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

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Abstract

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 in the hydrocarbon-rich, 1100m 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 lacking dispersant stimulated growth of natural hydrocarbon-degrading *Marinobacter*. Dispersants did not enhance heterotrophic microbial activity or hydrocarbon oxidation rates. Extrapolating this comprehensive data set 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 Statement

Oil spills resulting from anthropogenic activity, such as the explosion and sinking of the Deepwater Horizon drilling rig, are a significant source of hydrocarbon inputs into the marine environment. As a primary response to oil spills, chemicals are applied to disperse contiguous oil slicks into smaller droplets that may be more bioavailable to microorganisms. We provide compelling evidence that chemical dispersants applied to deep-sea waters in the Gulf of Mexico do not stimulate oil biodegradation. Direct measurements of alkane and aromatic hydrocarbon oxidation rates revealed instead that dispersants suppressed microbial activity. Dispersants impacted the microbial community composition and enriched bacterial populations with the ability to utilize dispersant-derived compounds as growth substrates, while oil-alone enriched for natural hydrocarbon degraders.

Crude oil enters marine environments through geophysical processes at natural hydrocarbon seeps (1) at a global rate of ~700 million liters per year (2). In areas of natural hydrocarbon seepage, such as the Gulf of Mexico (hereafter Gulf), exposure of indigenous microbial communities to natural oil fluxes can select for microbial populations that utilize oil-derived hydrocarbons as carbon and energy sources (3, 4). The uncontrolled deep-water oil well blowout that followed the explosion and sinking of the *Deepwater Horizon* (DWH) drilling rig in 2010 released more than 750 million liters of oil into the Gulf; roughly 7 million liters of chemical dispersants were applied at the sea surface and seabed (5) to disperse hydrocarbons and stimulate oil biodegradation. A deep-water (1000-1300 m) plume, enriched in aliphatic and aromatic hydrocarbons (6-11) and the anionic surfactant dioctyl sodium sulfosuccinate (DOSS) (12, 13) a major component of the dispersants (14), formed early in the discharge (7). The chemistry of the hydrocarbon plume significantly altered the microbial community (11, 15-17), driving rapid enrichment of low-abundance bacterial taxa such as *Oceanospirillum*, *Cycloclasticus*, and *Colwellia* (18). In contrast, the major hydrocarbon degraders from Gulf waters that are adapted to slow-diffusive natural hydrocarbon seepage were present in low-abundance or absent in DWH deep-water plume samples, suggesting an inability to cope with plume conditions (18).

Chemical dispersants break up surface oil slicks, reduce oil delivery to shoreline ecosystems (19), and increase oil dissolution in the water column, presumably making it more bioavailable (20) and potentially stimulating biodegradation (21). The efficacy of dispersants in achieving these aims remains poorly documented (22) and, in some cases, dispersant application led to substantial negative environmental effects (e.g. *Torrey Canyon* oil spill (23)). Dispersant application often requires ecological trade-offs (24) and little is known about the impacts of

dispersants on the activity and abundance of natural hydrocarbon-degrading microorganisms (25). This work addressed three key questions: 1) Do dispersants influence microbial community composition? 2) Is the indigenous microbial community as effective at oil biodegradation as microbial populations resulting from dispersants exposure? And, 3) Do dispersants and chemically dispersed oil affect hydrocarbon biodegradation rates?

Laboratory experiments were employed to unravel the effects of oil-only (supplied as a water-accommodated fraction; 'WAF'), Corexit 9500 ('dispersant-only'), oil-Corexit 9500 mixture (supplied as a chemically enhanced water-accommodated fraction; 'CEWAF') or a CEWAF with nutrients ('CEWAF+nutrients') (26) on Gulf deep-water microbial populations (*SI Appendix* Fig. S1 and S2). Experimental conditions (*SI Appendix* Table S1) mimicked those prevailing in the DWH deep-water hydrocarbon plume (6-13, 18). The results show that 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, when CEWAF (±nutrients) was added to deep seawater, microbial activity was not stimulated nor were microbial oil-degradation rates enhanced.

