Chemical dispersants can suppress the activity of natural oil-degrading microorganisms

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During the Deepwater Horizon oil well blowout in the Gulf of Mexico, the application of 7 million liters of chemical dispersants aimed to stimulate microbial crude oil degradation by increasing the bioavailability of oil compounds. However, the effects of dispersants on oil biodegradation rates are debated. In laboratory experiments, we simulated environmental conditions comparable to the hydrocarbon-rich, 1,100 m deep plume that formed during the Deepwater Horizon discharge. The presence of dispersant significantly altered the microbial community composition through selection for potential dispersant-degrading Colwellia, which also bloomed in situ in Gulf deep waters during the discharge. In contrast, oil addition to deepwater samples in the absence of dispersant stimulated growth of natural hydrocarbon-degrading Marinobacter.

In these deepwater microcosm experiments, dispersants did not enhance heterotrophic microbial activity or hydrocarbon oxidation rates. An experiment with surface seawater from an anthropogenically derived oil slick corroborated the deepwater microcosm results as inhibition of hydrocarbon turnover was observed in the presence of dispersants, suggesting that the microcosm findings are broadly applicable across marine habitats. Extrapolating this comprehensive dataset to real world scenarios questions whether dispersants stimulate microbial oil degradation in deep ocean waters and instead highlights that dispersants can exert a negative effect on microbial hydrocarbon degradation rates.

Significance

Oil spills are a significant source of hydrocarbon inputs into the ocean. In response to oil spills, chemical dispersants are applied to the oil-contaminated seawater to disperse surface slicks into smaller droplets that are presumed to be more bioavailable to microorganisms. We provide evidence that chemical dispersants applied to either deep water or surface water from the Gulf of Mexico did not stimulate oil biodegradation. Direct measurement of alkaline and aromatic hydrocarbon oxidation rates revealed either suppression or no stimulation of oil biodegradation in the presence of dispersants. However, dispersants affected microbial community composition and enriched bacterial populations with the ability to use dispersant-derived compounds as growth substrates, while oil-alone amendments enriched for natural hydrocarbon degraders.

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water-accommodated fraction, CEWAF) or a CEWAF with nutrients (CEWAF + nutrients) (SI Appendix) on Gulf deep-water microbial populations (SI Appendix, SI Text and Figs. S1 and S2). Experimental conditions (SI Appendix, Table S1) mimicked those prevailing in the DWH deep-water hydrocarbon plume (6–13, 18), the chemistry of which varied substantially over space and time (18). Amending samples with WAFs and CEWAFs assured that observed differences in microbial community composition and activity would be driven by compositional differences (e.g., the presence or absence of dispersants) in the dissolved organic carbon (DOC) pool rather than by differences in the bulk DOC concentration (26, 27). We developed an improved radiotracer method to directly quantify hydrocarbon oxidation rates. The microbial community composition was monitored over time using 16S rRNA amplicon sequencing. Dispersant application selected for specific microbial taxa and oligotypes with 16S rRNA gene sequences similar to those recovered in situ during the DWH discharge. Surprisingly, CEWAF (± nutrients) addition did not enhance microbial activity or microbial oil-degradation rates.

Results and Discussion

Dispersant Significantly Altered Microbial Community Composition. We hypothesized that dispersants would alter microbial community composition in the deepwater samples and that selection of one population over another would drive differences in hydrocarbon-degradation rates, altering the oil-degradation efficiency. We explored patterns in microbial abundance (Fig. 1A) using microscopy and community composition by Illumina paired-end sequencing of bacterial 16S rRNA gene amplicons (Fig. 1B). We resolved closely related bacterial taxa using oligotyping analysis (28) (Fig. 2 and SI Appendix, Fig. S3). We elucidated the ecological preference of specific taxa using statistical correspondence analysis (SI Appendix, Figs. S4–S8).

