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BIODEGRADATION

Hydrocarbon-Degrading Microbial Communities Are Site Specific, and Their Activity Is Limited by Synergies in Temperature and Nutrient Availability in Surface Ocean Waters

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ABSTRACT The objective of this study was to quantify the potential for hydrocarbon biodegradation in surface waters of three sites, representing geographic regions of major oil exploration (Beaufort Sea in the Arctic, northern Gulf of Mexico [GOM], and southern GOM), in a systematic experimental design that incorporated gradients in temperature and the availability of major nutrients. Surface seawater was amended in microcosms with Macondo surrogate oil to simulate an oil slick, and microcosms were incubated, with or without nutrient amendment, at temperatures ranging from 4 to 38°C. Using respiration rate as a proxy, distinct temperature responses were observed in surface seawater microcosms based on geographic origin; biodegradation was nearly always more rapid in the Arctic site samples than in the GOM samples. Nutrient amendment enhanced respiration rates by a factor of approximately 6, stimulated microbial growth, and generally elevated the taxonomic diversity of microbial communities within the optimal temperature range for activity at each site, while diversity remained the same or was lower at temperatures deviating from optimal conditions. Taken together, our results advance the understanding of how bacterioplankton communities from different geographic regions respond to oil perturbation. A pulsed disturbance of oil is proposed to favor copiotrophic r-strategists that are adapted to pointed seasonal inputs of phytoplankton carbon, displaying carbon and nutrient limitations, rather than oil exposure history. Further understanding of the ecological mechanisms underpinning the complex environmental controls of hydrocarbon degradation is required for improvement of predictive models of the fate and transport of spilled oil in marine environments.

IMPORTANCE The risk of an oil spill accident in pristine regions of the world's oceans is increasing due to the development and transport of crude oil resources, especially in the Arctic region, as a result of the opening of ice-free transportation routes, and there is currently no consensus regarding the complex interplay among the environmental controls of petroleum hydrocarbon biodegradation for predictive modeling. We examined the hydrocarbon biodegradation potential of bacterioplankton from three representative geographic regions of oil exploration. Our results showed that rates of aerobic respiration coupled to hydrocarbon degradation in surface ocean waters are controlled to a large extent by effects of temperature and nutrient limitation; hydrocarbon exposure history did not appear to have a major impact. Further, the relationship between temperature and biodegradation rates is linked to microbial community structure, which is specific to the geographic origin.

KEYWORDS biodegradation, microbial communities, hydrocarbons, oil, temperature, nutrients

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The *Deepwater Horizon* (DWH) catastrophe represents the largest accidental marine oil spill in human history (1, 2). Approximately 3.19 million barrel equivalents of oil were released into the Gulf of Mexico (GOM) (3), and an estimated 10% of the released oil formed slicks on the surface ocean (4). While most of the oil was rapidly dispersed, a persistent oil slick was present at the wellhead during and after the spill (5). From June to November 2010, the integrated surface oil concentrations in the northern GOM varied from nondetectable to over 10,000 ppm, with the highest concentrations being observed near the wellhead and in coastal areas (2).

Microbial biodegradation is considered to be the primary means by which spilled oil is eliminated from the environment (6). Previous studies suggested that petroleum hydrocarbon degradation is site specific and correlated with the hydrocarbon exposure history (7, 8). The GOM is a prolific hydrocarbon basin that receives approximately 604,150 liters of oil per year through natural seeps alone (9, 10). It has been suggested that, because of chronic hydrocarbon exposure, microbial communities in the GOM are primed for hydrocarbon degradation and would rapidly respond to oil input (11–13). Apart from oil exposure history, however, microorganisms are under strong selection pressure from *in situ* environmental conditions (14). Environmental factors have been shown to be key determinants of microbial community structure and function (15, 16), which suggests that resource limitation may outweigh any effects of oil exposure history on biodegradation. Moreover, relatively few rate measurements are available to support the "priming" hypothesis, and further studies are warranted (17).

Hydrocarbon biodegradation is coupled to aerobic respiration in oceanic surface waters (18). Like all respiration processes, biodegradation is limited by the availability of oxygen and nutrients, temperature, and the physiology of hydrocarbon-degrading microorganisms (19). Since hydrocarbons are often distributed in seawater as liquid droplets, the form and solubility of oil also limit biodegradation. A large body of research, including laboratory and field studies, has shown that the ocean environment dictates the efficiency and capacity of microbial communities to degrade hydrocarbons (13, 20, 21). Despite this extensive knowledge base, however, quantitative understanding is lacking, and we have yet to determine how environmental factors interact to regulate the fate and transport of spilled oil in the oceans. After the DWH disaster, many studies focused on the microbial responses to oil contamination in deepsea oil plumes and on shorelines (22–26). Less information is available on the microbial responses to oil slicks in oligotrophic surface seawater.

As traditional shallow oil reservoirs in temperate regions are being depleted, oil exploration is moving toward regions at high latitudes, including in the Beaufort Sea (27). Moreover, due to global climate change, an elongated ice-free season promotes marine transportation through the Northwest Passage (28). These phenomena may lead to increased risk in the potential for an oil spill in fragile, pristine, polar ecosystems (29). Unlike the GOM, which spans subtropical to tropical climates, cold temperatures in the Arctic region may alter the form/solubility of oil and inhibit biodegradation (30), thereby increasing oil longevity in the environment. A systematic understanding of the rates and controls of hydrocarbon biodegradation, as well as the microbial community response, will be critical for assessing the environmental risks of oil exploration in polar regions.

The objective of this study was to quantify the potential for hydrocarbon biodegradation in simulated oil slicks generated from the surface waters of three sites that represent geographical regions of major oil exploration, based on hydrocarbon exposure history, potential hydrocarbon spill risk, and climatic region (Beaufort Sea in the Arctic region, northern GOM, and southern GOM), in a systematic experimental design that incorporates gradients in temperature and the availability of major nutrients. We hypothesized that hydrocarbon exposure history along with nutrient availability would override temperature as biodegradation controls. In contrast, we observed the highest biodegradation potential in surface seawater from the permanently cold Arctic site, with approximately equal impacts of temperature and nutrient availability on degradation activity at all sites.

