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Rapid microbial respiration of oil from the *Deepwater Horizon* spill in offshore surface waters of the Gulf of Mexico

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Abstract

The *Deepwater Horizon* oil spill was one of the largest oil spills in history, and the fate of this oil within the Gulf of Mexico ecosystem remains to be fully understood. The goal of this study—conducted in mid-June of 2010, approximately two months after the oil spill began—was to understand the key role that microbes would play in the degradation of the oil in the offshore oligotrophic surface waters near the *Deepwater Horizon* site. As the utilization of organic carbon by bacteria in the surface waters of the Gulf had been previously shown to be phosphorus limited, we hypothesized that bacteria would be unable to rapidly utilize the oil released from the Macondo well. Although phosphate was scarce throughout the sampling region and microbes exhibited enzymatic signs of phosphate stress within the oil slick, microbial respiration within the slick was enhanced by approximately a factor of five. An incubation experiment to determine hydrocarbon degradation rates confirmed that a large fraction of this enhanced respiration was supported by hydrocarbon degradation. Extrapolating our observations to the entire area of the slick suggests that microbes had the potential to degrade a large fraction of the oil as it arrived at the surface from the well. These observations decidedly refuted our hypothesis. However, a concomitant increase in microbial abundance or biomass was not observed in the slick, suggesting that microbial growth was nutrient limited; incubations amended with nutrients showed rapid increases in cell number and biomass, which supported this conclusion. Our study shows that the dynamic microbial community of the Gulf of Mexico supported remarkable rates of oil respiration, despite a dearth of dissolved nutrients.

Keywords: microbial respiration, Deepwater Horizon, oil spill, hydrocarbon degradation, Gulf of Mexico, petroleum hydrocarbon

1. Introduction

On 20 April 2010 the *Deepwater Horizon* drilling platform experienced an explosion which resulted in 11 fatalities and an uncontrolled release of oil from the Macondo well at a depth of 1544 m. Ultimately, over 600 million liters of oil were

released (Deepwater Horizon Unified Command 2010). Oil released from the wellhead ascended through the water column forming both a hydrocarbon plume at approximately 1100 m and a vast slick on the surface. Several reports have focused on the microbial degradation of hydrocarbons in the deep plume (Camilli *et al* 2010, Hazen *et al* 2010, Valentine *et al* 2010,

Table 1. Core data from stations occupied in June 2010 near the *Deepwater Horizon* (DWH) site. A dashed line (—) denotes that samples were not collected for that particular measurement. The parameters measured are abbreviated as follows: P = phosphate, R = respiration, RI = respiration with inorganic nutrient amendments, PL = polar membrane lipid concentrations, PLI = polar membrane lipid concentrations in inorganic nutrient amendments, E = enzyme activity (lipase and alkaline phosphatase), H = hydrocarbon degradation incubations, C = cell counts, CI = cell counts in inorganic nutrient amendments. Station names are also given for reference to our previously published work from this cruise (Camilli *et al* 2010).

Station name	Station name (Camilli <i>et al</i> 2010)	Latitude (°N)	Longitude (°W)	Distance from well (km)	Date of sampling	Phosphate (nmol l ⁻¹)	Water temp (°C)	Parameters measured
DWH	DWH	28.74	88.39	0	—	—	—	—
O-1	DT1	28.22	87.44	109	06/18/10	—	—	R, E
O-2	DT2	28.35	88.38	43.0	06/18/10	—	—	R
O-3	EN01	28.37	88.39	41.0	06/19/10	8.89	28.5	P, PL, E, C
O-4	EN02	28.60	88.54	23.4	06/19/10	—	27.7	PL, E, C
O-5	EN03	28.63	88.56	22.3	06/20/10	11.3	27.8	P, C
O-6	EN04	28.67	88.58	22.5	06/20/10	5.33	26.8	P, C
O-7	EN21	28.36	88.39	41.6	06/28/10	15.4	29.6	P, R, PL, E, C
I-1	EN05	28.74	88.41	4.16	06/20/10	—	28.2	R, RI, H, PL, E, C, CI
I-2	EN07	28.75	88.38	1.84	06/21/10	12.1	29.3	P, PL, E, C
I-3	EN08	28.73	88.35	1.69	06/21/10	9.67	29.4	P, PL, C
I-4	EN10	28.74	88.43	6.62	06/22/10	—	29.9	R, RI, PL, PLI, E, CI
I-5	EN20	28.73	88.39	2.09	06/28/10	10.7	29.6	P, R, RI, PL, E

Kessler *et al* 2011), but the goal of this study was to examine the microbial degradation of oil in the surface waters in close proximity to the *Deepwater Horizon* site.

