Changes in Bacterial Communities During Treatment of Municipal Wastewater in Arctic Wastewater Stabilization Ponds

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Wastewater stabilization ponds (WSPs) are commonly used to treat municipal wastewater in the Canadian Arctic. Bacterial community structure and functionality remain mostly uncharacterized for arctic WSPs, yet are presumed important for treatment outcomes during the 3-month summer treatment season with open water in the WSPs. The objective of this study was to investigate treatment performance and related temporal and spatial changes in the structure and putative function of bacterial communities during treatment of municipal wastewater in the WSPs of Pond Inlet and Clyde River, Nunavut over two consecutive summer treatment seasons. Influent raw wastewater contained a high organic load and large bacterial communities (~9 log 16S rRNA copies/mL) belonging mainly to Proteobacteria. Although designed to be facultative ponds, both WSPs remained anaerobic with neutral pH values (7.5–7.8) throughout the summer treatment season. Water quality data showed that nutrients [measured as carbonaceous biological oxygen demand ($CBOD_5$)], total suspended solids, and total ammonia nitrogen were progressively reduced during treatment in the ponds as the summer progressed. The pond bacterial population size and species richness depended on the pond temperature (2–18°C), with 8.5 log 16S rRNA copies/mL and the largest alpha diversities (Shannon-Wiener index of 4-4.5) observed mid-season (late July). While the phylogenetic beta diversity in raw wastewater from the two locations remained similar, pond bacterial communities underwent significant ($p < 0.05$) changes to dominance of Comamonadaceae, Geobacteracea, and Porphyromonadaceae. Multivariate distance based redundancy analysis and predicted gene functionalities in the microbiota agreed with water quality results that microbial removal of nutrients (e.g., $CBOD_5$) peaked in the middle of the summer coinciding with the treatment period with the highest pond temperatures. Information from this study will be useful for further development of models to predict biological treatment outcomes, which could be used to size and assess the feasibility of WSPs in extreme climates. Higher pond temperatures resulted in

optimal biological processes and nutrient removal in the middle of the summer. While it is challenging to control environmental factors in a passive wastewater treatment system there are some design considerations that could be used to optimize temperature regimes, such as the depth of the pond.

INTRODUCTION

Wastewater stabilization ponds (WSPs) are commonly used to treat municipal wastewater in remote arctic communities in Canada. The WSPs consist of either naturally occurring lakes with uncontrolled release or engineered structures with a lining and berm for controlled release of the water. Engineered WSPs have typically been designed to be facultative (i.e., aerobic) and to hold the annual volume of wastewater generated within the community (Ragush et al., 2015).

These WSPs are passive (i.e., non-mechanical) treatment systems that depend on physical settling and biological processes and are strongly affected by the climatic conditions including ambient temperature (Heinke et al., 1991). Operations of arctic WSPs are unique in that the content will remain frozen for 9 months of the year limiting the treatment season to the three summer months with open water and temperatures that support biological activity (Balch et al., 2018). Also, due to the low water consumption per capita ($\sim$100 L/person $\times$ day) in arctic regions such as Nunavut compared to Southern Canada (average of 274 L/ person $\times$ day), the deposited sewage tends to be more concentrated (Daley et al., 2014; Ragush et al., 2015). This can lead to high organic loading rates (OLRs) and anaerobic conditions in the facultative ponds which may affect the microbiological processes negatively (Ragush et al., 2017). Past studies have shown that primary treatment can be attained in arctic WSPs with removal of around 25–40% of nutrients, oxygen demanding material, suspended solids, and 99% of fecal indicator *Escherichia coli* (Marais, 1974; Heinke et al., 1991), even in WSPs with high OLRs and anaerobic conditions (Ragush et al., 2015; Huang et al., 2018).

It is well-known that microbial communities play an important role in biological wastewater treatment (Wagner et al., 2002). Insights in the dynamics of the wastewater microbiome during treatment can therefore be useful in determining which organisms are present and how they relate to the treatment of the wastewater for the benefit of future process optimization.

Studies of full scale mechanical wastewater treatment facilities show that low temperature generally reduces the bacterial diversity and slows down the growth and metabolic activity of functional bacteria to the extent where the treatment is affected. Rodriguez-Caballero et al. (2012) observed that low wastewater temperatures during the winter (10–15°C) caused difficulties in maintaining the required rates of nitrogen removal in biological wastewater treatment plants (WWTPs) operated in Northern Sweden. Another cold-climate study (Ju et al., 2014) found that the relative abundances of functional groups in activated sludge communities were shaped by seasonal temperature

changes, i.e., when the temperature dropped during winter season, the abundances of nitrite-oxidizing bacteria (NOB) and hydrolysers decreased, leading to the incomplete nitrification and hydrolysis of organic material. The relative temperature-driven reduction in microbial diversity was also observed in a Finnish study conducted in the seven Northern communities, where wastewater was treated in bioreactors operated at low temperatures of 3–7°C resulting in good nutrient removal but variable performance for nitrogen removal (Gonzalez-Martinez et al., 2018).

In terms of microbial communities related to treatment in arctic WSPs, Gromala et al. (2021) recently examined water samples from WSPs located in Baker Lake (Qamani'tuaq), Cambridge Bay (Iqaluktuuttiaq), and Kugluktuk (Qurluktuk), all located in Western Nunavut, and reported temporal and spatial differences in the abundance of major bacterial phyla that were relatable to specific WSPs and the time after the Spring thaw. However, studies of the structure and function of microbial communities in relation to the treatment performance of arctic WSP systems throughout the summer treatment season have to the best of our knowledge not been performed.

The objective of this study was to investigate the temporal and spatial changes in the structure and putative function of bacterial communities and their link to treatment performance during treatment of municipal wastewater in arctic WSP systems located in the communities of Pond Inlet (Mittimatalik) and Clyde River (Kangiqtugaapik) in Nunavut, Canada. This study was conducted over two summer treatment seasons (June to early-September) and involved monitoring microbial populations in influent raw wastewater and the progressively treated water in the WSPs as well as selected environmental and water quality parameters. Information about the microbiology of these systems can help in understanding passive, biological wastewater treatment systems operated in cold climates.

MATERIALS AND METHODS

Study Sites

From June, 2013 to September, 2014, six and five sampling trips were made to Pond Inlet and Clyde River, Nunavut, Canada. Both communities are located on Qikitaaluk/Baffin Island. The area's polar climate is characterized by long cold winters ($\sim$9 months from mid-September to May) and short cool summers (June to mid-September).

Domestic wastewater is collected daily or several times a week from holding tanks located inside buildings (homes, library, schools, health clinic, municipal offices, etc.) and trucked to

the WSPs for treatment. Wastewater is loaded into the WSPs throughout the year, however, treatment mainly occurs after the Spring thaw and during summer months, where the content of the WSPs is liquid. None of the communities have any industries or agricultural activities that would contribute to the wastewater.

Pond Inlet (72° 41′ 57″ N, 77° 57′ 33″ W) has a population of 1612 (Statistics Canada, 2012). The average daily temperature is −34°C in February (coldest month) and 6°C in July (warmest month, Environment Canada, 2014a). The community's WSP system consists of one engineered pond with a surface area of ∼40,000 m^2 and an average depth of ∼1.9 m during the summer. The treated wastewater (effluent) is discharged once annually in September by pumping the water over the berm after which it goes through a 500 m channel and into the ocean (Eclipse Sound). The annual estimated wastewater generation for the community is 8.0×10^7 L (140 L/person × day).

Clyde River (70° 28′ 26″ N, 68° 35′ 10″ W) has a population of 1004 (Statistics Canada, 2012). The community experiences average daily temperatures of −30°C in February (coldest month) and 5°C in July (warmest month, Environment Canada, 2014b). The WSP system in Clyde River consists of a small primary pond (6,000 m^2) and a larger secondary pond (15,000 m^2). The estimated annual wastewater volume is 3.1×10^7 L (88 L/person × day). This two-cell WSP system is designed to be operated in a staged manner, where the raw wastewater is deposited in the primary pond. The pre-processed wastewater would then be transferred into the secondary pond for further treatment before the annual decant in September, where the water is pumped into a wetland area that leads into the ocean (Patricia Bay). In September 2013, operations deviated as untreated wastewater was deposited in both ponds.

Sampling Strategy

On each visit, grab samples of wastewater were obtained from the wastewater trucks (i.e., raw wastewater, $n \geq 3$), and from different locations ($n = 2$–5) and at varying depths (0.1, 1.1, and 1.9 m) in the ponds to examine spatial variability. Effluent samples were also obtained during the decant ($n = 3$) in Pond Inlet or from the secondary pond ($n = 3$) in Clyde River just prior to the annual decant.

Samples from the Pond Inlet WSP were obtained in the beginning (late June/early July, just after the Spring thaw,), middle (late July/early August), and end (early/middle September, time of the annual decant which is just before to the freeze-up) of the summer treatment season in 2013 and 2014. Sludge samples (one composite sample per visit) were also obtained from the Pond Inlet WSP in 2014.

Samples from Clyde River were collected three times in 2013, while sampling in 2014 took place at the beginning and end of the summer just prior to the decant event.

Wastewater samples were collected in 1-L or 500-mL sterile containers (Nalgene, Fisher Scientific, Nepean, ON, Canada) from an inflatable boat or from the shore of the pond using a subsurface pole sampler and then stored in a cooler (4°C). Within 24 h of the sampling event, samples were flown to the Northern Water Quality Laboratory (NWQL) at the Nunavut Research Institute in Iqaluit, NU, for further processing.