TABLE 1 Characteristics and results for each sampling site

Characteristic[a]	CB2	DWH01	IXTOC01
Latitude	75°47′N	28°43′N	19°22′N
Longitude	129°17′W	88°23′W	92°19′W
Total inorganic nitrogen level (μM)	6.16 ± 3.0	2.83 ± 3.8	2.54 ± 2.1
Phosphate level (μM)	2.08 ± 1.5	0.7 ± 0.1	1.72 ± 0.8
In situ temperature (°C)	0.7	31	35.9
T_{opt} (°C)	30	38	38
Activation energy (kJ/mol)	54.7 ± 11.3	83.1 ± 16.5	76.2 ± 12.0
Q_{10}	2.1	3.1	2.8
Rate constant (*k*) at 20°C (day^{-1})			
UN treatment	0.003 ± 0.0004	0.001 ± 0.0002	0.002 ± 0.0002
NA treatment	0.01 ± 0.002	0.008 ± 0.0006	0.009 ± 0.0002
$R_{Topt}/R_{4°C}$	5	99	55

[a]T_{opt} indicates the temperature at which maximum degradation occurred. Activation energy indicates the apparent temperature response of the degradation rates. Q_{10} indicates the degradation rate change with a 10°C increase in temperature at 20°C. $R_{Topt}/R_{4°C}$ is the ratio of degradation rates at the T_{opt} and at 4°C for each site.

RESULTS

Site characteristics. *In situ* nutrient concentrations and temperatures of the three sampling sites are provided in Table 1. As expected, the temperature at the time of sampling was highest at the tropical site (IXTOC01), intermediate at the subtropical site (DWH01), and much lower at the polar site (CB2). Major nutrient concentrations, including total dissolved inorganic nitrogen (the sum of nitrate, nitrite, and ammonium) and soluble phosphorus levels, were highest at CB2, but these differences were not statistically significant ($P = 0.23$ for total inorganic nitrogen levels and $P = 0.18$ for phosphorus levels) (Table 1).

Biodegradation rates. In the current study, surface seawater samples from three geographical regions of oil exploration (Beaufort Sea in the Arctic region, northern GOM in the subtropics, and southern GOM in the tropics) were incubated with amended crude oil in a systematic experimental design that included six temperatures and two nutrient treatments. The temperature responses of microbial respiration were determined in triplicate microcosms, with and without nutrient amendment, for all sites. Microbial respiration, as determined by CO_2 accumulation, was used as a proxy for hydrocarbon degradation, as in previous studies (Fig. 1) (31–34). This assumption is supported by the fact that little respiration was detected in control microcosms to which no oil was added (see Fig. S1 in the supplemental material). Dissolved oxygen concentrations were quantified at the final time point, and it was confirmed that at least 50% oxygen saturation remained in all incubations. In addition, known aerobic hydrocarbon-degrading bacteria dominated the seawater microbial communities in all oil-amended treatments.

Rapid biodegradation was observed as CO_2 production at 25°C, the optimal temperature (T_{opt}) for activity, in oil-amended incubations from all three sites, whereas little to no CO_2 production was observed in the control treatments to which no oil was added (representative controls are shown in Fig. S1), indicating that CO_2 production could be used an effective proxy for hydrocarbon degradation. Whereas no-oil controls showed little to no activity during the incubation, respiration began immediately in the oil-amended microcosm treatments, and no significant lag phase was observed. Respiration rates were nearly always higher at CB2 for both treatments (244.3 ± 27.0 μmol CO_2/liter/day for nutrient-amended [NA] treatment and 45.2 ± 4.8 μmol CO_2/liter/day for unamended [UN] treatment), in comparison with the GOM sites, except at the highest temperature studied (38°C), which is well above the ambient range for this permanently cold polar site. Maximum rates at the GOM sites were 144.6 ± 15.8 μmol/liter/day and 167.3 ± 1.6 μmol/liter/day for NA treatment in the DWH01 and IXTOC01 microcosms, respectively. Rates for the UN treatments were 29.6 ± 3.2 μmol CO_2/liter/

