative abundance of bacterial classes along the drinking water supply systems. (c) NMDS ordination illustrating differences in bacterial community composition of treated waters based on chlorination level (total chlorine measured at the chlorination point: CW or TW).

4. Discussion

4.1. Spatiotemporal variations in the bacterial composition of drinking water

The composition of DOM and nutrients (particularly the proportions of FDOM components, the DOM molecular size, and the concentrations of DOC, humic-like microbially processed FDOM compound, and total nitrogen) emerged as important drivers of microbial communities in source waters investigated in this study. These findings are consistent with previous studies reporting that the microbial composition of subarctic lakes and ponds was primarily driven by heterogeneous environmental conditions, especially concentrations in COD, nutrients, and FDOM (Comte et al., 2016; Roiha et al., 2016). Differences in FDOM component (including humic-like, microbially processed humic-like, and protein-like compounds) proportions can be associated with distinct bacterial communities that exhibit different metabolic capacities for organic matter utilization (Roiha et al., 2016). The relationship between DOM molecular weight and the abundance of microbially processed humic-like FDOM component and microbial composition may be driven by variation in DOM biolability (Fellman et al., 2010). Environmental changes that alter DOM composition seasonally or over longer timescales, such as water browning which particularly affects CDOM and FDOM fractions (Creed et al., 2018), are therefore likely to influence drinking water microbiological quality. Given the key role DOM plays in structuring bacterial communities in both source waters and along the drinking water supply systems, environmental changes affecting DOM characteristics in the catchment area are likely to influence microbiological water quality. In the short term, such changes may include meteorological events (e.g., precipitation or temperature shifts), while long-term drivers involve permafrost thaw and erosion, active layer deepening, and broader changes in hydrology and vegetation, such as the expansion of dwarf shrubs observed across the Arctic (Prowse et al., 2006; Wauthy et al., 2018).

In our study, temporal variations in both bacterial assemblages and composition of DOM and nutrients further indicate that microbiological water quality fluctuates seasonally. Seasonal changes in lake DOM composition (e.g., molecular weight and concentrations) have also been associated with fluctuations in the magnitude of disinfection by-product formation through reactions between chlorine and DOM, with important implications for drinking water safety (Zheng et al., 2025). Temporal variability in the microbiological quality may include episodic contamination events. For instance, we identified a seasonal occurrence of fecal contamination from birds at the Mittimatalik source, which coincided with the peak presence of migratory birds in the region and the proximity to the Bylot Island bird sanctuary. Collectively, these spatial and temporal patterns in bacterial composition suggest that drinking water quality differs not only between settlements but also over time, emphasizing the need for ongoing, site-specific monitoring to ensure safe water supply in northern communities.

4.2. Effect of water treatment on bacterial composition of treated waters

Our results demonstrate that bacterial composition changes along the drinking water supply systems of northern settlements, reflecting both treatment effect and shifts in chemical conditions. Bacterial assemblages were influenced by DOM and nutrient composition as well as by treatment procedures, which varied among settlements. The reduction in alpha diversity in treated waters indicates that several bacterial taxa were removed by treatment. However, the persistence or dominance of certain groups (e.g., Alphaproteobacteria and Gammaproteobacteria) reflects their capacity to withstand and proliferate despite initial chlorination.

Low residual chlorine concentrations, together with the persistence of nutrients and organic matter, likely enabled the regrowth of bacteria that survived the chlorine treatment, as observed in CB, QAM, and SAL. Most tap waters contained low chlorine concentrations, below the 0.2 mg L^{-1} guideline

recommended by Health Canada to limit bacterial regrowth (Health Canada, 2013). Such low chlorine levels, likely due to the time elapsed since application and the absence of secondary disinfection in most hamlets, represent a risk of microbiological contamination (Farenhorst et al., 2017; Daley et al., 2018). In addition, the pH range measured in treated waters (6.6–8.3) indicates that chlorine exists in equilibrium between hypochlorous acid (HOCl) and hypochlorite ion (OCl^-) in some waters, rather than predominantly as HOCl the more effective form for disinfection typically found around pH 6-6.5 (Health Canada, 2009). Notably, at CB, the pH > 7.5 suggests that more chlorine is present as OCl^-, which has lower disinfection efficacy. Sustained DOC and CDOM concentrations (as compared to source water) were measured in most hamlet waters, except at FGH where coagulation is applied to reduce organic matter from the Mackenzie River (see Table S4 in Herrera et al., 2025). Persistent DOM can react with chlorine, decreasing disinfection efficiency (Zheng et al., 2025). Consistent with this, our model revealed a positive correlation between bacterial abundance and consumed chlorine levels suggesting that high chlorine demand, driven by DOM and microbial loads, promotes bacterial regrowth through depletion of free chlorine (in addition to its decay over time after application). In contrast, the negative relationship between bacterial abundance and chlorination levels suggests that higher initial chlorination more effectively reduces microbial loads, both by lowering bacterial abundance during treatment and by maintaining higher free chlorine levels at taps to prevent regrowth. Although total bacterial abundance (including both viable and non-viable cells) was measured, these relationships are likely driven by the viable cell fraction, as chlorination primarily affects bacterial viability rather than total number of cells (Phe et al., 2005). The reduction in bacterial abundance with higher chlorination levels may also partly result from chlorination-induced damage to cellular structures and nucleic acids, which can impair stainability and detection in severely lysed cells (Phe et al., 2005). Filtration also reduced bacterial abundance, likely through the removal of DOM and larger bacterial cells, although it

452 was not an informative predictor in our best-supported model. Although bacterial abundance alone does
453 not indicate health risk, as most bacteria are non-pathogenic (Health Canada, 2012), monitoring
454 abundance along distribution systems remains useful for assessing treatment efficiency and detecting
455 increases linked to raw water quality changes or contamination events.

456 The greater dissimilarity observed in bacterial composition between treated and source waters also
457 reflects heterogeneous conditions along the supply system and between hamlets, due in part to
458 differences in treatment (e.g., filtration, chlorination levels; Table S2). Bacterial composition differed
459 significantly across treated waters sampled after chlorination, in distribution trucks, at domestic taps,
460 and at public taps, indicating system-specific effects. Notably, household taps exhibited distinct bacterial
461 profiles likely shaped by tank-storage conditions, such as low free chlorine levels and elevated
462 temperatures. Such conditions can favor persistence of bacterial taxa and biofilm formation, as observed
463 in previous studies (Fish & Boxall, 2018; Daley et al., 2018).

464 Although more targeted approaches could confirm their presence, amplicon sequence variants identified
465 in treated waters included potential environmental opportunistic pathogens (e.g., *Mycobacterium*,
466 *Legionella*, *Pseudomonas*, *Aeromonas*, and *Sphingomonas*) as well as taxa known to persist in
467 chlorinated water and form biofilms (e.g., *Hyphomicrobium*, *Methylobacterium*, *Acidovorax*, and
468 *Undibacterium*) (Li et al., 2017a; Luo et al. 2021; Szewzyk et al., 2000; Vaerewijck et al., 2005).
469 Notably, *Mycobacterium* and *Legionella* detected in tap waters here were also found in household
470 reservoirs in MIT (Gora et al., 2020a). Household tanks often had higher temperatures (average 19.9°C
471 vs. 12.8°C in source waters), which can promote *Legionella* growth and more generally enhance
472 bacterial regrowth at taps, as supported by the positive relationship between temperature and bacterial
473 abundance in our model.

