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Metagenomic survey reveals hydrocarbon biodegradation potential of Canadian high Arctic beaches

Esteban Góngora[1*], Antoine-O. Lirette[1], Nastasia J. Freyria[1], Charles W. Greer[1,2] and Lyle G. Whyte[1]

Abstract

Background Decreasing sea ice coverage across the Arctic Ocean due to climate change is expected to increase shipping activity through previously inaccessible shipping routes, including the Northwest Passage (NWP). Changing weather conditions typically encountered in the Arctic will still pose a risk for ships which could lead to an accident and the uncontrolled release of hydrocarbons onto NWP shorelines. We performed a metagenomic survey to characterize the microbial communities of various NWP shorelines and to determine whether there is a metabolic potential for hydrocarbon degradation in these microbiomes.

Results We observed taxonomic and functional gene evidence supporting the potential of NWP beach microbes to degrade various types of hydrocarbons. The metagenomic and metagenome-assembled genome (MAG) taxonomy showed that known hydrocarbon-degrading taxa are present in these beaches. Additionally, we detected the presence of biomarker genes of aerobic and anaerobic degradation pathways of alkane and aromatic hydrocarbons along with complete degradation pathways for aerobic alkane degradation. Alkane degradation genes were present in all samples and were also more abundant (33.8 ± 34.5 hits per million genes, HPM) than their aromatic hydrocarbon counterparts (11.7 ± 12.3 HPM). Due to the ubiquity of MAGs from the genus *Rhodococcus* (23.8% of the MAGs), we compared our MAGs with *Rhodococcus* genomes from NWP isolates obtained using hydrocarbons as the carbon source to corroborate our results and to develop a pangenome of Arctic *Rhodococcus*. Our analysis revealed that the biodegradation of alkanes is part of the core pangenome of this genus. We also detected nitrogen and sulfur pathways as additional energy sources and electron donors as well as carbon pathways providing alternative carbon sources. These pathways occur in the absence of hydrocarbons allowing microbes to survive in these nutrient-poor beaches.

Conclusions Our metagenomic analyses detected the genetic potential for hydrocarbon biodegradation in these NWP shoreline microbiomes. Alkane metabolism was the most prevalent type of hydrocarbon degradation observed in these tidal beach ecosystems. Our results indicate that bioremediation could be used as a cleanup strategy, but the addition of adequate amounts of N and P fertilizers, should be considered to help bacteria overcome the oligotrophic nature of NWP shorelines.

Keywords Hydrocarbon bioremediation, Baseline survey, Northwest Passage, Arctic marine shoreline

*Correspondence:
Esteban Góngora
esteban.gongora@mail.mcgill.ca
Full list of author information is available at the end of the article

Background

The continued reduction in sea ice area in the Canadian Arctic Ocean [1] is predicted to allow for open waters between July and October for most regions by the end of the century [2]. One of these regions is the Northwest Passage (NWP) which connects the Atlantic and Pacific Oceans through the Canadian high Arctic (Fig. 1). The predicted increase in shipping traffic through the NWP in the coming decades [3] will present environmental risks for the indigenous human populations and the marine and terrestrial environments of the area [4, 5]. This also increases the possibility of an accident leading to a hydrocarbon spill due to the movement of drifting sea ice from the northernmost part of the Canadian Arctic Archipelago and Greenland into the NWP [6] or a rise in storm frequencies [7]. Released hydrocarbons can also be entrapped in and under drifting sea ice which can then be transported away from the spill location [8, 9]. These factors increase the likelihood of marine hydrocarbon spills reaching NWP shorelines. Spill response in the Arctic by governments, industry, and other stakeholders will most likely be limited and slow due to the lack of equipment and resources, high costs, and poor accessibility in highly remote regions [8–10]. For these reasons, simpler remediation options should be considered due to their feasibility and lower cost.

One such remediation option is microbially-mediated hydrocarbon degradation, also known as bioremediation [11, 12]. Indeed, it has been observed that microorganisms capable of biodegrading hydrocarbons are ubiquitous in marine Arctic environments [13]. Previous experimental spills such as the Baffin Island Oil Spill (BIOS) project (1980–1984) [14, 15], the In situ Treatment of Oiled Sediment Shorelines (ITOSS) program in Svalbard (1996–1998) [16–19], as well as real-world cleanup efforts following the *Exxon Valdez* oil spill (1989) [20, 21] have shown that bioremediation can be used to clean hydrocarbons released on Arctic and subarctic marine beach ecosystems.

Despite their efficacy, bioremediation treatments will only be effective in the Arctic if the native microbiota already contains microorganisms capable of hydrocarbon degradation under the extreme environmental conditions encountered in polar environments. For example, at the *Bahía Paraiso* spill in the Antarctic Archipelago (1989), biodegradation was a negligible as determined by the low mineralization rates in hydrocarbon radiorespiration experiments [22, 23]. The cold and sub-zero temperatures present during most of the year, no sunlight during the

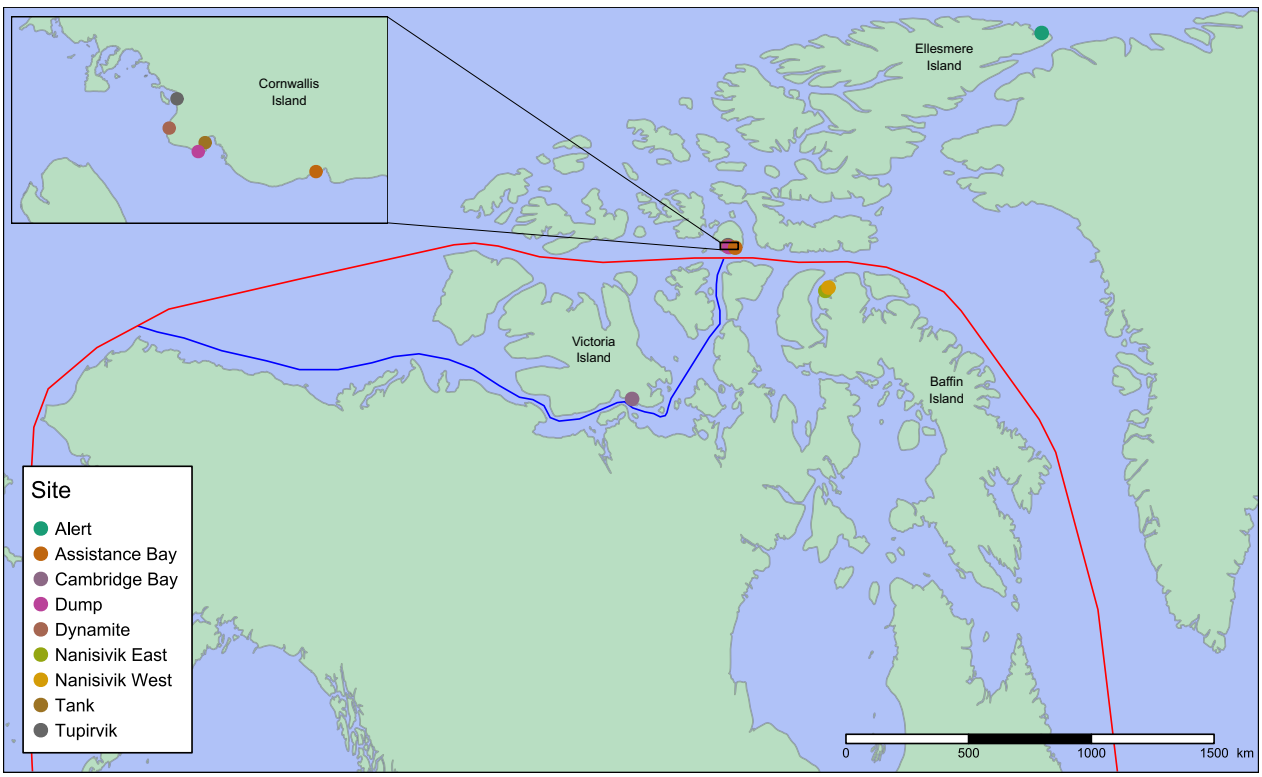

Fig. 1 Map of the Canadian high Arctic with the locations of the study sites. Lines show approximate current (blue) and future (red) routes that could be used by the shipping industry to transit the NWP

winter months, and the highly oligotrophic nature of the Arctic marine environment can slow down the biodegradative activity of the microbial communities [10, 24–26]. For example, on some experimental plots from the BIOS project, oil remains in beach sediments almost 40 years later [27, 28]. Hydrocarbon biodegradation can also be affected by increased salinity on the upper intertidal and supratidal zones of a shoreline due to the salinity tolerance of different types of microorganisms [29, 30]. Likewise, sediment heterogeneity makes it complicated to compare results between beaches [26]. Accordingly, stakeholders and response teams should have a baseline awareness of which Arctic regions have microbial communities with hydrocarbon degradation potential and which do not.