It is well documented that microbes can respond quickly to the addition of petroleum hydrocarbons, aiding in degradation and remediation (Cappello *et al* 2007 and references therein; Prince 2010 and references therein). However, while surface waters on the continental shelf of the Gulf of Mexico and the Mississippi river plume are rich in inorganic nutrients, the offshore waters of the Gulf of Mexico near the *Deepwater Horizon* site are oligotrophic. Bacterial production and respiration of organic carbon in this region have been observed to be limited by the availability of phosphate, an essential nutrient (Pomeroy *et al* 1995). In amendment experiments conducted by Pomeroy *et al* (1995) in the same region of the Gulf of Mexico as the *Deepwater Horizon* spill, microbes were unable to respire exogenous glucose without the addition of inorganic nutrients. These observations would predict that the microbial response to the oil from the Macondo well would be minimal, especially since the hydrophobic compounds that compose oil (Peters *et al* 2005 and references therein) play a comparatively less essential role in cellular physiology than the simple sugar glucose. Additionally, it has been recognized for several decades that the availability of nutrients can markedly affect the rate of microbial oil degradation (Atlas 1981, Leahy and Colwell 1990, Prince 2010 and references therein). Thus we hypothesized that the microbial community of the oligotrophic offshore surface waters of the Gulf of Mexico would be unable to rapidly respond to the large pulse of highly reduced organic carbon from the oil spill due to intense nutrient limitation.

Surface-water samples were collected at twelve stations near the *Deepwater Horizon* site in mid-June of 2010, seven stations outside the slick and five stations within the slick. These stations are the same as those reported in our previous publications on the subsurface plume of hydrocarbons (Camilli *et al* 2010, Reddy *et al* 2011; table 1). Community respiration

rates, lipase enzyme activities, microbial abundances, and microbial biomass were examined at stations inside and outside of the surface oil slick to better constrain how the microbial community responded to the introduction of crude oil. Alkaline phosphatase activities and phosphate concentrations were determined *in situ* to assess phosphate limitation. Inorganic amendment experiments were also conducted to test whether community respiration and microbial cell production in the slick was limited by nutrients as we had hypothesized. Additionally hydrocarbon degradation rates were determined in incubations, which in conjunction with community respiration rates, were used to further constrain the fate of oil released to surface waters.

2. Methodology

2.1. Sampling

Our cruise aboard the R/V *Endeavor* was conducted between 19 and 28 June 2010 (table 1). Water samples were collected from the surface mixed layer (<15 m) using Go-Flo bottles, which are underwater sampling bottles that stay closed until triggered at depth to prevent contamination from surface waters. These bottles were maintained free of oil contamination as described (Reddy *et al* 2011). Five stations were occupied within the oil surface slick (I-1 through I-5) and seven sites were occupied outside of the slick (O-1 through O-7). The stations inside the slick were generally in close proximity to the Macondo well (within 1.7–6.6 km; table 1) and were characterized by the presence of oil on or below the surface that was plainly identifiable by visual inspection over the rail of the ship. The stations outside the slick were south and southeast of the well site, which was generally upwind of the well site during our cruise, and no oil was visible at the surface.

2.2. Phosphate measurements

Water from the Go-Flo bottles was collected in 50 ml high-density polyethylene Nalgene bottles, and kept frozen until analysis. Samples were collected from four stations outside the slick and three stations within the slick (table 1). At several stations, sampling for other analyses took precedent and insufficient water was available to collect a sample for phosphate analysis. Water samples were thawed at room temperature, dissolved phosphate was pre-concentrated using magnesium induced co-precipitation (MAGIC), and then quantified using a molybdate-blue colorimetric method (Karl and Tien 1992).

2.3. Respiration rates

Respiration rates for whole seawater were determined using bottle incubations where oxygen concentrations were tracked over approximately 36 h. Incubations were conducted in triplicate at three stations outside the slick and three stations within the slick; incubations amended with nutrients were also conducted (section 2.8). In addition, a separate set of triplicate six-day incubations was conducted in parallel with incubations tracking hydrocarbons (section 2.4). Glass biological oxygen demand bottles were equipped with oxygen optode spot minisensors (PreSens) that were glued to the inside surface of the bottle using food-quality silicone cement. Prior to use on our cruise, bottles were soaked for nearly three months in distilled water that was exchanged every few weeks (in anticipation of a different, previously scheduled cruise). Immediately before the R/V *Endeavor* cruise, the bottles were rinsed three times with methanol, three times with 5% hydrochloric acid, and three times with 18 MΩ Milli-Q water. The bottles were then rinsed three times with sample seawater immediately prior to use. This rinsing routine was repeated at sea between each set of incubations. The incubations were initiated by transferring seawater to the glass bottles using standard gas sampling techniques. These bottles were then placed in a shaking incubator that was maintained at *in situ* temperatures in the dark. The oxygen minisensors were then monitored every few hours over the course of approximately 36 h using the manufacturer's Minisensor Meter (PreSens). To eliminate temperature changes that impact sensor stability, the bottles were left in the incubator with the lid closed and the fiber optic lead from the minisensor meter was threaded through a small hole in the incubator lid. Using this technique, we could confidently measure respiration rates of $<1 \mu\text{mol O}_2 \text{ l}^{-1} \text{ d}^{-1}$. At the beginning of the cruise, each sensor spot was calibrated and sensor stability was assessed over a 36 h incubation with Milli-Q water to assure drift of $<0.1 \mu\text{mol O}_2 \text{ l}^{-1} \text{ d}^{-1}$.