Continuous Monitoring of WSPs and Water Quality Measurements

Deployment of multi-parameter sondes (YSI Inc., Yellow Spring, OH) allowed for *in-situ* hourly measurements of wastewater temperature, pH, and dissolved oxygen (DO). Sondes were installed in the WSPs (0.1 m below the surface) during the first sampling trip and retrieved at the end of each treatment season.

Grab samples were analyzed for the content of carbonaceous biological oxygen demand (CBOD$_5$), total ammonia (TA), total phosphorus (TP), total nitrogen (TN), and total suspended solids (TSS) using standard methods. Briefly, CBOD$_5$ was analyzed in duplicate following the APHA standard method 5210 B (American Public Health Association, 1999). Dissolved oxygen was measured using a probe (Orion 83005MD, Fisher Scientific, Ottawa, ON, Canada) and an Orion StarTM series meters (Fisher Scientific). The APHA standard methods 2540 D (American Public Health Association, 1999) with Whatman TM934-AH 47 mm glass fiber filters (Fisher Scientific) were used for measurements of TSS. Measurements of total ammonia (TA) was done using a Thermo Scientific OrionTM High-Performance Ammonia Electrode with the addition of Ionic Strength Adjuster (Fisher Scientific). The ascorbic acid method *via* Hach® TNT™ or TNT plus™ test kits was used to test TP. TN was analyzed using Hach® TN Test 'N Tubes™ (0.5 to 25.0 mg/L N), according to the manufacturer's procedure.

DNA Extraction

Genomic DNA was extracted from samples immediately upon arrival to the NWQL. DNA was extracted from three biological replicates (separate samples) per sampling site whenever available, with two technical replicates per sample. Ten mL of each sample was centrifuged at $3,200 \times g$ for 10 min (min) to obtain a pellet, which was subjected to genomic DNA extraction using the MO BIO PowerSoil DNA isolation kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. Within 24–48 h, all DNA samples were transported to the laboratory at Dalhousie University in Halifax, NS in a cooler (4°C) and stored at −20°C until further analysis.

Size of Bacterial Communities (16S rRNA Copy Numbers)

Bacterial 16S rRNA gene copy numbers were quantified in a quantitative PCR (qPCR) assay with the BACT 2 primer set (1369F: 5′-CGGTGAATACGTTCYCGG-3′; 1492R: 5′-GGWTACCTTGTTACGACTT-3′) (Suzuki et al., 2000). The qPCR amplification was performed on a Bio-Rad CFX96 Touch™ Real-Time PCR detection system (Bio-Rad, Hercules, CA, USA). Each reaction consisted of: 20-μL total reaction volumes consisting of 4.0 μL of template DNA, 4.4 μL of sterile and nuclease-free water, 0.8 μL of each primer (10 μM), and 10 μL of 2× Power SYBR Green PCR master mix (ThermoFisher Scientific). The thermocycler program consisted of 10 min of initial denaturation at 95°C, followed by 40 cycles of 15 s denaturation at 94°C, 30 s annealing at 55°C, and 30 s extension at 72°C. Melt curve analysis (30 s at 55°C and then increasing by

0.5°C each cycle until reaching 95°C) showed that the melting temperature of the amplicon was 84.5 ± 0.5°C.

A standard curve was constructed using 10-fold dilutions of a plasmid DNA extracted from an *Escherichia coli* DH5α culture containing a positive control plasmid (pCR2.1, TOPO TA PCR 2.1 with an insertion of the 16S rRNA fragment, gift from Dr. C. Yost, University of Regina, Canada), with triplicate measurements of diluted standards. Assay efficiency was calculated to be 102%. The coefficient of determination values (R^2) of the standard curve was 0.999, the limits of detection (LOD) and quantification (LOQ) were 68.6 and 686 copies/reaction, respectively. Absence of PCR inhibitors was confirmed by comparison of the threshold cycle (Ct) values for 10-fold diluted DNA extracts.

Illumina MiSeq Paired-End Sequencing and Bioinformatics Analysis

The V6-V8 regions of the bacterial 16S rRNA gene amplicon library preparation and Illumina MiSeq Paired-End (PE) sequencing were performed following the established protocol (Comeau et al., 2017) at the Dalhousie University Integrated Microbiome Resource (IMR; http://cgeb-imr.ca). The demultiplexed PE reads were obtained in the fastq format from IMR and subjected to the bioinformatics analysis workflow briefly described in the following section.

The raw PE sequencing reads were preprocessed by stitching PE reads and removing low quality reads in PEAR (Zhang et al., 2014), and removing chimeras in VSEARCH (version 1.11.1, 28) in the Microbiome Helper pipeline at IMR (Comeau et al., 2017). As a result, an average of 73.1% of the reads (range 49.6–94.6% for individual samples) was generated as final high-quality sequences. The final high-quality sequences were then clustered into Operational Taxonomic Units (OTUs) at an identity level of ≥97% using the *de novo* UPARSE-OTU algorithm in the USEARCH pipeline (Edgar, 2013). OTUs from UPARSE were deposited in the Microbiome Helper pipeline to filter out spurious OTUs (Comeau et al., 2017), resulting in a total of 2,093 OTUs (from 139 samples). Subsequent analyses were carried out using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline version 1.9.1 (Caporaso et al., 2010a), unless otherwise noted. The taxonomic assignment was performed in the Ribosome Database Project (RDP) classifier version 2.2 (Wang et al., 2007) based on the Greengenes database (version 13_8) at the cut-off value of 60% (Claesson et al., 2009). The rarefied OTU table was generated by subsampling a minimal number of reads (10,539 sequences per sample), resulting in a total of 1,924 OTUs. The sequence alignment was built using the Python Nearest Alignment Space Termination (PyNAST) tool (Caporaso et al., 2010b). This alignment was used to build a phylogenetic tree in FastTree (version 2.1.10, http://www.microbesonline.org/fasttree/) which infers approximately-maximum-likelihood phylogenetic trees (Price et al., 2010). The rarefied OTU table and the phylogenetic tree were used in the downstream analysis, which included determining the core bacterial community that requires the core OTUs to be present in 100% of the samples (Huse

et al., 2012), alpha-diversity measures (number of the observed OTUs, Chao1, the Shannon-Wiener (SWI) and the Simpson evenness indexes), and phylogenetic beta-diversity measures, respectively. The phylogenetic beta-diversity was calculated using both the weighted and unweighted UniFrac metrics (Lozupone and Knight, 2005). Principal Coordinate Analysis (PCoA) and an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) tree were used to visualize the diversity trends based on the weighted UniFrac beta-diversity distance matrix. The trends revealed by the unweighted UniFrac analyses resembled the weighted UniFrac results and are therefore not presented. GenGIS (Parks et al., 2013) was applied to visualize information about bacterial communities representing different locations, times and years in a sampling map.

A distance-based redundancy analysis (dbRDA) was performed on the weighted UniFrac beta-diversity matrix and selected environmental variables for pond samples obtained from each of the two communities over the course of the 2-year study period. The models were assessed using the ANOVA function (R Core Team, 2021) to determine the significance of the environmental predictors (temperature, $CBOD_5$, TSS, TP, TN, and TA) on the beta-diversity among communities. The dbRDA was run using the capscale function in the R package vegan (Oksanen et al., 2020) and ordination plots were generated using the ggord package (Beck, 2020).

Predictions of Functions in Bacterial Communities

The Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt, version 1.1.0, Langille et al., 2013) in the Microbiome Helper pipeline (http://github.com/mlangill/microbiome_helper/wiki/PICRUSt-workflow) was used to predict gene contents based on 16S rRNA gene surveys. The PICRUSt analysis works from the observation that there is a good correlation between the phylogenetic information inferred from 16S rRNA marker gene sequences and genomic content when related reference genomes are available. Prior to the analysis, the accuracy of predictions is measured by the Nearest Sequenced Taxon Index (NSTI), with lower values indicating a closer relationship and availability of closely related reference genomes (Langille et al., 2013). Low NSTI values of 0.07 ± 0.01 and 0.08 ± 0.02 were calculated for wastewater samples from Pond Inlet and Clyde River, respectively. For comparison, it has been reported (Langille et al., 2013) that human-associated samples had the lowest (best) NSTI values (0.03 ± 0.02), while higher NSTI values (>0.14) were reported for other mammalian gut and soil samples. Thus, arctic municipal wastewater samples appeared to constitute a suitable data set to derive functional predictions by using PICRUSt and the recommended workflow.

Statistical Analysis

Significant differences ($p < 0.05$) in the log 16S rRNA copy numbers/mL and alpha diversity among sample groups were tested using the non-parametric Kruskal-Wallis test performed in Prism 7 (version 7.0b, Graph Pad Software, Inc., La Jolla, CA, USA). In the study of phylogenetic beta-diversity measures, the nonparametric statistical test through

TABLE 1 | Water quality in the Pond Inlet and Clyde River wastewater treatment systems.