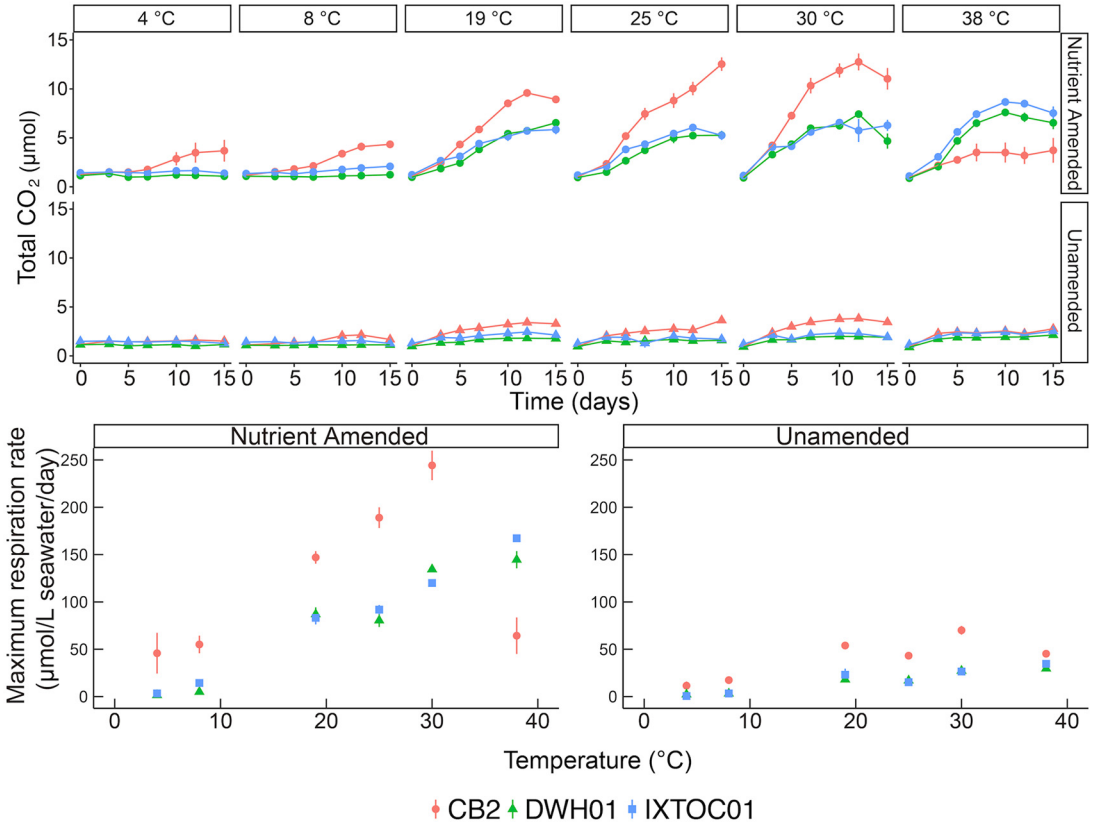

FIG 1 Biodegradation rates, as determined by carbon dioxide accumulation (upper), and estimated maximum respiration rates (lower) according to temperature and nutrient amendment in microcosms of surface seawater. Scatterplots show average values from triplicate measurements. Error bars indicate standard deviations.

day and 34.6 ± 5 μmol CO_2/liter/day for the DWH01 and IXTOC01 microcosms, respectively. At the T_{opt} for activity determined for samples from each site, GOM rates were 32 to 41% and 23 to 35% lower than CB2 rates for the NA and UN treatments, respectively.

According to permutational multivariate analysis of variance (PERMANOVA), nutrient amendment explained the greatest amount of variation in respiration (38%; $P = 0.01$), followed by temperature (12%; $P = 0.03$) and site (7%; $P = 0.064$) (Fig. 1). At most temperatures studied, activity was stimulated substantially by nutrient amendment, with an average 6-fold increase in respiration rates. By comparison, rates at the Arctic site increased an average of 3- to 5-fold across the temperature range studied. Interestingly, at cold temperatures well below the ambient range (4°C and 8°C), activities were suppressed at the GOM sites and the addition of nutrients did not enhance degradation rates. Rates increased with incubation temperature and showed maxima in the mesophilic range (at 30°C for CB2 and 38°C for the GOM sites). Our study might not have captured the T_{opt} for biodegradation for the GOM sites, as rates continued to increase throughout the range studied.

Microbial community analysis. Overall bacterial abundance was estimated at the end of each incubation using quantitative PCR (qPCR) of small-subunit (SSU) rRNA genes (Fig. S2). Nutrient amendment apparently stimulated microbial growth in all mesocosms, as bacterial abundance was 5- to 10-fold greater with the NA treatments than with the UN treatments. A positive correlation was observed from the linear regression between bacterial abundance and respiration rates at the T_{opt} ($R^2 = 0.44$, $P < 0.001$). For the GOM samples, bacterial abundance was much greater at the T_{opt} for microbial activity (30°C to 38°C) than at the lowest temperature studied (4°C). Bacterial abundance at 4°C in the CB2 incubations represented approximately one-half of that

determined at the T_{opt} for activity. Neither temperature nor nutrient availability significantly affected the cell-specific respiration rates.

The abundance of *nifH* (nitrogenase) genes, a proxy for the abundance of nitrogen-fixing or diazotrophic microorganisms, represented approximately 0.4% and 5% of the SSU rRNA copies in NA and UN treatments, respectively. Among all treatments for the same temperature and site, the *nifH* relative abundance increased approximately 32-fold ($P = 0.015$) (Fig. S2).

Over 3 million paired-end reads were generated on an Illumina MiSeq platform, and 2.4 million SSU rRNA gene sequences remained after quality control. Operational taxonomic units (OTUs) with a relative abundance of less than 0.05% of the total reads were removed. Samples that contained fewer than 6,000 reads were discarded. Beta diversity, as determined by the Bray-Curtis distance metric, showed a strong selection of microbial communities by sample site (Fig. 2a). Statistical analyses, using PERMANOVA, supported this interpretation. The primary parameter affecting community diversity was site, which accounted for 30% of the variation ($P < 0.001$), followed by temperature (14%; $P < 0.001$) and nutrient availability (5%; $P < 0.001$) (Fig. 2a). Communities were shown to cluster according to site and temperature.

The taxonomic (alpha) diversity of microbial communities in each microcosm was generally elevated with NA treatments within the optimal range of temperatures for activity at each site, while diversity remained the same or was lower at temperatures deviating from optimal conditions (Fig. 2b). The optimal range indicates the temperatures over which significant biodegradation was observed, i.e., 4°C to 30°C for CB2 and 19°C to 38°C for the GOM sites.

Attempts were made to characterize microbial communities in preincubation samples from all sites. However, only small volumes of seawater were available from each site (5 ml) and thus preincubation samples did not yield sufficient DNA for PCR amplification and sequencing of SSU rRNA genes. Because we did not have sufficient sample volume, we were not able to further test extraction methods (35, 36). The focus of the current study was to investigate the complex interplay of the environmental parameters that affect hydrocarbon degradation potential in different oil exploration areas. Therefore, the initial community composition, although important for determining hydrocarbon degradation potential, should not alter our interpretation of relative differences observed between sites.