2.4. Hydrocarbon degradation incubations

At station I-1, a longer-term incubation was conducted in order to constrain hydrocarbon degradation (table 1). We filled four 1 liter Teflon bottles with seawater, and three of these were immediately poisoned with mercuric chloride in order to afford replicate characterizations of the surface

slick. The remaining incubation was allowed to incubate for six days in the dark at approximately *in situ* temperatures before also being poisoned with mercuric chloride. Upon return to the laboratory, all four sample bottles were extracted with dichloromethane and analyzed with an Agilent 6890 gas chromatograph (GC) equipped with a flame ionization detector using standard protocols (Lemkau *et al* 2010). Since many compounds in oil are not amenable to GC analysis, the concentrations (mg l^{-1}) we report are effectively minimum estimates. Hence, we integrated and measured the total petroleum hydrocarbon signal as well as individual compounds such as octadecane (*n*-C₁₈) and phytane that are used as indicators of biodegradation. The degradation rate of total hydrocarbons ($\text{mg l}^{-1} \text{ d}^{-1}$) was converted to oxygen units ($\mu\text{mol O}_2 \text{ l}^{-1} \text{ d}^{-1}$) as described (Camilli *et al* 2010, Reddy *et al* 2011). Parallel six-day incubations for total respiration were also conducted, as described in section 2.3.

2.5. Enzymatic activity

Microbial alkaline phosphatase and lipase activities were assayed by observing the hydrolysis product of the commonly used fluorogenic substrates 4-methylumbelliferyl (MUF)-phosphate and MUF-butyrate (Sigma-Aldrich) in seawater incubations (Hoppe 1993). Aliquots of whole seawater were placed in 96-well plates, and the fluorescent substrates were added to initiate the assays. Fluorescence was measured at regular intervals with a CytoFluor Series 4000 multi-well plate reader, and increased linearly with time as expected. Using standard MUF additions and an external standard curve, fluorescence readings were converted to concentration units. Enzyme hydrolysis rates were calculated from the linear increase in MUF concentrations over time during the assays. The saturating concentrations of the enzyme substrates were determined at station O-2, and found to be $25 \mu\text{mol l}^{-1}$; this concentration was used in all assays conducted at all of the other stations. Enzyme activity was assessed at three stations within the slick and three stations outside the slick (table 1).

2.6. Polar membrane lipid concentrations

One liter seawater samples were filtered through a $0.22 \mu\text{m}$ pore size Durapore (Millipore) membrane filter under gentle vacuum. The filters were snap-frozen in liquid nitrogen and transported back to the laboratory in a cryogenic dry shipper. One sample was taken at three stations outside the slick, and five stations within the slick. In addition, samples for polar membrane lipids were collected from the amendment experiments conducted at station I-4 (section 2.8; table 1). Lipids were extracted from the filters using a modified Bligh and Dyer method as described (Van Mooy and Fredricks 2010). Phospholipid (phosphatidylglycerol, PG; phosphatidylethanolamine, PE; and phosphatidylcholine, PC) and sulfolipid concentrations (sulfoquinovosyldiacylglycerol, SQDG) were analyzed by high performance liquid chromatography/mass spectrometry (HPLC/MS) using a Hewlett Packard 1100 HPLC coupled to a Thermo TSQ triple quadrupole mass spectrometer as described (Pependorf *et al* 2011).

2.7. Cell counts

One milliliter seawater samples were collected in polypropylene cryovials and preserved with 3.7% filtered formaldehyde. After standing for 15 min at room temperature, the samples were snap-frozen in liquid nitrogen and transported back to the laboratory in a cryogenic dry shipper. The samples were defrosted, stained with SYBR Green (Molecular Probes) in triplicate, and cells were counted using a Guava 5HT (Millipore) flow cytometer as described (Tripp 2008). Bacterial cells and *Synechococcus* cells were differentiated and counted using plots of side scatter versus yellow fluorescence and red fluorescence. Samples for cell counts were taken at five stations outside the slick, three stations within the slick, and from two amendment experiments (section 2.8; table 1).

2.8. Amendment experiments

At stations I-1, I-4, and I-5, parallel sets of triplicate respiration incubations (section 2.3) were amended with inorganic nutrients. Inorganic nutrient amendments were made from concentrated stocks of ammonium nitrate (NH_4NO_3) and dipotassium phosphate (K_2HPO_4) dissolved in high purity water (HPLC grade, Sigma); final concentrations in the incubations were $32 \mu\text{mol l}^{-1} \text{NH}_4\text{NO}_3$ and $2 \mu\text{mol l}^{-1} \text{K}_2\text{HPO}_4$.

2.9. Analysis of slick area from satellite data

The area of the Gulf of Mexico covered by the *Deepwater Horizon* surface slick was estimated from publicly available satellite derived surface oil analysis products. Daily Experimental Marine Pollution Surveillance Reports (EMPSRs) produced by the National Environmental Satellite, Data, and Information service of the National Oceanic and Atmospheric Administration were downloaded as JPEG images for the dates 19 June to 28 June (www.ssd.noaa.gov/PS/MPS/deepwater.html). The EMPSRs had red pixels delineating the extent of the oil slick on each day. A mask covering the spatial extent of the red pixels was calculated in MATLAB (Mathworks). The area of the mask was computed using the 'bwarea' command from the image processing toolbox in MATLAB. We then scaled the number of red pixels in each image to the number of total pixels in a 1° latitude by 1° longitude square (approximately $10\,800 \text{ km}^2$ at 29°N) within each image, and thereby calculated the area of the slick.