4.3. Perspectives on monitoring microbiological water quality in northern communities

Given projections of continued climate warming and increased water browning, the risks associated with bacterial regrowth, reduced disinfection efficiency, and the persistence of potential opportunistic pathogens in northern drinking water systems are likely to be exacerbated in the future. As a result, treatment procedures (e.g., chlorination levels) may need to be adapted to ensure efficiency, potentially leading to increased treatment costs. Community-based monitoring is essential for tracking water quality at a frequency that captures temporal variability and allows rapid response to emerging risks.

Considering that environmental changes (e.g. increasing DOM) may reduce disinfection efficiency, a close monitoring of potential contamination sources is especially important in the context of climate change. For instance, monitoring should include indicators of fecal contamination by birds and other wildlife since seasonal animal migrations may increase the risk of contamination of northern drinking water sources, as observed here for birds. Although enterococci are not directly pathogenic, they are reliable indicators of fecal contamination due to their persistence and resistance to disinfectants (Health Canada, 2020). Quantifying enterococci in source waters and hamlet reservoirs can provide early warning of fecal inputs at the source or contamination along the distribution system, as well as insight into treatment efficiency and potential system infiltration. In combination with human and animal specific fecal markers, this approach enables microbial source tracking, as demonstrated for Mittimatalik in the present study. Comprehensive assessment should encompass all potential contamination sources, including zoonotic pathogens carried by Arctic wildlife such as *Trichinella*, *Echinococcus*, and *Giardia* (Daley et al., 2018). Local traditional knowledge of animal migration patterns can play a crucial role in identifying potential contamination threats.

In addition, our findings and previous work (e.g., Farenhorst et al., 2017; Gora et al., 2020a) show that household tanks provide conditions favorable for bacterial regrowth and biofilm formation (e.g., low

free chlorine levels and elevated temperature), which may facilitate persistence and opportunistic pathogens (Wingender & Flemming, 2011). This underscores the importance of monitoring microbiological quality at the point of use. Future efforts should include analyses of biofilm microbial communities (Gora et al., 2020a) and improved characterization of the pathogenic potential of opportunistic bacteria identified here to refine health risk assessments. We also recommend assessing changes in bacterial abundance using methods that allow the specific enumeration of viable cells, such as flow cytometry with dual staining (e.g., SYBR and propidium iodide) (Phe et al., 2005) and ATP-based bioluminescence (Liu et al., 2023).

As demonstrated in our study, by providing a more comprehensive overview of microbial communities of drinking water supply systems, microbial analyses through next-generation sequencing can help identify sources of changes in microbiological drinking water quality (e.g., changes in DOM composition of source water) and detect novel microbial threats in northern drinking water systems. While 16S rRNA gene sequencing has limited species-level resolution, combining it with quantitative PCR enables to accurately assess risks associated with pathogens. Emerging molecular approaches can therefore strengthen monitoring. In addition, although DNA-based approaches detect both viable and non-viable cells, potentially leading to overestimation of risk, it remains well-suited for application in northern settings relative to RNA sequencing, which is more reliable for detecting viable bacteria (Li et al., 2017b) but limited by RNA's instability and long transport times, which increase the risk of degradation.

5. Conclusion

This study showed that the bacterial composition of northern surface waters varies among hamlets and is influenced by DOM and nutrient composition (particularly the proportions of FDOM components, the DOM molecular size, and the concentrations of DOC, humic-like microbially processed FDOM

compound, and total nitrogen). Thus, the ongoing browning of surface waters is expected to alter lake and river microbiomes and, in turn, the microbiological quality of drinking water. The temporal variability observed in microbial assemblages highlights the need for regular monitoring of microbiological quality.

Differences in bacterial composition (both taxa and abundance) between source and tap waters also appear to be shaped by the local context, particularly by treatment procedures, which vary among hamlets. Increased bacterial abundance in treated water represents a potential health concern, as it may be associated with microbial contamination or biofilm formation in household tanks, conditions often associated with opportunistic pathogens.

Overall, our findings emphasize the importance of close source-to-tap monitoring of northern drinking water systems by local operators to ensure effective surveillance of water quality. This need is especially urgent in the context of climate change, which is expected to further alter the bacterial composition of water sources and, consequently, the safety of drinking water supplies.

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Availability of data

Sequence data were deposited in the Sequence Read Archive within BioProject PRJNA1338990.

References

Ahmed W., Hughes B., Harwood V. J. (2016). Current status of marker genes of Bacteroides and related taxa for identifying sewage pollution in environmental waters. *Water* 8 (6): 231. Doi: 10.3390/w8060231.

Anaviapik-Soucie T., L'Hérault V., Arreak T., Maktar E., Shirley J., Jamieson R., Truchon M.-H., and the Elders of Pond Inlet (2015). Building capacity to monitor fresh water quality in Pond Inlet-community needs assessment in a changing climate FINAL REPORT ON RESEARCH ACTIVITY 2014-15, Internal Report, Climate Change and Health Adaptation Program. 95 pp.

Bakaic M., Medeiros A.S. (2017). Vulnerability of northern water supply lakes to changing climate and demand. *Arctic Science* 3 (1): 1-16. Doi: 10.1139/as-2016-0029.

Bakaic M., Medeiros A.S., Peters J.F., Wolfe B.B. (2018). Hydrologic monitoring tools for freshwater municipal planning in the Arctic: the case of Iqaluit, Nunavut, Canada. *Environmental Science and Pollution Research* 25 (33), 32913-32925. Doi: 10.1007/s11356-017-9343-4.

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

Cassivi A., Covey A., Rodriguez M.J., Guilherme S. (2023). Domestic water security in the Arctic: A scoping review. *International Journal of Hygiene and Environmental Health* 247: 114060. Doi: 10.1016/j.ijheh.2022.114060.

Comte J., Monier A., Crevecoeur S., Lovejoy C., Vincent W. F. (2016). Microbial biogeography of permafrost thaw ponds across the changing northern landscape. *Ecography* 39 (7): 609-618. Doi: 10.1111/ecog.01667.

Cortes C. et Marcos R. (2018). Genotoxicity of disinfection byproducts and disinfected waters: A review of recent literature. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis* 831: 1-12. Doi: 10.1016/j.mrgentox.2018.04.005.

Creed I.F., Bergström A.-K., Trick C.G. et al. (2018). Global change-driven effects on dissolved organic matter composition: Implications for food webs of northern lakes. *Global Change Biology* 24 (8): 3692-3714. Doi: 10.1111/gcb.14129.

Daley K., Truelstrup Hansen L., Jamieson R.C., Hayward J. L., Piorkowski G. S., Krkosek W., Gagnon G. A., Castleden H., MacNeil K., Poltarowicz J., Corriveau E., Jackson A., Lywood J., Huang Y. (2018). Chemical and microbial characteristics of municipal drinking water supply systems in the Canadian Arctic. *Environmental Science and Pollution Research* 25: 32926–32937. Doi: 10.1007/s11356-017-9423-5.

Edge, T.A., R.J. Boyd, P. Shum, and J. Thomas. (2021). Microbial source tracking to identify fecal sources contaminating the Toronto Harbour and Don River watershed in wet and dry weather. *Journal of Great Lakes Research*. 47: 366-377. Doi: 10.1016/j.jglr.2020.09.002.

Farenhorst A., Li R., Jahan M., Tun H. M., Mi R., Amarakoon I., Kumar A., Khafipour E. (2016). Bacteria in drinking water sources of a First Nation reserve in Canada. *Science of the Total Environment* 575: 813-819. Doi: 10.1016/j.scitotenv.2016.09.138.

Fellman J.B., Hood E., Spencer R. G. M. (2010), Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review, *Limnology and Oceanography*, 55. Doi: 10.4319/lo.2010.55.6.2452.