Previous studies have used conventional microbiological methods such as plate counts to detect changes in oil-degrading microorganism abundances or CO_2 production indicating respiration [15, 31, 32]. Others used indirect methods such as changes in the oil composition relative to specific hydrocarbon chemical markers such as hopane, pristane, or phytane, among others [19, 33, 34]. More recent studies have taken advantage of advancements in molecular microbiology to help detect the presence and explain the activity of hydrocarbon-degrading microorganisms. For example, 16S rRNA gene, metagenomic, metatranscriptomic, and single-cell sequencing were all used by multiple research groups during the Deepwater Horizon oil spill to help describe the changes in the microbial communities in the water column, sediment, and shoreline and show how microbiomes responded to the spill at the functional level [35–37]. These techniques have also been applied to understand the microbial ecology of hydrocarbon biodegradation in Arctic soils [38–41], Arctic seawater and sea ice [42–46], Arctic beach sediments [47–49], and Arctic deep-sea sediments [50–52].

While these studies have increased our understanding of the ecology of hydrocarbon spills in Arctic terrestrial and marine environments, there is a scarcity of research on the interface connecting both areas, the shoreline. In this study, we performed a metagenomic survey of the microbial communities of 8 high Arctic beaches located along the NWP and an additional high Arctic beach that was impacted by a diesel spill. We aimed to understand the hydrocarbon biodegradation potential of NWP beach sediments from both natural and human-impacted shorelines. We also described the overall community composition to provide an overview of scenarios where bioremediation could be used as one of the main cleanup strategies in the case of a spill in one of these types of beaches due to the delayed and limited response expected under Arctic conditions, as mentioned above [8]. We also compared the results obtained from our metagenomes with microcosm experiments and genome sequences of isolates from some of the same sites of this study grown on hydrocarbons as the sole source of carbon [48, 49, 53]. The corroboration of our metagenomic survey results with the proven metabolic capacity of these isolates serves as evidence that metagenome sequencing can be used as an initial surveying tool to determine the feasibility of bioremediation as a cleanup strategy. Finally, we described other metabolic processes detected in these metagenomes to provide a better understanding of the microbial ecology of these shorelines, which can further help to determine if there are certain environmental conditions that could be limiting the hydrocarbon degradation potential of these microorganisms. Our results serve as a baseline description of the microbial communities of these sites that have not experienced any documented spills which could be used to focus contingency plans to the most vulnerable shorelines as well as to determine remediation endpoints [54].

Methods

Sampling sites: description and sample collection

The 9 sites used in this study were spread across four regions in the Canadian high Arctic: Cambridge Bay, Resolute, Nanisivik, and Alert (Fig. 1; Table S1). These sites were selected to represent both natural and human-impacted beaches around the NWP. The hamlet of Cambridge Bay on Victoria Island is one of the most frequented stopover sites for vessels around the current NWP route (blue line on Fig. 1); beach sediment was sampled by the docks (sample referred to hereafter as "Cambridge Bay"). The hamlet of Resolute on Cornwallis Island is expected to be a central stopover hub in the future once the ice on the most optimal route of the NWP has disappeared (red line on Fig. 1). For this reason, we sampled 5 beaches around Resolute. (1) "Dump beach", near where the waste from the hamlet is deposited and later incinerated; (2) "Dynamite beach", in close proximity to an abandoned dynamite storage site in the relatively pristine Allen Bay; (3) "Tank farm", adjacent to the fuel storage tanks that supply the Resolute community year-round; (4) Tupirvik, a territorial park on Allen Bay where local hunters often launch their boats; (5) Assistance Bay, an uninhabited beach approximately 17 km away from Resolute, was selected to represent a pristine location facing the NWP that is unlikely to be experiencing any kind of hydrocarbon contamination from anthropogenic sources. We additionally sampled two beaches on Baffin Island near the docks of the former company mining town of Nanisivik. The town is being converted into a refueling station for the Canadian Navy and government ships following an extensive decontamination project to

remove metal and fuel contaminants left behind by the mining operations. These beaches are located east and west of the docks. Finally, while not directly on the NWP, we sampled a beach at the Canadian Forces Station—Alert on Ellesmere Island, which is adjacent to areas that experienced diesel spills in 2006 and 2007 [38, 40]. A trench and pond were constructed shortly after a pipeline break to prevent the fuel from reaching the shoreline, but it has not been determined if any fuel was able to go past these barriers.

Between July and August 2018 (Table S1), beach sediment samples from the upper 5 cm of the intertidal zone of all beaches were collected aseptically into sterile Whirl–Pak bags and stored at −20 °C until processed. To evaluate the stability of the microbial communities with time, the sites around Resolute were also sampled in July 2019 (Table S1). For Assistance Bay, beach sediment samples were collected only in July 2019 from the upper 5 cm of both the intertidal and supratidal zones of the beach to evaluate how sediment heterogeneity within the same beach affects the microbial community of these two zones.

Physicochemical and hydrocarbon analyses

Salinity and dissolved oxygen were measured in situ on the pore water of the beach sediments using a YSI probe (Xylem) for the 2019 samples from the Resolute region. Nitrate, nitrite, ammonia, and phosphate were measured using the same pore water with CHEMetrics test kits (K-6933, K-7003, K-1503, K-8503, respectively) using a CHEMetrics V-2000 photometer. A sub-sample of the collected sediments from 2018 and from the 2019 Assistance Bay intertidal sediment were analyzed by SGS Canada Inc. to quantify petroleum hydrocarbons (PHCs), semi-volatile organic compounds (SVOCs) and volatile organic compounds (VOCs). PHCs were quantified using the Canadian Council of Ministers of the Environment Reference Method for the Canada-wide Standard for Petroleum Hydrocarbons in Soil—Tier 1. SVOCs were quantified using the USEPA methods 3541 and 8270D. VOCs were quantified using the USEPA methods 5035A, 5030B, 8260C.

DNA extraction, library preparation, and sequencing

We extracted internal DNA (iDNA) from intact viable cells and from extracellular/environmental DNA (eDNA) separately from the collected beach sediments. We used this approach to account for the fact that shorelines are an active environment receiving microbial inputs from both land and sea which leads to the possibility that we could detect eDNA from different sources that is not an active part of the shoreline microbiome [55–57]. For this we followed the methods described elsewhere [58, 59]. Briefly, the beach sediment was suspended in a sodium phosphate buffer and cells were separated from the eDNA by shaking and centrifuging the solution so that cells and the remaining sediment particles were collected in a pellet and eDNA was obtained from the supernatant. The pellet was resuspended in sodium phosphate buffer and lysed using a PowerBead tube (Qiagen). iDNA and eDNA were recovered from their respective supernatants using silica beads in a guanidine hydrochloride solution. Libraries were prepared using the Nextera XT DNA Library Prep Kit (Illumina) and indexed using the Nextera XT Index Kit v2 (Illumina) following the manufacturer's instructions. The indexed libraries were sequenced by Genome Québec in an Illumina HiSeq 4000 platform using a PE100 flow cell.

Bioinformatics and statistical analyses

Reads were first trimmed with Trimmomatic v0.11.5 [60] to remove low quality bases and sequencing adapters. iDNA and eDNA metagenomes were separately assembled with metaSPAdes v3.14.1 [61]. Reads were mapped to the assembled metagenomes with BBMap v38.87 [62]. Metagenomes were annotated and classified using MetaErg v1.2.0 [63]. We first tested for differences between the viable and potentially active (iDNA) and the inactive transient (eDNA) communities of the beaches based on the 16S rRNA gene sequences identified by MetaErg after removing reads classified as chloroplast or mitochondria (Fig. S1). The Shannon index was calculated for each metagenome using the phyloseq package v1.40.0 [64] and, after assessing the normality of the dataset with a Shapiro–Wilk test, a paired *t*-test was used to test differences in Shannon indexes between the two types of metagenomes (Fig. S2). 16S rRNA gene counts were transformed using relative abundances to account for read depth, then Bray–Curtis dissimilarities were calculated with phyloseq and visualized using non-metric multidimensional scaling (NMDS). PERMANOVA and PERMDISP were calculated with the vegan package v2.6–4 [65] to test for differences in community composition between iDNA and eDNA (Fig. S3). Alpha level for all tests was 0.05 and were performed in R v4.2.2.