Community	Year	Water type	Season	Temperature (°C)	CBOD$_5$ (mg/L)	TSS (mg/L)	TN (mg/L - N)	TP (mg/L)	TA (mg/L - N)
Pond Inlet	2013	Raw	All	19.5 ± 1.6[a]	591.8 ± 205.3	412.4 ± 177.0	160.4 ± 24.1	62.5 ± 17.1	136.5 ± 11.2
		WSP	Beginning	1.1 ± 0.1	191.4 ± 38.6	46.3 ± 27.3	84.4 ± 5.3	27.3 ± 1.0	82.6 ± 31.5
		WSP	Middle	15.8 ± 0.0	177.3 ± 14.0	21.7 ± 2.7	96.6 ± 3.5	31.1 ± 0.6	101.1 ± 16.3
		WSP	End	0.3 ± 0.0	112.6 ± 9.4	55.6 ± 10.9	101.9 ± 9.0	35.0 ± 0.6	89.9 ± 1.9
		Effluent	End	1.5 ± 0.7	103.3 ± 14.1	64.5 ± 8.9	109.1 ± 9.9	35.1 ± 1.0	87.1 ± 4.6
		WSP Beginning – WSP End	Removal (%)	41.2	−20.1	−20.7	−28.2	−8.8
		Raw to Effluent	Removal (%)	82.5	84.4	32.0	43.8	36.2
	2014	Raw	All	20.5 ± 4.6	347.4 ± 129.8	201.7 ± 91.4	128.6 ± 18.6	43.5 ± 6.1	83.3 ± 33.0
		WSP	Beginning	8.7 ± 0.0	226.7 ± 27.6	42.1 ± 35.6	119.2 ± 11.2	35.3 ± 2.9	106.3 ± 5.9
		WSP	Middle	18.8 ± 0.0	124.3 ± 26.5	76.3 ± 17.5	102.8 ± 7.6	32.0 ± 0.6	128.6 ± 2.5
		WSP	End	6.0 ± 0.0	114.8 ± 16.9	36.3 ± 6.7	87.8 ± 9.0	35.3 ± 0.2	90.8 ± 5.9
		Effluent	End	2.9 ± 0.1	113.3 ± 3.2	19.8 ± 0.4	117.0 ± 8.8	33.8 ± 0.4	86.4 ± 8.0
		WSP Beginning – WSP End	Removal (%)	49.4	13.8	26.3	0.0	14.6
		Raw to Effluent	Removal (%)	67.4	90.2	9.0	22.3	−3.7
Clyde River	2013	Raw	All	20.7 ± 2.6	389.6 ± 155.5	243.0 ± 119.4	129.6 ± 26.4	48.3 ± 8.3	113.0 ± 25.5
		WSP1	Beginning	3.4 ± 0.0	280.8 ± 10.8	64.8 ± 8.7	102.4 ± 8.0	33.6 ± 0.6	89.3 ± 8.4
		WSP1	Middle	11.5 ± 0.0	160.3 ± 31.6	51.0 ± 6.8	67.7 ± 0.8	23.4 ± 0.2	64.8 ± 2.8
		WSP1	End	3.0 ± 0.0	183.8 ± 20.0	61.8 ± 3.5	95.0 ± 3.6	29.0 ± 0.0	108.8 ± 2.1
		WSP2	Beginning	3.4 ± 0.0	105.0 ± 10.4	27.8 ± 2.9	76.1 ± 17.7	19.4 ± 0.2	51.3 ± 0.5
		WSP2	Middle	11.5 ± 0.0	92.3 ± 16.5	27.8 ± 2.9	60.1 ± 5.3	18.4 ± 0.2	45.5 ± 3.1
		WSP 2	End	3.0 ± 0.0	70.3 ± 25.7	37.8 ± 4.0	74.3 ± 1.7	19.6 ± 0.1	82.7 ± 2.4
		WSP1 Beginning – WP2 End	Removal (%)	75.0	41.7	27.4	41.7	7.4
		Raw – WSP2 End	Removal (%)	82.0	84.4	42.7	59.4	26.8
	2014	Raw	All	15.8 ± 1.8	277.7 ± 25.4	243.7 ± 107.4	90.0 ± 10.5	38.8 ± 5.8	104.9 ± 29.0
		WSP1	Beginning	6.4 ± 0.0	315.0 ± 56.8	16.0 ± 4.1	100.0 ± 0.0	43.9 ± 0.0	137.5 ± 8.3
		WSP1	End	4.3 ± 0.0	228.5 ± 87.9	41.8 ± 5.0	95.0 ± 5.4	26.7 ± 0.3	93.7 ± 6.1
		WSP2	Beginning	6.4 ± 0.0	105.3 ± 3.8	15.8 ± 1.3	77.0 ± 2.0	21.9 ± 0.0	62.4 ± 1.2
		WSP2	End	4.3 ± 0.0	69.0 ± 2.9	19.8 ± 5.7	68.1 ± 1.2	17.8 ± 0.1	68.9 ± 6.3
		WSP1 Beginning – WP2 End	Removal (%)	78.1	−23.8	31.9	59.5	49.7
		Raw – WSP2 End	Removal (%)	75.2	91.9	24.3	54.1	34.3

[a] Average value ± standard deviation (n-1).

permutations analysis of similarities (ANOSIM) approach was performed in the QIIME pipeline (Caporaso et al., 2010a) to test whether there were statistically significant differences among the trends that were observed in PCoA plots. In the study of inferring functional content in the bacterial communities, significant differences at a 5% level between two groups were tested using Welch's t-test with the Benjamini-Hochberg false-discovery rate (FDR) correction, while significant differences at a 5% level among three or more groups were tested using the Kruskal-Wallis H-test with FDR correction. These two tests were conducted in the statistical analysis of taxonomic and functional profiles (STAMP) software version 2.1.3 (Parks et al., 2014).
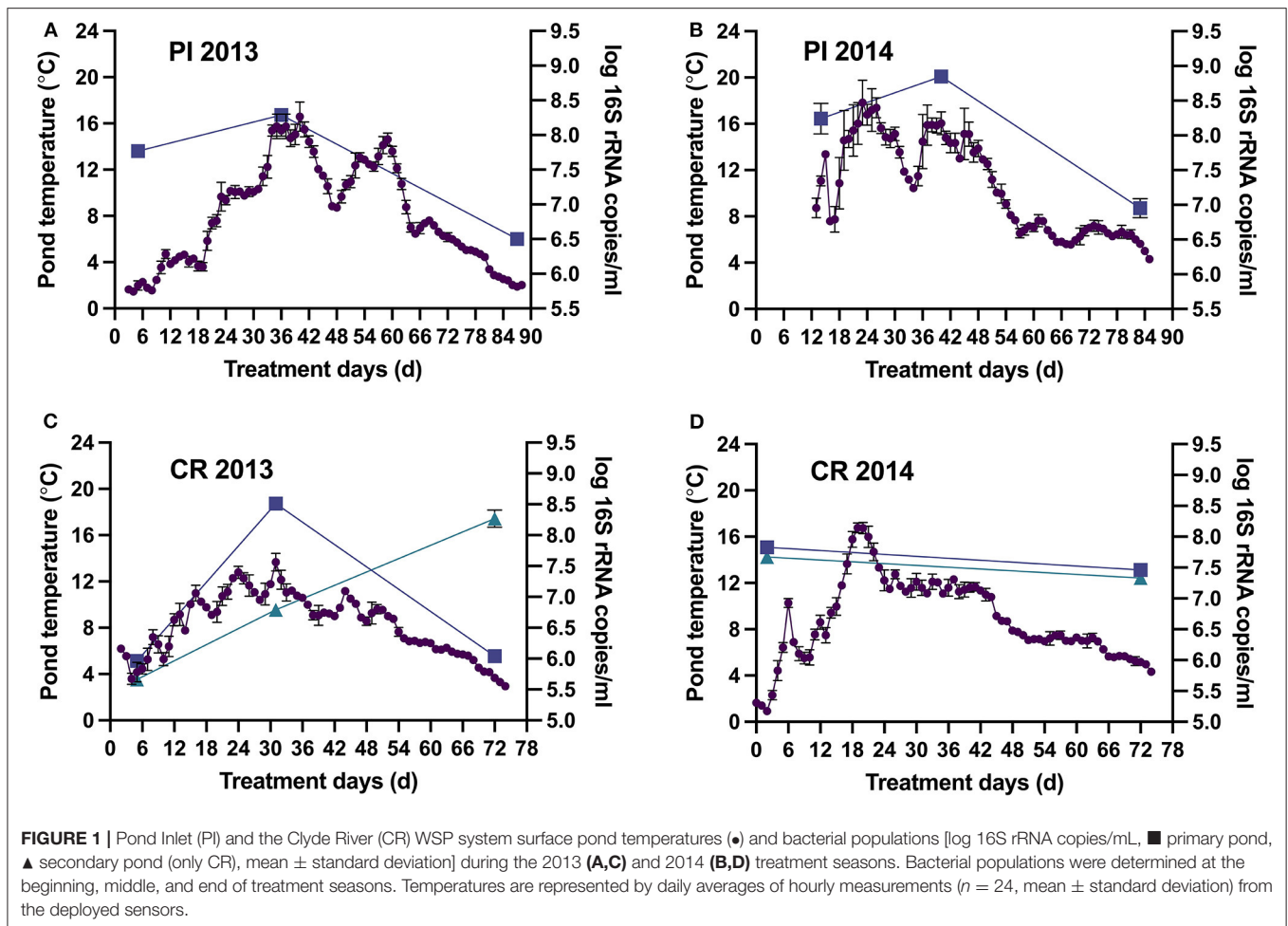
RESULTS

WSP Environment and Removal of Bacteria and Nutrients

Raw wastewater samples from both communities consistently contained an average of 9.0 log 16S rRNA copies/mL, a level which showed no change ($p > 0.05$) over the duration of the study (data not shown). The water quality varied but tended be contain higher levels of CBOD$_5$ and other nutrients in Pond Inlet compared to Clyde River (**Table 1**).