Fish K. E. et Boxall J. B. (2018). Biofilm microbiome (re)growth dynamics in drinking water distribution systems are impacted by chlorine concentration. *Frontiers in Microbiology* 9: 2519. Doi: 10.3389/fmicb.2018.02519.

Gora S. L., Anaviapik Soucie T., McCormick N. E., Ontiveros C. C., L'Hérault V., Gavin M., Trueman B.F., Campbell J., Stoddart A.K., Gagnon G.A. (2020) Microbiological water quality in a decentralized Arctic drinking water system. Environmental Science: *Water Research and Technology* 6 (7): 1855-1868. Doi: 10.1039/d0ew00019a.

Gora S. L., Trueman B. F., Anaviapik-Soucie T., Gavin M. K., Ontiveros C. C., Campbell J.,Gagnon, G. A. (2020). Source water characteristics and building-specific factors influence corrosion and point of use water quality in a decentralized Arctic drinking water system. *Environmental Science and Technology*, 54(4): 2192–2201. Doi: 10.1021/acs.est.9b04691.

Green, H.C., Haugland, R.A., Varma, M., Millen, H.T., Borchardt, M.A., Field, K.G., Walters, W.A., Knight, R., Sivaganesan, M., Kelty, C.A. and Shanks, O.C. (2014). Improved HF183 quantitative real-time PCR assay for characterization of human fecal pollution in ambient surface water samples. *Applied and Environmental Microbiology*. 80: 3086-3094. Doi: 10.1128/AEM.04137-13.

Hall-Stoodley L, Lappin-Scott H. (1998). Biofilm formation by the rapidly growing mycobacterial species Mycobacterium fortuitum. *Federation of European Microbiological Society Microbiology Letters*. 168:77–84. Doi: 10.1111/j.1574-6968.1998.tb13258.x

Harshman R. A. et Lundy M. E. (1994). PARAFAC: Parallel factor analysis. *Computational Statistics & Data Analysis* 18 (1): 39-72. Doi: 10.1016/0167-9473(94)90132-5.

Health Canada (2009) Guidelines for Canadian Drinking Water Quality: Guideline Technical Document—Chlorine. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. 45p.

Health Canada (2012). Conseils sur l'utilisation de la numération des bactéries hétérotrophes dans les approvisionnements d'eau potable au Canada. Bureau de l'eau, de l'air et des changements climatiques, Direction générale de la santé environnementale et de la sécurité des consommateurs, Santé Canada, Ottawa, Ontario. 22p.

Health Canada (2013). Conseils pour un approvisionnement en eau potable salubre dans les secteurs de compétence fédérale, version 2. Bureau de la qualité de l'eau et de l'air, Direction générale de la santé environnementale et de la sécurité des consommateurs, Santé Canada, Ottawa, Ontario. 87p.

Health Canada (2020). Guidance on the use of Enterococci as an indicator in Canadian drinking water supplies. water and air quality bureau, healthy environments and consumer safety branch, Health Canada, Ottawa, Ontario. 32p.

Herrera C. A., Comte J., Guilherme S., Rodriguez M. J., Moisan M.-A., Laurion I (2025). Tracking disinfection by-products in northern Canada: impacts of dissolved organic matter and regional variation. Preprint. https://espace.inrs.ca/id/eprint/16723.

ITK (2020). Access to drinking water in Inuit Nunangat. ITK Quarterly Research Briefing (2), 17p.

Koskey A.M., Fisher J.C., Traudt M. F., Newton R. J., McLellan S.L. (2016). Analysis of the gull fecal microbial community reveals the dominance of Catellicoccus marimammalium in relation to culturable Enterococci. *Applied and Environmental Microbiology* 80 (2). Doi: 10.1128/AEM.02414-13.

Legendre M., Bartoli J., Shmakova L., Jeudy S., Labadie K., Adrait A., Lescot M., Poirot O., Bertaux L., Bruley C., Couté Y., Rivkina E., Abergel C., Claverie J.-M. (2014). Thirty-thousand-year-old distant relative of giant icosahedral DNA viruses with a pandoravirus morphology. *Proceedings of the National Academy of Sciences* 111(11): 4274-4279. Doi: 10.1073/pnas.1320670111.

Lemieux JM., Fortier R., Talbot-Poulin MC., Molson J., Therrien R., Ouellet M., Banville D., Cochand M., Murray R. (2016). Groundwater occurrence in cold environments: examples from Nunavik, Canada. *Hydrogeology Journal* 24, 1497–1513 (2016). Doi:10.1007/s10040-016-1411-1.

Li Q., Yu S., Li L., Liu G., Gu Z., Liu M., Liu Z., Ye Y., Xia Q., Ren L. (2017). Microbial communities shaped by treatment processes in a drinking water treatment plant and their contribution and threat to drinking water safety. *Frontiers in Microbiology* 8. Doi: 10.3389/fmicb.2017.02465.

Li R., Tun H. M., Jahan M., Zhang Z., Kumar A., Dilantha Fernando W. G., Farenhorst A. et Khafipour E. (2017). Comparison of DNA-, PMA-, and RNA-based 16S rRNA Illumina sequencing for detection of live bacteria in water. *Scientific reports* 7(1): 5752. Doi : 10.1038/s41598-017-02516-3.

Liu S., Zhao J., Guo Y., Ma X., Sun C., Cai M., Chi Y., Xu K. (2023). Application of ATP-based bioluminescence technology in bacterial detection: A review. *Analyst* 148(15): 3452–3459. Doi: 10.1039/D3AN00576C.

Logue J.B., Stedmon C.A., Kellerman A.M., Nielsen N.J., Andersson A.F., Laudon H., Lindström E.S., Kritzberg E.S. (2016). Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter. *Journal of the International Society for Microbiological Ecology* 10: 533–545. Doi: 10.1038/ismej.2015.131.

Loiko S. V., Pokrovsky O.S., Raudina T. V., Lim A., Kolesnichenko L.G., Shirokova L.S. et al. (2017). Abrupt permafrost collapse enhances organic carbon, CO2, nutrient and metal release into surface waters. *Chemical Geology* 471:153–65. Doi: 10.1016/j.chemgeo.2017.10.002.

Luo L. W.,Wu Y.H., Yu T., Wang Y.H, Chen G.Q., Tong X., Bai Y., Xu C., Wang H., Ikuno N., Hu H.-Y. (2021). Evaluating method and potential risks of chlorine-resistant bacteria (CRB): A review. *Water Research* 188: 116474. Doi: 10.1016/j.scitotenv.2021.145986.

Martin D., Bélanger D., Gosselin P., Brazeau J., Furgal C. and Déry S. (2007). Drinking water and potential threats to human health in Nunavik: Adaptation strategies under climate change conditions. *Arctic* 60(2): 195-202. Doi: 10.14430/arctic244.

Mazoyer F., Laurion I., Rautio M. (2022). The dominant role of sunlight in degrading winter dissolved organic matter from a thermokarst lake in a subarctic peatland. Biogeosciences, 19, 3959–3977. Doi: 10.5194/bg-19-3959-2022.

Mayer, R.E., G.H. Reischer, S.K. Ixenmaier, J. Derx, A.P. Blaschke, J.E. Ebdon, R. Linke, L. Egle, W. Ahmed, A.R. Blanch, D. Byamukama, M. Savill, D. Mushi, H.A. Cristóbal, T.A. Edge, M.A. Schade, A. Aslan, Y.M. Brooks, R. Sommer, Y. Masago, M.I. Sato, H.D. Taylor, J.B. Rose, S. Wuertz, O.C. Shanks, H. Piringer, R.L. Mach, D. Savio, M. Zessner, and A.H. Farnleitner. (2018). Global distribution of human-associated fecal genetic markers in reference samples from six continents. *Environmental Science and Technology*. 52(9): 5076-5084. Doi: 10.1021/acs.est.7b04438.

