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ORIGINAL ARTICLE

Chemical dispersants enhance the activity of oil- and gas condensate-degrading marine bacteria

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Application of chemical dispersants to oil spills in the marine environment is a common practice to disperse oil into the water column and stimulate oil biodegradation by increasing its bioavailability to indigenous bacteria capable of naturally metabolizing hydrocarbons. In the context of a spill event, the biodegradation of crude oil and gas condensate off eastern Canada is an essential component of a response strategy. In laboratory experiments, we simulated conditions similar to an oil spill with and without the addition of chemical dispersant under both winter and summer conditions and evaluated the natural attenuation potential for hydrocarbons in near-surface sea water from the vicinity of crude oil and natural gas production facilities off eastern Canada. Chemical analyses were performed to determine hydrocarbon degradation rates, and metagenome binning combined with metatranscriptomics was used to reconstruct abundant bacterial genomes and estimate their oil degradation gene abundance and activity. Our results show important and rapid structural shifts in microbial populations in all three different oil production sites examined following exposure to oil, oil with dispersant and dispersant alone. We found that the addition of dispersant to crude oil enhanced oil degradation rates and favored the abundance and expression of oil-degrading genes from a *Thalassolituus* sp. (that is, metagenome bin) that harbors multiple alkane hydroxylase (*alkB*) gene copies. We propose that this member of the *Oceanospirillales* group would be an important oil degrader when oil spills are treated with dispersant.
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Introduction

Crude oil is introduced into the marine environment through natural geophysical processes at an estimated rate of 700 million liters per year (Kvenvolden and Cooper, 2003; Committee on Oil in the Sea: Inputs, Fates, and Effects *et al.*, 2003). Constant exposure of native microbes to low concentrations of hydrocarbons allows the maintenance in the environment of hydrocarbonoclastic bacteria that can readily use oil-derived compounds as carbon and energy sources (Head *et al.*, 2006; Widdel *et al.*, 2010). In the context of the recent Deepwater Horizon (DWH) MC252 oil spill in the Gulf of Mexico, which released 750 million liters of oil into the gulf, there is now scientific consensus that hydrocarbonoclastic microorganisms played a major role in the removal of hydrocarbons from the ecosystem (American Academy of Microbiology, 2011).

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Chemical dispersants act by lowering the interfacial tension between oil and water leading to oil emulsification into tiny droplets, which in turn increases bioavailability of crude oil to natural hydrocarbon degraders (Brakstad *et al.*, 2015b). For many decades, dispersants have been used in catastrophic oil spills to help increase oil degradation rates and minimize oil delivery to shorelines (Harris and Chris, 1995; Law and Carole, 2004; Henry and Charlie, 2005; Steen *et al.*, 2008; Bejarano *et al.*, 2013), but because of ecological trade-offs (Smith, 1968), their deployment is controversial and their effectiveness is debated (Committee on Understanding Oil Spill Dispersants: Efficacy and Effects *et al.*, 2005; Kleindienst *et al.*, 2015b).

Currently, Newfoundland and Labrador is home to three active offshore oil projects: Hibernia; Terra Nova; and White Rose, while the Scotian Shelf near Sable Island has a major gas condensate-producing platform. These platforms represent the major hydrocarbon-producing systems off eastern Canada. In this study, we simulated a crude oil spill at the Hibernia and Terra Nova platforms and a gas condensate spill at the Thebaud (Sable Island) platform. Recent studies have identified bacteria

from more than 79 genera that are able to degrade hydrocarbons, and several of these, including *Alcanivorax*, *Cycloclasticus*, *Oleiphilus*, *Oleispira*, *Thalassolituus* and some members of the genus *Planomicrobium (* Mason *et al.*, 2012; Reddy *et al.*, 2012; Redmond and Valentine, 2012; Valentine *et al.*, 2012; Kleindienst *et al.*, 2016), get their carbon almost exclusively from hydrocarbons (Prince, 2010; Prince *et al.*, 2010). Many DWH-related studies showed that microorganisms belonging to the order Oceanospirillales were involved in oil degradation (Hazen *et al.*, 2010; Valentine *et al.*, 2010; Kessler *et al.*, 2011; Redmond and Valentine, 2012; Gutierrez *et al.*, 2013) and that they possessed active oil degradation genes (Mason *et al.*, 2012; Rivers *et al.*, 2013), but little information was presented regarding the hydrocarbon-degrading activities of the different members of this order during an oil spill.

We applied shotgun metagenomic and metatranscriptomic analyses to evaluate the natural microbial community response following hypothetical crude oil or gas condensate spills. The biodegradation of physically and chemically dispersed gas condensate and crude oil was monitored by conducting nutrient-enhanced microcosm studies using summer and winter sea water (SW) freshly procured from the three different locations. The latest developments in bioinformatics algorithms enables the recovery of complete or draft bacterial genomes from metagenomic data sets, which is referred to as metagenome binning. In this work, we conducted a binning-centric instead of the more classical contig-based approach to analyze our sequencing data. A metagenome bin represents a draft version of a microbe's entire genome, which allows for unprecedented population dynamics resolution in the analysis and interpretation of genomics data. This approach is gaining traction (Sangwan *et al.*, 2016) and is increasingly being implemented for environmental sequence data analysis (Hugerth *et al.*, 2015; Hultman *et al.*, 2015; Evans *et al.*, 2015). Here we found that the diversity of rare resident marine microbes of this region enabled an efficient response to experimental gas condensate and crude oil spills, and that the addition of dispersant in microcosms improved the *n*-alkane, but not the polycyclic aromatic hydrocarbon (PAH), degradation rate. More importantly, we provide high-resolution analyses that for the first time put in contrast the relative contribution of various members of the Oceanospirillales group. We demonstrated that the presence of dispersant favored the proliferation of a *Thalassolituus* metagenome bin that showed high expression levels of the multiple *alkB* genes it harbored.

Materials and methods

Sampling and microcosm setup

SW samples, from locations in the vicinity of oil and natural gas production platforms in offshore Newfoundland and Labrador and Nova Scotia, were collected from the sea surface (3–5 m depth), using a Sea-bird Niskin rosette frame (24 10 l bottles) (Sea-Bird Scientific, Bellevue, WA, USA) cast from the Canadian Coast Guard research vessel, Hudson, in the summer and late fall/winter of 2013. Summer (10–19 July 2013) and winter (14 November–10 December 2013) SW was sampled from Hibernia (3 m depth in summer (46.707°N and −48.785°W) and 5 m depth in winter (43.858°N and −60.157°W)), Terra Nova (3 m depth in summer (46.431°N and −48.480°W) and 5 m depth in winter (47.000°N and −48.288°W)) and Thebaud (4 m depth in summer (43.858°N and −60.157°W) and 3 m depth in winter (47.000°N and −48.471°W)) production platforms (Supplementary Information File 3—*Water collection for microcosms*). Microcosm studies were initiated, onboard the ship, as soon as SW was procured from the reference sites. Triplicate SW samples were recovered from three separate bottles of the Niskin rosette at each sampling site using 20 l acid-washed jerricans for microcosm preparation. For microcosms, the SW (100 ml) was transferred to 250 ml baffled flasks. Biostimulation with nutrients is an effective method for augmenting the rate of oil bioremediation (Atlas and Bartha, 1972, 1973; Bragg *et al.*, 1994; Venosa *et al.*, 1996; Röling *et al.*, 2002; Coulon *et al.*, 2007; McKew *et al.*, 2007b). We learned from our experience that hydrocarbon degradation in the Arctic and Gulf of Mexico surface SW happens very slowly, if at all, when nutrients are limiting (Yergeau *et al.*, 2015 and unpublished observations). For the objective of having effective operational conditions to observe the effect of dispersant in an adequate time frame, nutrients were added to all microcosms. Therefore, Bushnell-Haas (Difco, Becton Dickinson and Company, Mississauga, ON, Canada) nutrients (2 ml), weathered oils, condensate and premixed dispersant (COREXIT EC 9500 A - Nalco Energy Services, Burlington, ON, Canada)) with oil or condensate were added to each flask/bottle. The final concentrations of COREXIT EC 9500 A in microcosms were as follows: oil-with-dispersant microcosms were setup at 5.4 p.p.m. (1:186, 281 v/v); condensate-with-dispersant microcosms had 6.0 p.p.m. (1:167, 364 v/v); and dispersant-only microcosms had 7.3 p.p.m. (1:137, 461 v/v). Oil and condensate concentrations were 107.4 (1:20 v/v) and 119.5 p.p.m. (1:20 v/v), respectively.