Surface pond temperatures in Pond Inlet gradually increased from 2 to 8°C to mid-season peak temperatures of 18°C followed by a decrease to below 5°C in the month of September (**Figures 1A,B**). The bacterial population size followed the changes in pond temperatures with midseason peaks of 8–8.9 log 16S rRNA copies/mL followed by significant ($p < 0.05$) decrease to 6.5–7 log 16S rRNA copies/mL at the end. The population size in 2014 was significantly ($p < 0.05$) higher than in 2013, which coincided with the higher pond temperatures recorded in 2014. Results from the deployed DO and pH sensors revealed negligible DO levels (<0.2 mg/L), except for two one-week mid-season spikes to measurable levels (see **Supplementary Figure 1**), and stable pH-values from 7.5 to 7.8 (**Supplementary Figure 2**). Absence of marked increases in pond pH and DO indicated that algal blooms were absent in 2013 and 2014.

FIGURE 1 | Pond Inlet (PI) and the Clyde River (CR) WSP system surface pond temperatures (●) and bacterial populations [log 16S rRNA copies/mL, ■ primary pond, ▲ secondary pond (only CR), mean ± standard deviation] during the 2013 **(A,C)** and 2014 **(B,D)** treatment seasons. Bacterial populations were determined at the beginning, middle, and end of treatment seasons. Temperatures are represented by daily averages of hourly measurements ($n = 24$, mean ± standard deviation) from the deployed sensors.

In Clyde River, the highest and lowest bacterial levels in the primary pond (∼8.5 and ∼6.0 log 16S rRNA copies/mL, respectively) also coincided with the highest (13.7°C) and lowest (3.7°C) pond temperatures in 2013 (**Figure 1C**). Normal operation of the Clyde River WSP systems in 2014 reduced populations in the secondary pond to 7.4 log 16S rRNA copies/mL at the end of the treatment season (**Figure 1D**). However, in September 2013, the secondary pond contained 8.3 log 16S rRNA copies/mL (**Figure 1C**), which was likely caused by direct discharge of raw wastewater into that pond. DO levels remained negligible (<0.2 mg/L) throughout both treatment seasons, and pH ranged from 7.5 to 7.8, indicating absence of algal blooms in both years (**Supplementary Figures 1, 2**).

Overall, arctic WSP treatment effected a >2 log reduction in bacterial numbers leading to an effluent with a content of 6–7.0 log 16S rRNA copies/mL ready to be released into the receiving environment (**Figure 1**). Water quality data showed that treatment in the WSPs removed 67–83, 84–92, 9–43, 22–59% and none to 36% of the $CBOD_5$, TSS, TN, TP, and TA, respectively, found in the raw wastewater (**Table 1**). The content of $CBOD_5$ in the ponds decreased over the summer treatment season, with the lowest values 70–90 mg/L observed at the end of

the summer in the secondary pond in Clyde River as opposed to 103–115 mg/L in Pond Inlet.

Alpha Diversity in Bacterial Communities in Arctic WSP Systems

The alpha diversity decreased during the treatment of the wastewater as shown by the significant ($p < 0.05$) decreases in the SWI from values of ∼5 for the raw wastewater to values ranging between 2.5 and 4.5 in the ponds of both communities (**Figure 2**). Comparison of pond samples across the treatment seasons showed that the alpha diversity was lower at the start and end of the treatment season (2.5–4) while SWI values peaked at 4.3–4.5 during the warmer mid-season. The alpha diversity tended to be lower in Clyde River samples, which coincided with pond temperatures overall being colder in Clyde River compared to Pond Inlet (**Figure 1**). Other alpha-diversity indices (observed_OTUs and chao1) revealed the same trends (**Supplementary Tables 1, 2**). However, the community evenness (Simpson_e), ranged from 0.02 to 0.06 regardless of the sample type or season or year (**Supplementary Tables 1, 2**).

The alpha diversity of bacterial community in Pond Inlet sludge samples, which were only obtained in 2014, increased over the treatment season, a change which occurred simultaneously
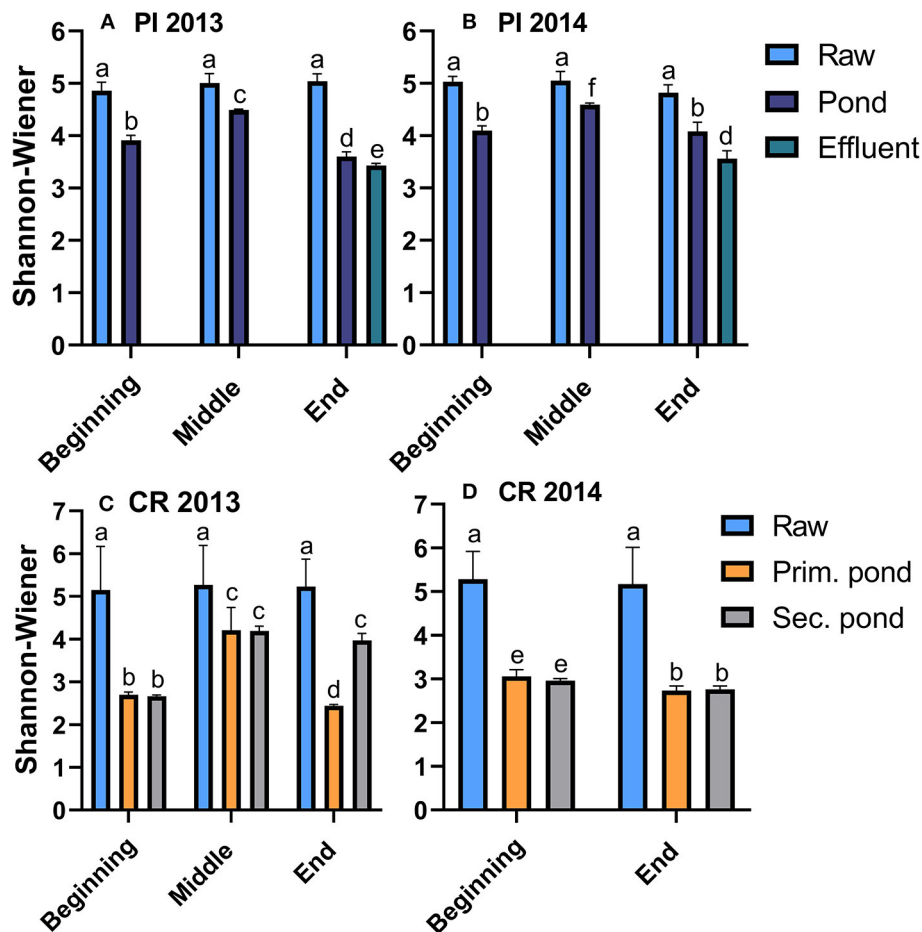
FIGURE 2 | Alpha diversity (Shannon-Wiener Index) in raw and treated wastewater samples from Pond Inlet [PI, **(A,B)**] and Clyde River [CR, **(C,D)**] in 2013 and 2014. Bars with different letters within the same community are significantly ($p < 0.05$) different from each other.
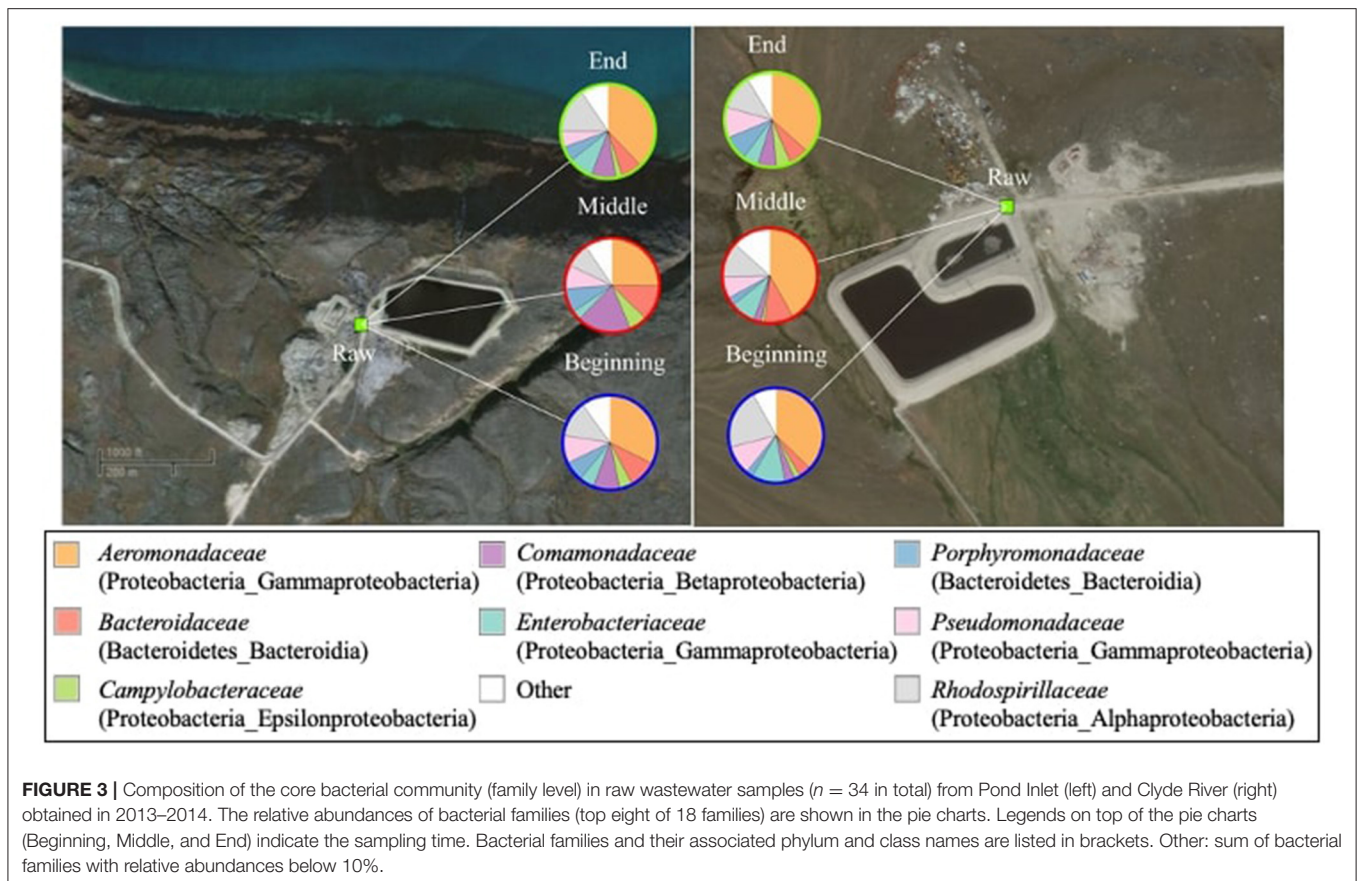
with growing bacterial populations with final levels of 9.9 log 16S rRNA copies/mL (**Supplementary Table 1**).