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. Doi: 10.1371/journal.pone.0061217

Medeiros A.S., Wood P., Wesche S. D., Baikac M., Peters J. F. (2016). Water security for northern peoples: review of threats to Arctic freshwater systems in Nunavut, Canada. *Regional Environmental Change* 16: 635-647. Doi: 10.1007/s10113-016-1084-2.

Murphy, K.R., Stedmon, C.A., Graeber, D., and Bro, R. 2013. Fluorescence spectroscopy and multi-way techniques. PARAFAC. *Anal. Methods*, 5: 6557–6566. Doi : 10.1039/c3ay41160e.

Oksanen J., Simpson G., Blanchet F., Kindt R., Legendre P., Minchin P., O'Hara R., Solymos P., Stevens M., Szoecs E., Wagner H., Barbour M., Bedward M., Bolker B., Borcard D., Carvalho G., Chirico M., De Caceres M., Durand S., Evangelista H., FitzJohn R., Friendly M., Furneaux B., Hannigan G., Hill M., Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J, Borman T (2025). vegan: Community Ecology Package. R package version 2.6-10. https://doi.org/10.32614/CRAN.package.vegan.

Phe M.H., Dossot M., Guilloteau H., Block J.C. (2005). Nucleic acid fluorochromes and flow cytometry prove useful in assessing the effect of chlorination on drinking water bacteria. *Water Research*. 39(15): 3618-28. Doi: 10.1016/j.watres.2005.06.002.

Prowse T.D., Wrona F.J., Reist J.D., Gibson J.J., Hobbie J.E., Lévesque L.M.J., and Vincent W. F. (2006). Climate change effects on hydroecology of arctic freshwater ecosystems. *Ambio* 35 (7): 347-358. Doi: 10.1579/0044- 7447.

R Core Team (2025). R: A Language and Environment for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ratelle M., Spring A., Laird B. D., Andrew L., Simmons D., Scully D., Skinner K. (2022). Drinking water perception and consumption in Canadian subarctic Indigenous communities and the importance for public health. *FACETS Journal* 7: 343-359. Doi: 10.1139/facets-2021-0094

694 Roiha T., Peura S., Cusson M. and Rautio M. (2016). Allochthonous carbon is a major regulator to
695 bacterial growth and community composition in subarctic freshwaters. *Scientific reports* 6: 34456. Doi:
696 10.1038/srep34456.

697 Ryu, H., Griffith, J.F., Khan, I.U.H., Hill, S., Edge, T.A., Toledo-Hernandez, C., Gonzalez-Nieves, J.
698 and Domingo, J.S. (2012). Comparison of gull feces-specific assays targeting the 16S rRNA genes of
699 *Catellicoccus marimammalium* and *Streptococcus* spp*. Applied and Environmental Microbiology*. 78:
700 1909-1916. Doi: 10.1128/AEM.07192-11.

701 Saleem F., Schellhorn H.E., Simhon A., Edge T.A. (2023). Same-day Enterococcus qPCR results of
702 recreational water quality at two Toronto beaches provide added public health protection and reduced
703 beach days lost. *Canadian Journal of Public Health*. 114(4):676-687. Doi: 10.17269/s41997-023-00763-
704 8.

705 Savichtcheva O, Okayama N, Okabe S. (2007). Relationships between Bacteroides 16S rRNA genetic
706 markers and presence of bacterial enteric pathogens and conventional fecal indicators. *Water Research*
707 41(16): 3615-28. Doi: 10.1016/j.watres.2007.03.028.

708 Szewzyk U., Szewzyk R., Manz W. et Schleifer K.-H. (2000) Microbiological safety of drinking water.
709 *Annual Review of Microbiology* 54: 81–127. Doi: 10.1080/1040-840291046669.

710 Tanentzap A. J., Fitch A., Orland C., Emilson E. J., Yakimovich K. M., Osterholz H., Dittmar T. (2019).
711 Chemical and microbial diversity covary in fresh water to influence ecosystem functioning. *PNAS* 116:
712 24689–24695. Doi: 10. 1073/pnas.1904896116.

713 Timofeev V., Bahtejeva I., Mironova R., Titareva G., Lev I., Christiany D., et al. (2019). Insights from
714 Bacillus anthracis strains isolated from permafrost in the tundra zone of Russia. *PLoS ONE* 14(5):
715 e0209140. Doi: 10.1371/journal.pone.0209140.

716 USEPA. (2015). Method 1609.1: Enterococci in water by TaqMan® quantitative polymerase chain
717 reaction (qPCR) with internal amplification control (IAC) assay. EPA-820-R-15–099. Available at:
718 https://www.epa.gov/sites/default/files/2015-08/documents/method_1609-1-enterococcus-
719 iac_2015_3.pdf.

720 Vaerewijck M. J., Huys G., Palomino J. C., Swings J.et Portaels F. (2005). Mycobacteria in drinking
721 water distribution systems: Ecology and significance for human health. *Fems Microbiology Reviews* 29:
722 911–934. Doi: 10.1016/j.femsre. 2005.02.001.

723 Vonk J. E., Tank S. E., Bowden W. B., Laurion I., Vincent W. F., Alekseychik P., Amyot M., Billet M.
724 F., Canário J., Cory R. M., Deshpande B. N., Helbig M., Jammet M., Karlsson J., Larouche J.,
725 MacMillan G., Rautio M., Walter Anthony K. M. and Wickland K. P. (2015). Reviews and syntheses:
726 Effects of permafrost thaw on Arctic aquatic ecosystems. *Biogeosciences* 12: 7129-7167. Doi:
727 10.5194/bg-12-7129-2015.

728 Walters W., Hyde E.R.,Berg-Lyons D., Ackermann G., Humphrey G., Parada A., Gilbert J. A., Jansson
729 J. K., Caporaso J. G., Fuhrman J. A., Apprill A., Knight, R. (2015). Improved bacterial 16S rRNA gene
730 (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community
731 surveys. *mSystems* 1(1): e00009-15. DOI: 10.1128/msystems.00009-15.

Wang F., Li W., Li Y., Zhang J., Chen J., Zhang W., Wu X. (2018). Molecular analysis of bacterial community in the tap water with different water ages of a drinking water distribution system. *Frontiers of Environmental Science & Engineering* 12: 6. Doi: 10.1007/s11783-018-1020-4

Wauthy M., Rautio M., Christoffersen K. S., Forsström L., Laurion I., Mariash H. L., Peura S., Vincent W. F. (2017). Increasing dominance of terrigenous organic matter in circumpolar freshwaters due to permafrost thaw. *Limnology and Oceanography Letters* 3(3): 186-198. Doi: 10.1002/lol2.10063.

Wingender J. and Flemming H.-C. (2011). Biofilms in drinking water and their role as reservoir for pathogens. *International Journal of Hygiene and Environmental Health* 214(6): 417-23. Doi: 10.1016/j.ijheh.2011.05.009.

Wright C. J., Farahbakhsh K., RICG, Shiwak I., Flowers C., IHACC Research Team et Harper S. (2018). Water quality and health in northern Canada: stored drinking water and acute gastrointestinal illness in Labrador Inuit. *Environmental Science and Pollution Research International* 25(33): 32975-32987. Doi: 10.1007/s11356-017-9695-9.