At the class level, *Gammaproteobacteria*, *Alphaproteobacteria*, and *Betaproteobacteria* dominated the microbial communities, constituting up to 98%, 22%, and 38% of the total sequences retrieved, respectively (Fig. 3). Equal or greater alpha diversities were observed in CB2 NA microcosms, compared to UN treatments, across the temperature range, and the Shannon entropy plunged in both treatments at 38°C (Fig. 2b). *Colwellia* dominated both CB2 treatments at low temperatures (4°C and 8°C) and was elevated, compared to GOM sites, according to linear discriminant analysis effect size (LEfSe) analysis. At mesophilic temperatures, *Thalassolituus* was strongly enriched in all CB2 microcosms, in comparison with GOM incubations. Both GOM sites demonstrated similar patterns in alpha diversity (Fig. 2b). Low microbial diversity was observed at low temperatures, with significantly greater relative abundances of *Alcanivorax*, *Sulfitobacter*, *Marinobacter*, and *Alteromonas* being observed at GOM sites. At mesophilic temperatures, the GOM NA treatments were enriched in *Alcanivorax* and *Marinobacter*, compared to CB2. The genus *Acinetobacter* constituted the majority of the microbial communities for all UN treatments at mesophilic temperatures and the NA treatment at 38°C for CB2.

DISCUSSION

The fate and transport of discharged oil in seawater are determined by a complex interplay among hydrocarbon chemistry, the microbial food web, and ambient oceanographic parameters (21). The complex interactions between environmental factors that regulate the efficiency of microbially mediated hydrocarbon degradation are not completely understood (13).

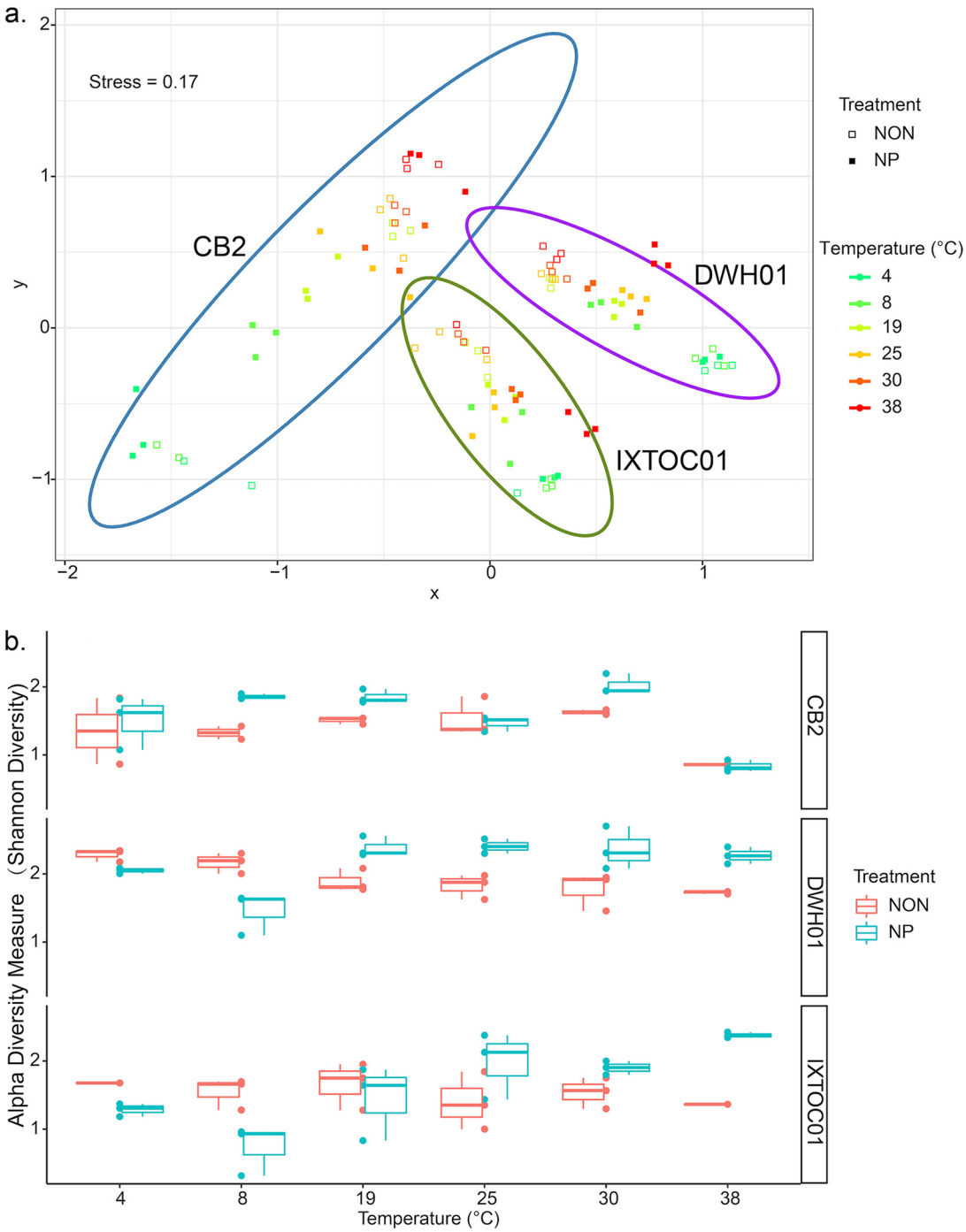

FIG 2 Beta (a) and alpha (b) diversity, determined for microbial communities in seawater microcosms, according to temperature and nutrient amendment. Beta diversity is displayed as the Bray-Curtis distance metric on a nonmetric multidimensional scaling (NMDS) plot, and alpha diversity is shown as Shannon entropy. Boxplots show average values of triplicate samples. Error bars indicate standard deviations. NON and NP refer to the unamended and nutrient-amended treatments, respectively.

