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## Nutrient Depletion as a Proxy for Microbial Growth in Deepwater Horizon Subsurface Oil/Gas Plumes

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# Nutrient depletion as a proxy for microbial growth in Deepwater Horizon subsurface oil/gas plumes

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#### Abstract

The Deepwater Horizon accident resulted in a substantial uncontrolled hydrocarbon release to the northern Gulf of Mexico, much of which was entrained in deep submerged plumes. While bio-degradation of the hydrocarbons has been inferred from microbial biomass and genetics, the amount of conversion of oil and gas carbon to biomass remains uncertain having only been estimated in modeling studies. Here we examine correlated depletions of nitrate, phosphate and oxygen in the submerged plumes and conclude that a substantial portion of hydrocarbons in these plumes was converted to biomass ( $0.8-2 \times 10^{10}$  mol C). This contrasts with nutrient-limited surface waters where other work has suggested hydrocarbon-induced microbial growth to have been minimal. Our results suggest the need for better monitoring of changes in nutrients as well as study of nutrient recycling in similar future hydrocarbon releases.

Keywords: Deepwater Horizon, hydrocarbon degradation, Gulf of Mexico, oil spill, nutrients, oxygen

S Online supplementary data available from [stacks.iop.org/ERL/7/045301/mmedia](http://stacks.iop.org/ERL/7/045301/mmedia)

#### 1. Introduction

The Deepwater Horizon blowout resulted in a release of ∼5× 10<sup>6</sup> bbl of crude oil (McNutt *et al* [2011\)](#page-5-0) and ∼10<sup>10</sup> moles of methane (Kessler *et al* [2011,](#page-5-1) Reddy *et al* [2011\)](#page-5-2) in the northern Gulf of Mexico. Essentially all of the methane and much of the oil entered deep water plumes (McNutt *et al* [2011\)](#page-5-0). These hydrocarbons constituted a pool of potentially oxygen-depleting organic matter (Joye *et al* [2011\)](#page-5-3), substrate for microbial growth (Hazen *et al* [2010,](#page-5-4) Redmond and Valentine [2011\)](#page-5-5), and an injection of toxic materials such as polycyclic aromatic hydrocarbons (Diercks *et al* [2010\)](#page-5-6). Based

on dissolved oxygen and methane distributions, Kessler *et al* [\(2011\)](#page-5-1) found that the plume hydrocarbons were primarily consumed by the microbial community. Implicit is that the hydrocarbon release stimulated a substantial deep water microbial bloom (Hazen *et al* [2010,](#page-5-4) Redmond and Valentine [2011,](#page-5-5) Kessler *et al* [2011\)](#page-5-1). A physical-metabolic model of the release (Valentine *et al* [2012\)](#page-5-7) yielded an estimate of the microbial bloom of  $10^{11}$  g C, equivalent to about half of the carbon injected into the Gulf during the blowout. And, an oxygen-based calculation yielded a similar estimate (Du and Kessler [2012\)](#page-5-8). Such estimates are important because they help provide an understanding of the fate of the hydrocarbons, including both the rapidity with which the methane disappeared (Kessler *et al* [2011\)](#page-5-1) as well as in what forms and to which geochemical reservoirs the released hydrocarbons were delivered. Herein, we examine nutrient distributions in deep waters in the vicinity of the wellhead

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<span id="page-3-0"></span>

Figure 1. Dissolved nutrient and oxygen profiles in four cruises to the vicinity of the Deepwater Horizon blowout site. For the May and October 2010 cruises, profiles were constructed from samples collected at different locations around the wellhead.

as a means of providing another estimate of how much the hydrocarbon consumption supported growth rather than just respiration to carbon dioxide.

#### 2. Methods and materials

Water sampling in the vicinity of the Deepwater Horizon blowout was conducted aboard the R/V *Pelican* (10–14 May 2010), R/V *F.G. Walton Smith* (26 May–1 June 2010), and R/V *Cape Hatteras* (11–20 October 2010 and 20–29 October 2011) using each ship's rosette-mounted Niskin bottles. During the first two cruises, samples were collected during multiple hydrocasts in a southwesterly trend within 27 km of the wellhead. During October 2010, samples were also collected during multiple hydrocasts, this time in all directions and ranging to over 100 km away from the wellhead. For October 2011, the data are from one hydrocast at a station within 5 km of the wellhead. Station locations and hydrocast data are listed in the supplementary data tables (available at [stacks.iop.org/ERL/7/045301/mmedia\)](http://stacks.iop.org/ERL/7/045301/mmedia).