Wright C. J., Sargeant J. M., Edge V. L., Ford J. D., Farahbakhsh K., Shiwak I. (2018). How are perceptions associated with water consumption in Canadian Inuit? A cross-sectional survey in Rigolet, Labrador. *Science of the Total Environment* 618: 369–378. Doi: 10.1016/j.scitotenv.2017.10.255

Zhou L., Zhou Y., Yao X., Cai J., Liu X., Tang X., Zhang Y., Jang K. S., Jeppesen E. (2020). Decreasing diversity of rare bacterial subcommunities relates to dissolved organic matter along permafrost thawing gradients. *Environment International* 134: 105330. Doi: 10.1016/j.envint.2019.105330.

Supplementary material

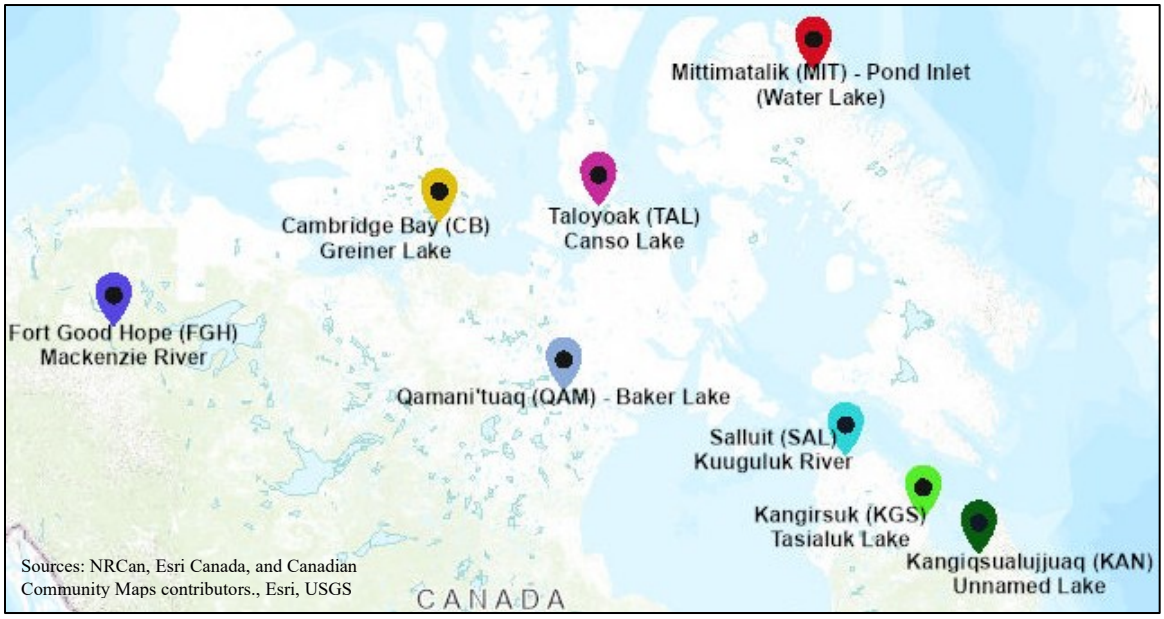

Figure S1. Location of the northern settlements included in this study and their primary drinking water sources.

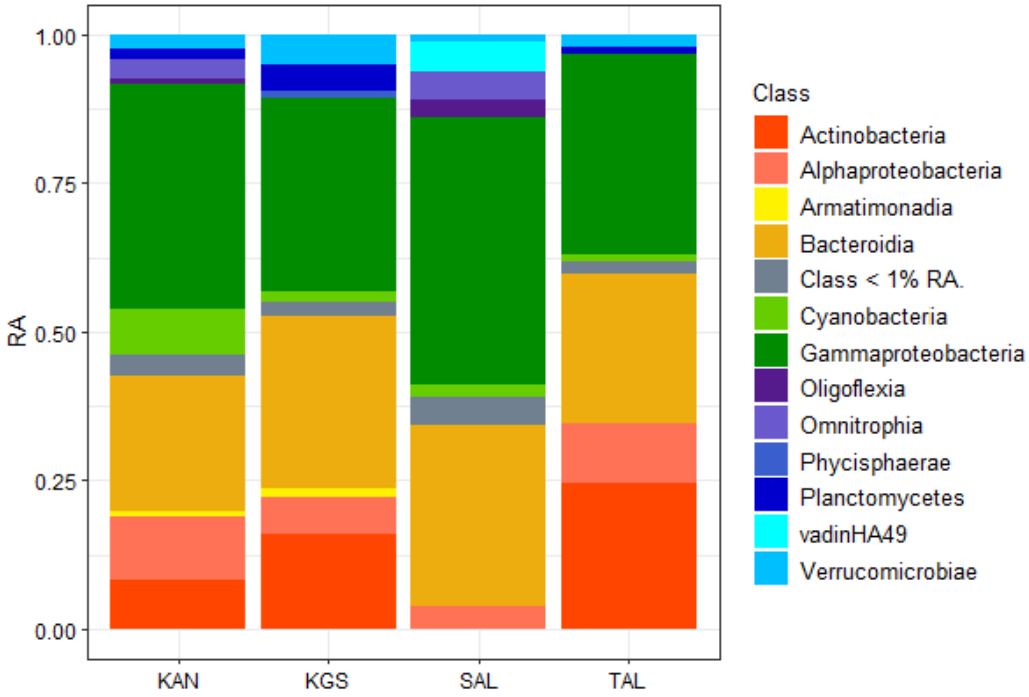

Figure S2. Bacterial composition of alternative water sources (AW) shown as relative abundance (RA) of bacterial classes.