Environmental controls of biodegradation. Since the GOM is exposed to a substantial amount of natural oil seepage, it was suggested immediately after the DWH disaster that microbial communities are primed or adapted for an intrinsically high potential for oil biodegradation (22). Conversely, based on analysis of SSU rRNA gene amplicons, numerous studies have shown that hydrocarbon-degrading bacteria are likely to be ubiquitous, albeit rare, in GOM ecosystems, including areas that are not immediately exposed to natural seepage (21, 37). Known petroleum hydrocarbon-

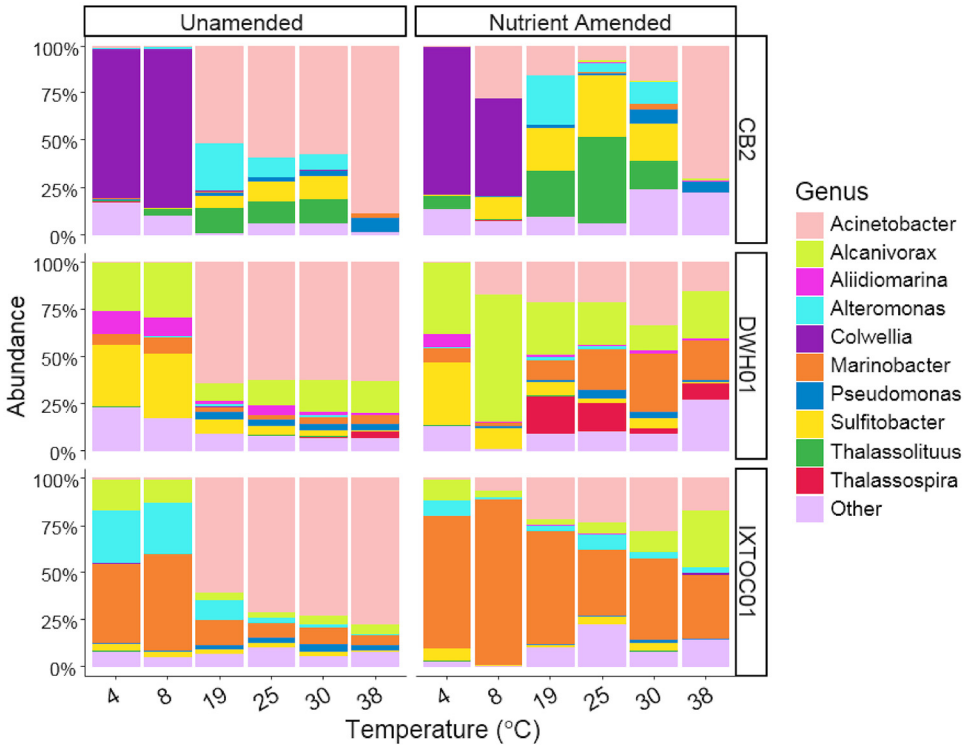

FIG 3 Microbial community compositions in surface seawater microcosms from CB2 (upper), DWH01 (middle), and IXTOC01 (lower) sites. Barplots show the mean relative abundance of triplicate microcosms. Taxa are grouped at the genus level, and relative abundance was calculated relative to total sequences retrieved.

degrading bacteria may be separated into two categories, based on their ranges in the utilization of carbon substrates (38). Generalist taxa, such as *Acinetobacter* and *Marinobacter*, have the potential to use a wide range of substrates other than hydrocarbons (39, 40). In contrast, specialists, including *Alcanivorax* (41) and *Thalassolituus* (42), almost exclusively use hydrocarbons as substrates for growth (18). In the absence of substantial petroleum hydrocarbon input, these organisms may utilize analogous compounds produced by phytoplankton. For the specialists, there are multiple sources of hydrocarbons in the ocean. Indigenous microbial communities in the immediate vicinity of natural hydrocarbon seeps are physiologically adapted to the processing of released hydrocarbons (37). However, natural hydrocarbon seepage occurs in all of the world's ocean basins. Moreover, phytoplankton represent a widespread source of hydrocarbons, such as alkanes, throughout the world's oceans (17, 43), and hydrocarbon-degrading bacteria are likely to be equally distributed. Evidence, in the form of potential rates, to support the hypothesis that GOM microbial communities are inordinately primed for hydrocarbon degradation, in comparison to other ocean basins, is lacking.

In this study, the activity and growth of microorganisms in oil-amended microcosms of pristine Arctic seawater, which was collected far from any oil production areas along the coast of northern Canada and Alaska, nearly always exceeded those of seawater sampled from the northern and southern GOM at sites of major oil spills. These data should be verified by direct measurements of petroleum hydrocarbons, and clearly further sampling across ocean basins is needed to assess spatiotemporal variations in hydrocarbon degradation potential. Nonetheless, our results contrast with the paradigm that the GOM is primed for degradation, in comparison to other ocean basins. Rather, this study demonstrates strong selection for hydrocarbon-degrading microbial communities by site, depending on factors other than the history of exposure to high levels of hydrocarbons. Beta diversity analysis showed that microbial communities were

most strongly separated by the site of origin, from the polar to tropical sites. Alpha diversity variance reflected microbial adaptation to the respective preferential temperature conditions. Finally, the compositions of major hydrocarbon-degrading communities were clearly distinct among sites, as suggested by LEfSe analysis, especially considering the ranges of activity.

One concern with our experimental design is that samples were stored at 4°C for 1 to 2 months before incubation. The systematic design of this experiment required that large numbers of tubes be incubated in incubators set to different temperatures. Initiation of the experiment was delayed for this period so that all incubations could be conducted in parallel under identical conditions in various incubators. We acknowledge that sample storage should always be performed with caution, especially when refrigeration is employed to slow microbial metabolism. However, comparable studies showed that sample integrity was maintained using the same refrigeration-based storage methods and potential rates were comparable even after 3 months of storage at 4°C (44). In more extreme cases, a 2-month period of frozen storage did not induce permanent damage to microbial activity, with rates comparable to *in situ* measurements being observed (45). Further, Stenberg et al. reported that, although long-term refrigeration (13 months) reduced organic matter content in samples, substrate-induced microbial activity remained the same after storage (46). In the present study, microbial communities immediately responded to oil amendment without a lag phase, suggesting no suppression of microbial activity. Thus, we contend that sample integrity was maintained to the best of our ability and relative differences in potential rates likely trend with the properties of the different sites. The community composition of the microcosms might have been affected by sample storage, however.