Since we observed no statistical differences in Shannon index or Bray–Curtis dissimilarities between the iDNA and eDNA metagenomes (Figs. S2 and S3), we merged the iDNA and eDNA metagenomes of each sample into a total DNA metagenome. The total DNA metagenomes contained 40,735,858 ± 14,414,163 paired reads per sample. After combining the two datasets for each sample, reads were co-assembled using metaSPAdes. Reads were mapped to the co-assembled metagenomes with BBMap, the metagenomes were annotated and classified with MetaErg. We used the 16S rRNA gene sequences

annotated from the co-assembled dataset by MetaErg to determine differences in alpha diversity and community composition among the beaches. We tested differences in Shannon index using a paired Wilcoxon signed ranked test for differences between years and ANOVA to test differences among regions. Differences in community composition among regions and between years were analyzed with PERMANOVA and PERMDISP based on Bray–Curtis dissimilarities. Given that we obtained a significant result for the region PERMANOVA, we then tested for pairwise differences in community composition among regions using a pairwise PERMANOVA [66]. To further corroborate the community composition stability between years, we compared the Bray–Curtis dissimilarities for all pairs of sites collected in 2018 and 2019 (e.g., Tank farm—2018 vs. Tupirvik—2018, etc. and Dynamite beach—2019 vs. Dump beach—2019, etc.) and with the Bray–Curtis dissimilarities between years for all samples (e.g., Tupirvik—2018 vs. Tupirvik—2019, etc.) using a Kruskal–Wallis rank sum test. As we observed the presence of aromatic and anaerobic degradation genes only for a few samples (see Results below), we tested for differences in the overall community composition of these samples with a PERMANOVA against those which did not contain these types of genes.

Genome binning was performed with MetaBAT2 v2.12.1 [67] after which the quality of the produced metagenome-assembled genomes (MAGs) was improved with RefineM [68]. Overall bin statistics were estimated with CheckM [68] and MAG completeness and contamination was determined with CheckM2 [69]. MAGs were classified with GTDB-Tk v2.1.0 [70] using the Genome Taxonomy Database (GTDB) r207 and individually annotated with MetaErg. A phylogenomic tree of the final MAG collection was created by first obtaining the aligned and concatenated amino acid sequences of single-copy core genes of the anvi'o (v7.1) Bacteria_71 collection [71]. The phylogenomic tree was then inferred by maximum likelihood using FastTree [72] within anvi'o and the resulting tree was manually midpoint rooted.

To further understand the microbial ecology in the absence of hydrocarbons, we manually explored the KEGG annotations obtained from MetaErg to detect the presence of nitrogen, sulfur, and carbon metabolisms in the MAGs (Table S2). We used CANT-HYD (–cut_nc) [73] to identify the presence of 37 marker genes involved in aerobic and anaerobic degradation pathways of a wide variety of aliphatic and aromatic hydrocarbons. To complement the CANT-HYD results, if we obtained a CANT-HYD hit for a given hydrocarbon degradation gene, we looked at the KEGG annotation to determine if the rest of the genes in the respective degradation pathway were present in selected high-quality MAGs. The MAG1 and MAG12 cell diagram used to exemplify the genomic potential of the NWP beach microbiomes was created with Biorender.com.

Because we observed a high abundance of MAGs assigned to *Rhodococcus*, we created a pangenome of Arctic *Rhodococcus* to determine the ubiquity of hydrocarbon degradation genes in genomes from this genus. The pangenome was created with anvi'o using *Rhodococcus* MAGs obtained in this study along with *Rhodococcus* genomes from NWP beach isolates capable of fuel oil degradation [53]. The genome sequences of these *Rhodococcus* isolates are available on NCBI under the BioProject accession number PRJNA945214.

Results

Taxonomic composition of metagenomes and MAGs

Based on average relative abundances of the 16S rRNA gene sequences extracted from the metagenomes of all samples, the microbial communities of the studied NWP beaches are dominated by *Pseudomonadota* (43.4% ± 14.45), *Actinomycetota* (36.3% ± 27.9), and *Bacteroidota* (13.4% ± 8.1); fewer than 1% of 16S rRNA gene reads were classified as *Archaea* (Fig. 2). Similar patterns were observed for the taxonomic classification of the metagenomic reads based on the MetaErg annotation (Fig. S4). The 20 most abundant genera all belonged to the same three dominant phyla (Fig. S5, Table S3) with *Rhodococcus* having the highest average relative abundance among samples (24.7% ± 20.3).

We recovered 82 bins which recruited 48.9% of the total metagenomic reads. From these, we obtained 19 high-quality (> 90% completeness and < 5% contamination) and 23 medium-quality (> 50% completeness and < 10% contamination) MAGs (Table S4). The MAGs were classified into 5 different phyla (Fig. 3A), with most of them belonging to *Pseudomonadota* (59.5%, 25 MAGs) and *Actinomycetota* (23.8%, 10 MAGs). We assessed genome novelty of these MAGs based on the taxonomic classification rank assigned with the GTDB (Fig. 3B; Table S4). Most of the MAGs (71.4%, 30 MAGs) were unclassified at the species level and 4.8% (2 MAGs) further showed novelty at higher taxonomic ranks: 1 at the genus level (MAG26; family *Porticoccaceae*) and 1 at the family level (MAG38; order *Woeseiales*).

Beach communities are stable over time and among NWP regions

We observed no statistical differences in the Shannon index (Wilcoxon signed rank test: $V = 1$, $p = 0.25$; Fig. S6) or Bray–Curtis dissimilarities (PERMANOVA: pseudo-$F = 0.616$, $R^2 = 0.093$, $p = 0.857$; Kruskal–Wallis: $H = 1.550$, $p = 0.461$; Fig. S7) for the beaches in the Resolute region sampled in 2018 and 2019. For differences

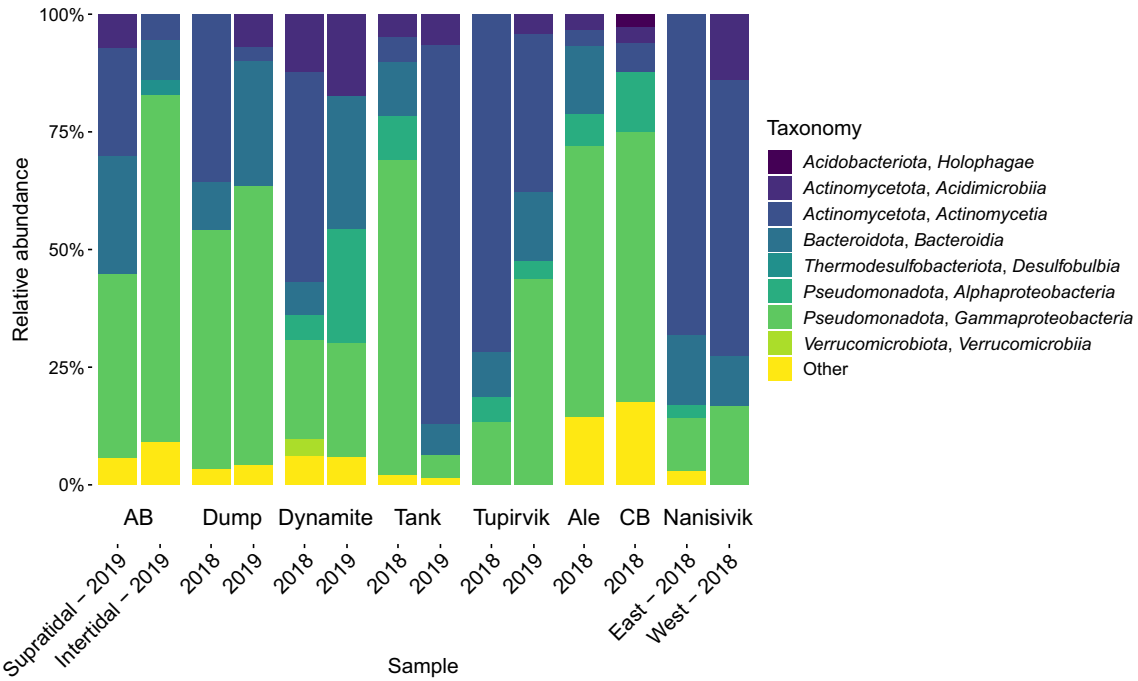

Fig. 2 Taxonomy of the microbial communities of the studied NWP beaches. The taxonomic ranks included were Phylum and Class, respectively. Relative abundances were calculated using metagenome-extracted 16S rRNA gene sequences. Taxa with a relative abundance lower than 2.5% were pooled into "Other". AB, Assistance Bay; Ale, Alert; CB, Cambridge Bay

among the studied regions (Resolute, Alert, Nanisivik, and Cambridge Bay), we detected no statistical differences in the Shannon index (ANOVA: $F=2.529$, $p=0.116$; Fig. S8). PERMANOVA did detect statistically significant differences in community composition among regions (PERMANOVA: pseudo-$F=1.496$, $R^2=0.310$, $P=0.012$), but after pairwise comparisons were performed with a pairwise PERMANOVA, the statistical differences were no longer observed (Table S5; Fig. S9). We observed a higher proportion of *Gammaproteobacteria* in the intertidal zone of the Assistance Bay sediment compared to the supratidal zone sediment of the same beach. On the other hand, there was a higher proportion of *Actinomycetia* and *Bacteroidia* 16S rRNA gene reads in the supratidal zone compared to the intertidal zone (Fig. 2).