The flasks were incubated at the ambient surface SW temperature, at the time of collection, and continually mixed on Thermo MaxQ 2000 orbital shaker tables (Thermo Scientific, Ottawa, ON, Canada) at 150 r.p.m. Microcosms were set up directly onboard ship where experiments were conducted at 6 and 11 °C for winter and summer conditions, respectively. Setup and sampling of microcosms are described in detail in Supplementary Information File 3—*Microcosms experimental designs* (Supplementary Table SM3). Treatments (SW control, SW+Bushnell-Haas +oil (or condensate)) and SW+Bushnell-Haas+oil

(or condensate)+dispersant (COREXIT EC 9500 A), SW +dispersant were run in triplicates for each time point. Oil and dispersant concentration calculations are detailed in Supplementary Methods (Supplementary Information File 3—Supplementary Tables SM4–SM6). Two parallel sets of microcosms were prepared: one set for molecular analyses of DNA and RNA, hereby termed molecular microcosms and another set for chemistry analyses, termed chemistry microcosms. A total of 130 molecular microcosms and 234 chemistry microcosms were prepared (Supplementary Information File 3—Supplementary Table SM3).

Chemical and molecular analyses

Hydrocarbon concentrations were monitored during the course of the experiment at the specified time points ($T = 3$, 10, 15, 28 and 42 days) from chemistry microcosms. Water samples were processed using liquid–liquid extraction (modified version of US Environmental Protection Agency Method 3510C). Further details can be found in previous studies (Cole *et al*., 2007; King *et al*., 2015b). Purified extracts of water from the microcosms were analyzed using high-resolution gas chromatography (Agilent 6890 GC) coupled to an Agilent 5973 N mass selective detector (Wilmington, DE, USA) operated in the selective ion-monitoring mode using the following GC (MDN-5 S column 30 m × 0.25 mm inner diameter, 0.25 μm film thickness, Supelco, Mississauga, ON, Canada) conditions: cool on-column injection with oven track mode (tracks 3 °C higher than the oven temperature program); 80 °C hold 2 min; ramp at 4 °C min^{-1} to 280 °C; hold 10 min.

Quantification criteria for PAH included retention time matching, that is, within ± 0.010 min of the retention time of the standard, and comparing the relative abundance of the qualifying ion(s) (±10%), the molecular ion and one or more qualifier ions in the mass spectrum of the compound with the commercial standard. Seven levels of PAH standards were used to calibrate the system. The Auto-Quant software (Agilent, Palo Alto, CA, USA) was used to interpret and quantify analytes detected in the extracts. All processed data were compared to established limits to pass quality control. These limits have been set at ± 30% of the true value compared to experimental values. If results fell outside the limits they were repeated. The same was true for reference materials used to track analytical quality.

Initial SW samples (2 l) were filtered (< 10 psi) in triplicates onto Millipore 0.22 μM polyethersulfone membranes for $T = 0$ collection of genomic material for total nucleic acid extraction. The filter was transferred into a 50 ml Falcon tube and immediately flash frozen by submersion in liquid nitrogen and stored in a − 20 °C freezer onboard the ship before being transferred to a − 80 °C freezer. The genomics microcosms were killed at $T = 5$ and 42 days for the summer study and $T = 7$ and 42 days for the winter study by filtering (⩽10 psi) the contents of each flask through a 0.22 μM filter (as described above).

Total nucleic acids were recovered using a modified version of the hexadecyl trimethyl ammonium bromide method of Ausubel (2002). The modifications were as follows: the incubation time for the Tris-EDTA/lysozyme treatment was reduced substantially from 1 h to 15 min and the temperature for this step was increased from 37 to 56 °C. The hexadecyl trimethyl ammonium bromide/NaCl incubation was followed by phenol/chloroform/isoamyl alcohol (25:24:1) then a chloroform/isoamyl alcohol extraction steps. DNA was quantified using Quant-iT PicoGreen assay (ThermoFisher Scientific, Mississauga, ON, Canada) (Invitrogen, Life Technologies, Burlington, ON, Canada) and 1 ng of gDNA was used as a template to construct the sequencing library, using the Illumina (San Diego, CA, USA) Nextera XT library preparation protocol following the manufacturer's instructions. However, the 'library normalization' step was omitted and normalization was instead performed by pooling equal amounts of libraries after Quant-iT PicoGreen quantification. The quality of the pooled library was assessed (http://support.illumina.com/sequencing/sequencing_kits/nextera_xt_dna_kit/documentation.html) using an Agilent 2100 Bioanalyzer with a High Sensitivity DNA Kit. A similar methodology was used for RNA workflow with the exception that RNA was quantified using the Quant-iT RiboGreen assay and that Ribosomal RNAs were removed and library preparation was performed using ScriptSeq Complete Kit Bacteria Low-Input Library Prep protocol according to the manufacturer's (Epicentre, Madison, WI, USA)) instructions starting with 100 ng total RNA.

Genomics and bioinformatics

Metagenomic and metatranscriptomic libraries were prepared and sequenced on an Illumina HiSeq2000 system on a 2 × 100 bp configuration. A total of 203 and 126 samples were submitted for metagenome and metatranscriptome sequencing, respectively. Sequencing data (456 Gb for metagenome and 560 Gb for metatranscriptome) was processed through our metagenomics and metatranscriptomics bioinformatics pipelines (Supplementary Information File 3—Supplementary Figure SM1). Read count summaries and insert sizes are provided for both metagenome (Supplementary Information 2 Data Sets—Supplementary Table S5) and metatranscriptome (Supplementary Information 2 Data Sets—Supplementary Table S6) sequencing libraries. Sequencing adapters were removed from each read and bases at the end of reads having a quality score < 30 were cutoff (Trimmomatic v0.32; Bolger *et al*., 2014) to generate quality-controlled reads.

Quality control-passed reads from each sample were co-assembled using Ray software v2.3.1 (Boisvert *et al*., 2012) with a kmer size of 31 (see Supplementary Information 2 Data Sets—Supplementary Table S7 for

assembly statistics). Gene prediction was performed by calling genes on each assembled contig using MetageneMark v1.0 (Tang *et al.*, 2013). Genes were annotated following the JGI's guidelines (Huntemann *et al.*, 2016): (1) RPSBLAST (v2.2.29+) (Camacho *et al.*, 2009) against COG database (for example, CDD v3.11); (2) RPSBLAST (v2.2.29+) against KOG database (for example, CDD v3.11). The best hit having at an *e*-value ⩽1e-02 was kept for each query; (3) HMMSCAN (v3.1b1) (Eddy, 2011) against PFAM-A (v27.0) database (Finn *et al.*, 2013). Best hit having at least an *e*-value ⩽1e-02 was kept for each query; (4) TIGRFAM database (v15.0). Best hit having at least an *e*-value ⩽1e-02 was kept for each query; (5) BLASTP (v2.2.29+) against KEGG database v71.0; and (6) BLASTN (v2.2.29+) against the nucleotide database (version of 16 May 2013) of National Center for Biotechnology Information. Contig (and not gene) sequences were blasted against the NCBI's nucleotide database as well for taxonomic assignment. For each of these database comparisons, the best hit having at least an *e*-value ⩽1e-02 and alignment length ⩾90 bp was kept for each query. Quality control-passed reads were mapped (BWA mem v0.7.10) (unpublished—http://bio-bwa.sourceforge.net) against contigs to assess quality of metagenome assembly and to obtain contig abundance profiles. Alignment files in bam format were sorted by read coordinates using samtools v1.1 and only properly aligned read pairs were kept for downstream steps. Each bam file (containing properly aligned paired read only) was analyzed for coverage of called genes and contigs using bedtools (v2.17.0) (Quinlan and Hall, 2010) using a custom bed file representing gene coordinates on each contig. Only paired reads both overlapping their contig or gene were considered for gene counts. Coverage profiles of each sample were merged to generate an abundance matrix (rows = contig and columns = samples) for which a corresponding counts per million (CPM) abundance matri x (edgeR v3.10.2) (Robinson *et al.*, 2010) was generated.