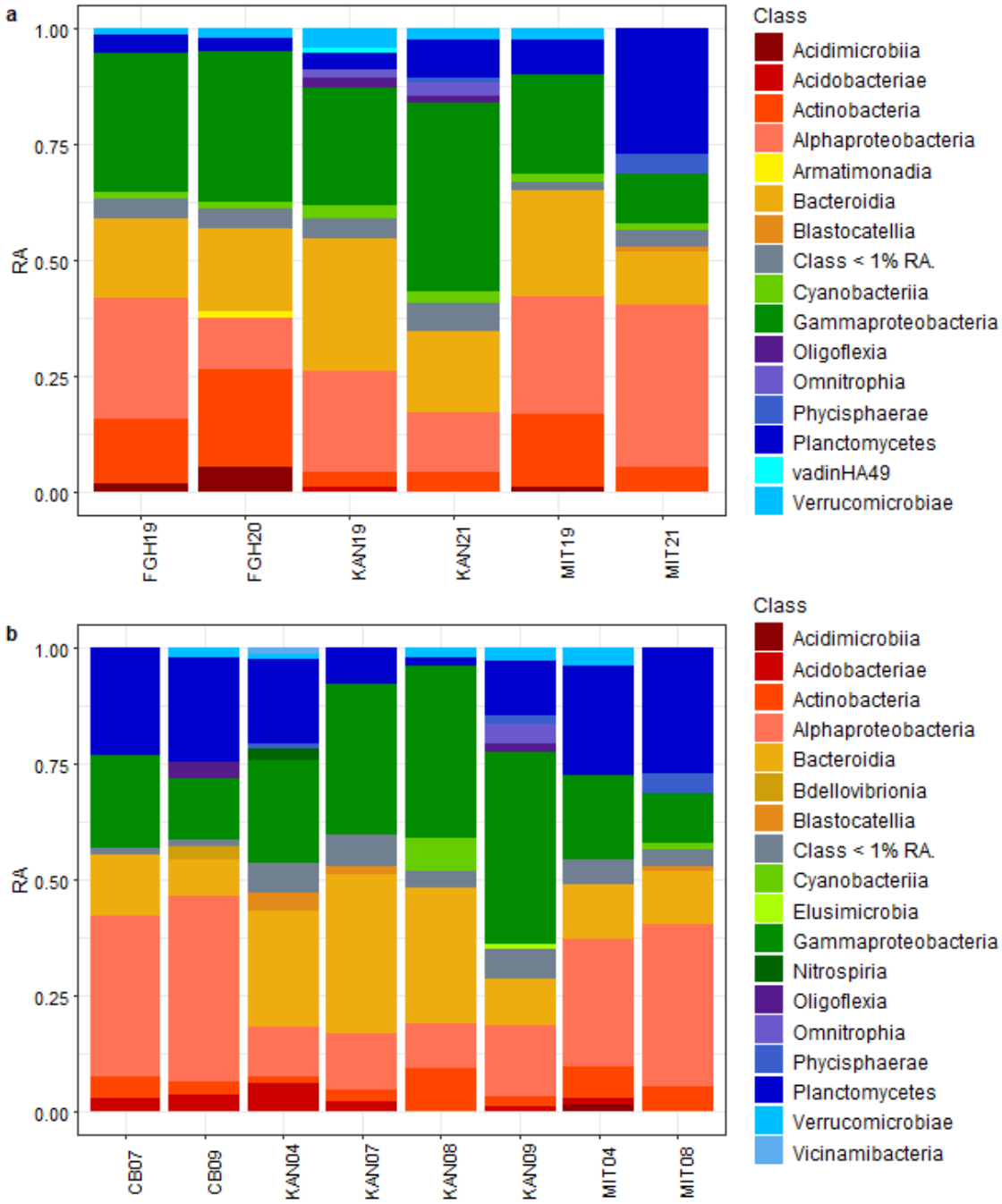

Figure S3. Bacterial composition of water in the drinking water systems of northern settlements, shown as relative abundance (RA) of bacterial classes, across years (a) and months (b).

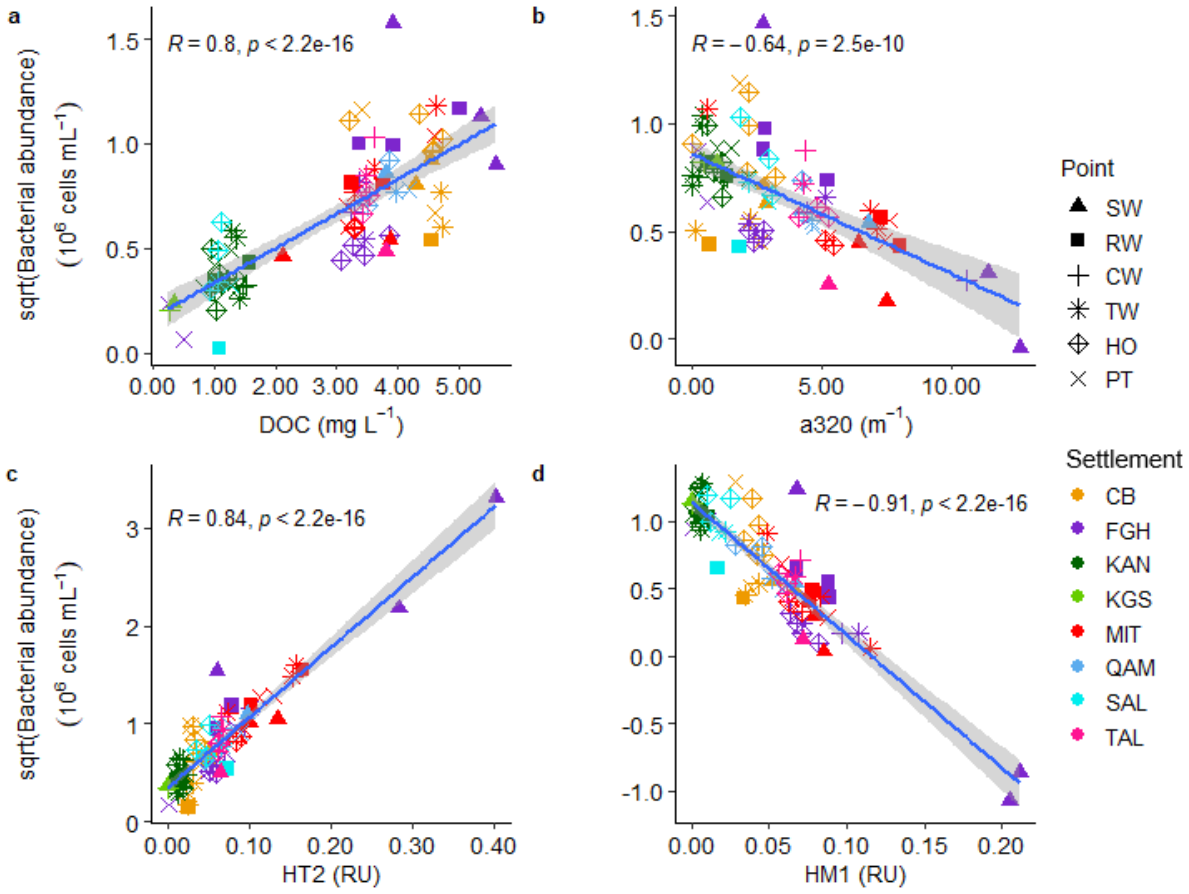

Figure S4. Conditional relationships between bacterial abundance in water (10^6 cells mL^{-1}) and concentrations of DOC (a), cDOM (b), and FDOM components HT2 (c) and HM1 (d). Relationships are based on the regression model, with Pearson correlation coefficients (R) indicated.