Results and Discussion

Dispersant significantly altered microbial community composition

We hypothesized that dispersants would alter microbial community composition and that the selection of one population over another would drive differences in hydrocarbon-degradation rates, altering the oil-degradation efficiency. We therefore explored patterns in microbial abundance (Fig. 1a) using microscopy and community composition using Illumina paired-end sequencing of bacterial 16S rRNA gene amplicons (Fig. 1b). We resolved closely related

bacterial taxa that would otherwise group into a single operational taxonomic unit (OTU) using oligotyping analysis (27) (Fig. 2). We furthermore highlighted the ecological preference of specific microbial taxa using statistical correspondence analysis (CA) (*SI Appendix* Fig. S3-7).

All dispersant-amended treatments showed ingrowth of *Colwellia* (*SI Appendix* Fig. S3), a group containing both hydrocarbon and dispersant degraders (28). After one week of incubation, the relative abundance of *Colwellia* compared to other Bacteria 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 could indicate that dispersant components served as growth substrates. Detailed analysis revealed that the relative abundance of *Colwellia* oligotypes 01, 02, and 05 increased in dispersant treatments (Fig. 2a, *SI Appendix* Fig. S4). 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. S8), verifying the environmental relevance of these organisms.

Though *Colwellia* oligotypes 03 and 10 increased in WAF treatments, the dominant microbial responder to WAF addition was *Marinobacter*, whose relative abundance increased from 2% to 42% of all Bacteria after 4 weeks (Fig. 1b). In contrast, in dispersant-only and CEWAF (±nutrients) treatments, *Marinobacter* comprised only 1-5% of all sequences. The CA analysis emphasized the dominance of *Marinobacter* in WAF samples (*SI Appendix* Fig. S5) and the same *Marinobacter* oligotypes occurred across all treatments, illustrating that dispersants did not select for specific *Marinobacter* oligotypes, as was the case for *Colwellia* (Fig. 2b). The *Marinobacter* (*SI Appendix* Fig. S9) degrade a wide variety of hydrocarbons, including pristane, hexadecane, octane, toluene, benzynes, phenanthrene, etc. (29-31) 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 (26).

Like *Marinobacter*, the abundance of *Cycloclasticus* increased primarily in the absence of dispersants. In WAF treatments, the relative abundance of *Cycloclasticus* increased from 12% to 23% after 1 week and an oligotype (type 03) closely related to *Cycloclasticus pugetii* (Fig. 2c and *SI Appendix* Fig. S10), which degrades naphthalene, phenanthrene, anthracene, and toluene as sole carbon sources (32), 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 (a.k.a. DWH *Oceanospirillum* (33)) abundance decreased consistently across treatments and their abundance did not correlate with the presence or absence of WAF, dispersant or CEWAF (±nutrients) (Fig. 1b, 2d, and *SI Appendix* Fig. S7). The *Oceaniserpentilla* oligotypes closely resembled those observed *in situ* during the DWH incident (18) (*SI Appendix* Fig. S11). The DWH *Oceanospirillum* oxidize *n*-alkanes and cycloalkanes (17); the latter were lacking in the microcosms because they are absent in surrogate Macondo oil, possibly explaining the low abundance of *Oceanospirillum* in these experiments.

Stimulation of cell growth and exopolymer formation

At the start of the experiment, all treatments exhibited similar cell abundance (3×10^5 cells mL^{-1}; Fig. 1a). At the end of the experiment, microbial abundance in the WAF treatment increased by a factor of 60, which was significantly higher (T_4: $p < 0.0001$) relative to microbial abundance in CEWAF (±nutrients) treatments. Microbial abundance in dispersant-only

148 treatments increased by a factor of 29, far below levels in WAF treatments but clearly showing
149 stimulation of microbial growth by dispersant alone.