All dispersant-amended treatments showed ingrowth of Colwellia (SI Appendix, Fig. S4), a group containing both hydrocarbon and dispersant degraders (29). After 1 wk, the relative abundance of Colwellia increased from 1% to 26–43% in dispersant-only and CEWAF (± nutrients) treatments (Fig. 1B). In contrast, Colwellia was a minority (1–4%) in WAF treatments. Selective enrichment of Colwellia in dispersant-only treatments indicates that dispersant components served as growth substrates (29). The relative abundance of Colwellia oligotypes 01, 02, and 05 increased in dispersant treatments (Fig. 2 and SI Appendix, Fig. S5), whereas oligotypes 03 and 10 increased in treatments receiving oil only, underscoring the role of dispersants in driving variation in Colwellia taxa. Phylogenetic analysis of the 16S rRNA gene amplicons confirmed that these oligotypes were closely related to species detected in DWH plume samples in situ (9, 16, 18) (SI Appendix, Fig. S9), verifying the environmental relevance of these organisms during the DWH discharge.

The dominant microbial responder to WAF addition was Marinobacter, whose relative abundance increased from 2% to 42% after 4 wk (Fig. 1B). In contrast, Marinobacter comprised only 1–5% of all sequences. The correspondence analysis emphasized the dominance of Marinobacter in WAF samples (SI Appendix, Fig. S4) and the same Marinobacter oligotypes occurred across all treatments, illustrating that dispersants did not select for specific Marinobacter taxa, as was the case for Colwellia (SI Appendix, Fig. S3A). Marinobacter (SI Appendix, Fig. S10) degrade a wide variety of hydrocarbons, including pristane, hexadecane, octane, toluene, benzynes, and phenanthrene (30–32) and are likely dominant hydrocarbon degraders under natural conditions. However, their abundance clearly declined in the presence of dispersants. Whether...
Colwellia outcompetes Marinobacter or whether Marinobacter is inhibited by some component of Corexit 9500 or the CEWAF remains to be resolved (SI Appendix).

Like Marinobacter, the abundance of Cycloclasticus increased primarily in the WAF treatments, where their relative abundance increased from 12% to 23% after 1 wk and an oligotype (type 03) closely related to Cycloclasticus pugetii (SI Appendix, Figs. S3B and S11), which degrades naphthalene, phenanthrene, anthracene, and toluene as sole carbon sources (33), increased substantially. Cycloclasticus also increased slightly in relative abundance in the CEWAF + nutrients treatment (Fig. 1B), but less so than in the WAF treatment.

Oceaniserpentilla (also known as DWH Oceanospirillum) (34) abundance decreased consistently across treatment, regardless of the presence or absence of WAF, dispersant, or CEWAF (± nutrients) (Fig. 1B and SI Appendix, Figs. S3C and S8). The observed oligotypes closely resembled those observed in situ during the DWH incident (18) (SI Appendix, Fig. S12). The DWH Oceanospirillum oxidize n-alkanes and cycloalkanes (17); cycloalkanes are absent in surrogate Macondo oil, possibly explaining the low abundance of Oceanospirillum in the microcosms.

Cell Growth and Exopolymer Formation. Initially, cell abundance was similar across treatments (3 × 10^5 cells·mL^-1; Fig. 1A). At the experiment’s termination, microbial abundance in WAF treatments had increased by a factor of 60, which was significantly higher (T3, P < 0.0001) than microbial abundance in CEWAF (± nutrients) treatments. Microbial abundance in dispersant-only treatments increased by a factor of 29, less than in WAF treatments but showing clear stimulation of growth by dispersant alone.