2.10. Statistical analyses

Non-parametric statistical tests were conducted using Statistica software (version 6.1; StatSoft). The Mann–Whitney test was chosen for comparisons because the number of data points in compared cases was generally small ($n \leq 5$). Differences were deemed significant when p-levels returned by Statistica were < 0.05 .

3. Results and discussion

3.1. Phosphorus stress

Phosphate was scarce at all of the stations where phosphate was measured, and there was no difference in concentrations

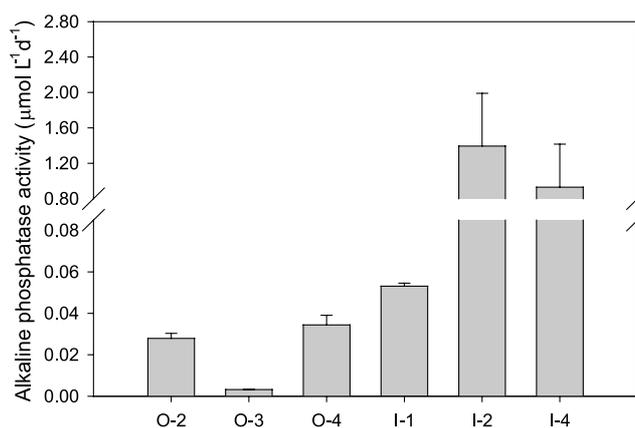


Figure 1. Alkaline phosphatase activity at sampling stations within the slick and outside of the slick. Error bars indicate the magnitude of the range of analytical triplicates. Data from inside the slick and outside the slick are statistically distinct (Mann–Whitney, $p < 0.05$).

between stations inside and outside of the slick (Mann–Whitney, $p \geq 0.05$, $n_{\text{in}} = 3$, $n_{\text{out}} = 4$; table 1). Concentrations were on the order of 10 nmol l^{-1} and were similar to those observed in the northern Sargasso Sea (Cavender-Bares *et al* 2001, Ammerman *et al* 2003), an ecosystem where the growth of bacteria has been shown to be phosphate limited (Cotner *et al* 1997). However, alkaline phosphatase activity was significantly higher within the slick (Mann–Whitney, $p < 0.05$, $n_{\text{in}} = 3$, $n_{\text{out}} = 3$; figure 1). Alkaline phosphatases are enzymes used by marine microbes in an attempt to access dissolved organic phosphorus when phosphate is scarce. Elevated rates of alkaline phosphatase activity are generally interpreted as an indication of enhanced phosphorus stress of marine microbes (Perry 1972, Ammerman *et al* 2003, Sylvan *et al* 2007, Dyhrman *et al* 2007 and references therein). Thus our observations show that microbes within the slick were more stressed than microbes outside of the slick; this was presumably due to enhanced phosphorus demand being driven by the availability of organic carbon from oil within the slick.

3.2. Microbial respiration and enzymatic activity

We observed that microbial respiration rates and enzyme activities varied considerably over relatively short spatial scales ($< 1 \text{ km}$) near the *Deepwater Horizon* site. Community respiration rates were significantly higher within the oil slick compared to outside the slick (Mann–Whitney, $p < 0.05$, $n_{\text{in}} = 4$, $n_{\text{out}} = 3$; figure 2), indicating enhanced oxidation of organic carbon. A study conducted in June 1993 found that the mean respiration rate observed in this region of the Gulf of Mexico ranged from 2.4 to $4.3 \mu\text{mol O}_2 \text{ l}^{-1} \text{d}^{-1}$ (Pomeroy *et al* 1995), which is very similar to the mean rate of $2.2 \mu\text{mol O}_2 \text{ l}^{-1} \text{d}^{-1}$ (range 0.8 – $4.4 \mu\text{mol O}_2 \text{ l}^{-1}$) that we found at stations outside of the slick. Thus we can reasonably assert that the stations outside of the slick reflected pristine conditions (i.e. similar to conditions before the spill). However, respiration rates inside the slick averaged $12.1 \mu\text{mol O}_2 \text{ l}^{-1} \text{d}^{-1}$, (range 4.8 – $16.3 \mu\text{mol O}_2 \text{ l}^{-1}$), which

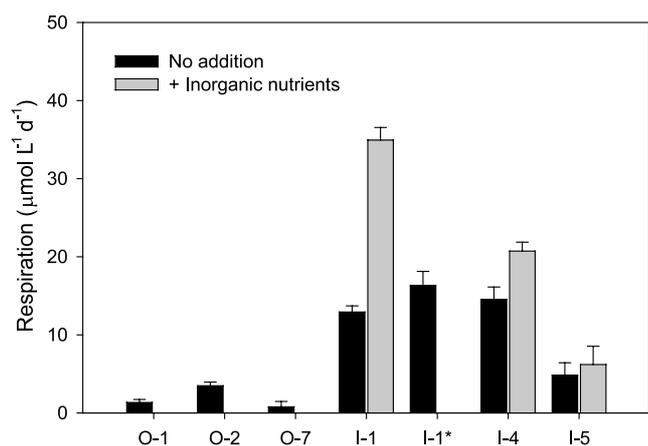


Figure 2. Community respiration rates at sampling stations within the slick and outside of the slick after incubations of approximately 36 h; except for 'I-1*', which was incubated for six days in parallel with the hydrocarbon degradation experiment. Parallel sets of incubations at stations within the slick were amended with inorganic nutrients. Error bars indicate the magnitude of the range of respiration rates for triplicate incubations. Data from inside the slick and outside the slick are statistically distinct (Mann–Whitney, $p < 0.05$).