NWP beach microbiomes contain genes from multiple pathways associated with hydrocarbon degradation

We were able to detect 15 out of the 37 hydrocarbon degradation marker genes analyzed by CANT-HYD in our metagenomes (Fig. 4) with the most prevalent genes being associated with the aerobic degradation of alkanes. Genes associated with aerobic alkane degradation were highly prevalent with *alkB* (associated with the degradation of C_5–C_{22} hydrocarbons [74, 75]), *cyp153* (associated with the degradation of C_5–C_{10} hydrocarbons [75]), and *ladAα* (associated with the degradation of C_{15}–C_{36} hydrocarbons [76]) being present in all 9 beaches. Genes coding for the large (*prmA*) and small (*prmC*) subunits of the propane monooxygenase were also present in 7 beaches (77.8%). Genes associated with the degradation of mono- (MAH alpha/beta and *tmoA/E*) and polycyclic (*nboB/C* and non-*ndoB*) aromatic hydrocarbons were much less abundant with 62.5% of the sampled beaches (5 beaches) containing at least one gene from these pathways. Anaerobic hydrocarbon degradation genes were much less prevalent as they were only found in the Alert beach sediment. We also detected hydrocarbon degradation genes in 23 (54.7%) of our MAGs and 12 (30%) of our low-quality bins (Fig. 5). Most of these contained one or more aerobic alkane degradation genes with *cyp153* and *alkB* being the most abundant and present in 22 (52.3%) and 8 (20%) of the MAGs and bins, respectively. Aerobic aromatic degradation genes were detected in 6 (7.3%) of the MAGs and bins, whereas genes involved in the anaerobic degradation pathway of alkanes (*ahyA*) and ethylbenzene (*ebdA*) were only present in MAG12. We observed statistical differences in the community composition of samples where we detected aromatic and anaerobic degradation genes (Dynamite beach—2019, Tank farm—2018, Alert—2018, Cambridge Bay—2018, Nanisivik East—2018) compared

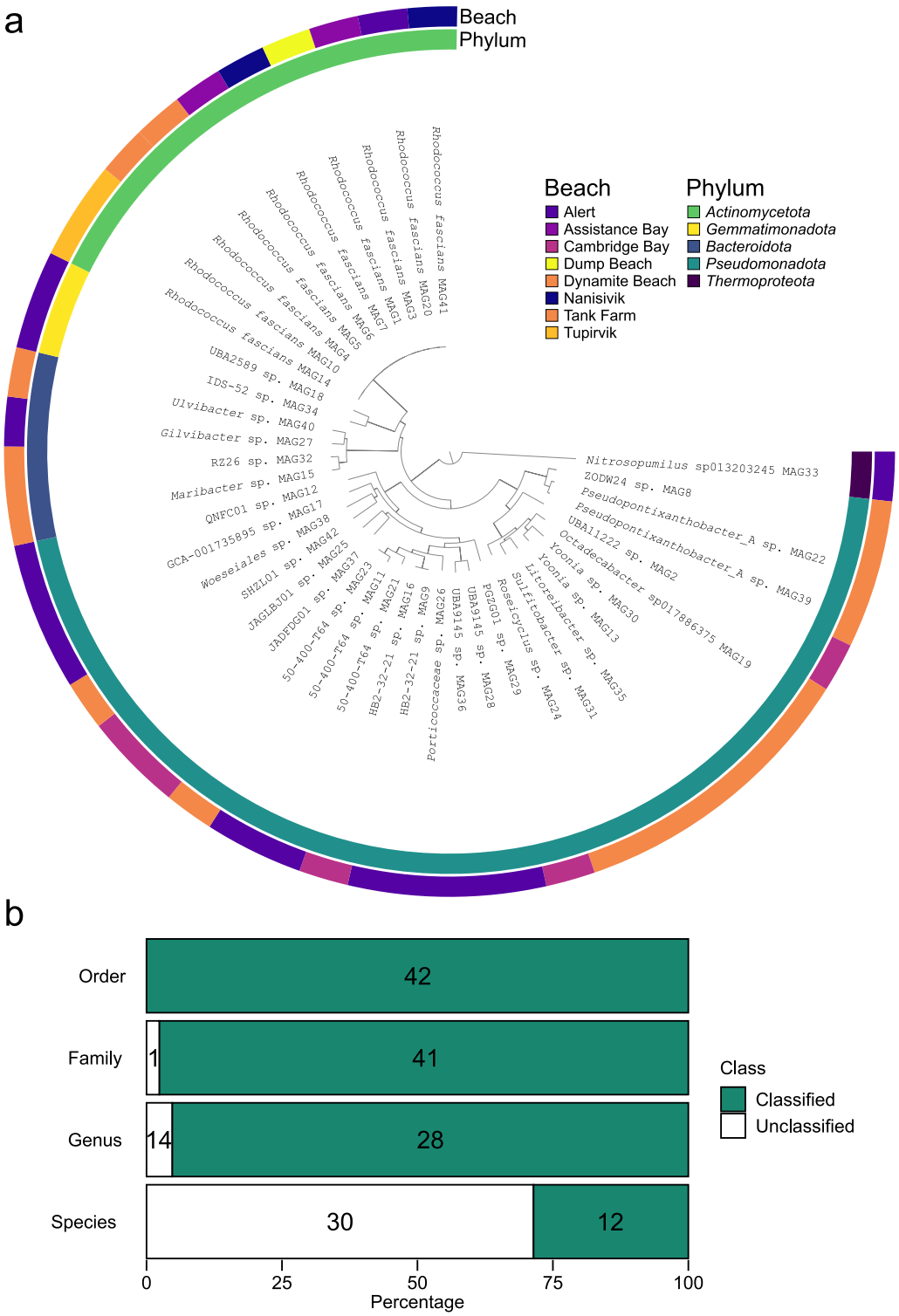

Fig. 3 Taxonomy and novelty of NWP metagenome-assembled genomes (MAGs). **A** Maximum-likelihood phylogenomic tree of the medium- and high-quality MAGs obtained from the sampled Arctic beaches. Samples are grouped by beach and phylum. **B** Percentage of MAG taxonomic novelty at various ranks based on their GTDB-Tk classification. Numbers on the boxes represent the number of MAGs classified/unclassified for a given rank