150 Marine snow, here defined as particles >0.5 mm in diameter, formed in WAF, dispersant-
151 only and CEWAF (±nutrients) microcosms, but differed in appearance, size and abundance
152 across treatments (*SI Appendix*, *Supplementary Results and Discussion*). Microbial exopolymeric
153 substances, including transparent exopolymer particles (TEP) serve as the matrix for marine
154 snow formation (34). Oil-degrading bacteria produce copious amounts of TEP as biosurfactants
155 (35). TEP production increased in the WAF microcosms relative to controls, underscoring the
156 metabolic activities of oil-degrading bacteria (*SI Appendix* Table S1). The abundance of TEP
157 could not be quantified in dispersant treatments (26) but massive formation of oil snow was
158 observed in the CEWAF+nutrients treatments (*SI Appendix*, *Supplementing Results and*
159 *Discussion*), inferring that TEP levels were likely elevated. The different types of macroscopic
160 particles that formed resembled marine oil snow observed *in situ* during the DWH oil spill (*SI*
161 *Appendix* Fig. S12 f, g). Fluorescence *in situ* hybridization in combination with catalyzed
162 reporter deposition (CARD-FISH) revealed that *Gammaproteobacteria* and *Alteromonadales,*
163 including *Colwellia* dominated micro-aggregate populations in CEWAF+nutrients treatments (*SI*
164 *Appendix* Fig. S12q-r and *SI Appendix*, *Supplementary Results and Discussion*). These findings
165 point towards *Colwellia's* involvement in marine oil snow formation when dispersants were
166 present.

167 **Microbial activity and oil and dispersant degradation**

168 Addition of dispersants did not enhance bacterial oil degradation or general microbial activity as
169 reflected by rates of hydrocarbon oxidation, bacterial protein production, and exoenzyme
170 activities. Radiotracer assays allowed direct quantification of alkane ([1-^{14}C]-hexadecane) and

polycyclic aromatic hydrocarbon (PAH; [1-^{14}C]-naphthalene) oxidation rates across treatments (26) (Fig. 3 a, b). These two hydrocarbon classes are chemically distinct and PAHs are inherently toxic and mutagenic (36). Naphthalene concentrations in the WAF treatments exceeded hexadecane concentrations, as expected given the relative solubility of the two compounds (e.g. naphthalene and hexadecane solubility at 25ºC are 31.6 and 9×10^{-4} mg L^{-1}, respectively).

Hexadecane oxidation rates were significantly (T_3 and T_4: $p = 0.004$) lower in dispersant-only and CEWAF (±nutrients) treatments (Fig. 3a), implying that dispersants suppressed hexadecane degradation. Similarly, naphthalene oxidation rates in the WAF treatments were significantly (T_3 and T_4: $p < 0.0001$) higher than those in dispersant-only and CEWAF (±nutrients) treatments, indicating that dispersants inhibited also microbial naphthalene degradation (Fig. 3b). Biodegradation of other *n*-alkanes and PAHs could be similarly decreased or inhibited by dispersants.

Rates of ^{3}H-leucine incorporation showed that bacterial protein synthesis was highest in WAF treatments, particularly at later time points (Fig. 3c; *SI Appendix* Table S1), underscoring that dispersant-only and CEWAF (±nutrients) did not stimulate bacterial production to the same degree (T_3 and T_4: $p < 0.001$). We observed similar patterns for exoenzyme activities indicative of potential bacterial degradation rates of carbohydrate- and protein-rich exopolysaccharides (EPS). All enzyme assays exhibited up to one order of magnitude higher activities in the WAF and dispersant-only treatments compared to the CEWAF (±nutrients) treatments (Fig. 3d-f, *SI Appendix* Table S1).