Beta Diversity and Core Microbiome in Raw Wastewater

The weighted and unweighted UniFrac measures showed that the bacterial beta diversity was not significantly ($p > 0.05$) different in raw wastewater samples obtained from the two communities during the study period. The raw wastewater core microbial community was comprised of 18 bacterial families mainly belonging to the *Proteobacteria* phylum. Dominant *Proteobacteria* families were *Aeromonadaceae* (25.2–41.8%), followed by *Rhodospirillaceae* (8.5–20.9%), *Comamonadaceae* (2.7–18.6%), *Enterobacteriaceae* (3.3–11.5%), *Pseudomonadaceae* (5.4–10.5%), and *Campylobacteraceae* (1.1–6.2%) (**Figure 3**). Within the *Bacteroidetes* phylum and *Bacteroidia* class, two families were present; *Bacteroidaceae* with a prevalence of 4.2–12.3% and *Porphyromonadaceae* with abundances ranging from 2.4 to 8.5%. The genus level composition of the core microbiomes in the raw wastewater samples is shown in **Supplementary Figure 3**.

Treatment and Changes in Phylogenetic Beta Diversity in Arctic WSP Systems

Treatment in the WSP systems in Pond Inlet and Clyde River significantly ($p < 0.05$) altered the phylogenetic beta diversity of bacterial communities. For Pond Inlet, this observation is exemplified by a comparison of raw, pond, sludge and effluent samples from September 2014, which showed different distributions of bacterial families along the treatment process (**Figure 4A**). Here, the *Aeromonadaceae* family (40.0%) dominated in the raw wastewater samples but became a minor group (1.8%) in the pond samples. Sludge samples were dominated by the *Pseudomonadaceae* family (29.7%). Pond and effluent samples resembled each other with the most abundant group being *Comamonadaceae* (~41%), *Campylobacteraceae* (~15%), and *Geobacteraceae* (~13%). The above mentioned families were represented at the genus level by *Rhodoferax*, *Arcobacter*, and *Geobacter* in the pond and effluent samples (**Supplementary Figure 4**). The resulting PCoA plot (**Figure 4B**) from the weighted UniFrac analysis shows that 53.3% of the variation in microbial beta diversity depended on the sample type while another 24.0% of the variation was due

FIGURE 3 | Composition of the core bacterial community (family level) in raw wastewater samples ($n = 34$ in total) from Pond Inlet (left) and Clyde River (right) obtained in 2013–2014. The relative abundances of bacterial families (top eight of 18 families) are shown in the pie charts. Legends on top of the pie charts (Beginning, Middle, and End) indicate the sampling time. Bacterial families and their associated phylum and class names are listed in brackets. Other: sum of bacterial families with relative abundances below 10%.

to the difference between raw wastewater vs. samples from the WSP. Results from Clyde River showed similar changes in the composition of bacterial communities along the WSP treatment train with *Geobacteraceae*, *Campylobacteraceae*, and *Comamonadaceae* making up 50% of the bacterial community in the secondary pond (**Supplementary Figure 5**).

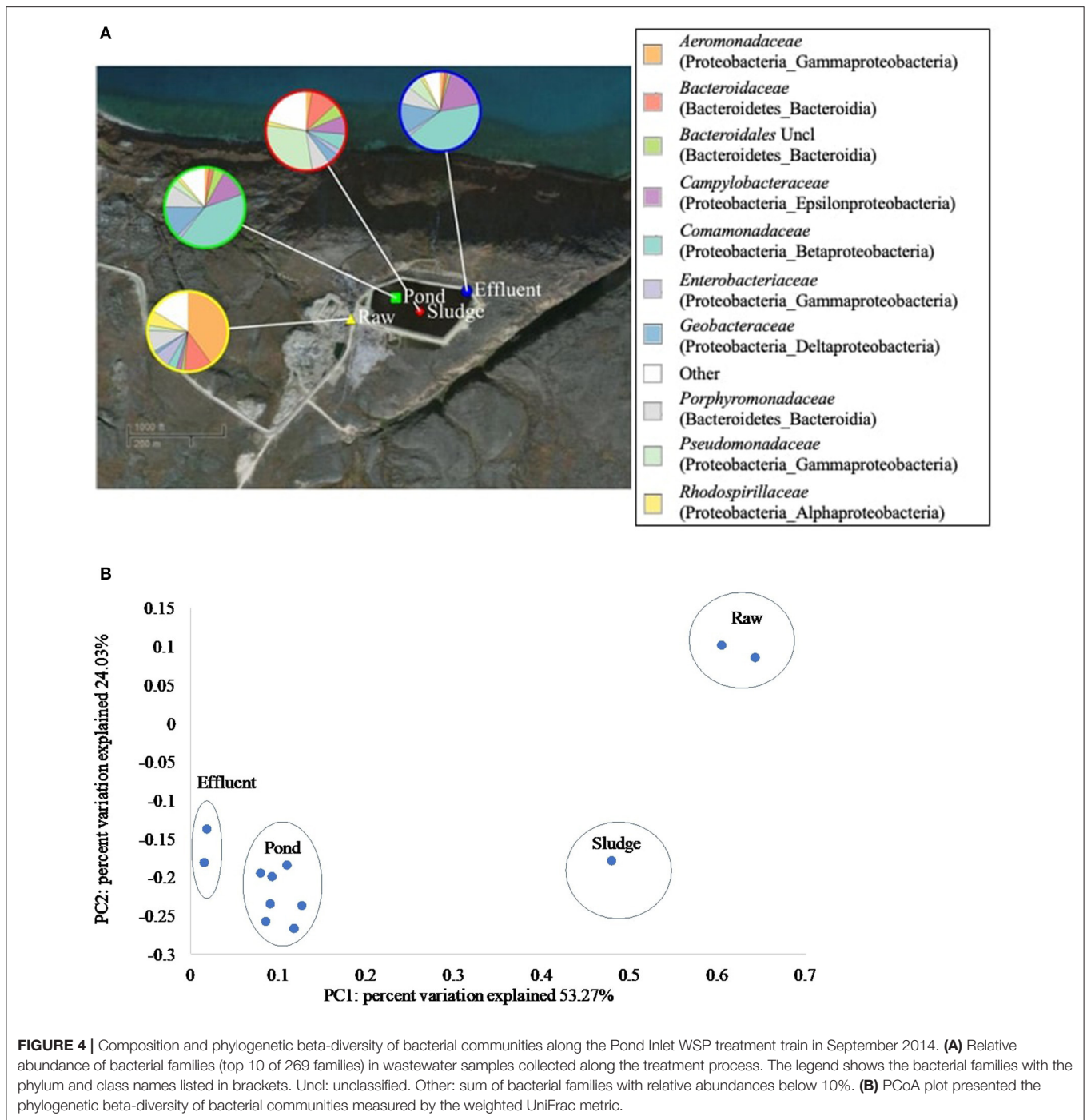## Structure of Bacterial Communities in Relation to Wastewater Treatment in the WSPs

The time of sampling within the treatment season significantly ($p < 0.05$) influenced the phylogenetic beta diversity of bacterial communities in the arctic WSPs as did the sampling year. These seasonal and annual differences in pond bacterial communities are shown for Pond Inlet in **Figure 5**. Results from Clyde River showed similar trends and the results from 2014 are presented in **Figure 6**.