Marine oil snow, here defined as particles >0.5 mm in diameter, formed in WAF, dispersant-only, and CEWAF (± nutrients) microcosms, but differed in appearance, size, and abundance across treatments (SI Appendix). Microbial exopolymeric substances, including transparent exopolymer particles (TEP), are a matrix for marine snow formation (35). Oil-degrading bacteria produce TEP as biosurfactants (36). TEP production increased in the WAF microcosms relative to controls, underscoring the metabolic activities of oil-degrading bacteria (SI Appendix, Table S1). The abundance of TEP could not be quantified in dispersant treatments (SI Appendix) but extensive formation of oil snow was observed in the CEWAF + nutrients treatments (SI Appendix), inferring that TEP levels were likely elevated. The macroscopic particles observed in these experiments resembled marine oil snow observed in situ during the DWH oil spill (SI Appendix, Fig. S13 F and G). Catalyzed reporter deposition in combination with fluorescence in situ hybridization (CARD–FISH) revealed that Gamma-proteobacteria and Alteromonadales, which includes the Colwellia, dominated microaggregate populations in CEWAF + nutrients treatments (SI Appendix, Fig. S13 P–R and SI Text). These findings suggest that Colwellia plays an important role in marine oil snow formation in the presence of dispersants.

Microbial Activity and Oil and Dispersant Degradation. Dispersant addition did not enhance bacterial oil degradation or microbial activity in general, as reflected in rates of hydrocarbon oxidation, bacterial protein production, and exoenzyme activities. Radiotracer assays allowed direct quantification of alkane ([1-14C]-hexadecane) and poly cyclic aromatic hydrocarbon (PAH) ([1-14C]-naphthalene) oxidation rates across treatments (SI Appendix) (Fig. 3 A and B and SI Appendix). Hexadecane oxidation rates were significantly reduced (T3 and T6: P = 0.004) in dispersant-only and CEWAF (± nutrients) treatments (Fig. 3A), implying that dispersants suppressed hexadecane degradation. Similarly, naphthalene oxidation rates in the WAF treatments were higher than those in dispersant-only and CEWAF (± nutrients) treatments (T3 and T6: P < 0.0001), inferring that dispersants did not stimulate microbial naphthalene degradation (Fig. 3B). When substrate turnover constants instead of concentration-dependent rates were considered, inhibition of hexadecane turnover remained apparent, whereas naphthalene turnover was comparable between WAF and CEWAF treatments (SI Appendix, Fig. S14). Together, these data show a clear concentration-independent inhibition of hexadecane oxidation by dispersants and further show that dispersants did not stimulate naphthalene biodegradation rates.

To validate the patterns of rates in these deepwater samples in another Gulf habitat, we determined hydrocarbon turnover of hexadecane and naphthalene in highly oil-contaminated (SI Appendix) surface seawater samples with and without dispersant addition (dispersant to seawater dilution was 1:100,000 vol/vol). Application of the radiotracer assay demonstrated that hexadecane turnover was ameliorated significantly by dispersant treatment, whereas naphthalene turnover was inhibited significantly by dispersant treatments compared with the WAF (± nutrients) treatments (Fig. 3 D–F and SI Appendix, Table S1), underscoring that dispersant-only and CEWAF (± nutrients) did not stimulate bacterial production (T3 and T6: P < 0.001) relative to the WAF treatments.

Results from gas chromatography-mass spectrometry (GC-MS) and excitation/emission matrix spectra (EEMS) in deepwater samples further confirmed the patterns of hydrocarbon degradation across deepwater treatments. Concentrations of n-alkanes and hexadecane decreased more significantly in WAF treatments (SI Appendix, Fig. S16). In the WAF treatment, microorganisms preferentially degraded low molecular weight n-alkanes (<C20) relative to high molecular weight (>C21) compounds and the isoprenoids, pristane and phytane. In the dispersant treatments, this pattern was not observed (SI Appendix, Fig. S17). The temporal changes in n-alkane concentration (SI Appendix, Fig. S16) supported the rate data (SI Appendix, Table S1) and emphasized the fact that oil degradation was highest in WAF treatments and that addition of CEWAF, even in the presence of additional nutrients, did not generate higher overall hydrocarbon degradation compared with WAF treatments.