Samples were filtered (0.45  $\mu$ m) and frozen (−20 °C) until analysis. For the first three cruises, nutrient analyses were performed at USM using an Astoria-Pacific A2+2 nutrient auto-analyzer (Astoria-Pacific International, OR, USA). For samples from October 2011, nutrients were analyzed by auto-analyzer at the Geochemical and Environmental Research Group (GERG; Texas A&M University). A subset of frozen samples from the first three cruises was also analyzed at the same time at GERG to make sure that results from all four cruises were properly intercalibrated. Dissolved oxygen data were taken from the ship's *in situ* oxygen sensor mounted on the rosette sampling system. Oxygen calibrations were performed by standard Winkler titrations. Failure of the titration system during the late May 2010 cruise probably accounts for the slight  $(<10 \mu$ mol kg<sup>-1</sup>) overall negative shift of those oxygen data relative to October 2011, but did not affect the slope of the oxygen–nutrient relationship, which was nearly identical in the 2 May 2010 cruises despite the data being obtained at different times and aboard different ships.

#### 3. Results and discussion

During the May 2010 cruises, profiles of *in situ* fluorescence and beam attenuation showed peaks in the depth range of 600–1300 m, which were subsequently identified as subsurface plumes of oil and gas (e.g., Diercks *et al* [2010,](#page-5-6) Camilli *et al* [2010\)](#page-5-9). We observed slight decreases in dissolved nitrate and phosphate as well as oxygen in this depth range of the subsurface plumes during the 2 May 2010 cruises (figure [1\)](#page-3-0). In contrast, the two post-blowout cruises (October 2010 and 2011) showed no evidence of submerged oil/gas plumes and likewise did not show the nutrient or oxygen decreases (figure [1\)](#page-3-0). Nutrient and oxygen values for samples outside of the submerged plumes were comparable among all cruises. Also, for dissolved silica, no depletions were observed. Dissolved nitrite and ammonium concentrations were less than 1% of the nitrate in the May 2010 nutrient-depleted samples, indicating that nitrate decreases were not reflected in proportionate increases in other forms of dissolved inorganic nitrogen. Interestingly, although elevated levels of polycyclic aromatic hydrocarbons (PAHs) were found at plume depths, no correlation was found between PAH concentrations and nutrient depletions. All of these observations lend confidence to the interpretation of the nutrient depletions as not resulting from analytical or sampling artifacts, but rather from biological uptake. Our results can be contrasted with those of Hazen *et al* [\(2010\)](#page-5-4), who found a significant decrease in nitrate concentrations inside of submerged plume waters (mean decrease  $6 \mu M$ ) in late May 2010, but found no significant decrease in plume phosphate concentrations (mean decrease  $0.03 \mu M$ ).

We also examined other chemical parameters in the water samples (e.g., trace elements) to verify that the nutrient depletions were not caused by sample bottles accidentally tripping at a shallow depth. Pre/post-tripped bottles would not have affected the oxygen data since these were obtained from the CTD oxygen sensor. A few samples were eliminated from our dataset because all examined water parameters suggested that the Niskin bottle closed at a shallower depth than expected. In order to quantify the extent of the oxygen and nutrient depletions, profiles of oxygen and nutrients in the 600–1600 m depth range from October 2011 were fitted

<span id="page-4-1"></span>

Figure 2. Dissolved nutrient and oxygen anomalies in the subsurface near the Deepwater Horizon blowout site during two cruises in May 2010. (A) Oxygen anomaly versus depth. (B) Oxygen anomaly versus nitrate anomaly. (C) Phosphate anomaly versus nitrate anomaly.

<span id="page-4-3"></span>

Figure 3. Dissolved nitrite versus the nitrate anomaly in the subsurface near the Deepwater Horizon blowout site during May 2010.

<span id="page-4-0"></span>Table 1. Coefficients for third order polynomial fits to oxygen and nutrient data, Oct. 2011, 600–1600 m. Depth (*z*) in meters; concentrations in  $\mu$ M.

|        | Oxygen                   | Nitrate                  | Phosphate                 |
|--------|--------------------------|--------------------------|---------------------------|
| $z^3$  | $-8.6567 \times 10^{-8}$ | $1.6612 \times 10^{-8}$  | $-1.7679 \times 10^{-10}$ |
| $z^2$  | $2.1681 \times 10^{-4}$  | $-5.3416 \times 10^{-5}$ | $6.6621 \times 10^{-7}$   |
| Z      | $-5.5765 \times 10^{-2}$ | $4.6401 \times 10^{-2}$  | $-1.2431 \times 10^{-3}$  |
| Const. | $1.0662 \times 10^{2}$   | $1.7482 \times 10^{1}$   | $2.5665 \times 10^{0}$    |

to third order polynomials (table [1\)](#page-4-0) in order to estimate concentrations at depths that were not sampled in 2011. Thus, the May 2010 oxygen and nutrient anomalies are differences relative to the concentrations determined in the October 2011 profile.