Taxonomy of each contig was assigned using the NCBI taxonomy database (Sayers *et al.*, 2009; Benson *et al.*, 2009) (ftp://ftp.ncbi.nih.gov/pub/taxonomy/taxdump.tar.gz) (as downloaded on 5 June 2015). Each Geninfo Identifier (GI) resulting from BLASTN against nucleotide were used to retrieve full taxonomic lineages (when available) from the NCBI taxonomy database. Taxonomic lineages were integrated to the contig abundance of read counts matrix to generate an Operational Taxonomic Unit (OTU) table format file (with contigs replacing OTUs as rows). Taxonomic summaries were performed using a combination of in-house Perl and R scripts and Qiime v.1.9.0 (Caporaso *et al.*, 2010). Bin abundance tables along with their taxonomic lineages are included in Supplementary Table S3 (Supplementary Information 2 Data Sets).

Binning was performed using Metabat (v0.26.1) (Kang *et al.*, 2015a) and an abundance matrix was generated using the jgi_summarize_bam_contig_depths software (Kang *et al.*, 2015b) with –minContigLength 1000 –minContigDepth 2 and –minContigIdentity 95 parameters. Bins obtained from Metabat were further processed/decontaminated by splitting each bin into three sub-bins based on the assigned taxonomic lineage at the order level as each bin typically had a significant amount of contigs associated with the same order taxon. For instance in our data set, the bin labeled '1' had 947 contigs assigned to the Oceanospirillales order level, 3 contigs to the *Alteromonadales* and 1 contig to the *Burkholderiales*. Consequently, three sub-bins were generated and labeled 1-Oceanospirillales, 1-Alteromonadales and 1-Burkholderia, respectively. Sub-bins were kept only if they had a cumulative contig length of at least 100 kb and contained at least 10 contigs. To focus on the most relevant bins we kept a selection of the 20 most abundant bins out of the 601 total bins. Quality assessment of bins was done using CheckM v1.0.4 (Parks *et al.*, 2015) and detailed in Supplementary Information 2 Data Sets (Supplementary Table S2).

For metatranscriptomic data processing, quality control-passed reads were mapped (BWA mem v0.7.10) against contigs from the metagenome assembly following alignment sorting (samtools v1.1) and gene abundance computation (bedtools v.217.0). According to our experimental design, differentially expressed genes were assessed with edgeR (v3.10.2) using its generalized linear model approach detailed by the authors (https://www.bioconductor.org/packages/3.3/bioc/vignettes/edgeR/inst/doc/edgeRUsersGuide.pdf—section 3.2.3, page 29) with metatranscriptomic raw count matrices as input. Genes having a log fold-change ratio $\geqslant |1.5|$ and false discovery rate <0.05 were considered as differentially expressed. Metadata for all samples reported in this study are available in Supplementary Table S8 (Supplementary Information 2 Data Sets).

To increase detection power in searching for alkane 1-monooxygenase genes (*alkB*), we made multiple alignments (MUSCLE v3.8.31) (Edgar, 2004) of each accession number previously reported (Nie *et al.*, 2014). Markov models (named *alkB*, *alkB*multi2 and *alkB*multi3) were generated (hmmbuild followed by hmmpress, both with default parameters) from these alignments and gene sequences were compared against these training sets with hmmscan (Hmmer v3.1b1). Hits were integrated with Pfam-A database search described above and hits having an *e*-value ⩽1e-10 and query length ⩾100 and alignment length ⩾100 were kept. Among these matches, hits matching FA_desaturase (PF00487.19) or our newly added *alkB* models were considered as potential *alkB* genes. We applied the same procedure for genes *mcp*, *cheR*, *gspE*, *gspF* and *ompS* previously reported (Wang and Shao, 2014).

Availability of data

Raw sequence reads of the shotgun metagenomic and metatranscriptomic data were submitted to the

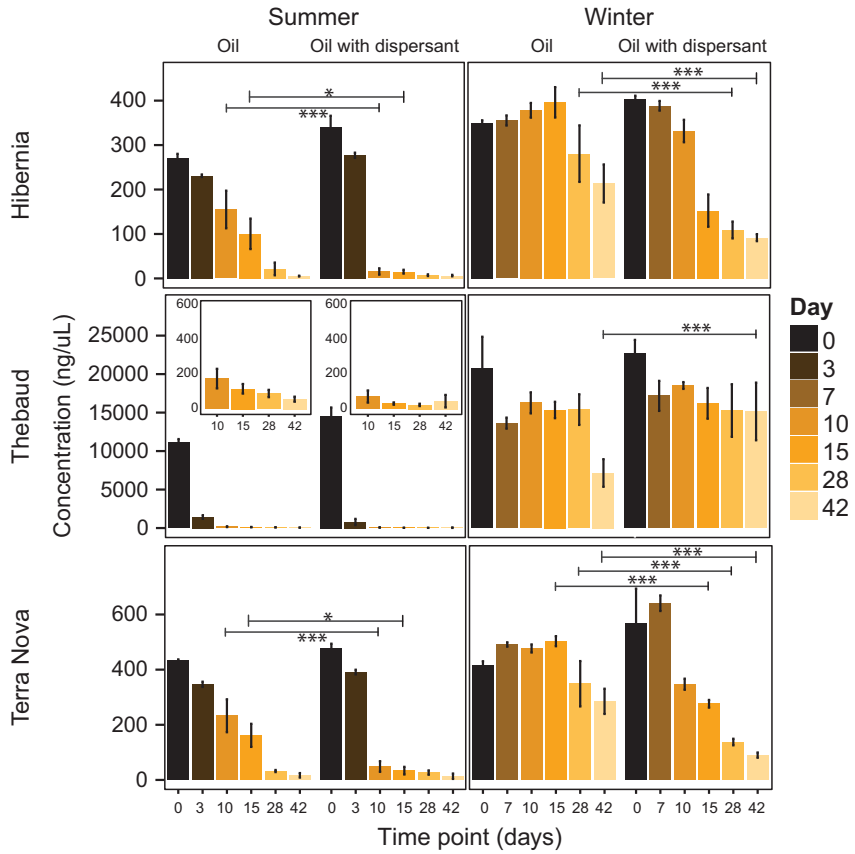

Figure 1 Total residual alkane concentration in Hibernia, Thebaud and Terra Nova summer and winter microcosms. Concentrations are normalized to hopane. Treatments are SW plus oil and SW plus oil with dispersant. All microcosms received Bushnell-Haas nutrients. Time of killing is shown by colored bars. Statistical significance was assessed with an analysis of variance followed by a *post hoc* Tukey test. ***Adjusted *P*-value <0.001; *Adjusted *P*-value <0.05.

sequence read archive under accession no. SRP079000 under Bio Project PRJNA329908. Metagenome bin sequences can be found under NCBI accession no. SAMN072553[31-50].

Results and discussion

Oil degradation in microcosms

The degradation rates in both summer and winter for each of the three sites (Hibernia, Terra Nova and Thebaud), in addition to the half-lives of the different hydrocarbon components (alkanes, PAHs and alkylated PAHs) are summarized in Figure 1; Supplementary Information File 1—Supplementary Figures S1 and S2; and Supplementary Information 2 Data Sets—Supplementary Table S1. Degradation rates were typically higher in the summer than in the winter for all hydrocarbon components at all three sites. On the basis of the observed degradation kinetics, alkanes were degraded faster at all three sites in comparison to PAHs and alkylated PAHs, which is in agreement with the general order of hydrocarbon compound degradation (Perry, 1984) and with what was observed during the DWH spill (Hazen *et al.*, 2010; Valentine *et al.*, 2010; Kessler *et al.*, 2011; Mason *et al.*, 2012; Redmond and Valentine, 2012; Rivers *et al.*, 2013; Gutierrez *et al.*, 2013). The positive effect of dispersant application was most noted for *n*-alkanes, especially in the winter, whereas no clear impacts were identified with PAH and alkylated PAH degradation rates.