In Pond Inlet in 2013, early season samples were dominated by *Pseudomonadaceae* (49.5%) and *Campylobacteraceae* (13.6%) (**Figure 5A**). Communities then shifted to a mid-season mix dominated by *Comamonadaceae* (36.8%) and *Campylobacteraceae* (24.5%) and finally to an end-season blend with *Comamonadaceae* (31.7%), *Geobacteraceae* (16.8%), and *Porphyromonadaceae* (10%) dominating. For the annual

differences, the mid-season dominating family *Comamonadaceae* varied between 36.8 and 70.0% in 2013 and 2014, respectively. When switching to the end-season, the relative abundance of *Comamonadaceae* decreased to 31.7% in 2013 and 44.3% in 2014 (**Figure 5A**).
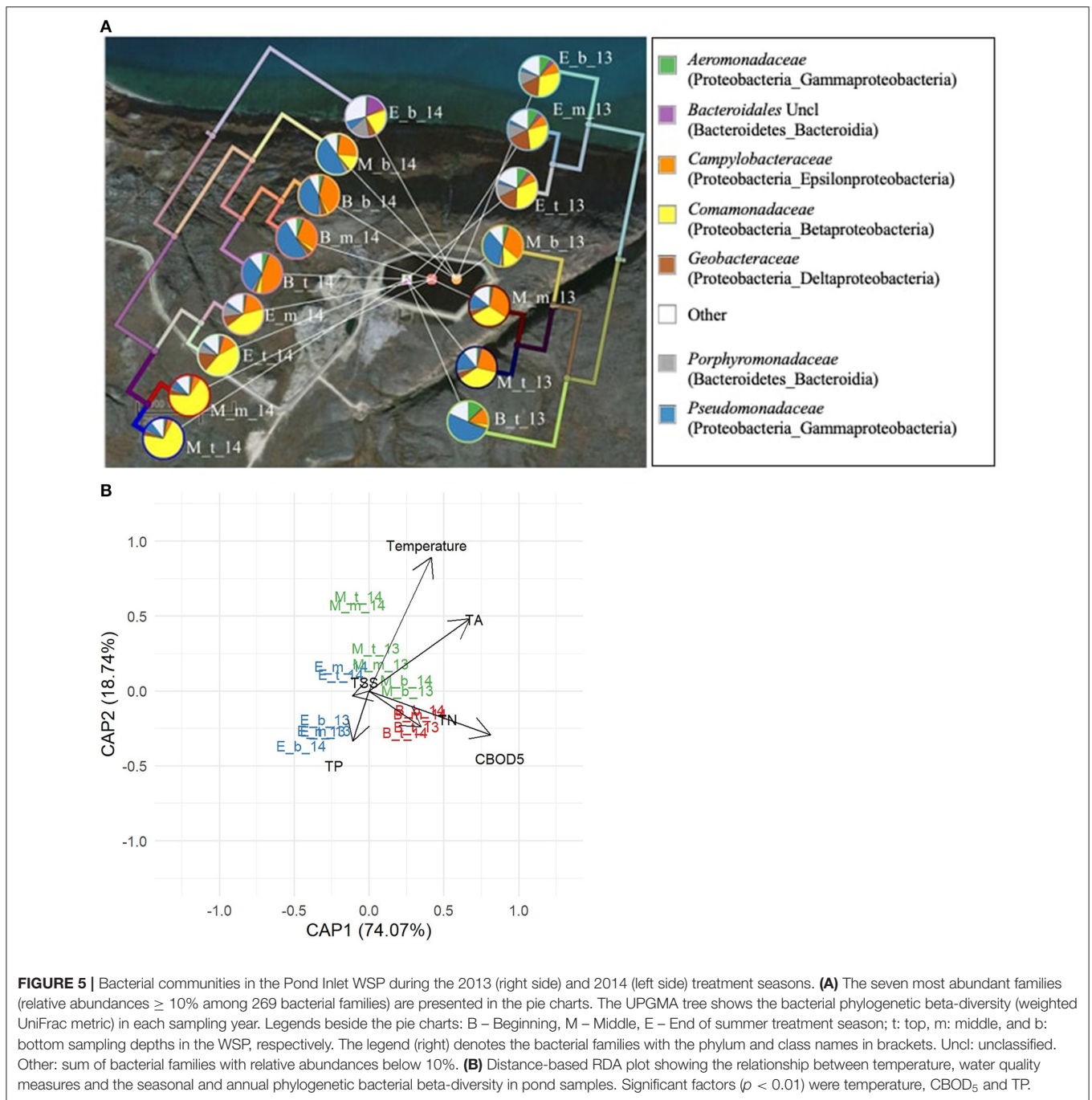
The dbRDA plot relating the measured environmental and water quality factors to the weighted UniFrac beta diversity among pond communities in Pond Inlet revealed that temperature, $CBOD_5$ and TA were significant factors ($p < 0.05$) driving differences in the bacterial communities (**Figure 5B**). The $CBOD_5$ content and *Pseudomonadaceae* and *Campylobacteraceae* correlated positively with the beginning of the treatment season, while the negative correlation at the middle and end of the treatment season showed the role of the microbiota (i.e., *Comamonadaceae*) in removing $CBOD_5$, a development that correlated with the higher temperatures in the middle of the season and its effect on the bacterial communities. The decrease in TP correlated with increases in *Geobacteraceae*, and members of the anaerobic *Bacteroidales* (incl. *Porphyromonadaceae*) at the end of the treatment season. The dbRDA plot also revealed differences between 2013 and 2014 microbial communities obtained in the middle and end of the season supporting the marked effect of the ambient temperature on the microbiota and wastewater treatment in the Pond Inlet WSP system.

FIGURE 4 | Composition and phylogenetic beta-diversity of bacterial communities along the Pond Inlet WSP treatment train in September 2014. (A) Relative abundance of bacterial families (top 10 of 269 families) in wastewater samples collected along the treatment process. The legend shows the bacterial families with the phylum and class names listed in brackets. Uncl: unclassified. Other: sum of bacterial families with relative abundances below 10%. (B) PCoA plot presented the phylogenetic beta-diversity of bacterial communities measured by the weighted UniFrac metric.

For Clyde River, *Pseudomonadaceae* dominated in the primary pond at the beginning of the 2014 season, while late season primary pond samples contained a diverse community including *Campylobacteraceae*, *Geobacteraceae*, *Rhodopirillaceae*, and *Comamonadaceae* (**Figure 6A**). A similar change from a *Pseudomonadaceae* dominated (about 50%) community to a highly diverse community at the end of the season was seen in the secondary pond samples, where the proportion of

Porphyromonadaceae and *Comamonadaceae* increased together with *Geobacteraceae*. These seasonal trends were reflected in the phylogenic UPGMA tree with the first branch splitting at the beginning/end of season followed by a second split into the primary/secondary pond. The dbRDA plot relating the measured environmental and water quality factors to the beta diversity among pond communities in Clyde River revealed that temperature, $CBOD_5$, TA, TSS, TN, and TP were
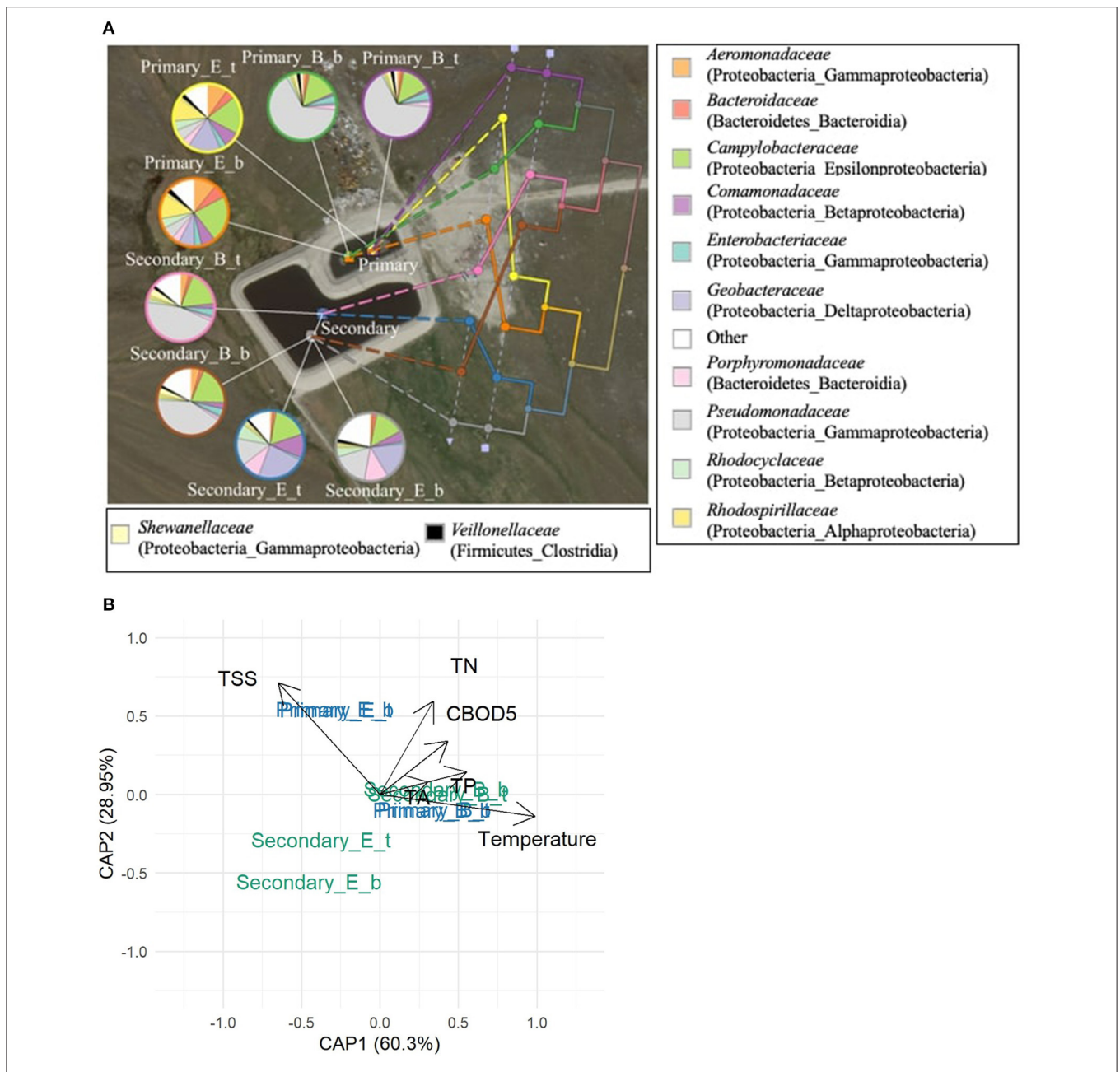
FIGURE 5 | Bacterial communities in the Pond Inlet WSP during the 2013 (right side) and 2014 (left side) treatment seasons. **(A)** The seven most abundant families (relative abundances $\geq$ 10% among 269 bacterial families) are presented in the pie charts. The UPGMA tree shows the bacterial phylogenetic beta-diversity (weighted UniFrac metric) in each sampling year. Legends beside the pie charts: B – Beginning, M – Middle, E – End of summer treatment season; t: top, m: middle, and b: bottom sampling depths in the WSP, respectively. The legend (right) denotes the bacterial families with the phylum and class names in brackets. Uncl: unclassified. Other: sum of bacterial families with relative abundances below 10%. **(B)** Distance-based RDA plot showing the relationship between temperature, water quality measures and the seasonal and annual phylogenetic bacterial beta-diversity in pond samples. Significant factors ($p < 0.01$) were temperature, CBOD$_5$ and TP.

significantly ($p < 0.05$) correlated to the observed differences (**Figure 6B**).