Phosphate and nitrate anomalies had a positive correlation with the oxygen anomaly, and the phosphate and nitrate anomalies were also well correlated (figure [2\)](#page-4-1). Again, these observations suggest that the nutrients were removed together with the oxygen in the subsurface plume. The molar oxygen/nitrate anomaly ratio was approximately 8, while the N/P depletion ratio was 12–13 (table [2\)](#page-4-2). Scatter in the graphs of  $O_2$ , N and P anomalies makes it difficult to

<span id="page-4-2"></span>Table 2. Submerged plume oxygen and nutrient molar anomaly ratios determined during two cruises in the vicinity of the Deepwater Horizon wellhead during May 2010.

|                    | $O_2/NO_2^-$  | $NO_3^- / PO_4^{3-}$ | n  |
|--------------------|---------------|----------------------|----|
| 10–14 May 2010     | $7.7 \pm 1.9$ | $12.0 \pm 1.6$       | 7  |
| 26 May–1 June 2010 | $8.3 \pm 1.4$ | $12.8 \pm 0.7$       | 29 |

predict which could have theoretically been depleted to zero first (i.e., which was most limiting); however, our empirical relationships suggest that all three would have run out at nearly the same time. Nonetheless, given that reported oxygen depletions throughout the blowout period did not exceed ∼25% of ambient concentrations (e.g., Kessler *et al* [2011,](#page-5-1) Du and Kessler [2012\)](#page-5-8), it seems unlikely that anything close to N or P limitation would have occurred during or after the blowout. We also observed that the highest deep water values of nitrite were associated with the highest nitrate depletions (figure [3\)](#page-4-3), suggesting the possibility of nutrient recycling associated with the nutrient depletions.

Assuming that nutrient depletion dominantly reflects microbial growth, the correlated nutrient and oxygen depletions reported here provide a way to estimate the overall microbial production in the submerged oil/gas plumes. We do this by starting with the total integrated subsurface oxygen depletion from Du and Kessler [\(2012\)](#page-5-8), who estimated  $1.9 \text{ (+0.4)} \times 10^{10}$  mol of O<sub>2</sub> were respired in the plume during the blowout. Assuming our oxygen/nitrate depletion ratio (table [2;](#page-4-2) figure [2\)](#page-4-1) held for the entire blowout, then dividing that ratio into the integrated oxygen depletion yields a total blowout nitrate uptake of  $1.6-4.0 \times 10^9$  mol N. (The range in this estimate is determined by both the uncertainty in the oxygen depletion and the uncertainty in the  $O_2/NO_3^$ anomaly ratio.) We then multiply this result by a bacterial  $C/N$ molar ratio of 5 (Goldman *et al* [1987\)](#page-5-10), yielding 0.77–2.0 ×  $10^{10}$  mol microbial C produced. This is similar in magnitude to the  $10^{11}$  g C of bacterial productivity (i.e.,  $0.8 \times 10^{10}$  mol C) estimated in the modeling study of Valentine *et al* [\(2012\)](#page-5-7). To put these numbers into a local context, the estimated bacterial carbon production in the submerged oil/gas plume is about one tenth of the annual primary production associated

with the outflow of the Mississippi River (e.g., Lohrenz *et al* [1997,](#page-5-11) Chen *et al* [2000\)](#page-5-12). While the time, specific location, and areal scales of these two events are clearly different, the comparison nonetheless indicates that the bloom associated with the blowout was not outside of the range of organic matter fixation occurrences in the northern Gulf of Mexico. Toxic effects associated with the crude oil, however, are another matter.

While all of the numbers in our calculation have significant uncertainties, the result is similar to the total moles of C released in the blowout (2.0–2.4  $\times$  10<sup>10</sup> mol C: Kessler *et al* [2011\)](#page-5-1), supporting the notion of a massive bloom response (i.e.,  $66 \pm 33\%$  conversion of hydrocarbon-C to biomass C). Because we did not obtain samples during later stages of the blowout nor have nutrient data been reported as part of the government response [\(www.nodc.noaa.](http://www.nodc.noaa.gov/General/DeepwaterHorizon/support.html) [gov/General/DeepwaterHorizon/support.html\)](http://www.nodc.noaa.gov/General/DeepwaterHorizon/support.html), we cannot be certain that microbial growth occurred throughout the period of uncontrolled hydrocarbon release. This would suggest our estimate is an upper bound. We also have no information on the extent of nutrient recycling, which would result in our oxygen/nitrate depletion ratio underestimating the extent of nitrate uptake. And, we cannot state whether the sole C source for the bloom was hydrocarbons. However, methanotrophs are known to use methane as their sole carbon source, though not all species may be obligate in that regard (Dedysh *et al* [2005\)](#page-5-13). We do note that isotopic evidence from shallower nearshore regions affected by the blowout supports the hypothesis of transfer of hydrocarbon-C to biomass, at least in that environment (Graham *et al* [2010\)](#page-5-14). We conclude, therefore, that hydrocarbons in the subsurface plume were likely substantially converted to biomass. In contrast, in surface waters contaminated with Deepwater Horizon crude oil, Edwards *et al* [\(2011\)](#page-5-15) found evidence of hydrocarbon-supported respiration but with only limited microbial growth due to low nutrient concentrations.

The results presented here suggest the need for and utility of nutrient measurements in studies of similar future hydrocarbon releases. Such studies, including concentration measurements throughout the course of the event, as well as estimates of the extent of nutrient recycling, have the potential to shed light on the biological response to and the initial fate of the hydrocarbons.

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