Results from gas chromatography-mass spectrometry (GC-MS) and excitation/emission matrix spectra (EEMS) confirmed variable rates of oil-derived hydrocarbon degradation across treatments. Concentrations of *n*-alkanes and hexadecane decreased more significantly in WAF

194 treatments (*SI Appendix* Fig. S13). However, addition of dispersant led to changes in degradation
195 patterns for individual compounds. In the WAF treatment, microorganisms preferentially
196 degraded low molecular weight *n*-alkanes (<C20) relative to high molecular weight (≥C21)
197 compounds and the isoprenoids, pristane and phytane. In the dispersant treatments, this pattern
198 was not observed (*SI Appendix* Fig. S14). The temporal changes in *n*-alkane concentration (*SI*
199 *Appendix* Fig. S13) supported the rate data (*SI Appendix* Table S1), and underscored the fact that
200 oil degradation was highest in WAF treatments and that addition of CEWAF+nutrients did not
201 generate higher overall hydrocarbon degradation rates.
202 Liquid chromatography tandem mass spectrometry (LC–MS/MS) enabled quantitative
203 detection of distinct dispersant compounds: the anionic surfactant DOSS and the nonionic
204 surfactants Span 80, Tween 80, Tween 85, as well as, α/β-ethyhexylsulfosuccinate (EHSS), the
205 hydrolysis products of DOSS (13, 37). Biodegradation of DOSS to EHSS occurs under aerobic
206 conditions (37). In the dispersant-only treatment, a significant (p <0.05) decrease (8%) of DOSS
207 and an increase of EHSS (15%) was detected at T_3 (*SI Appendix* Fig. S15a, b). At all other time
208 points, no significant (p <0.05) change in DOSS or EHSS was observed in the dispersant-only
209 treatments (*SI Appendix* Fig. S15a, b). However, the nonionic surfactants were consumed within
210 1 week driving concentrations below detection (20 µg L^{-1}; *SI Appendix* Fig. S15c, d). Though the
211 carrier solvent dipropylene glycol butyl ether (DGBE) was not analyzed, it could have served as
212 an additional growth substrate for microorganisms (38) in the dispersant treatments.
213 In the CEWAF (±nutrients) treatments, DOSS decreased significantly (p <0.05) after
214 6 weeks (*SI Appendix* Fig. S15a). No significant change in EHSS concentrations was observed in
215 CEWAF (±nutrients) treatments (*SI Appendix* Fig. S15 b), indicating that DOSS was converted
216 to other products. This observation was supported by the formation of sulfur-containing

compounds detected by ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) (39) (Fig. 4f and 4g). In the CEWAF (±nutrients) treatments, the nonionic surfactants were at or below detectable levels at time zero, inferring that they probably associated with residual organic phase that was removed during CEWAF preparation. However, similarly to dispersant-only setups, low concentrations of nonionic compounds and DGBE could have served as additional microbial growth substrates in CEWAF (±nutrients) amended treatments.

Molecular characterization of dissolved organic matter

Most compounds remaining in weathered oil-contaminated fluids fall outside the GC-amenable analytical window (40), and conventional GC analysis (41) did not detect roughly 60% (on a mass basis) of compounds in Macondo crude oil. The FT-ICR-MS analysis further supported the conclusion that significantly more oil-derived dissolved organic molecules were degraded in the WAF compared to CEWAF (±nutrients) treatments, underscoring a more extensive degree of oil biodegradation in the absence of dispersant (Fig. 4).

Between 50 and 74% of the degraded compounds were highly unsaturated CHO molecular formulae (Fig. 4a, b), which include the common aromatic hydrocarbons abundant in Macondo crude oil (41). Oil-derived nitrogen-containing dissolved organic matter (DOM) compounds also decreased during the incubations (between 26 and 43% of the decreasing formulae, Fig 4c, d), agreeing with previous studies reporting that crude oil (42), including Macondo oil (41), contains numerous biodegradable polar and water-soluble organic nitrogen compounds. The WAF incubations exhibited the highest rates of degradation of oil-derived nitrogen-containing compounds (ca. 8% of the initially present formulae vs. ~1% in the CEWAF treatment, respectively) (39). In the WAF treatments, protein synthesis rates significantly exceeded those in

240 the dispersant-amended treatments (T_4: $p = 0.0002$), and a 31% decrease of seawater- and oil-
241 derived dissolved organic nitrogen (DON) concentrations in these treatments indicates that the
242 generation of microbial biomass was supported by significant rates of nitrogen uptake (*SI*
243 *Appendix* Table S1). The enhanced uptake of oil-derived organic nitrogen underscores that oil
244 can serve as an important nitrogen source when oil-degrading microbial communities are
245 nitrogen limited (43).