For both seasons, dispersant had a positive effect on alkane degradation, which contains *n*-alkanes of various lengths (C_{10}–C_{35}). In the summer, conditions appeared to be more conducive to degradation even without dispersant, but the degradation rate of the alkane fraction was still positively affected by the presence of dispersant. Gas condensate (Thebaud) on the other hand, contained more readily bioavailable short *n*-alkanes (C_{15} and shorter) that were quickly degraded without the apparent need for dispersant (both seasons) and in the winter it appeared that dispersant might actually impair the degradation of condensate compounds (Supplementary Information File 1—Supplementary Figures S1 and S2). The effectiveness of chemical dispersants in low-temperature SW has shown conflicting results with studies reporting improved degradation of crude oil with dispersant, while other studies have shown a neutral to negative effect (reviewed in Cowton and Michael (2016)). More recently, dispersant was found to have a negative impact on the hydrocarbon-degrading activity of indigenous

bacteria (Kleindienst *et al*., 2015b). We did not see a negative impact of dispersant, especially on alkane degradation at our study sites. On the contrary, our data indicated that dispersant had a positive effect on *n*-alkane degradation rates. However, we did not find any clear benefit for dispersant use in gas condensate where longer *n*-alkanes are absent. One possibility for the difference observed between our results and those of other studies may be due to the different dispersant concentrations that were used. For instance, Kleindienst and co-workers (2015b) set up their microcosms to have a final COREXIT concentration of 15 p.p.m. (1:66, 538 v/v) and 26.8 p.p.m. (1:37, 362 v/v) for their dispersant-only and oil-with-dispersant microcosms, respectively (Lewis, 2015; Kleindienst *et al*., 2015b). In this study, the COREXIT concentration was significantly lower with values of 7.3 p.p.m. (1:137, 461 v/v) and 5.4 p.p.m. (1: 186, 281 v/v) for dispersant and oil-with-dispersant microcosms, respectively. Except for the 42-day incubation time point in Thebaud winter microcosms, for which dispersant seemed to have a negative effect on short-chain alkane degradation, our results are generally in agreement with others (Lunel *et al*., 1997; Baelum *et al*., 2012; Lee *et al*., 2013; Prince and Butler, 2014) regarding the use of dispersant to stimulate oil degradation in SW. However, our data also suggest that while dispersant favors the degradation of *n*-alkanes, it does not affect the degradation of aromatic compounds. Our microcosms were all supplemented with inorganic nutrients (Bushnell-Haas), which was reported to select against *Cycloclasticus sp*. (Singh *et al*., 2014), an aromatic compound-degrading microbe. In consequence, presence of inorganic nutrients may also be impacting the degradation of aromatic compounds.

Effects of oil on microbial community structures

Our metagenomic analysis pipeline gave us 601 metagenome bins (Supplementary Information 2 Data Sets—Supplementary Tables S2 and S3). From a total of 943 147 contigs, 91 847 made it into these bins, representing an integration rate of 9.74%. Community taxonomic profiles were highly similar (Supplementary Information File 1—Supplementary Figure S3) between contig-centric and bin-centric approaches, which suggests that our bins captured most of the microbial diversity present in our samples as assessed by a contig-based approach. To obtain a global image of the community dynamics in our microcosms, we computed alpha diversity (observed species/bins and Shannon indexes) and beta diversity (Bray–Curtis) metrics (Supplementary Information File 1—Supplementary Figures S4 and S5). As expected, T0 conditions usually harbor more diverse communities than T5 or T42 (analysis of variance: $F = 18.99$, $P < 000.1$) with relatively high observed bins and Shannon index values of more than 200 and 6, respectively. Microcosms treated with oil, dispersant and oil with dispersant showed similar diversity index values. Microcosms with dispersant only presented intermediate diversity indexes: lower than the control conditions, but higher than oil or oil with dispersant. Alpha diversity indexes drop more rapidly in treatment conditions in the summer than in the winter except for the Terra Nova site, where oil with dispersant had more diverse communities in the winter. Beta diversity plots and Permanova tests performed on bin abundance (Supplementary Information File 1—Supplementary Figure S5) showed that samples cluster primarily by treatment (that is, substrate and incubation time) indicating that these two variables are the main drivers in the formation of distinct communities. Interestingly, the control conditions of all sites clustered together as well, suggesting a similar community at time zero across all sites.

We narrowed down our analysis by selecting the 20 most abundant bins across all samples for downstream analyses (Supplementary Information 2 Data Sets—Supplementary Table S2) and established their corresponding microbial community profiles (Figure 2). *Colwellia* bins were mostly observed at Terra Nova at day 5 in the summer and winter with oil and oil with dispersant, and were also highly enriched at day 5 in Hibernia summer microcosms with oil only. *Cycloclasticus* bins were highly abundant at Thebaud at 42 days in oil with dispersant in winter and to a lesser extent in the summer for the same time point. In contrast to the crude oil substrate used at the other two stations, the gas condensate from the Thebaud station contained several orders of magnitude higher PAH and alkylated PAH concentrations (Supplementary Information File 1—Supplementary Figures S1 and S2), which probably provided an ideal environment for *Cycloclasticus*, a well-known PAH degrader (Harayama *et al*., 2004; Head *et al*., 2006; Yakimov *et al*., 2007; Coulon *et al*., 2007; McKew *et al*., 2007b; Cui *et al*., 2008; Niepceron *et al*., 2010). Our results are consistent with DWH spill microbial community succession where *Cycloclasticus* and *Colwellia* were shown to be abundant (Valentine *et al*., 2010) and active (Redmond and Valentine, 2012; Gutierrez *et al*., 2013) during the spill. *Colwelliaceae* OTUs were also reported to increase in abundance in the later stages of microbial succession in microcosm experiments (Baelum *et al*., 2012). Our data suggest that *Cycloclasticus* is also well adapted for cold temperature PAH degradation.

Glaciecola was observed in tandem with either a *Pseudoalteromonas* (Terra Nova; winter with dispersant) or an *Alteromonas* (Terra Nova and Thebaud at 5/7 days with oil and oil with dispersant) bin. *Alteromonas* species are metabolic generalists capable of rapidly adapting to environmental disturbances (reviewed in Dang and Lovell (2016)) and were also seen (Hazen *et al*., 2010; Valentine *et al*., 2010; Dang and Lovell, 2016) and isolated (Gutierrez *et al*., 2013) in the DWH water column. *Glaciecola* and *Pseudoalteromonas* are associated with