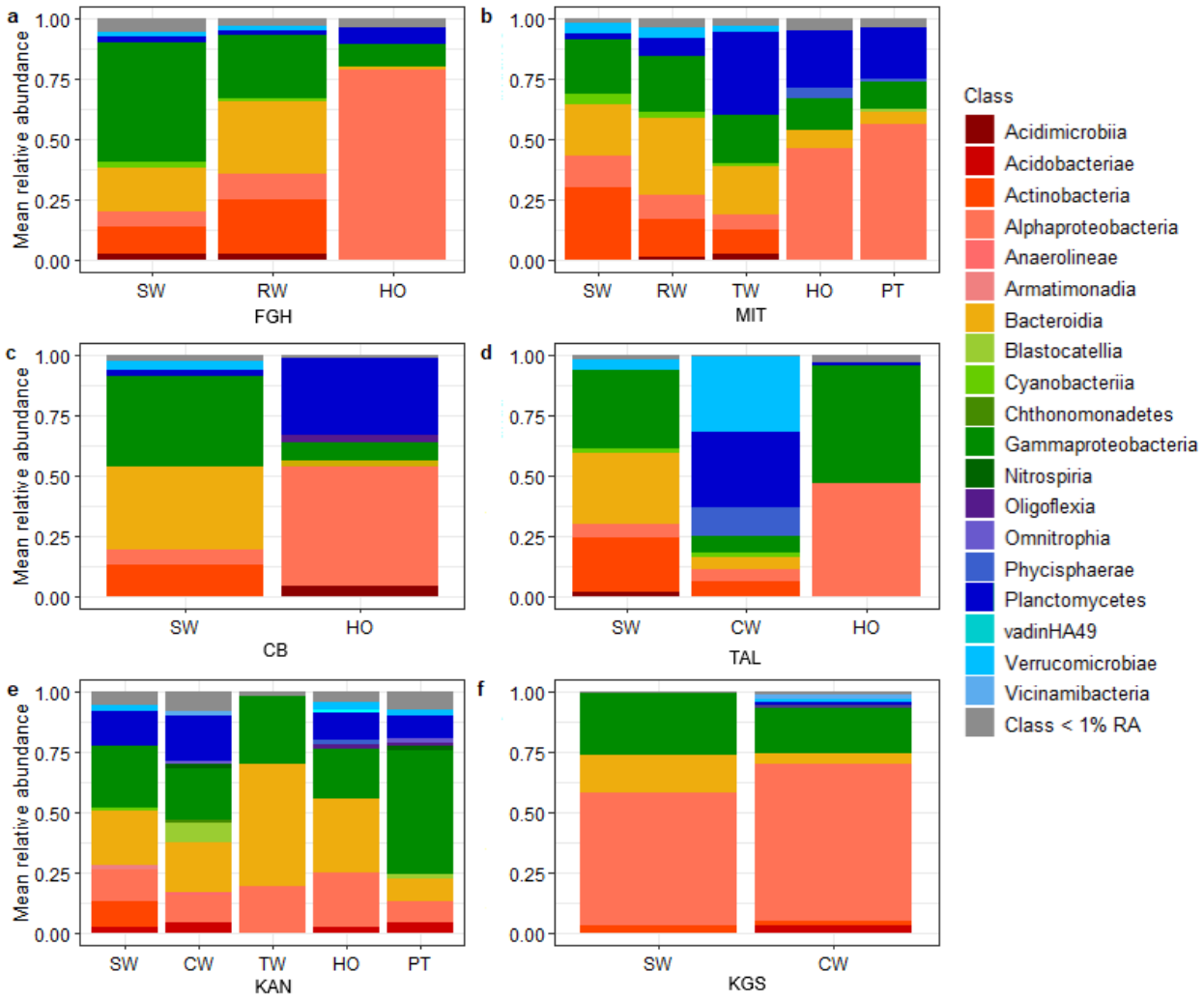

Figure S5. Bacterial composition (relative abundance of bacterial classes) of water samples collected along the drinking water supply systems of the studied northern settlements: FGH (Fort Good Hope), MIT (Mittimatalik), CB (Cambridge Bay), QAM (Qammani'tuaq), TAL (Taloyoak), KAN (Kangiqsualujjuaq), KGS (Kangirsuk), and SAL (Salluit).

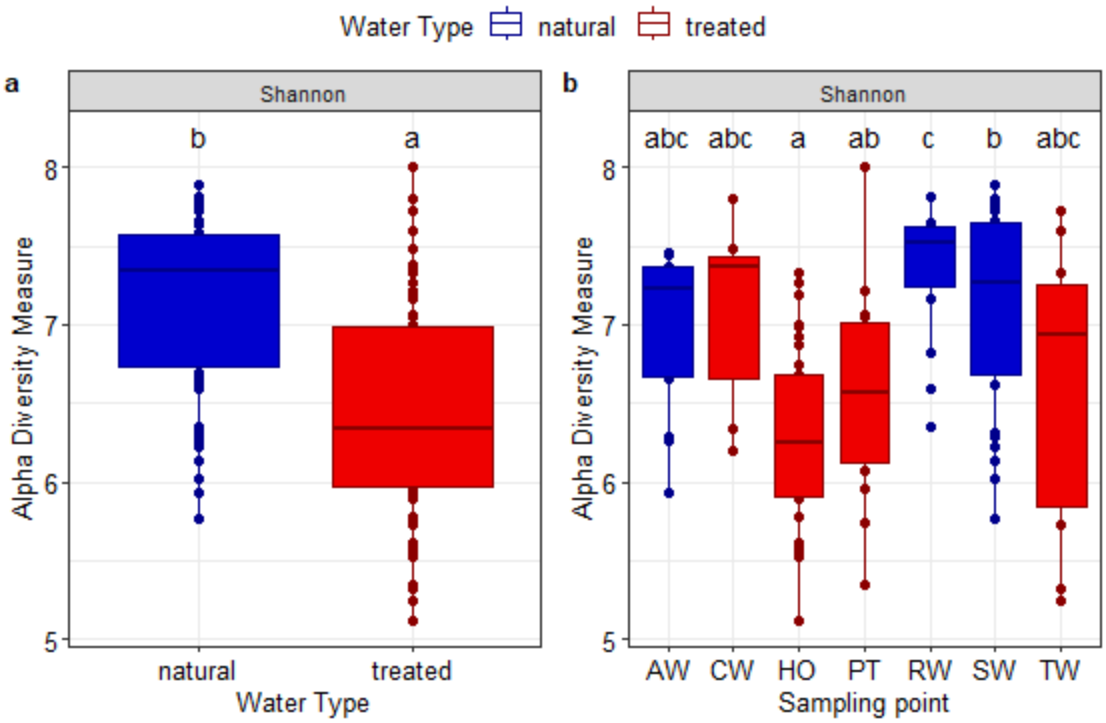

Figure S6. Alpha diversity (Shannon index) of bacterial communities of water along the drinking water supply system, shown by water type (natural vs. treated) (a) and by sampling points (b). Different letters indicate groups that differ significantly according to pos-hoc tests.

Table S1. Year and month of sampling in each settlement, along with the sampling points in the drinking water distribution network where water was collected and molecular data were obtained. The number of houses where tap water samples were collected is also indicated.

Northern settlement	Year	Month	SW	RW	CW	TW	OH	PT	AW
Fort Good Hope (FGH)	2019	June	X	X			1		
	2021	August							
Cambridge Bay (CB)	2021	July	X				3		
	2021	Sept.	X				3		
Qamani'tuaq (QAM)	2019	August	X						
Taloyoak (TAL)	2019	July	X		X		2		X
Mittimatalik (MIT)	2019	July	X	X			1	X	
	2021	April		X		X	1	X	
	2021	August	X	X		X	3	X	
Salluit (SAL)	2019	May	X						X
Kangirsuk (KGS)	2021	August	X		X				
Kangiqsualujjuaq (KAN)	2019	Sept.		X		X	2	X	
	2021	April		X	X	X	1	X	
		June		X	X	X	1	X	
		Sept.		X	X	X	1	X	X

Table S2. Water treatment steps in the eight settlements.

Northern settlement	Coagulation	Membrane filtration	UV disinfection	Chlorination
Fort Good Hope (FGH)	X	X		X
Cambridge Bay (CB)		X	X	X
Qamani'tuaq (QAM)				X
Taloyoak (TAL)			X	X
Mittimatalik (MIT)				X
Salluit (SAL)			X	X
Kangirsuk (KGS)			X	X
Kangiqsualujjuaq (KAN)			X	X

Table S3. Results of PERMANOVA assessing the effects of settlement, month, year, and water type on microbial composition in natural and treated waters.