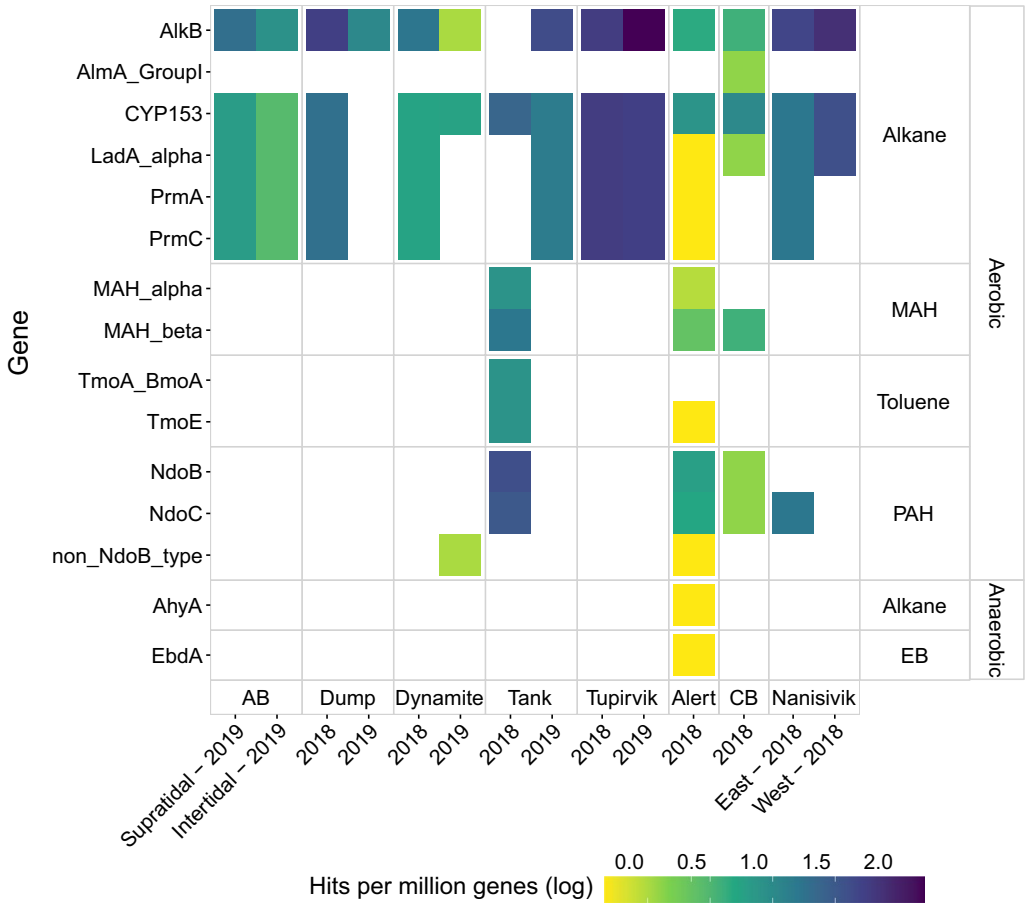

Fig. 4 Abundances of CANT-HYD marker hydrocarbon degradation genes present in the NWP metagenomes. CANT-HYD gene counts were normalized to hits per million coding genes per sample and then transformed using the natural logarithm. AB, Assistance Bay; CB, Cambridge Bay; MAH, monocyclic aromatic hydrocarbon; PAH, polycyclic aromatic hydrocarbon; EB, ethylbenzene

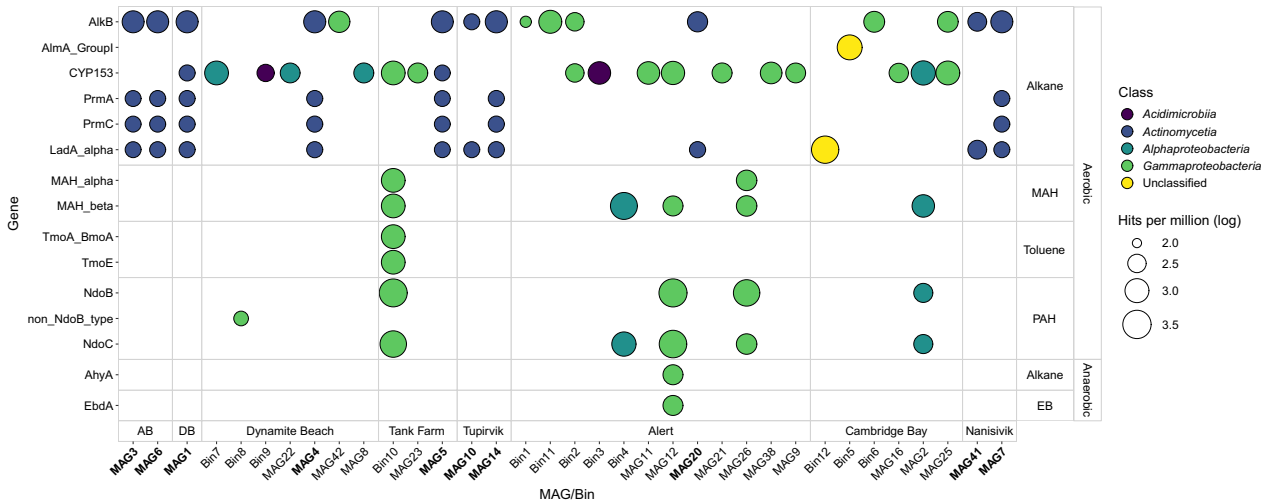

Fig. 5 Abundances of CANT-HYD marker hydrocarbon degradation genes in the recovered MAGs and selected bins. CANT-HYD gene counts were normalized to hits per million coding genes per sample and then transformed using the natural logarithm. AB: Assistance Bay; DB: Dump beach; MAH: monocyclic aromatic hydrocarbon; PAH: polycyclic aromatic hydrocarbon; EB: ethylbenzene. MAGs with their name highlighted in bold were classified as *Rhodococcus fascians*. The detailed taxonomy of each MAG/bin is listed in Table S4

to the samples that did not contain these genes (PERMANOVA: pseudo-F = 1.8141, R2 = 0.131, $P = 0.043$).

High-quality MAGs reveal the functional potential of NWP beaches

Taxonomic classification showed that the genus *Rhodococcus* were highly prevalent in most beaches with an average relative abundance of 26.1% ± 22.4 of 16S rRNA gene reads, 30.3% ± 27.8 overall metagenome reads, and 10 (23.8%) of the MAGs were classified as *Rhodococcus fascians* with 5 of them having 100% completion and less than 0.5% contamination. Additionally, we also observed the presence of 13 (30.9%) MAGs that belonged to various taxa within the phylum *Pseudomonadota* with 6 (14.3%) having over 90% completion and less than 1% contamination. Therefore, we studied these MAGs in more detail to look for the presence of a larger variety of the genes comprising these degradative pathways. One of the limitations of the CANT-HYD pipeline is that it detects only one or a few key marker genes per pathway [73], usually associated with the first enzyme involved in the pathway, but it cannot determine whether the full biodegradative pathway is present. By complementing the CANT-HYD results with the KEGG annotations obtained from MetaErg, we were able to reconstruct hydrocarbon degradation pathways with a higher resolution for MAG1, classified as *R. fascians* and representing the highly prevalent *Rhodococcus* clade, and MAG12, one of the *Pseudomonadota* MAGs classified to the putatively novel genus QNFC01 of the family *Immundisolibacteraceae* (Fig. 6). Through this method, we observed that MAG1 and MAG12 encoded a complete pathway for alkane degradation. The CANT-HYD results showed that MAG1 contains three copies of *alkB* and one copy each of *cyp153*, *ladAα*, *prmA*, and *prmC*, while MAG12 contains two copies of *cyp153*. Alkanes are converted to fatty acids followed by β-oxidation (ko00071) and the resulting acetyl-CoA molecules are further metabolized via the TCA cycle (ko00020). MAG12 also contained a copy of the putative anaerobic alkane hydroxylase *ahyA* for which there is still not a concrete metabolic pathway defined after the hydroxylation step [77].

The CANT-HYD results showed no MAH or PAH genes in MAG1, but we did detect one copy each of the MAH beta subunit and *ebdA*, 7 copies of *ndoB*, 6 copies of *ndoC* in MAG12. While we did not obtain complete MAH and PAH degradation pathways, we detected a larger number of genes from the KEGG annotations