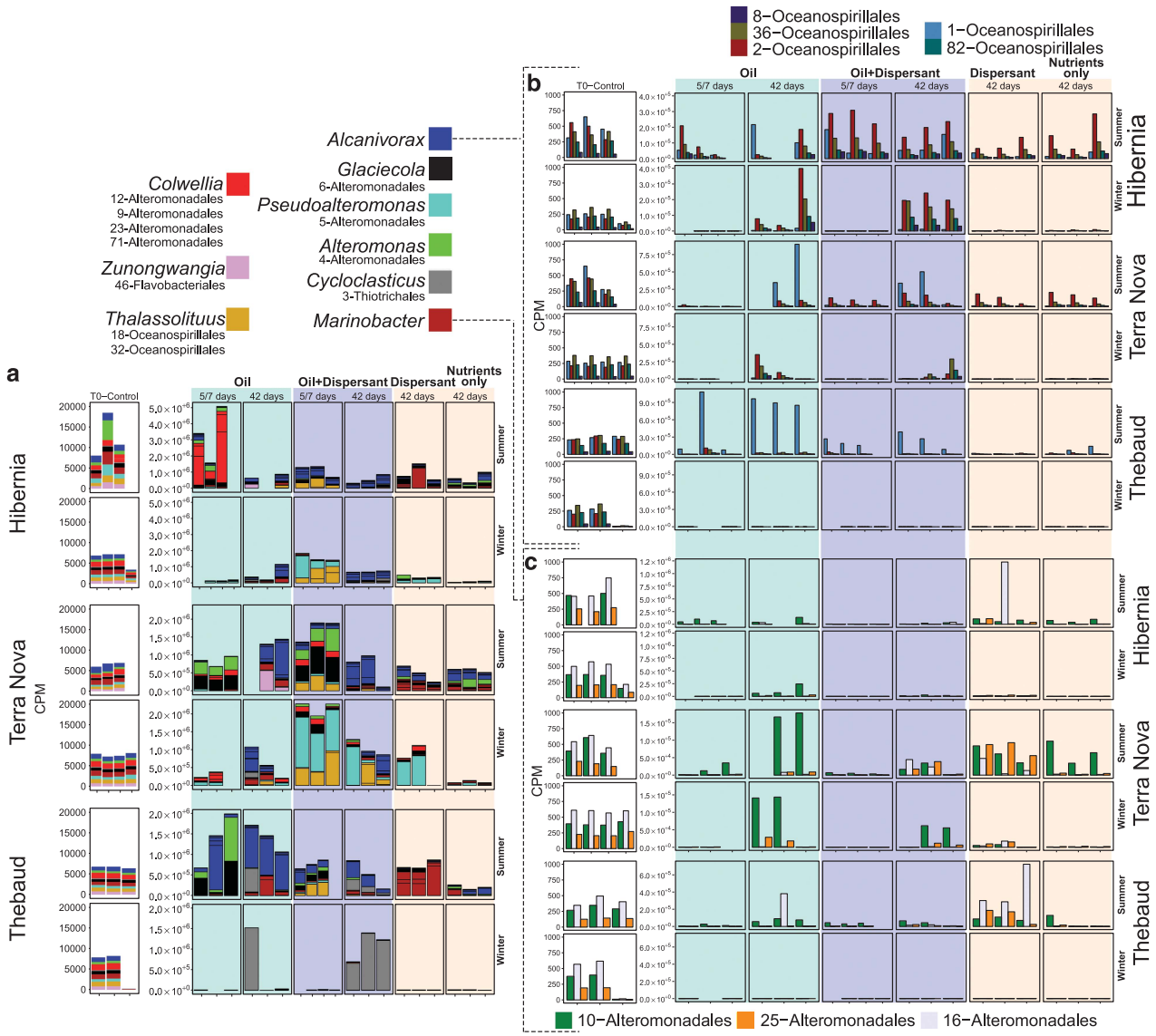

Figure 2 (**a**) Microbial community profiles of the most abundant 20 metagenome bins in Hibernia, Thebaud and Terra Nova summer and winter microcosms. Treatments are SW plus oil (light blue) at 5 or 7 days and 42 days; oil with dispersant (light purple) at 5 or 7 days and 42 days; and dispersant only (dark white) at 42 days. Summer microcosms were killed at 5 and 42 days and winter microcosms were killed at 7 and 42 days. Each color represent a taxon, each delimited box represent a bin and each column represent a sample. Bin identities are listed below each of their corresponding taxonomic assignment. (**b**) Microbial community profiles of *Alcanivorax* bins found in Hibernia, Thebaud and Terra Nova summer and winter microcosms. Treatments are SW plus oil (light blue) at 5 or 7 days and 42 days; oil with dispersant (light purple) at 5 or 7 days and 42 days; and dispersant only (light pink) at 42 days. Summer microcosms were killed at 5 and 42 days and winter microcosms were killed at 7 and 42 days. Each color represents an *Alcanivorax* metagenome bin. (**c**) Microbial community profiles of *Marinobacter* bins found in Hibernia, Thebaud and Terra Nova summer and winter microcosms. Treatments are SW plus oil (light blue) at 5 or 7 days and 42 days; oil with dispersant (light purple) at 5 or 7 days and 42 days; and dispersant only (light pink) at 42 days. Summer microcosms were killed at 5 and 42 days and winter microcosms were killed at 7 and 42 days. Each color represents a *Marinobacter* metagenome bin and each column represents a sample.

biodegradation in cold environments (Yakimov *et al.*, 2004; Deppe *et al.*, 2005; Brakstad and Kristin, 2006), which our data supports, as *Pseudoalteromonas* thrived in crude oil with dispersant at Hibernia and Terra Nova in the winter. *Glaciecola* was also well represented in the winter at these two locations, but seemed to be more successful in the summer.

Alcanivorax is a widely distributed hydrocarbonoclastic bacterium observed in oil-impacted environments around the globe (Head *et al.*, 2006), including the recent DWH oil spill (Joye *et al.*, 2014). This was reflected in our metagenomic data, where *Alcanivorax* bins were well represented in summer microcosms (and winter microcosms to a lesser extent) containing oil and oil with dispersant at day 42 (Figure 2a). At Hibernia, *Alcanivorax* bins are affluent at 5 days in oil-with-dispersant, but not in oil-only summer microcosms. *Alcanivorax* includes five distinct bins that are differentially abundant

depending on the location and treatment (Figure 2b). The most abundant bin was clearly 2-Oceanospirillales, usually followed by 36-, 82- and 1-Oceanospirillales while at Terra Nova and Thebaud, 1-Oceanospirillales stands out as the most dominant bin. A significant proportion of 2- and 36-Oceanospirillales bins were also observed at these two locations. At Thebaud, *Alcanivorax* bins are mainly represented by 1-Oceanospirillales in contrast to other locations that contain a more diversified *Alcanivorax* population, possibly because it contained gas condensate instead of crude oil. In the aftermath of the DWH oil spill, key microorganisms were identified to be intimately associated with oil degradation, one of which were members of the Oceanospirillales order. This large group comprises many subgroups at lower taxonomic levels (that is, family, genus and species) and despite many studies characterizing plume microbial populations, little information is available on the exact identity of these Oceanospirillales. Phylochip 16S rRNA gene surveys at about 1.5 km from the well head revealed that the plume microbial community was dominated by Oceanospirillales (Hazen *et al.*, 2010). Hazen *et al.* (2010) also observed a *Thalassolituus* (also belonging to Oceanospirillales), but did not indicate its abundance; according to their results, the vast majority of their phylochip OTUs belonged to an uncultured Oceanspirillales. The 16S rRNA gene Sanger clone surveys reported the involvement of *Colwellia* and *Cycloclasticus* in propane, ethane (Valentine *et al.*, 2010) and methane (Kessler *et al.*, 2011) degradation. Kessler *et al.* (2011) also observed Oceanospirillales in low overall abundance. Mason *et al.* (2012) thoroughly characterized microbial communities using various nucleic acid data types. They reported 16S rRNA gene amplicon data showing high abundance of Oceanospirillales, but did not obtain enough resolution to further characterize that OTU at the genus level. They also reported a modest amount of reads associated with *Colwellia* and *Cycloclasticus*. Their shotgun metagenome data also showed that the majority of their reads were assigned to the Oceanspirillales and metatranscriptomic analyses revealed Oceanospirillales to be transcriptionally active. From their samples, they did single-cell sequencing and using partial 16S rRNA gene alignment, found that one particular single cell, carrying oil degradation gene functions, had similarity to both *Oleispira antarctica* and *Thalassolituus oleivorans*, but did not assign a definitive taxon and labeled their single-cell isolate as belonging to Oceanspirillales using 16S rRNA gene amplicons mostly classified at the class or order level. Another study observed that Oceanospirillales OTUs were very dominant in a set of plume samples (Redmond and Valentine, 2012). Rivers *et al.* (2013) performed metatranscriptomic and 16S rRNA gene amplicon sequencing and found Oceanspirillales OTUs in high abundance in plume samples. More specifically, they were assigned to the genera *Neptuniibacter* and *Bermanella*. From their metatranscriptome data set, transcripts of the *alkB* gene were found in high abundance. They reported that the majority of their transcripts mapped to six genomes, of which two were Oceanospiralles: *Bermanella marisrubri* and *Neptuniibacter caesariensis*. *Alcanivorax* and *Marinobacter* OTUs were specifically identified to be dominant in the presence of *n*-hexadecane and were also found in fairly high abundance in DWH plume samples (Redmond and Valentine, 2012; Gutierrez *et al.*, 2013).