Hydrocarbon-degrading bacteria are ubiquitous in the world's oceans, as well as in terrestrial environments (47, 48). Since organic matter produced by extant photosynthetic organisms contains compounds analogous to petroleum hydrocarbons, such as alkanes (43), it is likely that microbial communities in any ecosystem exposed to such compounds are primed or adapted to hydrocarbon degradation to some extent. Thus, the potential for oil biodegradation is more likely limited by the complex interplay of environmental parameters unique to each ecosystem. We suggest that the site-specific responses can be explained by the ecological strategy employed by indigenous planktonic microbial communities (49). Previous studies have suggested that marine microorganisms can be separated into two categories according to their responses to resource limitation, namely, copiotrophic r-strategists, which are well adapted to pulsed disturbances of high nutrient and carbon levels interspersed with long periods of starvation, and oligotrophic K-strategists, which are adapted to consistently low inputs of carbon and nutrients, with less ability to respond to disturbances (50, 51). Indeed, r-strategists have been associated with colder ecosystems such as the deepsea and polar regions exposed to pulse disturbances (52–54). We posit that hydrocarbon degraders in Arctic bacterioplankton adopt an r-strategy, which derives from rapid responses to pulses of organic matter over an abbreviated seasonal cycle, whereas communities in the GOM are K-strategists adapted to a more stable, nutrient-poor environment. During the spring bloom in the Arctic Ocean, phytoplankton carbon is injected into surface waters in a short period (55–57), and heterotrophic bacterioplankton respond rapidly to the carbon input through a series of physiological modifications, including shifting kinetic parameters to those more suitable for higher substrate concentrations (52). Despite the significantly increased biomass, community structure appeared to be stable (58), indicating that the majority of the microbial community adopted an r-strategy. Similarly, Arctic zooplankton demonstrated r-strategy-like behavior by sustaining low metabolic rates for much of the year and rapidly becoming more active during spring phytoplankton blooms (59). Thus, it is reasonable to speculate that Arctic surface waters are replete with r-strategist copiotrophs capable of rapidly responding to intense carbon inputs, such as that during an oil spill. In contrast, GOM bacterioplankton experience more consistent conditions of warmer temperatures, sunlight, and organic matter inputs from rivers, leading to more stable resource

availability (60, 61). Thus, contrasting ecological strategies to resource utilization may account for the observed differences in biodegradation potentials. Our conclusions are corroborated by previous studies that hypothesized that hydrocarbon degraders employing an r-strategy could respond more quickly during oil spills than those using a K-strategy (37). In studies of *in situ* microbial communities, it remains challenging to distinguish r-strategists from K-strategists.

Temperature has long been recognized as a critical parameter that regulates hydrocarbon biodegradation (6). It influences biodegradation through effects on the physicochemical properties of oil and on microbial structure/function (62). Many studies, primarily conducted in the laboratory, indicate that temperature strongly regulates the capacity and efficiency of petroleum hydrocarbon degradation in seawater (63). Results are equivocal, however, and kinetic constraints may not be as important as previously thought (64–68).

In this study, distinct temperature responses were observed in surface seawater microcosms based on geographical origin, from the subtropical and tropical GOM sites (DWH01 and IXTOC01) to the Arctic Beaufort Sea (CB2). We observed more rapid degradation in surface seawater samples between 4°C and 30°C in the Arctic samples, compared to subtropical and tropical samples, in corroboration of the findings by Bagi et al., who found faster hydrocarbon degradation in Arctic Ocean seawater than in temperate Atlantic Ocean seawater (32). Results from CB2 microcosms indicated that the *in situ* microbial community from a permanently cold Arctic environment could maintain relatively rapid degradation across a large range in temperatures, indicating cold adaptation (28, 63, 69–72). In contrast, little degradation activity was observed at lower temperatures (4°C and 8°C) in microcosms from the GOM sites, suggesting adaptation to mesophilic temperatures (73). Our observations are corroborated by the fact that the CB2 site had slightly elevated nutrient concentrations, relative to the GOM sites (74). Generally, nitrogen and phosphorus levels are depleted at the surface by extensive photosynthetic production in low-latitude tropical oceans (74), while low temperatures and light deficiencies lead to higher nutrient levels in the polar region. Thus, higher inorganic nutrient levels may support a greater potential for hydrocarbon degradation in polar waters (75).

Multiple lines of evidence were provided for temperature adaptation. A comparison of both apparent activation energy values and the ratios of the degradation rate at the T_{opt} for activity (R_{Topt}) to the rate at the lowest temperature (4°C) ($R_{4°C}$) revealed large differences between the Arctic and GOM sites. Lower activation energies and $R_{Topt}/R_{4°C}$ ratios for CB2 microcosms pointed to less change in microbial activity across the range in temperatures, while higher values for both GOM sites indicated more variation with temperature (76). These results reflect the differences in temperatures at the sampling sites. Whereas the average annual sea surface temperature in the GOM is approximately 25°C (77), the temperature at CB2 in the Beaufort Sea permanently remains at $<$1°C (78). The change in rates with a 10°C increase (Q_{10}) values also diverged between Arctic and GOM sites and fell within the range of findings from previous work (63). In corroboration of evidence from rate measurements, endpoint biomass, as indicated by the abundance of rRNA genes, in Arctic seawater incubations equaled or exceeded biomass measured in GOM incubations, and cell-specific respiration rates did not differ substantially between sites. Differences in the degradation potential observed across temperatures indicate a critical role of microbial adaptation to the indigenous conditions. Microorganisms native to the cold environment showed greater activity at low temperatures, compared to microorganisms from warm environments. The consensus appears to be that it is not possible to interpret rates of oil biodegradation as first order with respect to temperature, due to confounding factors such as nutrient limitations, the solubility of hydrocarbons, and microbial community compositions in various studies (63). Variations in methodology and experimental conditions also likely contribute to uncertainty.