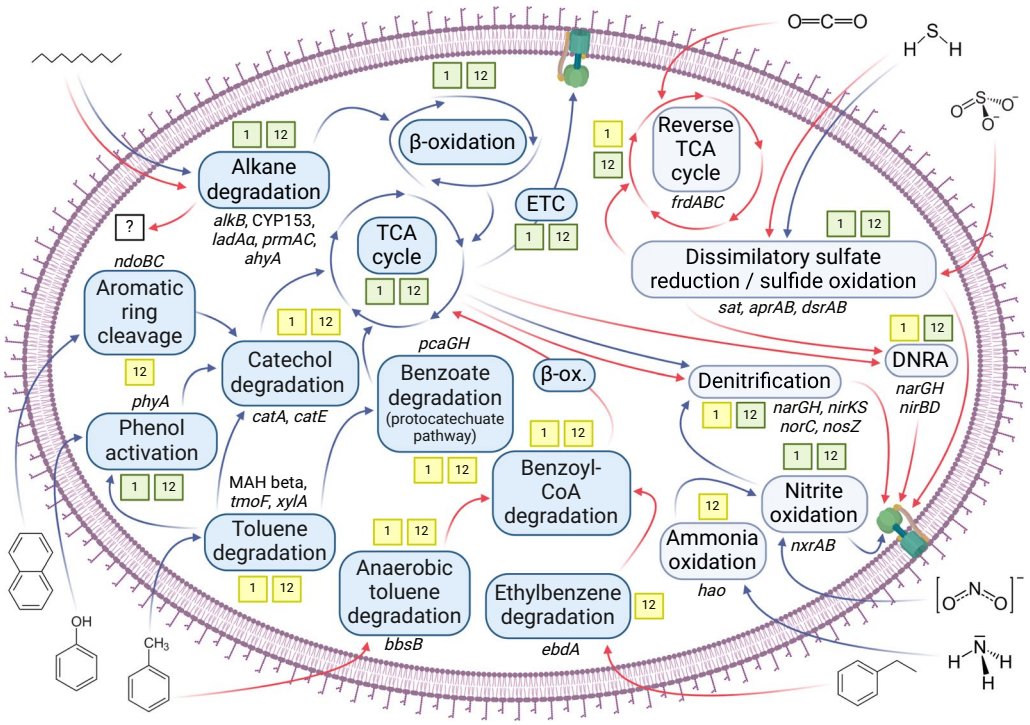

Fig. 6 Cell schematic illustrating the diverse metabolisms of MAG1 and MAG12. MAG1 was classified as *Rhodococcus fascians* and was recovered from the 2018 Dump beach metagenome. MAG12 was classified as inside the putatively novel genus QNFC01 in the family *Immundisolibacteraceae* and was recovered from the 2018 Alert metagenome. Genes next to their pathways were detected using CANT-HYD and KEGG annotations. Green and yellow boxes represent complete and incomplete metabolic pathways for each MAG, respectively. Blue and red arrows represent aerobic and anaerobic pathways, respectively

compared to the standalone CANT-HYD results. The KEGG annotations detected the presence of genes involved in the initial activation of the aromatic rings (*phyA*, *tmoF*, and *xylA*) as well as genes belonging to three intermediate pathways: the catechol ortho- (*catA*) and meta-cleavage (*catE*), and the protocatechuate (*pcaGH*) pathways [78]. Additionally, we observed the presence of a gene (*bbsB*) involved in an intermediary step of the anaerobic degradation of toluene [79].

On the other hand, complete and partial pathways for various anaerobic respiration metabolisms as well as other metabolisms that can be performed in the absence of hydrocarbons were present in MAG1 and MAG12 as well as in other high- and medium-quality MAGs (Fig. 6; Tables S8 and S9). Nitrite oxidation and DSR were the most prevalent pathways we observed with 31 (73.8%) and 30 (71.4%) MAGs having complete pathways for these processes, respectively. Anaplerotic pathways were also prevalent with 18 (42.9%) MAGs possessing the complete set of genes for these pathways and 24 (57.1%) having partial pathways. DNRA (39 MAGs, 92.9%), denitrification (41 MAGs, 97.6%), and the rTCA cycle (34 MAGs, 81.0%) were partially present in most MAGs. Ammonia oxidation was the least prevalent metabolism with 2 (4.8%) and 18 (42.9%) of MAGs possessing a complete or partial pathway, respectively.

The pangenome of *Rhodococcus* reveals the ubiquity of alkane degradation in the Arctic

Given the high prevalence of *Rhodococcus* MAGs in our dataset, we conducted a pangenomic analysis for NWP *Rhodococcus* (Fig. 7) by comparing our 10 *Rhodococcus* MAGs with the genomes of 7 *Rhodococcus* strains isolated from Tupirvik beach sediments capable of growing on ultra-low sulfur fuel oil (ULSFO) as the sole carbon source [53]. Aerobic alkane degradation appears to be part of the core pangenome of Arctic *Rhodococcus* as *alkB* was present in all the MAGs and isolates with more than one copy detected in all but two MAGs (MAG10 and MAG41). In addition, *ladAα* was present in 14 (82.4%) of the studied genomes (Table S6). Three other alkane degradation genes (*cyp153* and *prmA/C*) were also detected in 52.9% of the genomes.

Discussion

To our knowledge this is the first metagenomic survey of Canadian high Arctic beaches describing their community composition along with their functional potential, particularly with regards to hydrocarbon biodegradation. Our statistical comparison of the metagenomes of beaches sampled in two different years suggests that the microbial communities of these beaches might remain relatively constant throughout the years. This could indicate that the results in this study, as well as future metagenomic surveys on other Arctic beaches along the

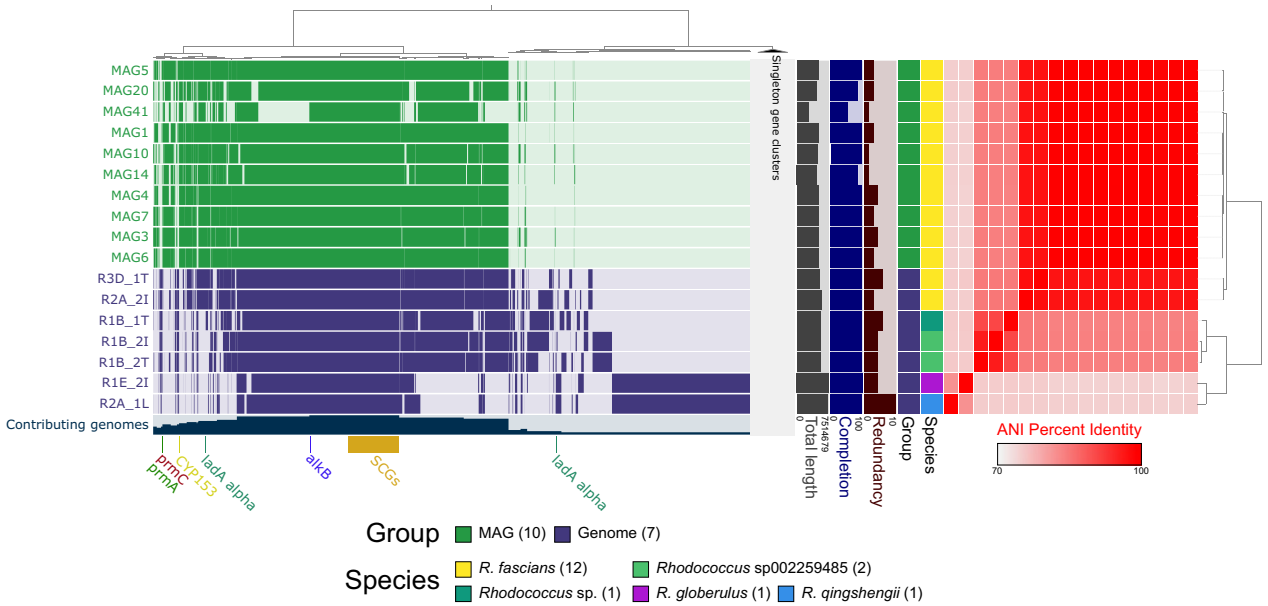

Fig. 7 Pangenome of Arctic *Rhodococcus*. MAGs recovered in this study were compared to the genomes of *Rhodococcus* strains isolated from Tupirvik beach sediment and grown using ultra low sulfur fuel oil (ULSFO) as the sole carbon source. The pangenome shows that the alkane monooxygenase (*alkB*) is part of the core pangenome and that other alkane degradation pathways part of the accessory pangenome are prevalent in many of these genomes. SCGs, single-copy core genes; ANI, average nucleotide identity

NWP, could be valid longitudinally over moderate (several years) timescales. The creation of a genomic database of NWP beach microbial communities, including those studied here, would be of value for the Canadian government and other stakeholders to define baseline microbiome profiles, high-risk shorelines, and safer travel routes, as well as improving preparedness and contingency plans in case of an oil spill [80–82]. With regards to the comparison of the microbial community among the four sampled regions, we observed no statistical differences either. However, it should be noted that the limited number of samples for certain regions drastically reduces the statistical power of this analysis. This can also cause non-homogeneous multivariate dispersions among groups (PERMDISP: $F=25.53$, $P=0.001$) that could lead to overly conservative PERMANOVA results [83]. Differences in the taxonomic composition of the supratidal and intertidal zone sediments from Assistance Bay were observed, but it was not possible to produce statistical evidence of these differences given that we only had one sample per zone. However, we did observe similar abundances of alkane degradation genes for both zones which suggests that bioinformatic analyses based solely on taxonomy are not sufficient to determine hydrocarbon degradation potential. Obtaining a larger number of samples from sites in these regions could help to determine more clearly the similarities and differences in the marine beach microbiomes of the various areas of the NWP. Future studies should also include robust environmental and hydrocarbon concentration data to be able to associate patterns in the microbial community to these variables.