Bin abundance of *Marinobacter* was dependant on platform location and season, but initial communities (T0) showed remarkably similar abundances across all three sites (Figure 2c) with 16-Alteromonadales being the most abundant followed by 10- and 25-Alteromonadales. Generally, at 42 days, *Marinobacter* is fairly abundant in the winter in oil-only and dispersant-only microcosms, while it is in lower abundance in oil-with-dispersant microcosms. A previous study found that COREXIT mixed with either marine broth or marine broth with oil was toxic to *Marinobacter hydrocarbonoclasticus* (Hamdan and Fulmer, 2011). More recently, it was reported that microcosms containing relatively high dispersant concentrations prevented the dominance of *Marinobacter* OTUs (Kleindienst *et al.*, 2015b; Lewis, 2015). Our results further expand on these observations and suggest that certain *Marinobacter* species are less competitive in a substrate containing both oil and dispersant, but can otherwise thrive when only one or the other of these two substrates is present. This is particularly apparent at Terra Nova where 10-Alteromonadales is highly abundant in oil-only microcosms at 42 days while 25- and 16-Alteromonadales are found in low abundance. In contrast, in dispersant-only microcosms for the same time point, 10- and 25-Alteromonadales are similarly abundant (Figure 2c), which suggests that certain *Marinobacter* species (for example, metagenome bins) may actually grow very well in the presence of dispersant.

Our results indicated a succession of microorganisms over time with more easily degradable compounds such as short-chain alkanes being the first to be depleted. This type of succession was also observed during the DWH oil spill (Atlas and Hazen, 2011; Joye *et al.*, 2014; Kostka *et al.*, 2014; King *et al.*, 2015a) and more generally in marine environments (Röling *et al.*, 2002; Head *et al.*, 2006; Yakimov *et al.*, 2007; Berthe-Corti* and Nachtkamp, 2010; Greer, 2010; McGenity *et al.*, 2012). This may have been modulated by the presence of dispersant, the nature of the added substrates (gas condensate or crude oil), the sampling season and the incubation temperature. Although crude oil was added to both Hibernia and Terra Nova microcosms, they presented different successional patterns over time, which is especially noticeable in the abundance profiles of *Alcanivorax* bins (Figure 2b). While the community structures were very similar under control conditions ($T=0$) these communities were

not truly identical and their initial state may have had an outcome on population composition later under the different treatments. However, *Alcanivorax* and *Marinobacter* bins had very similar abundance profiles at T0 across all sites and seasons (Figures 2b and c), which does not suggest a correlation between initial abundance profiles and later population composition. From a population dynamics perspective, bacteria appearing as dominant after oil addition were initially found to constitute $<0.5\%$ of the total bin abundance in the controls (Supplementary Information 2 Data Sets—Supplementary Table S4) and could be considered as rare microorganisms. The microbial assemblages in a given environment are thought to be governed, in part, by stochastic processes in the early phases of community succession (Dini-Andreote *et al.*, 2015) and this could explain, to a certain degree, the community differences we observed between Hibernia and Terra Nova at 5/7 and 42 days.

Effect of dispersant on microbial community structure

The addition of dispersant to oil had an apparent effect on the structure of microbial communities at day 5 (summer), but not so much at 42 days (Figure 2; Supplementary Information File 1—Supplementary Figure S5). The addition of dispersant in winter microcosms also seemed to positively influence growth of certain microbes. In oil-only microcosms, microbes were usually in very low abundance at 7 days in the winter while they were already thriving at 5 days in the summer (cumulative CPM values in Figure 2). In contrast, in oil-with-dispersant winter microcosms, microbial communities were already in high abundance at day 7. We included nutrients and nutrients-with-dispersant controls at 42 days for all microcosm series and found microbial community profiles to be very different between these two conditions (Figure 2a). In general, nutrient-only microcosms had comparable (summer) or lower (winter) overall microbial abundance compared to nutrients-with-dispersant microcosms. Notably, at Terra Nova in the winter, the 25-Alteronomonas bin is abundant in nutrients-with-dispersant microcosms but virtually absent in nutrient-only microcosms (Figure 2c). Similarly, *Marinobacter* (10-, 25- and 16-Alteromonadales) are highly abundant in nutrients-with-dispersant microcosms, but in very low abundance in nutrient-only microcosms at Thebaud in the winter (Figure 2c). Conventional wisdom suggests that dispersant stimulates oil biodegradation by increasing its bioavailability in water, but it could also act as a carbon source boosting microbial growth (Mulkins-Phillips and Stewart, 1974; Lindstrom and Braddock, 2002; Chakraborty *et al.*, 2012) and ultimately leading to faster oil degradation. Alternatively, it is possible that microbes growing on dispersant are producing metabolites that are in turn consumed by oil degraders as previously suggested (Röling *et al.*, 2004). At Hibernia and Terra Nova in the winter, where the alkane degradation rate was clearly enhanced by dispersant, microbial profiles at 7 days are consistently dominated by a *Pseudoalteromonas* bin (5-Alteromonadales) in oil-with-dispersant microcosms (Figure 2). For the same winter time point in Hibernia, this *Pseudoalteromonas* bin is always accompanied with a *Thalassolituus* bin (18-Oceanospirillales) while at Terra Nova, it is associated with *Colwellia*, *Alteromonas* and *Glaciecola* bins, and the absence of *Thalassolituus*. *Alcanivorax* bins eventually dominated at 42 days in oil-with-dispersant winter microcosms at Hibernia while no clear picture emerged from Terra Nova because of the variation in biological replicates.

Interestingly, differences in population profiles in oil compared to oil with dispersant is mainly explained by the proliferation of a *Thalassolituus* bin (18-Oceanospirillales), which is consistently seen in high abundance at 5 days in oil-with-dispersant microcosms at all three sites in the summer (Figure 2). The only winter condition where the presence of a *Thalassolituus* bin was dominant was at Hibernia at 7 days. The near absence of *Thalassolituus* at other sites in the winter was possibly due to the generally slower oil degradation rate occurring in the winter (Figure 1). This bin was observed at similar abundance levels in all control microcosms regardless of the sampling season. This *Thalassolituus* bin would likely have been observed in the winter had our genomic microcosms been killed and sequenced at 15 or 28 days instead of 7 days. Bin 18-Oceanospirillales showed high similarity with *Thalassolituus oleivorans*, a microorganism that is known to have oil-degrading capabilities (Yakimov, 2004; McKew *et al.*, 2007a) and has been observed at oil spill sites (Mason *et al.*, 2014; Brakstad *et al.*, 2015a). While there is strong evidence that Oceanospirillales in general are involved in oil degradation (Hazen *et al.*, 2010; Valentine *et al.*, 2010; Kessler *et al.*, 2011; Mason *et al.*, 2012; Redmond and Valentine, 2012; Rivers *et al.*, 2013; Gutierrez *et al.*, 2013) very little information is available regarding the precise function of *Thalassolituus* in oil-degrading microbial communities. A recent 16S rRNA gene amplicon survey comparing oil and oil-with-dispersant mesocosms showed shifts in microbial population structure, but did not report the presence of Oceanospirillales (Meng *et al.*, 2016).

Alkane 1-monooxygenase gene expression

The 20 most abundant bins reported in this study were not only abundant, but transcriptionally active as well (Supplementary Information File 1—Supplementary Figure S6), which is consistent with previous findings (Mason *et al.*, 2012; Rivers *et al.*, 2013). Ratios of RNA:DNA for each gene of each bin were fitted with a non-parametric smoothing (Loess) and generally followed a linear or logarithmic trend: the more abundant a gene was, the more important