is approximately five times greater than the respiration rates outside of the slick. Thus the presence of oil stimulated an enhanced respiration rate of $9.9 \mu\text{mol O}_2 \text{ l}^{-1} \text{ d}^{-1}$ (figure 2). It should be noted that we do not believe that this enhanced respiration is an artifact of the slightly elevated temperature within the slick (table 1). Published temperature dependence relationships for bacterial respiration (Q_{10} of approximately 2.3 (Lomas *et al* 2002)) would predict a rate increase of only 11% in response to the 1.2°C average temperature elevation; the difference in average rates we observed was 455%. The respiration data support the conclusion that oil from the Macondo well served as an organic carbon substrate for respiration by microbes in surface water, and that these microbes responded despite intense phosphorus stress.

The enhanced respiration rates inside the slick suggest that the microbes rapidly acclimated to breaking down hydrocarbon substrates. This is consistent with other studies showing that deep-sea microbes within the subsurface plume were also able to readily respond to the addition of hydrocarbons to their environment (Hazen *et al* 2010, Valentine *et al* 2010, Kessler *et al* 2011). Indeed, we observed a decrease in the $n\text{-C}_{18}$ /phytane ratio in our degradation experiments from 2.2 to 1.5, which is a hallmark of natural microbial degradation (e.g. De Jonge *et al* 1997). We also found that approximately 0.4 mg l^{-1} of the observed 1.9 mg l^{-1} total petroleum hydrocarbons were degraded over the course of six days. The absolute degradation rate of $0.07 \text{ mg l}^{-1} \text{ d}^{-1}$ yields a steady-state hydrocarbon turnover rate of 0.04 d^{-1} ($0.07 \text{ mg l}^{-1} \text{ d}^{-1}$ divided by 1.9 mg l^{-1}). This turnover rate is slower than reported in the deep-sea plume (Hazen *et al* 2010) but nonetheless shows a half-life of hydrocarbons of only 26 days with respect to microbial degradation. Clearly, additional factors (e.g. evaporation, photo-oxidation, physical dispersion) contributed to shortening the overall half-life of the slicks.

Converting the hydrocarbon degradation rate to units of oxygen equates to $7.1 \mu\text{mol O}_2 \text{ l}^{-1} \text{ d}^{-1}$, which corresponds to 71% of the enhanced respiration rate. Given that our hydrocarbon concentrations are minimum estimates of the concentration of total oil in the water and in contact with microbes, it is remarkable that the reported hydrocarbon degradation rate accounted for such a large fraction of the enhanced respiration.

Although our data are limited to the specific time and location of our study, extrapolating the excess rates of respiration within the slick has the potential to yield much-needed constraints on the total potential rates at which microbes degraded oil released to surface waters from the *Deepwater Horizon* spill. Our estimates of the size of the slick on the days during our cruise ranged from 5600 to 24 000 km^2 . Given an excess respiration rate of $9.9 \mu\text{mol O}_2 \text{ l}^{-1} \text{ d}^{-1}$ and a mixed layer depth of 15 m, we calculate that the total microbial hydrocarbon degradation capacity across the surface slick ranged from approximately 6×10^8 to $3 \times 10^9 \text{ mol C d}^{-1}$. This calculation assumes that the hydrocarbon degradation rate remained constant with time, which was probably not the case as microbes are known to preferentially degrade more labile hydrocarbons first, leaving more recalcitrant polar molecules behind. However, the rate of oxygen drawdown (i.e. respiration) remained linear over the course of the six-day hydrocarbon degradation experiment (ordinary least squares linear regression, $R^2 = 0.99$, $n = 35$; not shown). Given a mean flow rate from the Macondo well of 62 000 barrels d^{-1} (McNutt *et al* 2011) and a carbon content of $1.1 \times 10^5 \text{ g C barrel}^{-1}$ (Reddy *et al* 2011), the total flux of carbon from the well was also on the order of $6 \times 10^8 \text{ mol C d}^{-1}$, although some oil components dissolved at depth before reaching the surface (Camilli *et al* 2010, Reddy *et al* 2011). Nonetheless, our calculations suggest that the microbes in the surface waters of the Gulf of Mexico possessed the metabolic potential to degrade oil at a rate that was of the same order as the delivery rate of oil. Of course other competing processes (e.g. evaporation, photo-oxidation, physical dispersion) would also have contributed to the removal of oil from the surface slicks, and thus it is unlikely that this potential was completely realized.