Our interpretation of nutrient limitation in surface waters is supported by the quantification of nitrogen-fixing or diazotrophic microbial communities in the microcosms. The abundance of diazotrophs was estimated by qPCR of the most commonly

used molecular marker for nitrogen fixation, *nifH* (nitrogenase). Nitrogen fixation transforms nitrogen gas into bioavailable ammonia, which provides a competitive advantage for a large diversity of prokaryotes under nutrient-limited conditions (79, 80). In the current study, diazotroph abundance was 32 times higher for UN treatments than for NA treatments, suggesting that nitrogen fixers were responding to limited nutrient availability in the presence of a simulated oil slick. These results are corroborated by field observations during the DWH oil spill. Previous studies reported elevated nitrogen fixation potential in response to oil contamination in GOM waters and sediments (81, 82). Nitrogen fixation was suggested to be a primary nitrogen source in oiled environments during the DWH spill (83), and the findings described above indicate that nutrient limitation likely inhibits oil degradation.

Microbial community dynamics across site, temperature, and nutrient availability. The dominance of the class *Gammaproteobacteria* was expected, as most well-known marine hydrocarbon-degrading bacteria reside in this group (22, 25, 28, 84). Although both generalists (*Acinetobacter* [39], *Colwellia* [85], and *Marinobacter* [40]) and specialists (*Alcanivorax* [41] and *Thalassolituus* [42]) were present in the microcosms, the microbial populations were likely shaped by the petroleum hydrocarbon amendment rather than other natural carbon sources. Petroleum hydrocarbons represented the only carbon source available in the incubations, as indicated by comparison to control treatments. Previous work indicated that members of the *Gammaproteobacteria* are well adapted to changing environmental conditions and respond rapidly to pulses in organic matter input (50, 86). At higher taxonomic resolution, shifts in community composition reflected adaptation in response to environmental conditions. At cold temperatures closest to ambient conditions in polar surface waters, the genus *Colwellia* was selected in all microcosms. The genus *Colwellia* was often found in oil-contaminated seawater under cold conditions (22, 32, 48, 87). In the current study, abundant *Colwellia* OTUs showed the highest sequence identity to the psychrophilic strains *Colwellia maris* and *Colwellia rossensis*, both of which were isolated from cold environments (88, 89); this may explain the rapid degradation that occurred in CB2 microcosms at 4°C and 8°C. At higher temperatures in incubations of Arctic waters, other genera (*Thalassolituus* and *Sulfitobacter*) dominated the communities with nutrient addition. While the genus *Thalassolituus* is an obligate hydrocarbon-degrading group (42), members of *Sulfitobacter* have not yet been shown to degrade hydrocarbons. Both microbial groups were often associated with oil-contaminated sites under various conditions (22, 33, 90–92).

Unlike the Arctic microcosms, no distinct microbial group at the genus level was selected by low temperature in incubations of GOM seawater, and the dominant OTUs were all most closely affiliated with mesophilic microorganisms isolated at warm temperatures (93–96). This finding suggests that the minimum degradation observed was due to dysbiosis and a lack of cold-adapted microorganisms in GOM microcosms pushed to temperature extremes. The enrichment of known hydrocarbon degraders, including *Alcanivorax*, *Alteromonas*, *Marinobacter*, and *Thalassospira*, indicated that these groups are adapted to high oil concentrations and elevated nutrient concentrations. These findings are corroborated by previous studies of planktonic ecosystems in the GOM, where these genera were enriched under heavily oiled conditions either *in situ* (44, 97) or *ex situ* (98).

At mesophilic temperatures, all UN microcosms were dominated by the genus *Acinetobacter*, regardless of the site sampled, and the group persisted in all NA microcosms. *Acinetobacter* species are well known to degrade a range of hydrocarbons, including long-chain alkanes (99) and polyaromatic hydrocarbons such as phenanthrene and pyrene (100). The high relative abundance of *Acinetobacter* in UN treatments suggests that it outcompetes other hydrocarbon degraders in heavily oiled and nutrient-depleted environments. In agreement with this finding, *Acinetobacter* was observed at high abundance in oil slicks and heavily oiled beach and saltmarsh sediments (25, 101, 102) but not in the dispersed oil plumes resulting from the DWH oil spill in May 2010 (44).

Here we demonstrate that the microbial community responses to oil contamination in surface waters of major oil exploration regions are site specific and dependent on ambient conditions, temperature, and nutrients. The activity, diversity, composition, and growth of microbial communities were all strongly selected by site in seawater microcosms, suggesting adaptation to deterministic environmental parameters. Surprisingly, the highest potential hydrocarbon degradation rates were observed in pristine polar waters, with approximately equal effects of temperature and nutrient availability on degradation activity at all sites. The results call into question the role of chronic oil pollution in the priming of GOM waters for oil biodegradation. We hypothesize that the adoption of different ecological strategies for resource utilization provides a basis for hydrocarbon degradation potential in surface seawater. It is important to note that this study represents only a snapshot of the likely spatiotemporal variation of the three sites and should not be extrapolated to the entire geographic region. Future studies should incorporate direct measurements of hydrocarbons and should focus on further verification of the complex interplay among environmental controls of biodegradation.