The UPGMA tree for Pond Inlet revealed that bacterial communities in the bottom layer differed significantly ($p < 0.05$) from the top or middle layers during the 2014 treatment season, but not in 2013 (**Figure 5A**). Interestingly, the different sampling depths in Clyde River two-cell WSP did not affect ($p > 0.05$) microbial community diversity in 2014 (**Figure 6A**).

Functional Content Predictions for Bacterial WSP Communities

The two most significant ($p < 0.05$) KEGG pathways, which were predicted to be present in the bacterial communities in WSP samples, are associated with carbohydrate and energy metabolism. As shown in **Figure 7A**, the predicted proportion of sequences associated with carbohydrate metabolism was significantly higher ($p < 0.05$) during the middle of the season compared to the beginning and end of the 2014 treatment season
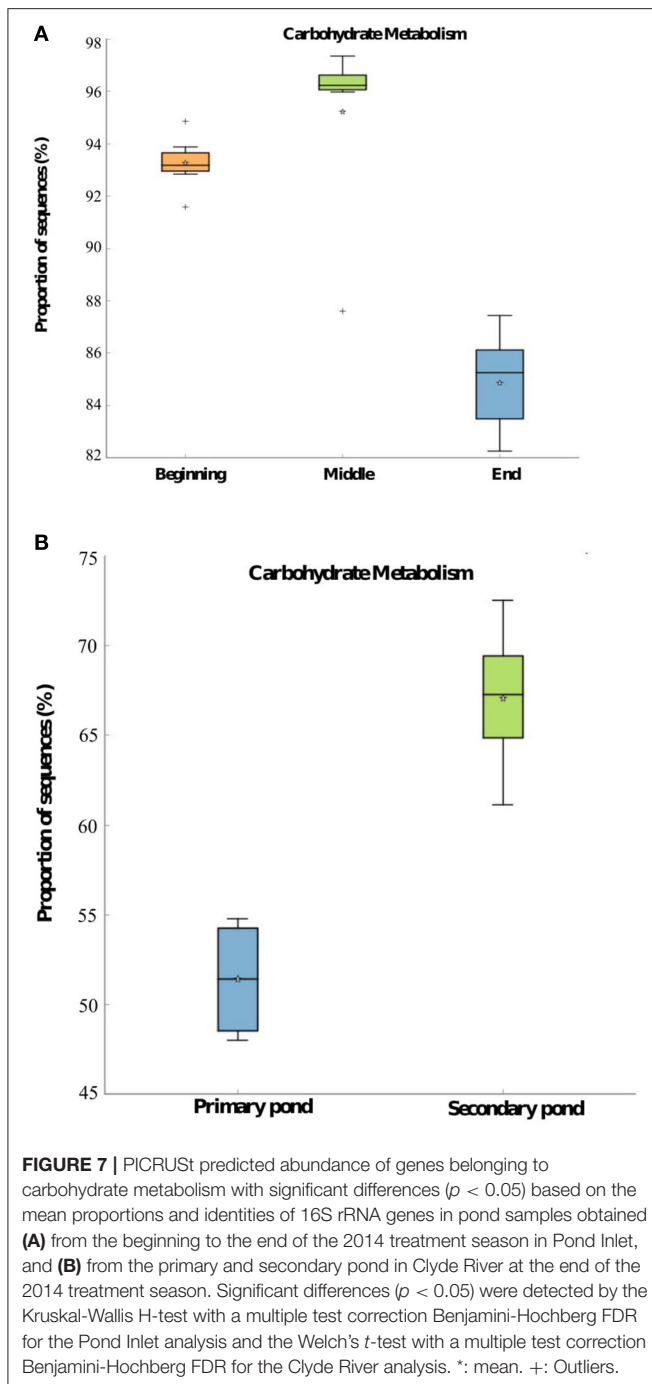
FIGURE 6 | Bacterial communities in Clyde River WSP system during the 2014 treatment season. **(A)** The UPGMA tree shows the phylogenetic beta-diversity of bacterial communities measured by the weighted UniFrac metric. The 12 most abundant bacterial families (relative abundances ≥ 10%, total of 269 bacterial families) are presented in the pie charts. Legends beside the pie charts: Primary – Primary pond, Secondary – Secondary pond; B – Beginning, E – End of summer treatment season; t – top and b – bottom indicates the sampling sites in the WSP, respectively. The legend beside the map shows bacterial families: the phylum and class names are listed in the bracket of each family name. Other: sum of bacterial families with relative abundances <10%. **(B)** Distance-based RDA plot showing the relationship between temperature, water quality measures (TSS, TN, CBOD$_5$, TA, TP, all significant, $p < 0.05$) and the seasonal phylogenetic bacterial beta-diversity in the pond samples.

in Pond Inlet. The carbohydrate metabolism was predicted to be significantly ($p < 0.05$) higher in the bacterial community of the secondary pond in Clyde River compared to the primary pond (**Figure 7B**). The energy metabolism in Pond Inlet and Clyde River bacterial WSP communities followed the same trend (data not shown). The predicted abundance of genes responsible for the carbohydrate and energy metabolism in mid-season pond

samples were significantly ($p < 0.05$) higher in 2014 compared to 2013 (data not shown).

In both Pond Inlet and Clyde River WSPs, PICRUSt identified low proportions (0.002–0.006%) of 16S rRNA genes associated with the three KEGG pathways that encode ammonia monooxygenase, an enzyme which is involved in microbial removal of ammonia. This agrees with the relative low abundance

FIGURE 7 | PICRUSt predicted abundance of genes belonging to carbohydrate metabolism with significant differences ($p < 0.05$) based on the mean proportions and identities of 16S rRNA genes in pond samples obtained **(A)** from the beginning to the end of the 2014 treatment season in Pond Inlet, and **(B)** from the primary and secondary pond in Clyde River at the end of the 2014 treatment season. Significant differences ($p < 0.05$) were detected by the Kruskal-Wallis H-test with a multiple test correction Benjamini-Hochberg FDR for the Pond Inlet analysis and the Welch's t-test with a multiple test correction Benjamini-Hochberg FDR for the Clyde River analysis. *: mean. +: Outliers.

(0.01%) of both the *Nitrosomonadaceae* and *Crenotrichaceae* families, which are associated with the three pathways, in the WSPs bacterial communities.

DISCUSSION

The treatment of the wastewater resulted in removal of 70–90% of the content of TSS and CBOD$_5$ and reduction of *E. coli* levels to 10^{4-6} MPN/100 mL (Huang et al., 2018) leading to treated water qualities (effluent in Pond Inlet and water in the secondary pond

at the end of the season in Clyde River), which complied with the criteria set by the licensing Nunavut Water Board of there being $<10^4$-10^6 *E. coli* MPN/100 mL, 180 mg/L TSS, and 120 mg/L CBOD$_5$ in water released into the recipient (Nunavut Water Board, 2014). These criteria have been set in consideration of the climate, nature of the treatment systems, receiving environment and population density in Nunavut and are different from the Canadian Wastewater Systems Effluent Regulations (WSERs), which require maximum contents of 25 mg/L CBOD$_5$, 25 mg/L TSS, and 1.25 mg/L un-ionized ammonia for wastewater effluents in Southern Canada (Environment Canada, 2015). The observed removal of nutrients is likely due to a combination of biological processes, physical settling processes, dilution (due to snow and rainfalls) and evaporation events following the disposal of the wastewater into the WSPs (Heinke et al., 1991; Ragush et al., 2015).

The bacterial communities loaded into the WSPs were similar as the influent raw wastewater contained a core set of bacteria in spite of coming from the two geographically separated arctic communities. It may be that low per capita consumption of drinking water and absence of industrial or agricultural activities in both communities led to the presence of this core bacterial community dominated by *Proteobacteria* (**Figure 3**). Treatment subsequently led to significant changes to the composition of bacterial communities and a 2-log reduction in 16S rRNA gene copies/ml in the treated water at the end of the treatment season. This reduction was similar to the 2-3 log removal of total coliform bacteria and *Escherichia coli* previously reported for these WSPs (Huang et al., 2018). This suggests a preferential reduction in enteric bacteria, i.e., a disinfection effect, which was supported by a shift from dominance of fecally related *Aeromonadaceae*, *Bacteroidaceae*, *Campylobacteraceae*, and *Enterobacteriaceae* in influent wastewater toward *Comamonadaceae, Porphyromonadaceae* (*Paludibacter*), and *Geobacteraceae* in the treated water (**Figure 4** and **Supplementary Figure 5**). A similar reduction in the content of enteric bacteria and shift in bacterial communities was observed in a comparative study of the influent and effluent of a German WWTP (Numberger et al., 2019). The disinfection effect may be due to pond environmental factors (temperature, DO, pH, etc.), microbial completion and/or antagonism and solar irradiation although the underlying mechanisms remain unknown (Ho and Goethals, 2020).