The microbial communities of these NWP beaches were dominated by *Pseudomonadota*, *Actinomycetota*, and *Bacteroidota* which is consistent with a previous study which described the community composition of the same beaches using 16S rRNA gene sequencing [48] and with a metagenomic study of the same area in Alert where our sample originated [38]. We observed a larger proportion of *Actinomycetota* in our metagenomes, mostly due to the high abundance of *Rhodococcus* sequences, compared to the composition of a clone library obtained from a beach in Spitsbergen, Norway [16] as well as for sea ice and seawater 16S rRNA gene and metagenomic libraries of samples taken around Cornwallis Island [84, 85] and for 16S rRNA gene sequencing of Labrador Sea seawater [42]. The higher proportion of this phylum has been associated with low (< 10%) organic matter in Arctic and sub-Arctic soils [39, 41]. This is consistent with the oligotrophic nature of the beaches in this study which had organic matter contents ranging from 0.26 to 0.95% [48]. Our MAG novelty at the genus level is consistent with a compendium of marine environments [86], but lower than previous metagenomic studies conducted using water from the Baltic Sea (90.9%) [87] and from various sites across the Arctic Ocean (83.2%) [88].

The taxonomic classification obtained from our metagenomic survey suggests that the microbial communities of the studied NWP shorelines have the genomic capacity to bioremediate a hydrocarbon spill. Among the 20 most abundant genera, bacteria belonging to 13 of those genera are known to be capable of degrading various types of hydrocarbons and the abundance of another 4 genera has been positively correlated with the presence of hydrocarbons (Table S3). Previous studies have also observed the increase in abundance of the genera *Rhodococcus*, *Flavobacterium*, and *Psychrobacter* in microcosms grown using Tank farm beach sediment and ULSFO as the sole source of carbon as well as Tupirvik beach sediment amended with marine diesel [48, 49]. We recovered MAGs belonging to known hydrocarbon-degrading taxa such as the families *Alcanivoracaceae* [89] and *Immundisolibacteraceae* [90] and a bin from the family *Cycloclasticaceae* [91], as well as MAGs belonging to 8 genera associated with hydrocarbon degradation (Table S4). Also among our MAGs are those classified to *Ilumatobacter*, *Rhodococcus*, *Sulfitobacter*, *Cycloclasticus*, *Loktanella*, and *Granulosicoccus*; genera that are present in the Tupirvik and Tank farm microcosm studies using fuels as a carbon source [48, 49]. This was further corroborated with our CANT-HYD and KEGG annotation results which showed the presence of one or more key hydrocarbon-degrading genes as well as complete degradation pathways in our metagenomes and MAGs. We obtained similar abundances of CANT-HYD biomarker genes compared to seawater samples taken by the TARA Oceans survey from marine environments around the world, including various polar sites [73].

Hydrocarbon analyses showed that PHCs, SVOCs, and VOCs were below the detection limit for most beaches, with the exception of Dump beach, Tank farm, and Nanisivik—East (Table S7). However, hydrocarbon concentrations detected for those three beach sediments were still below the Canada-wide Standards for petroleum hydrocarbons in soil for industrial use [92]. Based on this, our metagenomic results are consistent with previous studies that have shown the ubiquity of hydrocarbon degradation pathways in marine environments in the absence of a hydrocarbon spill [93, 94]. The presence of these organisms and their pathways suggest that there is a natural hydrocarbon cycle occurring in marine environments that is sustaining hydrocarbon degrading populations in pristine environments. The first explanation for this phenomenon is that some "obligate" hydrocarbon degraders can grow using non-hydrocarbon organic compounds such as dissolved organic carbon and cellular components

of lysed marine cells [95, 96]. The second explanation is presence of hydrocarbon seeps that release short-chain gaseous alkanes and liquid alkanes and aromatic hydrocarbons [52, 97]. A hydrocarbon seep has been found in the Canadian Arctic near Scott Inlet (~900 km from Cornwallis Island), but it was observed that hydrocarbon concentrations decrease with distance from the seep and background methane levels are observed in the upper regions of the water column [97]. The third explanation is the existence of a cryptic marine alkane cycle in which cyanobacteria and eukaryotic phytoplankton produce long-chain alkanes and alkenes which are then quickly metabolized by hydrocarbon degraders that are closely associated with these photosynthetic organisms [94, 98, 99]. Hydrocarbon biosynthesis is suggested to be a universal process in cyanobacteria [100] and alkanes and alkenes appear to be required to maintain their membrane flexibility, which is required for cell division and growth [101]. Alkane-synthesizing cyanobacteria have been isolated and detected in metagenomic studies of Arctic ponds [98, 102]. However, we did not find any 16S rRNA gene sequences or MAGs assigned to cyanobacteria in our dataset. We did observe cyanobacterial sequences in the MetaErg classification, but they only accounted for 0.2% ± 0.11 of the overall metagenomic reads.

Mono- or polycyclic aromatic hydrocarbon degradation pathways were less prevalent compared to the highly prevalent alkane metabolism detected in the shoreline metagenomes. MAHs and PAHs are generally more recalcitrant to biodegradation due to their greater size or complexity, thus requiring multi-operon metabolic pathways [11, 103]. The low prevalence of these complex pathways in the NWP beach microbiomes could explain why PAHs tend to remain in Arctic environments for longer periods of time compared to their aliphatic counterparts [27, 28, 46, 50]. This is in line with the results of the microcosm experiment carried out previously using beach sediment from Tank farm which showed relatively higher rates of alkane biodegradation compared to the PAH degradation rates [48]. This study also performed radiorespiration assays using Tank farm, Nanisivik, and Cambridge Bay sediments supplemented with ^{14}C-labelled hexadecane and naphthalene and confirmed that respiration rates were higher in the hexadecane microcosms [48], which could be explained by the lower prevalence of genes in these pathways that we observed in the metagenomes of these beaches. Anaerobic hydrocarbon degradation genes were only observed in the Alert beach sediment. While we did not quantify dissolved oxygen for the Alert sample, we did observe high oxygen concentrations in the Resolute samples. It has been observed that oxygen diffusion decreases in soils as water freezes [104] which, combined with the close to freezing temperatures of NWP shorelines, could result in microscopic particles of frozen beach sediment where anaerobic conditions could be occurring. Nonetheless, we observed the presence of genes encoding for other anaerobic metabolic pathways in multiple MAGs from the studied beaches (Tables S8, S9). The reduced number of reference sequences that were used to create the HMMs for the anaerobic pathways in CANT-HYD could cause divergent sequences to not be detected [73], which could explain why we only observed anaerobic hydrocarbon degradation genes at Alert. Additionally, increasing the sequencing depth could help detect the presence of low abundance genes, such as those for an anaerobic metabolism in well oxygenated beaches.

It is worth noting that we found genes related to MAH and PAH biodegradation for the beaches that appear to have the highest baseline levels of hydrocarbon contamination. Similar to Alert [38, 40] there is a known history of hydrocarbon contamination at Nanisivik, but we did not detect elevated levels of hydrocarbon contamination in the sampled sediments (Table S7). There are other sites where past and current human activity could be causing smaller undocumented releases of hydrocarbons. For example, there is a relatively high volume of shipping activity at the main dock where the sample from Cambridge Bay was taken and the Tank farm is an active fuelling station. This suggests that hydrocarbon-degrading microorganisms inhabiting NWP shorelines could thrive when hydrocarbons are released into their environment. This was demonstrated in the microcosm experiments using Tank farm and Tupirvik beach sediment in which a higher abundance of hydrocarbon-degrading bacteria and genes were observed for samples incubated with fuels compared to the unoiled controls [48, 49].

The 16S rRNA gene and MAG taxonomies indicated a high prevalence of *Rhodococcus* in NWP shorelines. This is consistent with previous studies showing that *Rhodococcus* appears to be an abundant genus in Arctic and Antarctic marine and terrestrial environments with the genus comprising up to 34% of the community [105–108]. While we detected sequences belonging to other *Rhodococcus* species in our metagenomes, we were only able to obtain MAGs classified as *R. fascians*. Previous studies have shown that *R. fascians* is often present in large proportions in Arctic marine environments [105, 108] and seasonal dominance of this phytopathogenic species has been observed in a Norwegian fjord following the collapse of phytoplankton blooms [107].