MATERIALS AND METHODS

Sample collection. Surface water samples were collected in Niskin bottles at 10 m below the sea surface, from research cruises aboard the Canadian Coast Guard icebreaker *Amundsen* (10 September 2015), the R/V *Weatherbird II* (20 August 2015), and the R/V *Justo Sierra* (2 August 2015) for the polar site CB2, the subtropical site DWH01, and the tropical site IXTOC01, respectively. Site characteristics are presented in Table 1. DWH01 and IXTOC01 represent subtropical and tropical sites, respectively, where the largest accidental marine oil spills in history occurred in the GOM (11). The Beaufort Sea represents a pristine, permanently cold, polar site that is being considered for offshore drilling platforms on the continental slope. Site CB2 was chosen because it had many oceanographic similarities to DWH01. Like DWH01, CB2 is a pelagic site located on the continental slope, remote from shore. In addition, the region surrounding CB2 is socioeconomically and ecologically relevant as a highly productive marine ecosystem in the path of the Northwest Passage (28). Samples for nutrient analysis were immediately filtered through 0.2-μm polycarbonate filters (MoBio Laboratories, Carlsbad, CA, USA) and stored at −20°C. Samples for incubation were stored in Nalgene bottles at 4°C until use. Major inorganic nutrient (nitrite/nitrate, ammonia, and soluble phosphate) levels were determined using established methods (103–105).

Microcosm experiments. For each site, microcosms were constructed by amending 5 ml of seawater with 5 μl of surrogate MC252 oil (106) in 30-ml sealed glass tubes. The surrogate oil is a sweet light crude with analytical and toxicological properties similar to those of the MC252 oil discharged during the DWH disaster; it was set aside by BP as a tractable model oil for experimentation. Experimental treatments included UN microcosms, to which no nutrient was added, and NA microcosms, which received 32 μM ammonium (NH_4Cl) and 2 μM phosphate (K_2HPO_4) (final concentrations) (31). Microcosms with no oil addition were constructed and incubated at 25°C to indicate the amount of respiration supported by recently produced natural organic matter present in seawater at the time of sampling. Triplicate microcosms were incubated in the dark for 15 days at six different temperatures, spanning the temperature range of polar to tropical climates (4°C, 8°C, 19°C, 25°C, 30°C, and 38°C). Further reduction to subzero temperatures could not be achieved with the available laboratory equipment. Microcosms were sampled at regular intervals for respiration rate measurements.

Respiration was used as a proxy for oil biodegradation, as established in previous work (24). Respiration rates were quantified as CO_2 accumulation by sampling the microcosm headspace with a gastight syringe and immediately injecting the sample into a gas chromatograph with a flame ionization detector that was equipped with a methanizer (Shimadzu Scientific, Kyoto, Japan). Total carbon dioxide production was calculated as the sum of the gas phase and the dissolved phase using Henry's law, with temperature compensation (107). Maximum respiration rates of the linear growth stage were calculated using the R package grofit, with default settings (108). Calculation of activation energy and temperature coefficient (Q_{10}) values was carried out following the methods described by Bagi et al. (32). Rate coefficients (k) were calculated using the pseudo-first-order equation $dC/dt = -kt$, where C is the residual carbon concentration (in micromolar) and t is the incubation time (in days). Dissolved oxygen concentrations were determined using a Presens Microx 4 optode system with a PSt7 needle-type sensor (Presens, Regensburg, Germany).

DNA extraction and sequence analysis. After incubation, all of the seawater volume was pelleted and extracted with the Quick-DNA fungal/bacterial microprep kit (Zymo Research, Irvine, CA). Extracted DNA was quantified with the Qubit HS assay kit (Invitrogen, Carlsbad, CA, USA), and 10 ng per reaction was used to generate SSU rRNA amplicons. Established next-generation sequencing protocols were applied for profile prokaryotic community compositions. V4 variable regions of the SSU rRNA gene were amplified using the primers CS1_515F and CS2_806R (109, 110). The amplicons were further barcoded with commercial 10-base barcodes (Fluidigm Corp., South San Francisco, CA, USA), concentrations were normalized, and sequencing was performed on an Illumina MiSeq 2000 platform at the DNA services facility of the University of Chicago (111–113).

Bioinformatic tools were used for downstream analysis of the sequence libraries. PEAR was used for merging of pair-end reads. The merged sequences were processed through vsearch and mothur for

demultiplexing and trimming, respectively. Chimeras were eliminated from the libraries using vsearch. Dereplicated sequences were grouped into OTUs using SWARM (with $d = 1$) (114). Clustered OTUs were then assigned against the SILVA database (115, 116). Downstream analyses were conducted with R packages, including phyloseq (117), vegan, DESeq2, and ggplot2 (117). Multivariate analysis was performed for statistical analysis of microbial community dissimilarity. LEfSe analysis was performed to identify significantly affected taxa (by uploading the abundance table to http://huttenhower.sph.harvard.edu/galaxy).

Quantitative PCR was employed for quantification of SSU rRNA genes and dinitrogenase (*nifH*) genes on a StepOnePlus platform (Applied Biosystems, Foster City, CA, USA), using PolF/PolR (118) and 331F/518R (119) primers, respectively. Standard curves were obtained with standard plasmids containing target *Escherichia coli* K12 SSU rRNA genes (2.76×10^3 to 2.76×10^8 copies). The running conditions were as follows: 2 min at 50°C, 2 min at 95°C, and 40 cycles of 95°C for 15 s, 55°C for 15 s, and 72°C for 1 min. The *nifH* genes were amplified using established methods (120). Standard curves were obtained with standard plasmids containing target *Azotobacter vinelandii nifH* gene fragments as the insert (3.2×10^2 to 3.2×10^7 copies). The running conditions were as follows: 2 min at 50°C, 2 min at 95°C, and 45 cycles of 95°C for 15 s and 63°C for 1 min. In all experiments, negative controls containing no template DNA were subjected to the same qPCR procedure, to exclude or to detect any possible DNA contamination.

Data availability. Data are publicly available through the Gulf of Mexico Research Initiative Information and Data Cooperative (https://data.gulfresearchinitiative.org/data/R4.x267.179:0009 [DOI: https://doi.org/10.7266/n7-df3b-bq71]) (121). The generated sequence data are available at the NCBI under BioProject no. PRJNA434326.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM.00443-19.

SUPPLEMENTAL FILE 1, PDF file, 0.5 MB.

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All data are publicly available through the Gulf of Mexico Research Initiative information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org/data/R4.x267.179:0009 (DOI: https://doi.org/10.7266/n7-df3b-bq71).

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