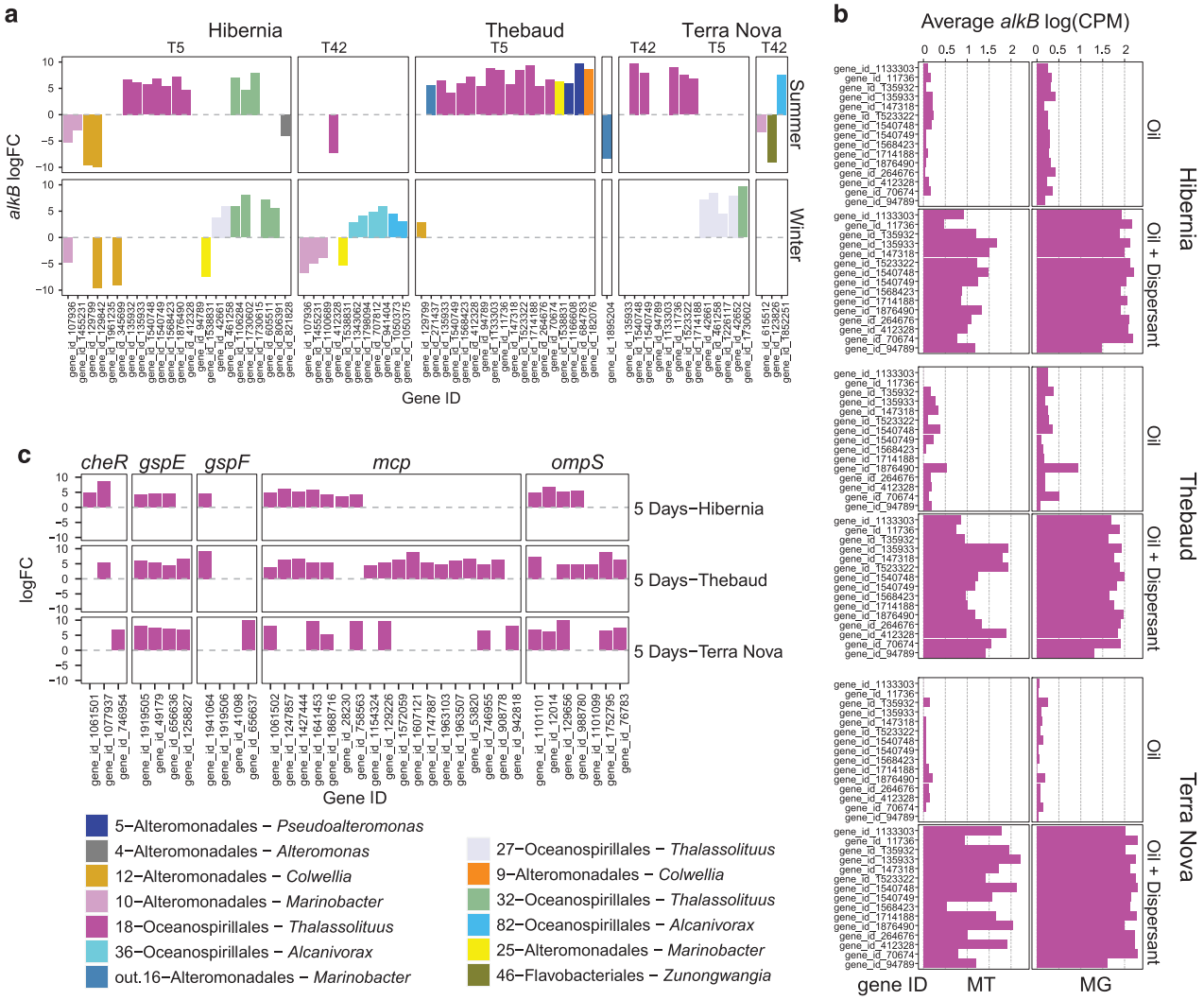

Figure 3 Fold change (log_2) of significantly differentially expressed (**a**) *alkB* and (**c**) *cheR*, *gspE*, *gspF*, *mcp* and *ompS* genes in oil with dispersant vs oil alone. Values >0 means the corresponding gene is upregulated in oil with dispersant compared to the oil control. (**b**) Average log(CPM) values of *alkB* genes harbored by the 18-Oceanospirillales *Thalassolituus* bin at 5 days in oil and oil with dispersants in metatranscriptomics and metagenomics data types.

was its expression. We focused our analyses on the expression of alkane 1-monooxygenase (*alkB*) a key enzyme in the degradation of *n*-alkanes (van Beilen *et al.*, 2006; Rojo and Fernando, 2009) and thus an appropriate marker gene for oil degradation. We performed an extensive search of *alkB* genes in our assembled metagenome contigs and listed their occurrence on a per-bin basis (Supplementary Information 2 Data Sets—Supplementary Table S2). We found that out of our most 20 abundant bins, 11 harbored a total of 121 distinct *alkB* genes. The number of distinct *alkB* genes was surprisingly high: 18 for 18-Oceanospirillales, 11 for 36-Oceanospirillales and 10 for 16-Alteromonadales. A metagenome bin is an abstract unit that takes into account contig abundance covariance and tetranucleotide frequencies across samples (Kang *et al.*, 2015b). Bins we report in this study could very well comprise two or more closely related species, explaining the high number of *alkB* genes we observed. However, contamination levels were relatively low (average of 5.93%) for the 20 most abundant bins we obtained in our study. Bins having 8 or more copies of *alkB* (16-Alteromonadales, 36-Oceanospirillale and 18-Oceanospirilalle, 3-Thiotrichales, 1-Oceanospirillale and 82-Oceanospirillales) had 17.99%, 14.96%, 11.36%, 2,7% and 1.72% contamination, respectively (Supplementary Information 2 Data Sets—Supplementary Table S2). We then obtained abundance profiles from our metatranscriptomic data, where for each microcosm location, we extracted *alkB* genes having a transcribed log fold change $\geqslant 1.5$ with a false discovery rate <0.05 between oil and oil-with-dispersant microcosms (Figure 3). As each treatment microcosm contained oil, it was expected to retrieve a fairly high number of upregulated *alkB* genes. However, because oil biodegradation was more efficient in microcosms containing oil-with-dispersant compared to oil-only microcosms, we were interested

in determining what *alkB* gene transcripts were differentially abundant based only on the addition of dispersant. Consequently, we obtained differentially expressed potential *alkB* genes in oil-with-dispersant vs oil-only microcosms (Figure 3a). We found that at all locations in summer microcosms, *alkB* genes harbored by the 18-Oceanospirillales *Thalassolituus* bin were consistently more abundant in oil-with-dispersant microcosms (vs oil-only) at 5 days. At Hibernia in the summer, another *Thalassolituus* bin (32-Oceanospirillales) had three *alkB* genes with more transcripts in the oil-with-dispersant condition compared to oil-only.

This *Thalassolituus* 18-Oceanospirillales bin clearly stands out, accounting for the majority of differentially abundant *alkB* genes in all oil-with-dispersant microcosms in the summer. As log fold-change values do not indicate the magnitude of gene abundance, but rather the abundance relative to another treatment, we also computed the average CPM values of each of these 18-Oceanospirillales *alkB* genes for the day 5 time point (Figure 3b), which confirmed that both metagenome and metatranscriptome data sets showed higher *alkB* read abundance in oil-with-dispersant (average values of ~1.5 log(CPM)) compared to oil-only microcosms (average values between 0 and 0.5 log(CPM)). We also looked for genes directly involved in alkane degradation (Wang and Shao, 2014). Of particular interest were genes coding for the chemotactic system components sensing *n*-alkanes, for which we generated hidden Markov models. We mined our metagenome data set with these models and found putative *mcp*, *cheR* and *ompS* genes. From our metatranscriptome data, we found that many of these genes were significantly more abundant in 18-Oceanospirillales in the presence of oil with dispersant vs oil only (Figure 3c). OmpS regulates expression of chemotactic genes, including *mcp* and *cheR*, is involved in the expression of *alkB* and is thought to be a signal transmitting protein and potentially the first alkane sensor outside the cell (Wang and Shao, 2014). Why *Thalassolituus* thrives at T5 in oil-with-dispersant microcosms is subject to speculation, but it could be that its particular OmpS and Mcp chemotactic receptors are highly responsive to emulsified oil or dispersant. From the genes reported by Wang and Shao (2014), we also found two *Thalassolituus* genes (*gspE* and *gspF*) showing high similarity to an ATPase involved in a type II/IV secretion system that were also positively regulated in oil-with-dispersant microcosms. *Thalassolituus* was found to be in competition with *Alcanivorax* (McKew *et al*., 2007a) and while the nature of this competition remains elusive, it was speculated that *Thalassolituus* might actively release bioactive compounds to inhibit competitors (McKew *et al*., 2007a; McGenity *et al*., 2012). Abundance of *gspE* and *gspF* transcripts will require further investigation, but it provides support that *Thalassolituus* actively uses its type II/IV secretion apparatus during degradation of emulsified oil.