Biodegradation of anionic surfactant DOSS to α,β-ethyhexyl-sulfosuccinate (EHSS) occurs under aerobic conditions (37). In the dispersant-only treatment, a significant (P < 0.05) decrease (8%) of DOSS and an increase of EHSS (15%) was observed at T3 (SI Appendix, Fig. S18 A and B). The nonionic surfactants were

![Fig. 2. Different microbial oligotypes respond to dispersants or oil (WAF). Oligotyping enabled the interpretation of 16S rRNA gene sequence diversity at the level of specific oligotypes. Relative abundance (averaged across bi-ological triplicates) of Colwellia oligotypes in microcosms, simulating DWH spill-like plumes.](image-url)
concentrations was observed in CEWAF (cyclotron resonance mass spectrometry (FT-ICR-MS) (38) (Fig. 4). The FT-ICR-MS results further suggest that significantly more oil-derived dissolved organic molecules were degraded in the WAF compared with CEWAF (± nutrients) treatments, again leading to the conclusion that more extensive biodegradation occurred in the absence of dispersant (Fig. 4 and SI Appendix, Fig. S19). Between 50% and 74% of the degraded compounds were highly unsaturated molecular formulae containing only the elements C, H, and O (SI Appendix, Fig. S19 A and B), which include the common aromatic hydrocarbons abundant in Macondo crude oil (39).

Oil-derived nitrogen-containing dissolved organic matter (DOM) compounds also decreased during the incubations (between 26% and 43% of the decreasing formulae, Fig. 4 A and B), agreeing with previous studies reporting that crude oil (40), including Macondo oil (39), contains numerous biodegradable polar and water-soluble organic nitrogen compounds. The WAF treatments exhibited the highest rates of degradation of oil-derived nitrogen-containing compounds (~8% vs. ~1% in the CEWAF treatment, Fig. 4 A and D) (38). In the WAF treatments, protein synthesis rates significantly exceeded those in the dispersant-amended treatments (T: P = 0.0082), and a 31% decrease of seawater- and oil-derived dissolved organic nitrogen (DON) concentrations showed that the generation of microbial biomass required significant rates of nitrogen assimilation (SI Appendix, Table S1). The enhanced uptake of oil-derived organic nitrogen illustrates that oil can serve as an important nitrogen source when oil-degrading microbial communities are nitrogen limited (41).

Organic sulfur compounds are abundant in Macondo oil (39). The FT-ICR-MS results imply complex processing of sulfur-containing oil-derived and dispersant-derived DOM, including degradation of oil-derived sulfur compounds and formation of new organic sulfur compounds (Fig. 4 C–E). The FT-ICR-MS detected DOSS (molecular formula C₂₀H₃₈O₇S; see arrow in Fig. 4 D and E) in all dispersant-amended treatments after 6 wk of incubation. The formation of new organic sulfur-compounds was particularly pronounced in the CEWAF (± nutrients) samples (circled area in Fig. 4 D and E), signaling that their formation was stimulated by dispersant addition. Elevated relative abundances of Colwellia in post-DWH discharge seawater along with enhanced expression of genes involved in the degradation of sulfur-containing organic matter (e.g., alkanesulfonate monooxygenase) (42) infer a role for Colwellia in organic sulfur cycling in situ during the DWH incident. The genome of C. psychrerythraea strain 34H has a remarkable potential for sulfur metabolism (43). Thus, we hypothesize that Colwellia played an important role in the observed turnover of DOSS-derived sulfur compounds as a result of their capability to metabolize the organic sulfur compounds in dispersants; they may have exhibited similar metabolic abilities in situ during the DWH incident.

Molecular Characterization of Dissolved Organic Matter. High-resolution FT-ICR-MS analysis provides a much more robust way to assess the molecular diversity of hydrocarbons in oil than does conventional GC-MS analysis (39, 40). The FT-ICR-MS analysis further suggest that significantly more oil-derived dissolved organic molecules were degraded in the WAF compared with CEWAF (± nutrients) treatments, again leading to the conclusion that more extensive biodegradation occurred in the absence of dispersant (Fig. 4 and SI Appendix, Fig. S19). Between 50% and 74% of the degraded compounds were highly unsaturated molecular formulae containing only the elements C, H, and O (SI Appendix, Fig. S19 A and B), which include the common aromatic hydrocarbons abundant in Macondo crude oil (39).