	Df	SumOfSqs	R^2	F	Sign.
Natural waters					
Spatial variability					
Settlement	7	3.70	0.36	1.62	***
Inter-annual variability (Year)					
Year	1	0.51	0.10	1.57	ns
Settlement	2	1.14	0.22	1.74	**
point	2	0.65	0.12	1.00	ns
Year: Settlement	2	0.68	0.13	1.04	ns
Intra-annual variability (Month)					
Settlement	2	1.04	0.23	1.67	***
Month	3	1.24	0.27	1.32	*
Settlement:Month	2	0.72	0.16	1.15	ns
Natural and treated waters					
Spatial variability and water type					
Water.Type	1	2.37	0.08	6.17	***
Settlement	7	4.49	0.16	1.67	***
Water.Type: Settlement	5	2.59	0.09	1.35	***
Inter-annual variability (Year)					
Year	1	0.57	0.04	1.58	*
Settlement	2	1.46	0.11	2.04	**
Sampling point	5	3.40	0.26	1.89	*
Year: Settlement	2	0.72	0.06	1.00	ns
Intra-annual variability (Month)					
Month	4	2.68	0.17	1.93	**
Settlement	2	1.56	0.10	2.24	**
Sampling point	6	3.85	0.24	1.85	**

Month: Settlement	1	0.38	0.02	1.09	ns
Treated waters					
Spatial variability and sampling point					
Settlement	5	3.38	0.22	1.70	***
Sampling point	3	1.91	0.12	1.60	***
Water treatment effect					
Filtration	1	0.59	0.06	1.43	*
UVdisinfection	1	0.50	0.05	1.22	.
ChlorinationLevel	1	0.59	0.06	1.43	*
FreeChlorine	1	0.54	0.06	1.32	*

p-value ≤ 0.001: ***; p-value ≤ 0.01: **; p-value ≤ 0.05: *; p-value ≤ 0.1: **.** ; p-value > 0.1: ns

Table S4. Results of Mantel tests (correlation coefficient R and significance according to adjusted p-values) showing Spearman correlations between bacterial composition and DOM-nutrient variables (TP, TN, DOC, a_{320}, S_{285}, SUVA, FDOM components HT1-HT4, HM1, Try) as well as chemical characteristics (pH, temperature, and chlorine) in natural and treated waters.

	All waters		**Natural waters**		**Treated waters**	
	R	Sign.	R		R	Sign.
DOC	0.24	***	0.63	**	0.37	***
a320	0.06	ns	0.19	ns	0.14	*
S285	0.20	**	0.34	*	0.24	*
SUVA	0.01	ns	-0.01	ns	0.07	ns
Ftot	0.08	ns	0.21	ns	0.33	***
HT1	0.10	.	0.19	ns	0.28	**
HT2	0.07	ns	0.19	ns	0.22	**
HT3	0.06	ns	0.17	ns	0.23	**
HT4	0.13	*	0.20	ns	0.39	***
HM1	0.17	**	0.44	*	0.37	***
Try	0.07	ns	0.05	ns	0.22	**
%HT1	0.15	*	0.40	*	0.07	ns
%HT2	0.27	***	0.47	*	0.19	*
%HT3	0.14	*	0.42	*	0.17	*
%HT4	0.32	***	0.42	*	0.16	.
%HM1	0.21	***	0.55	**	0.39	***
%Try	0.16	*	0.59	**	0.14	.
TP	3.04E-03	ns	-0.04	ns	0.05	ns
TN	0.10	*	0.25	*	0.26	**
Temp	0.25	**	0.20	ns	0.05	ns
pH	0.13	.	-0.12	ns	0.31	**
Free chlorine	NA	NA	NA	NA	0.12	ns

p-value ≤ 0.001: ***; p-value ≤ 0.01: **; p-value ≤ 0.05: *; p-value ≤ 0.1: **.** ; p-value > 0.1: ns

Table S5. Results of qPCR for enterococci detection and dPCR-based microbial source tracking for Gull and Human fecal markers in drinking water sources (SW) and reservoir water (RW) of Kangiqsualujjuaq (KAN), Mittimatalik (MIT), Cambridge Bay (CB), Fort Good Hope (FGH), Qamani'tuaq (QAM), and Kangirsuk (KGS). Target Sequence Copies (TSC) detected in 2 µl of DNA were converted to total sequences in DNA recovered from 100 mL of water. For samples with replicates, means and standard deviations are reported. Nd indicates no detection.

Samples	Enterococci TSC/100ml ±SD	Gull4 TSC/100ml	HF183 TSC/100ml
KAN21_04_RW	10	Nd	Nd
KAN21_07_RW	Nd	Nd	Nd
KAN21_09_RW	13 ±9	Nd	Nd
MIT19_07_SW	139 ±121	2	Nd
MIT19_07_RW	824 ±129	25	Nd
MIT21_04_RW	4 ±7	Nd	Nd
MIT21_08_SW	77 ±98	Nd	Nd
MIT21_08_RW	101 ±88	Nd	Nd
CB21_07_SW	573	Nd	Nd
CB21_09_SW	7 ±12	Nd	Nd
FGH19_06_SW	25	Nd	Nd
FGH20_07_RW	Nd	Nd	Nd
FGH21_08_RW	225	Nd	Nd
QAM19__08_SW	5	Nd	Nd
KGS21_08_SW	428	Nd	Nd

Table S6. Results of Kruskal-Wallis tests assessing the effects of Settlement, month, year, water type (natural vs. treated), and sampling point (for treated waters) on bacterial abundance.

	X^2	Df	p-value	Significance
Effect of Settlement				
Natural waters	10.8	7	0.15	ns
Natural and treated waters	30.5	7	7.80E-05	***
Effect of month				
Natural and treated waters	11.4	4	0.02	*
Effect of year				
Natural and treated waters	3.8	2	0.15	ns
Effect of water type				
MIT	0.6	1	0.44	ns
CB	1.0	1	0.38	ns
FGH	11.0	1	3.00E-04	***
KAN	0.2	1	0.70	ns
Effect of sampling point (treated water)				
MIT	1.6	4	0.82	ns
CB	6.5	4	0.15	ns
FGH	14.4	5	1.00E-04	***
KAN	6.1	4	0.19	ns

p-value ≤ 0.001: ***; p-value ≤ 0.01: **; p-value ≤ 0.05: *; p-value ≤ 0.1: **.** ; p-value > 0.1: ns

Table S7. Parameters of the linear regression models: LM1, predicting bacterial abundance in water along the supply system based on DOM-nutrient composition; and LM2, predicting bacterial abundance in treated water based on treatment procedures.

LM1 Bacterial abundance of natural and treated waters vs DOM-nutrient composition				
Adjusted R^2	0.57			
p-value	1.91E-09			
Df	62			
F	7.61			
Res SE	0.21			
Predictors	Estimate	SE	t	Sign.
FGH	0.18	0.12	1.49	ns
KAN	0.22	0.15	1.48	ns
KGS	0.29	0.23	1.26	ns
MIT	0.36	0.13	2.88	**
QAM	0.04	0.13	0.28	ns
SAL	0.02	0.19	0.12	ns
TAL	0.59	0.13	4.65	***
HO	0.17	0.10	1.71	.
PT	0.05	0.11	0.50	ns
RW	0.12	0.11	1.06	ns
SW	0.43	0.12	3.70	***
TW	-0.01	0.11	-0.12	ns
DOC	0.17	0.04	3.93	***
a320	-0.06	0.02	-3.11	**
HT2	7.19	1.38	5.20	***
HM1	-9.85	2.52	-3.90	***

LM2 Bacterial abundance of treated waters and treatment procedure				
Adjusted R^2	0.86			
p-value	1.32E-10			
Df	26			
F	23.82			
Res SE	0.13			
Predictors	Estimate	SE	t	Sign.
FGH	-0.38	0.08	-4.81	***
KAN	-0.72	0.11	-6.72	***
KGS	-0.36	0.09	-4.07	***
MIT	0.17	0.07	2.44	*
QAM	0.27	0.12	2.33	*
SAL	0.15	0.15	0.98	ns
TAL	0.11	0.08	1.38	ns
Temperature	0.01	0.01	2.35	*
ChlorinationLevel	-2.15	0.23	-9.43	***
ConsumedChlorine	0.79	0.20	4.01	***

p-value ≤ 0.001: ***; p-value ≤ 0.01: **; p-value ≤ 0.05: *; p-value ≤ 0.1: **.** ; p-value > 0.1: ns