Lipase activity was also enhanced inside the slick (Mann–Whitney, $p < 0.05$, $n_{\text{in}} = 3$, $n_{\text{out}} = 3$; figure 3) suggesting that the population of microbes within the slick had enzymatically acclimated to hydrolyze ester bonds within lipids. There are few ester linkages in crude oil, and therefore high lipase activity does not necessarily indicate a response to or degradation of crude oil. It is conceivable that the lipase activity could have been a response to the ester linkages in dispersant molecules. Dioctyl sodium sulfosuccinate (DOSS) contains multiple ester bonds and is a chemical component of the dispersants used in response to the *Deepwater Horizon* oil spill. It has been shown that dispersants can be utilized as a sole carbon source by marine bacterial communities (Mulkins-Phillips and Stewart 1974). Additionally, a predecessor to modern dispersants, Corexit 8666, was shown to support enhanced growth of marine bacteria compared to un-amended controls (Mulkins-Phillips and Stewart 1974). Therefore, it is possible that the enhanced respiration that was not accounted

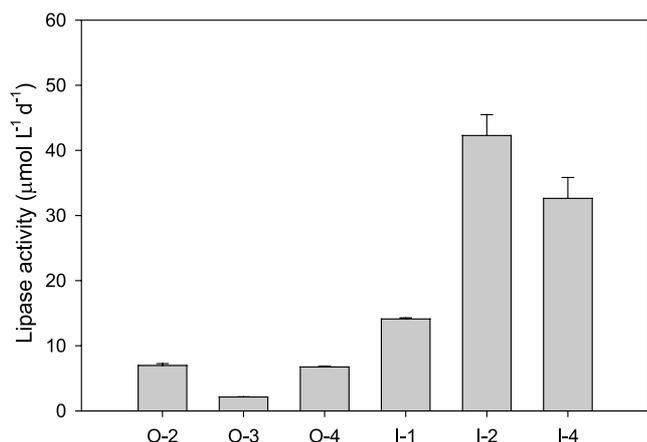


Figure 3. Lipase activity at sampling stations within the slick and outside of the slick. Error bars indicate the magnitude of the range of analytical triplicates. Data from inside the slick and outside the slick are statistically distinct (Mann–Whitney, $p < 0.05$).

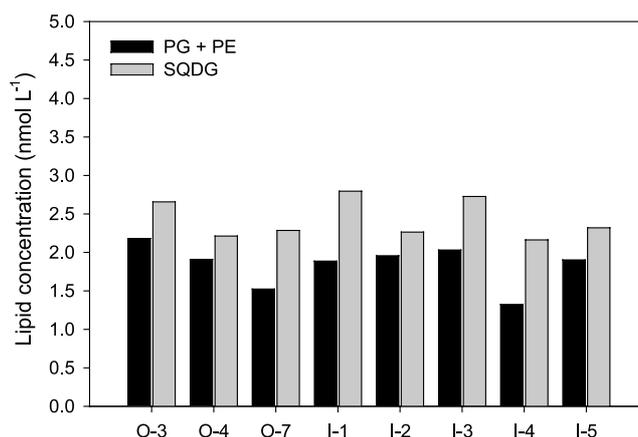


Figure 4. Polar membrane lipid concentrations. Phosphatidylglycerol and phosphatidylethanolamine (PG + PE) are thought to be derived primarily from heterotrophic bacteria, while sulfoquinovosyldiacylglycerol (SQDG) is thought to be derived from the membranes of cyanobacteria and eukaryotic phytoplankton. Results are from one analysis of surface waters at each station.

for by hydrocarbon degradation could have been partially supported by oxidation of dispersants.

Kujawinski *et al* (2011) detected DOSS in surface waters at a station near the *Deepwater Horizon* site, but the goal of their study was not to rigorously map DOSS concentrations within the surface slick. So although data compiled by Kujawinski *et al* (2011) indicate that dispersants were present in surface waters in June 2010, it is not absolutely certain that the slicks we sampled had been recently treated with dispersants. Nonetheless, if a total of 5.3×10^6 l (Kujawinski *et al* 2011) of dispersant was applied to the sea surface over the course of the spill (84 days), then we can estimate the approximate carbon flux from dispersants. For Corexit 9500A, Kujawinski *et al* (2011) reported a density of 1.0 kg l^{-1} and a DOSS content of 10% (DOSS is 54% carbon; g C (g DOSS)^{-1}). The exact formulations of the dispersants are proprietary information, and the carbon contents are unknown. However, if we assume that the remaining 90% was composed of equal parts light petroleum distillates (approximately 80% carbon) and sorbitan (40% carbon) then the carbon content of the bulk dispersant liquid was approximately 60% or 0.6 kg C l^{-1} . This brings the delivery of carbon from dispersants to approximately $3 \times 10^6 \text{ mol C d}^{-1}$, which is small compared to the flux of oil. Nonetheless, it is possible that the high lipase activities we observed were local responses to the introduction of dispersants to surface waters.

3.3. Microbial biomass and abundance

Despite enhanced respiration, lipase activity and relatively rapid oil degradation within the slick, we did not observe greater concentrations of microbial polar membrane lipids within the oil slick (Mann–Whitney, $p \geq 0.05$, $n_{\text{in}} = 5$, $n_{\text{out}} = 3$; figure 4). In the surface ocean, PG and PE are the major constituents of heterotrophic bacterial membranes and SQDG is the dominant polar membrane lipid found in the photosynthetic membranes of cyanobacteria and other phytoplankton (Oliver and Colwell 1973, Van Mooy *et al* 2006,

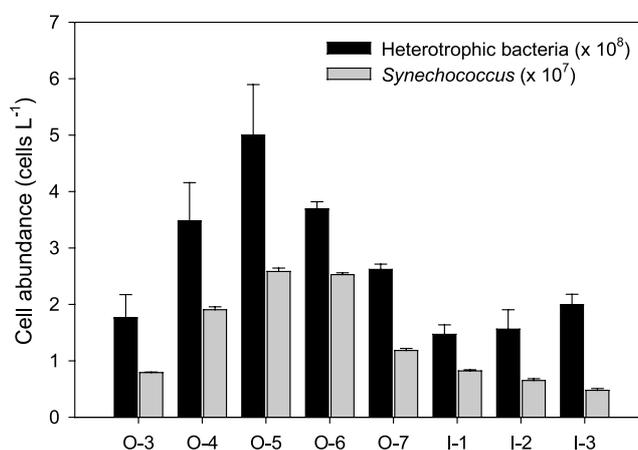


Figure 5. Heterotrophic bacteria and *Synechococcus* cell abundances at stations O-3 through O-7 outside of the slick and at stations I-1 through I-3 inside of the slick. Error bars are the magnitude of the range of analytical triplicates.