Monitoring of the WSP environment throughout the 2013 and 2014 summer treatment season showed that the intended facultative ponds stayed anaerobic with very low levels of oxygen, neutral pH-values and therefore no signs of algae growth (**Supplementary Figures 1, 2**). This was different from 2011 where a reduced operational depth in the pond, yielding a lower OLR (kg/m^2/day), and an unusual warm summer led to an algal bloom in the Pond Inlet WSP (Ragush et al., 2015). The lack of algae in the WSPs could affect the wastewater treatment as normal WSP treatment is based on growth of algae to supply oxygen to the aerobic decomposing bacteria, sequester phosphorous and disinfection due to the increased alkalinity of the water during algal blooms (Amengual-Morro et al., 2012; Ho and Goethals, 2020). The absence of algae growth may be related to the high OLRs and low temperatures in the

ponds as demonstrated in model experiments (Ragush et al., 2017). Since the WSPs remained anaerobic and with neutral pH throughout the treatment season, temperature became an important driver of changes in the bacterial communities over the treatment season. When the pond temperatures peaked mid-season, the pond bacterial community became more abundant and diverse. This coincided with improved removal of fecal indicator and pathogenic bacteria as well as nutrients (**Table 1**; Huang et al., 2018), suggesting that disinfection and nutrient removal are correlated to bacterial richness and diversity in WSPs. The correlation between temperature, nutrient removal and bacterial richness and alpha-diversity was similarly observed in seasonal studies of sewage batch reactors (Ebrahimi et al., 2010) and WWTPs operated in colder climates (Rodriguez-Caballero et al., 2012; Ju et al., 2014; Kang et al., 2018). The multivariate dbRDA supported that largely anaerobic biological treatment of the wastewater was closely related to bacterial community shifts and temperature in the WSPs (**Figures 5B, 6B**). This potentially makes the treatment success unpredictable due to annual differences in the ambient summer temperatures. However, when comparing a cold (2013) vs. a warm (2014) year, treatment outcomes in terms of effluent $CBOD_5$, TSS, and TA were still comparable (**Table 1**), indicating some robustness of the biological processes as well as concurrent impact of the physical processes.

Decomposing microorganisms with a wide range of metabolisms are important for removal of $CBOD_5$ and other nutrients in WSP systems (Wagner et al., 2002; Ferrera and Sánchez, 2016; Ho and Goethals, 2020). Bacterial community changes along the treatment process (raw to pond, and season) led to reductions in alpha diversity and increases in *Comamonadaceae*, *Geobacteraceae*, and *Porphyromonadaceae*. Similar impacts of the treatment process were observed in the study of several full-scale municipal WWTPs (Hu et al., 2012; Gao et al., 2016; Numberger et al., 2019; Xue et al., 2019). The members of the bacterial communities were not evenly composed in the arctic WSP systems as indicated by low Simpson evenness measures (0.02–0.06) in all samples. However, the unevenness of bacterial community composition in arctic WSPs was not unexpected as *Proteobacteria* was by far the most abundant bacterial phylum with a relative abundance of 70–90% in all samples. The same dominance of *Proteobacteria* was also reported by Gromala et al. (2021) for three arctic WSPs located in Western Nunavut and in seven Finnish WWTPs operated above the Arctic Circle (Gonzalez-Martinez et al., 2018). Members of this bacterial phylum are known to be responsible for removal of the organic waste in municipal wastewater (Wagner et al., 2002). *Comamonadaceae* (member of the *Burkholderiales* order in *Betaproteobacteria*) was the most dominant group in pond and treated effluent samples from Pond Inlet and was in the dbRDA related to removal of nutrients ($CBOD_5$). This family of bacteria has previously been reported to be involved in the biological nutrient removal processes, especially carbon and nitrogen containing compounds when the oxygen supply was limited (Sato et al., 2016; Usharani, 2019) as was the case in the present study. At the genus-level, *Rhodoferax* spp. (*Comamonadaceae*) became the dominant group in the pond and effluent samples (**Supplementary Figure 4**). This genus was recently reported

to be present in the WSP in Baker Lake (Gromala et al., 2021), indicating its presence in the arctic sewage system, and is known to decompose organic material (Zhang et al., 2012). The *Geobacter* genus [*Geobacteraceae, Deltaproteobacteria* or the proposed *Desulfobacterota* (Waite et al., 2020)], which started as a minor group in the raw wastewater and rose in abundance in the WSP samples, is associated with cold anaerobic soil and aquatic environments and an ability to reduce iron and sulfate, and can complete oxidize organic compounds (Lovley et al., 1987), and was observed in the anaerobic tank of industrial wastewater treatment systems operating at low temperatures (8–16°C) in China (Zhang et al., 2017). *Porphyromonadaceae* and other members of the anaerobic *Bacteroidetes* are known to degrade complex organic substances in WWTPs (Gao et al., 2016; Zhang et al., 2017).

In 2014 samples from the Pond Inlet WSP, the bacterial diversity depended on the sampling depth, which may be due to the solids settling processes, temperature shifts between the top and bottom layer of the pond, DO gradients, and other unmonitored wastewater and operational factors. However, this depth-related difference was not observed in samples from 2013 or from Clyde River, which may indicate variable mixing of the wastewater in the ponds depending on the year and WSP design. Sludge is known to accumulate in arctic WSPs (Miyamoto and Heinke, 1979). An examination of the sludge in Pond Inlet during the 2014 treatment season revealed increases in sludge community size and diversity (**Figure 4** and **Supplementary Table 1**), indicating the role of the sludge community in treatment of the wastewater in arctic WSPs should be the subject of further investigations.

The predicted functional gene content of wastewater bacterial communities pointed to higher levels of carbohydrate and energy metabolism in the middle of the treatment season in Pond Inlet WSP, concurring with the $CBOD_5$ removal observed between the beginning and middle of the season in 2014 (**Table 1**). In the two-cell WSP system in Clyde River, the predicted higher carbohydrate and energy metabolism of the secondary pond bacterial communities similarly coincided with the further reduction of $CBOD_5$ levels following initial treatment in the primary pond (**Table 1**). Gromala et al. (2021) also reported presence of KEGG pathways associated with carbohydrate and energy metabolisms in the Western Nunavut WSPs. Taken together, it seems likely that optimal $CBOD_5$ removal can be related to higher pond temperatures, larger bacterial populations, greater bacterial diversity, and enhanced carbohydrate and energy metabolic activity levels in mid-season WSPs.

The low proportion of genes associated with ammonium oxidizing pathways matched the low relative abundance of ammonia oxidizing bacteria in both WSPs. This finding was supported by the water quality data (**Table 1**), which showed a modest ammonia (TA) removal in both WSPs during the study period. Interestingly, Gromala et al. (2021) reported absence of ammonia oxidation pathways in WSPs in Baker Lake, and Cambridge Bay, while the genes in the pathway were detected in Kugluktuk. These findings overall suggest that the current design of arctic WSPs does not promote growth of microbial communities, which substantially contribute to the removal of ammonia.

CONCLUSIONS

The study explored the composition and putative function of bacterial communities in arctic WSPs treating municipal wastewater during two consecutive summer treatment seasons. The WSPs received raw wastewater which had a high nutrient content and similar bacterial communities despite the geographical separation. Although designed to be facultative, the ponds stayed anaerobic with neutral pH-values, and temperature in the WSPs became a major determinant of the size, composition and diversity of the bacterial communities. The multivariate dbRDA showed correlations between temperature, nutrient removal and increased levels of *Comamonadaceae, Geobacteraceae,* and *Porphyromonadaceae.* The predicted gene functions (KEGG pathway study) supported that increased microbial removal of nutrients occurred mid-season and in the secondary pond of the two-cell Clyde River WSP system.

While it is challenging to control environmental factors in a passive wastewater treatment system there are some design considerations that could be used to optimize temperature regimes, such as the depth of the pond. Also, use of ponds in sequence would improve treatment. Information from this study would also be useful for further development of models that predict biological treatment, which could be used to size and assess the feasibility of WSPs in extreme climates.

RESEARCH PERMITS

The permit for this work is registered with the Nunavut Research Institute under the following title:

"Northern Municipal Wastewater Effluent Discharge Quality Objectives in the Context of Canadian Council of Ministers of the Environment Strategy and Environment Canada's Wastewater Systems Effluent."

DATA AVAILABILITY STATEMENT

The sequencing dataset presented in this study has been deposited at NCBI as a Bioproject PRJNA630784. The remaining data can be found in the **Supplemental Material**.

AUTHOR CONTRIBUTIONS

YH: conception, execution of field work and experiments, data analyses, bioinformatics analysis, and writing of manuscript. CR: execution of field work, analysis of wastewater quality, and review of manuscript. LJ: statistical analyses, graphing, and review of manuscript. MH: bioinformatics analysis and review of manuscript. RB: conception, supervision, bioinformatics analysis, and review of manuscript. RJ: funding acquisition, conception, supervision, and writing and review of manuscript. LT: funding acquisition, conception, supervision, and writing and review of manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

ACKNOWLEDGMENTS

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/frwa. 2021.710853/full#supplementary-material

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