To the best of our knowledge, this is the first time that the presence of dispersant with oil has been shown to favor *Thalassolituus* growth and transcription of its alkane degradation genes (*alkB*, *cheR*, *ompS*, *mcp*, *gspE* and *gspF*). Interestingly, *Thalassolituus* was virtually absent in microcosms that contained dispersant or oil alone: it needed both oil and dispersant to thrive. Moreover, another *Thalassolituus* bin (32-Oceanospirillales) had some differentially abundant *alkB* genes at Hibernia (summer and winter) and one at Terra Nova at 5 days in the winter. We found far fewer differentially abundant *alkB* genes in the winter, which is likely due to the fact that the oil degradation rate in oil-with-dispersant microcosms occurred around 15 and 28 days, later than what was observed in the summer. At this point, we can only speculate why *Thalassolituus* thrived in oil-with-dispersant microcosms, but our data strongly suggest an important role for this microbe. *Thalassolituus* was abundant near the oil degradation rate peak (5 days in summer microcosms), but did not remain as a significant part of the community by 42 days. In contrast, *Alcanivorax* bins were found in high abundance at both 5 and 42 days. The short window during which *Thalassolituus* bins were detectable might explain why this microbe has been under the radar in other studies investigating the effects of dispersants on oil degradation. In addition, the vast majority of microbial surveys in oil degradation studies were conducted with short 16S rRNA gene amplicons, which results in a lower resolution for microbial identification and has possibly contributed to its underrepresentation in the literature.

The *Thalassolituus* bin we identified contained 18 potential *alkB* genes. To gain insight into why *alkB* genes hosted by *Thalassolituus* are more efficient in an oil-with-dispersant context, we generated a multiple alignment of all 121 *alkB* genes contained in our 20 most abundant bins and generated a phylogenetic tree (Figure 4a), which showed that *alkB* genes clustered together according to their host bin. Each *alkB* sequence of each bin was aligned such that we obtained one representative consensus *alkB* sequence for each bin. We then searched for motifs in the NCBI-CDD database and found that three particular domains (NCBI-CDD:255516, 234750 and 253129) were present in the *alkB* representatives of the 18-Oceanospirillales *Thalassolituus* bin we identified, but not in *alkB* sequences belonging to other bins. These three motifs match the consensus sequence region located upstream of the FA_desaturase between position 482–567 (Figure 4b). Interestingly, one of these motifs (253129) belongs to the *Arabidopsis* phospholipase-like protein (PEARLI 4) super family. Additional work will be needed to elucidate this *Thalassolituus* AlkB structure, but our data suggest the possible existence of a yet uncharacterized fatty acid desaturase extended with a phospholipid domain, which could somehow show specificity toward emulsified oil, but not oil or emulsifier alone.

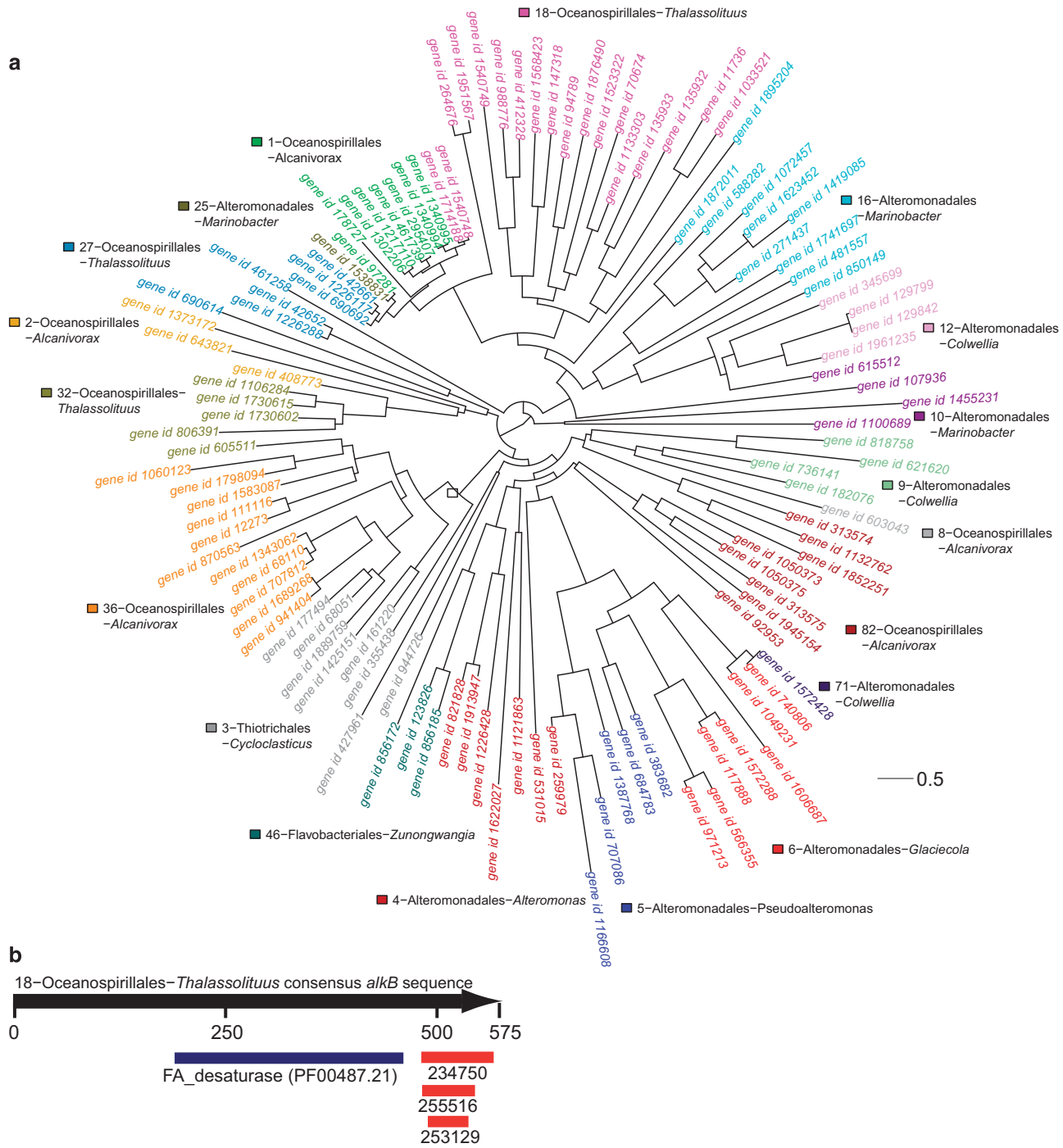

Figure 4 (**a**) All putative AlkB amino-acid (aa) sequences were aligned into a multiple alignment (MUSCLE v3.8.31), which was used to generate a phylogenetic tree (FastTree v2.1.8). Gene IDs are colored by their associated metagenome bin. (**b**) A multiple sequence alignment (MUSCLE) was generated using only AlkB aa sequences belonging to the 18-Oceanospirillales *Thalassolituus* bin. A consensus was generated (Jalview v2.9.0b2) from this multiple sequence alignment and analyzed for its motif/domain composition. Blue: Pfam annotations; red: NCBI's CDD annotations.

Conclusions

Dispersants have been used on a large scale for many oil spills over the last several decades, the most recent case being the DWH spill of 2010. However, there is no scientific consensus on the benefits of dispersants for stimulating hydrocarbon degradation (recently reviewed Kleindienst *et al.* (2015a)) with conflicting results from previous studies likely partially attributed to the absence of standardized protocols. Our data support that after 5 or 7 days of incubation in both summer and winter, the differences in oil and oil-with-dispersant microbial communities are mainly driven by the presence and

activity of two *Thalassolituus* metagenome bins. We also found that dispersants are effective at stimulating *n*-alkane biodegradation in marine waters and does not act solely as a preferred substrate for microbial growth. However, regardless of the presence or absence of dispersant, under all conditions, bacteria were able to respond to oil and condensate spills and rapidly degrade hydrocarbons. Indigenous bacterial populations in the regions examined were highly diversified and included an array of known hydrocarbon degraders. Providing that there are sufficient essential nutrients, these hydrocarbon-degrading bacteria could have an important role in attenuating potential oil spills.

Conflict of Interest

The authors declare no conflict of interest.

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Author contributions

JT wrote software, analyzed data and wrote manuscript. CWG and EY planned experimental design, analyzed data and edited manuscript. NF carried out the microcosms experiments. SC performed chemical analyses of microcosms. ME performed DNA extraction and prepared sequencing libraries. TLK and KL participated in the design of the study.

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