2009, Van Mooy and Fredricks 2010). While sulfolipids were only recently identified in the surface ocean, phospholipids have long been applied as a proxy for microbial biomass (e.g. White *et al* 1979). Our data suggest that both bacterial and phytoplankton biomass were relatively constant throughout our study area regardless of the presence of oil slicks (figure 4).

In contrast to polar membrane lipids, cell abundances of heterotrophic bacteria and the cyanobacteria of the genus *Synechococcus* were about a factor of two lower inside the slick than outside the slick (Mann–Whitney, $p < 0.05$, $n_{\text{in}} = 3$, $n_{\text{out}} = 5$; figure 5). But we suggest that this reflects a bias introduced during sampling. The difference in microbial abundances could be explained by a mechanism where cells became adsorbed to oil flocs (Hazen *et al* 2010), and these oil flocs adsorbed to the sides of the polypropylene vials used to store the flow cytometry samples, effectively excluding oil-adsorbed cells from counting. The tight correlation between the abundances of heterotrophic

bacterial cells and *Synechococcus* cells (Spearman Rank, $R^2 = 0.6173$, $p < 0.05$, $n = 16$; not shown) suggests that the adsorption of cells was a largely indiscriminate process. The polar membrane lipid analyses were conducted from samples filtered at sea and would have included both free-living microbes and microbes adsorbed to oil flocs. If biomass estimates from polar membrane lipids provide a more faithful indication of microbial abundance than flow cytometry, then our observations of consistent microbial standing stocks inside and outside of the slicks agree with studies of the 1979 *IXTOC I* oil spill in the Bay of Campeche (Atlas *et al* 1980) and the 2002 *Prestige* oil spill off the coast of Portugal (Bode *et al* 2006), where there were no differences in pre- and post-spill bacterial abundances. An alternative explanation is that since polar membrane lipids were similar inside and outside the slick, the abundance data are indicating that there was more biomass per cell inside the slick than outside the slick (i.e. the cells inside the slick were larger). However, the differences in median side scatter and green fluorescence of microbial cells inside and outside of the slick were $<10\%$ (data not shown), indicating that there were no major differences in the distribution of cell size or DNA content of the microbial community. The DNA content of microbial cells as revealed by flow cytometry has shown to be correlated with microbial growth rate (Longnecker *et al* 2006). Thus our available evidence indicates that while microbial respiration and enzyme activity were enhanced within the slick, microbial growth appeared to be arrested within the slick.

3.4. Controls on microbial activity, abundance and biomass within surface slicks

The lack of increased microbial numbers or biomass within the slick indicates that the enhanced respiration, alkaline phosphatase activity, and lipase activity in the slick were in effect ‘futile’ attempts by microbes to grow in response to the injection of organic matter into their environment. We propose two possible mechanisms for this futile microbial activity within the slicks: (1) growth rates were limited by nutrients other than organic carbon, or (2) respiration fueled an increase in microbial growth but top-down processes suppressed the accumulation of microbial stocks. It should be noted that a review by Gasol *et al* (2002) concluded that top-down control of bacterial communities was more prevalent than nutrient limitation in oligotrophic settings. However, the oil introduced by the *Deepwater Horizon* spill resulted in an unprecedentedly perturbed system—phosphate depleted but with an abundance of organic carbon—where conditions differed markedly from typical offshore oligotrophic settings.

3.4.1. Nutrient limitation of microbial growth within slicks.

As mentioned previously, bacterial growth and respiration in the central Gulf of Mexico were observed to be primarily limited by phosphate availability (Pomeroy *et al* 1995). Therefore, we focused on studying the impact of inorganic nutrient amendments on the microbial degradation of oil within the slicks. We observed a distinct increase in respiration rates in nutrient amendment experiments at two of the three