Factors Regulating Microbial Activity. To further unravel factors that regulate activity of key bacterial taxa, we determined statistically significant relationships between experimental conditions (geochemistry, cell counts, and microbial activity) and oligotype abundances. Distinct trends were apparent for Colwellia, Marinobacter, Oceanissp.entellia, and Cycloclasticus, as were correlations for specific oligotypes (SI Appendix, Table S2). Of the 24 detected Colwellia oligotypes, many correlated positively with concentrations of DOC (88%), ammonium (50%), cell counts (46%), and bacterial production (79%) as well as peptidase, glucosidase, and lipase (38–79%) activities. The majority of Colwellia oligotypes correlated negatively with concentration of total n-alkanes, hexadecane, naphthalene, and phenanthrene (71–79%), supporting the hypothesis that oligotypes of this taxon are predominantly responsible for dispersant breakdown. A considerable number of the 24 Marinobacter oligotypes correlated positively with cell counts (79%), bacterial production (79%), as well as peptidase and lipase (67–71%) activities. In contrast to Colwellia,
Mininobacter oligotypes correlated positively to total petroleum concentrations (83%) and hexadecane oxidation (71%), highlighting a key role for these microorganisms in hexadecane degradation in the absence of dispersants. Oceaniserpentilla and Cycloclastus oligotypes (30 and 31 types, respectively) correlated positively with nitrate and total n-alkanes, hexadecane, naphthalene, and phenanthrene (71–20%) concentrations. In addition, Cycloclastus abundance positively correlated with naphthalene oxidation (61%), supporting their involvement in PAH degradation.

**Evaluating the Utility of Dispersants.** Dispersants are used regularly as a response action after oil spills to disperse oil slicks, enhance the relative oil surface area in water, and to stimulate microbial hydrocarbon degradation. During the DWH incident, the deep-sea application of dispersants was unprecedented. Prior studies about microbial dispersant impacts generated confounding results (for review see ref. 25) most likely because nonspecific metrics were used, e.g., microbial cell counts or the production of CO₂. Though changes in these two metrics reflect changes in microbial growth or activity, they do not specifically signify changes in hydrocarbon degradation rates. Further, it is quite possible that microorganisms stimulated by dispersant addition may outcompete natural hydrocarbon degraders. Thus, a direct quantification of hydrocarbon oxidation, accomplished here by direct determination of hydrocarbon degradation rates, is necessary to elucidate the impacts of dispersants on microbial populations and activities. The data obtained do not support dispersant stimulation of oil biodegradation, questioning the utility of dispersant application to pelagic ocean ecosystems.

Dispersant impacts on pelagic environments that are not impacted by natural oil seepage remain largely unknown. However, it seems unlikely that dispersants would stimulate hydrocarbon degradation in a system that lacks a substantial population of hydrocarbon degraders when they had no stimulatory effect in samples from a system that was primed for oil degradation (e.g., oil degraders account for 7–10% of the natural microbial population at site GC600) (18). In fact, the presence of dispersant selected against the most effective hydrocarbon degrading microorganisms (i.e., Mininobacter). This multidisciplinary data set strongly suggests that dispersants did not stimulate microbial hydrocarbon-degradation rates, as maximal oil-degradation rates were observed in the WAF treatments. Though we quantified degradation rates of only two hydrocarbons, hexadecane and naphthalene, biodegradation of other n-alkanes and PAHs could be similarly affected by dispersants. Quantification of the total crude oil also showed that the highest levels of oil biodegradation occurred in treatments without dispersants.

Whereas microbial activities in CEWAF (+ nutrients) microcosms were comparable for 1 wk, rates were stimulated by nutrients in the later time points (e.g., CEWAF + nutrient hydrocarbon oxidation rates after 4 and 6 wk), suggesting progressive nutrient limitation. Clearly, the Gulf’s deepwater microbial community is able to degrade oil efficiently in the absence of dispersant. Therefore, caution is advised when considering dispersant applications as a primary response for future oil spills in deepwater environments similar to the Gulf. A full understanding of dispersant impacts on microbial populations requires immediate and careful evaluation of dispersant impacts across a variety of habitats.