Our hybrid annotation approach in which we complemented CANT-HYD hits with KEGG orthologs allowed us to detect the presence of multiple marker genes for alkane degradation along with the complete downstream pathways required to fully metabolize these

hydrocarbons. This suggests that MAG1 is capable of degrading short-, medium-, and long-chain (C_3, C_5–C_{13}, and C_{15}–C_{36}) alkanes [74, 75, 109, 110] and MAG12 has the genetic potential to degrade short- and medium (C_5–C_{13}) alkanes aerobically [75] and anaerobically [77]. We observed the presence of the first step of the degradation of phenols (*phyA*; K03380) in both MAGs and the catalytic subunits of the naphthalene 1,2-dioxygenase (*ndoBC*) in MAG12. However, given that we only detected a subset of the genes required for complete degradation of aromatic compounds with this hybrid approach, we cannot conclusively state that these two MAGs can perform these metabolisms. *R. fascians* has been grown using various types of MAHs and PAHs [111]. *R. fascians* can also produce biosurfactants capable of solubilizing anthracene [112] and emulsifying kerosene [113]. Multiple *Rhodococcus* isolates, including *R. fascians*, obtained from Tupirvik beach sediment have been grown using ULSFO as the carbon source at 5 °C [53]. The genetic similarity of these isolates with our MAGs (Fig. 7) supports the hydrocarbon degradative potential of the *R. fascians* MAGs of NWP beaches even at cold temperatures. *Immundisolibacter cernigliae*, the only described species from *Immundisolibacteraceae*, is capable of growing on a wide range of PAHs at mesophilic temperatures [90], but its ability to grow under cold conditions has not been reported. The genus QNFC01 was first detected from deep oceanic sediments close to a hydrothermal vent [114], which is in accordance with the cold tolerance and anaerobic metabolism we see for MAG12. This is important as the low temperatures encountered in the Arctic can limit hydrocarbon biodegradation rates [25].

Similar to the aerobic aromatic degradation pathways, we detected a limited number of genes for their anaerobic counterparts. While not direct evidence of anaerobic hydrocarbon biodegradation potential, we did detect pathways that could be coupled with these metabolisms. For example, denitrification, DNRA, and DSR were all present in the two studied MAGs and these processes often occur as anaerobic alternatives to aerobic respiration of hydrocarbons when oxygen conditions are limited, using nitrate or sulfate as the terminal electron acceptors [77, 115]. We observed high levels of oxygen saturation in pore water from the beach sediments in the Resolute region (Table S1), which suggests that anaerobic processes are not occurring at high rates during the Arctic summer.

Our metabolic reconstruction of MAG1 and MAG12 also revealed that these strains not only have potential as hydrocarbon degraders, but also have pathways for other aerobic and anaerobic metabolic processes. These metabolisms include nitrite oxidation, denitrification, DNRA, DSR, sulfide oxidation, and carbon fixation through the rTCA cycle (Fig. 6). *Rhodococcus* strains are capable of performing simultaneous heterotrophic nitrification and aerobic denitrification [116] and we observed the genomic potential for both processes in our MAGs. Autotrophic denitrification and DNRA can be coupled with sulfide [117] and sulfite [118, 119] oxidation, but these processes have not yet been shown to occur in *Rhodococcus*. For *I. cernigliae*, no growth under anaerobic conditions has been observed [90], but there is the potential for anaerobic metabolism in QNFC01 MAGs that were recovered from deep ocean sediments [114].

Finally, both sulfide and nitrite oxidation can be coupled to CO_2 fixation in chemolithoautotrophic microbes [120]. We observed the presence of genes encoding fumarate reductase (*frdABC*), 2-oxoglutarate synthase (*korAB*), and ATP-citrate lyase (*ACLY*) in MAG12 and only *frdABC* in MAG1. These genes encode the key non-reversible enzymes involved in the reverse TCA cycle [121] and further corroborate the genomic capacity of MAG12 to perform various anaerobic metabolisms. Citrate synthase, which performs the opposite reaction to the ATP-citrate lyase in the TCA cycle, is also able to operate reversibly in a process that is not easily detectable bioinformatically but still present in many organisms not thought to be capable of CO_2 fixation [122], which could be the case for MAG1. A related species, *Rhodococcus erythropolis* N9T-4, is capable of growth using trace CO_2 as a carbon source with a novel CO_2 fixation pathway which has not yet been fully described [123]. Various *Rhodococcus* strains can perform heterotrophic CO_2 fixation as part of the propane and propylene degradation pathways [123–125] and to replenish TCA metabolites as part of anaplerotic pathways [126, 127]. MAG1 does possess a propane monooxygenase (*prmAC*) and the carboxylases involved in the anaplerotic pathways (pyruvate carboxylase, *pycAB*; phosphoenolpyruvate carboxylase, *ppc*; and malate dehydrogenase, *maeB*) and we detected *pycAB* and *maeB* in MAG12. Anaplerotic pathways appear to be ubiquitous in Arctic soils, particularly in permafrost, which tends to be the most carbon-poor soil horizon [128].

These carbon and nitrogen metabolisms are also present in other medium- and high-quality MAGs (Tables S7 and S8). We quantified nitrate, nitrite, and ammonia concentrations in the pore water from beaches of the Resolute region with ammonia being the most abundant form present in these sediments (Table S1). This could suggest that ammonia has been produced which could indicate that processes such as ammonification or DNRA could be occurring at high rates in these environments. We have also observed the presence of sulfide at Assistance Bay in 2022 (unpublished data) which supports

the findings in the present study of a high prevalence of DSR genes in our metagenomes. Future research should use expression-based molecular techniques to determine whether these mechanisms are indeed occurring in the studied Arctic beaches.

The capabilities of these MAGs to perform such a wide variety of metabolic processes that tend to be associated with alternative sources of energy and nutrients are consistent with the oligotrophic conditions encountered across NWP beaches (Table S1). It is then very likely that bioremediation efforts on NWP shorelines will probably require the addition of N and P fertilizers in order to stimulate the microbial communities enough so that they can overcome their nutrient limitations, especially of decreased N and P concentrations expected after a hydrocarbon spill due to the increased metabolic activity from the growing hydrocarbon-degrading microbial populations colonizing the area of the spill [36]. The side effects of fertilizer use on the microbial communities besides stimulation of hydrocarbon degraders, for example eutrophication or anoxia [129], should also be evaluated before applying these products during a hydrocarbon spill. Further studies using the isolates we have obtained from these environments [53] will evaluate the physiological capabilities of Arctic *R. fascians* and other microorganisms to perform the multiple metabolisms that we described in MAG1 and MAG12. This will improve our understanding of the microbial ecology of these sites and help guide the optimization of bioremediation strategies aimed at enriching these organisms in contaminated shorelines so they can be used to clean up impacted beaches.

Conclusions

In this study, we described the microbial communities of marine beaches across the Canadian high Arctic based on a metagenomic survey focusing on the genomic potential for hydrocarbon biodegradation in these microbiomes. Our results showed that the microbial communities on these beaches harbour various hydrocarbon degradation pathways, mostly for the degradation of alkanes. This suggests that the microbial communities of Arctic beaches may be able to adapt and respond in the case of a hydrocarbon spill, making bioremediation a potential clean up strategy. We also described the presence of other nitrogen and sulfur metabolisms, such as nitrite oxidation, DNRA, and DSR, which these microbes might be performing in their environment using other sources of carbon. Future studies should focus on in situ and laboratory studies that confirm whether the microorganisms from these beaches are in fact capable of carrying out the metabolic processes described here under the cold and oligotrophic conditions that are observed across NWP shorelines.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40793-024-00616-y.

Additional file1. Supplementary figures (Figs S1–S8), supplementary tables (Tables S2, S4 and S6), and legends for supplementary tables in Additional file 2 (Tables S1, S3, S5, S7–S10) in PDF format.

Additional file 2. Supplementary tables (Tables S1, S3, S5, S7–S10) in XLSX format.

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Author contributions
EG and LGW conceived the study. EG designed the study and methodology. EG, LGW, and CWG collected the sediment samples. EG performed and interpreted the data analysis and wrote the manuscript. All the authors reviewed, revised, and approved the manuscript.

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Availability of data and materials
The metagenome and MAG datasets supporting the conclusions of this article are available in the NCBI Sequence Read Archive (SRA) repository under the BioProject accession number PRJNA1046404. The scripts used for the bioinformatics analyses were deposited on GitHub (https://github.com/estebangongora/NWP_beach_metagenomes).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Natural Resource Sciences, McGill University, 21111 Lakeshore Road, Sainte-Anne-de-Bellevue, QC, Canada. [2]Energy, Mining and Environment Research Centre, National Research Council Canada, 6100 Royalmount Avenue, Montreal, QC, Canada.

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