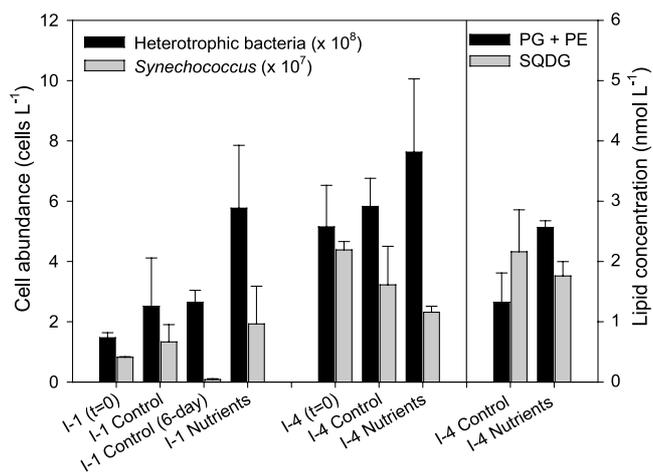


Figure 6. Heterotrophic bacterial cell abundance and *Synechococcus* cell abundance, for inorganic amendment experiments conducted at stations I-1 and I-4. Bacterial polar membrane lipid concentrations inorganic amendment experiments were determined at station I-4 only. The ‘t = 0’ results are from the seawater used to set up the incubations immediately prior to dispensing into replicate incubation bottles. The ‘control’ and ‘nutrients’ results are from incubations conducted for 36 h of incubation. The ‘(6-day)’ results are from incubations conducted in parallel with the hydrocarbon degradation experiment. Error bars are the magnitude of the range of triplicate incubations.

amendment stations within the surface slick (station I-1 and I-4; Mann–Whitney, $p < 0.05$; figure 2). At station I-1, we observed that respiration rates approximately tripled in response to inorganic nutrients (Mann–Whitney, $p < 0.05$). At station I-4, the rate of respiration increased by 42% in the inorganic nutrient treatments compared to the control (Mann–Whitney, $p < 0.05$). The increase in respiration in response to nutrient amendments is consistent with Atlas’ (1981) conclusion from the review of sixteen different studies that the addition of N and P to oil contaminated seawater yielded faster degradation rates of hydrocarbons.

The abundance of heterotrophic bacteria more than doubled in response to nutrients at station I-1, while at station I-4 there was no significant increase (figure 6). However, polar membrane lipids were quantified at station I-4, and nutrient amendments resulted in a 93% increase in the concentration of PG + PE compared to the control (Mann–Whitney, $p < 0.05$; figure 6). Overall, these results suggest that growth of heterotrophic bacteria, either in terms of abundance or biomass, was limited by the availability of nutrients. As might be expected in incubations conducted in the absence of sunlight, the concentration of *Synechococcus* did not increase significantly in response to nutrient amendments, nor did the concentration of SQDG (figure 6).

The additional physiological demands associated with surviving under conditions of nutrient stress (e.g. expression of alkaline phosphatase enzymes) are energetically expensive and have the potential to drive down bacterial growth efficiency and increase the cellular carbon demand (Teixeira-de-Mattos and Neijssel 1997, Del Giorgio and Cole 1998). Such an increase in maintenance energy could account for the enhanced

respiration rates within the slick, which occurred in the absence of increased microbial standing stocks.

3.4.2. Grazing control of microbial standing stocks within slicks. Although past studies and our own amendment incubations indicate that microbial growth was probably limited by inorganic nutrients within the slick, we did not have the opportunity to actually measure growth rates; implementing the radiotracer methods used to make bacterial production (BP) measurements was not possible within the time and logistical constraints of rapidly mobilizing for our research at sea. Our flow cytometry results provide some evidence that growth rates were not enhanced in the slick. Yet we cannot unequivocally exclude the possibility that enhanced microbial growth was simply being checked by enhanced top-down controls (i.e. mortality) within the slick, which prevented the accumulation of microbial standing stocks.

Grazing and viral lysis are important top-down control mechanisms in microbial ecosystems (Proctor and Fuhrman 1990, Gasol *et al* 2002), but grazing often outweighs viral lysis as the dominant mechanism of microbial mortality in oligotrophic systems (Guixa-Boixereu *et al* 1999). Indeed, a study that traced the carbon isotopic signatures of the planktonic food web in the Gulf of Mexico after the *Deepwater Horizon* spill concluded that there was trophic transfer of carbon from oil to bacteria to higher levels in the food web (Graham *et al* 2010). In addition, previous research on the response of the microbial community to the *Prestige* oil spill off the coast of Spain suggests that grazing control resulted in an increase in bacterial production and respiration within oil contaminated waters without a subsequent increase in bacterial biomass (Bode *et al* 2006). However, it must be noted that these two studies were conducted in nearshore environments that were unlikely to have been as nutrient depleted as waters we studied in proximity to the *Deepwater Horizon* site.

4. Conclusions

Microbes within the surface slick showed higher rates of alkaline phosphatase activity, indicating enhanced phosphate stress. Despite this, rates of microbial respiration and lipase activity were also higher within the slick, and the degradation of hydrocarbons was fairly rapid and supported the majority of respiration. However, we observed no increase in microbial abundances or biomass within the slick. Amendments with inorganic nutrients led to increased respiration and bacterial biomass, which suggests that microbial growth was limited by nutrients as we had hypothesized and had been observed previously (Pomeroy *et al* 1995).

Our observations suggest that the microbial community possessed the potential to respire hydrocarbons at an unprecedented rate for the oligotrophic Gulf of Mexico; our estimate suggests that this rate was potentially great enough to keep pace with the flux of oil reaching the surface from the Macondo well. Given our proximity to the *Deepwater Horizon* site it is likely that the oil, and possibly dispersants, had been introduced to the surface community only shortly before we sampled the water and made our observations. Thus,

the observed differences in microbial respiration and activity between stations within the slick and outside the slick is a testament to the rapid response of the microbes in the surface waters of the Gulf of Mexico to oil from the *Deepwater Horizon* spill.

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