**Materials and Methods.**

**Microcosm Setup and Sampling.** Seawater (160 L) was sampled from 1,178 m at an active natural hydrocarbon seep in the northern Gulf on March 7, 2013 (site GC600, latitude 27.3614, longitude −90.6018; SI Appendix, Fig. S1). After sampling, seawater was transferred to 20 L carboys and stored at 4 °C onboard the ship for 3 d. The carboys were transported at 4 °C to the laboratory at University of Georgia where the experiment and sampling were conducted in an 8°C cold room. Setup and sampling of microcosms are described in detail in SI Appendix, Materials and Methods. In brief, 72 2-L glass bottles (1.8-L sample per bottle) were incubated on a roller table (SI Appendix, Fig. S2). Treatments (WAF, dispersant-only, and CEWAF + nutrients) microcosms were performed after 0 (T₀), 1 wk (T₁), 2.5 wk (16 d; T₂), 4 wk (T₄), and 6 wk (T₆). CEWAF + nutrients treatments were sampled at T₀, T₁, and T₆. CEWAFs were prepared by mixing pasteurized seawater with oil and/or dispersants for 48 h at room temperature and subsequently subsampling CEWAFs, excluding contamination by oil or dispersants phases (SI Appendix). In addition, hydrocarbon turnover was determined in oil-contaminated surface seawater samples obtained along a transect from the Taylor Energy oil platform to the Mississippi River plume. Oil-contaminated surface seawater samples were used directly (untreated samples) or amended with dispersants (SI Appendix). Hydrocarbon turnover was analyzed using the newly adapted radiotracer assays (SI Appendix).

**Molecular, Microbiological, and Geochemical Analyses.** Nutrients (nitrate, nitrite, phosphate, and ammonium), dissolved inorganic carbon, and oxygen as well as hydrocarbons (44) and dispersant concentrations were monitored throughout the course of the experiment (SI Appendix). Microbial community evolution and cell numbers were investigated for each sample using 16S rRNA amplicon illumina sequencing (Bioproject accession PRJNA235405), computational oligotyping analysis (28), and total cell counts (SI Appendix). Activity measurements were performed using enzyme assays (peptidase, glucosidase, lipase) (45), [3H]-leucine incorporation analysis (46), as well as the newly developed method for the analysis of 14C-hexadecane and 14C-naphthalene oxidation (SI Appendix). TEP analyses were carried out for controls and oil-only treatments (47) and CARD–FISH analysis (48) were performed in particular for microbial aggregate formations in nutrient treatments (SI Appendix). Oil-derived hydrocarbons were extracted from water samples using a mixture of

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**Fig. 4.** Dispersants impact microbial turnover of dissolved organic matter. Analysis of molecular-level patterns in Van Krevelen diagrams (hydrogen-to-carbon, H/C, and oxygen-to-carbon, O/C ratios; each circle represents a molecular formula). (A and B) Van Krevelen diagrams showing nitrogen-containing formulae (color scale depicts N/C ratios; open circles, formulae contain no nitrogen). (C–E) Van Krevelen diagrams presenting changes in the presence or absence of sulfur-containing compounds (red circles, produced compounds, i.e., absent at T₀ but present at T₆; blue circles, degraded compounds, i.e., absent at T₀ but present at T₆; open circles, common compounds present at T₀ and T₆). DOSS (molecular formula C₂₀H₃₈O₇S₅, marked by arrow) was present at T₀ and T₆. Several sulfur-containing compounds were exclusively produced in the dispersant-amended treatments (molecular formulae marked by an ellipse).
hexane/dichloromethane (1:1, vol/vol). After concentration, hydrocarbon compounds were identified and quantified by GC/MSD using hexane:dichloromethane (1:1, vol/vol). After concentration, hydrocarbons at the Deepwater Horizon site. Nat Environ Sci 300:601:201-204.

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