246 Organic sulfur compounds are abundant in Macondo oil (41). The FT-ICR-MS results
247 imply complex processing of sulfur-containing oil-derived and dispersant-derived DOM,
248 including degradation of oil-derived sulfur compounds and formation of new organic sulfur
249 compounds (Fig. 4e-g). The FT-ICR-MS detected DOSS (molecular formula $C_{20}H_{38}O_7S$; see
250 arrow in Fig. 4f, g) in all dispersant-amended treatments after six weeks of incubation. The
251 formation of new organic sulfur-compounds was particularly pronounced in the CEWAF
252 (±nutrients) samples (circled area in Fig. 4f, g), signaling that their formation was stimulated by
253 dispersant addition. Elevated relative abundances of *Colwellia* in post-DWH discharge seawater
254 along with enhanced expression of genes involved in the degradation of sulfur-containing
255 organic matter (e.g., alkanesulfonate monooxygenase) (44) infer a role for *Colwellia* in organic
256 sulfur cycling *in situ*. The genome of *C. psychrerythraea* 34H has a remarkable potential for
257 sulfur metabolism (45). Thus, we hypothesize that *Colwellia* were important in the observed
258 turnover of DOSS-derived sulfur compounds as a result of their capability to metabolize the
259 organic sulfur compounds in dispersants; they may have exhibited similar metabolic abilities *in*
260 *situ* during the DWH incident.

261 **Factors regulating microbial activity**

Substantial variations in the inorganic nitrogen-containing compounds were observed throughout the experiment. Nitrite (NO_2^-) concentrations increased from below detection limit to 0.6 µM (*SI Appendix* Table S1) while nitrate (NO_3^-) concentrations decreased significantly in the WAF (from 23 µM to 2 µM; $p < 0.0001$) and dispersant-only (from 23 µM to 14 µM; $p = 0.002$) microcosms (*SI Appendix* Table S1), implying active nitrate uptake and potentially incomplete denitrification. While denitrification is generally considered to occur under anoxic or suboxic conditions, *Marinobacter hydrocarbonoclasticus* is classified as an aerobic denitrifier and may have denitrified in the presence of oxygen (46) in the WAF treatments. Likewise, *Colwellia psychrerythraea* has the genetic potential to denitrify. Genes for hydrocarbon degradation under nitrate-reducing conditions (*bbs*) as well as genes for denitrification (*narG, nirS, nirK and nosZ*) were observed *in situ* in the DWH deep-water plume (43). The presence of mucus-rich, microbial aggregates could further promote denitrification through formation of anoxic microzones (47). Microbial communities, especially in WAF treatments, assimilated phosphate but were never phosphate limited (*SI Appendix* Table S1).

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, Oceaniserpentilla,* and *Cycloclasticus* as were correlations for specific oligotypes (*SI Appendix* Table S2). Of the 24 detected *Colwellia* oligotypes, many correlated positively with concentrations of dissolved organic carbon (DOC) (88%), NH_4^+ (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*, *Marinobacter* 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 *Cycloclasticus* oligotypes (30 and 31 types, respectively) correlated positively with nitrate and total *n*-alkanes, hexadecane, naphthalene, and phenanthrene (71-80%) concentrations. In addition, *Cycloclasticus* abundance positively correlated with naphthalene oxidation (61%), supporting their involvement in PAH degradation.

Evaluating the utility of dispersants

Dispersants are used globally 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, the deep-sea application of dispersants was unprecedented. The data shown here do not support dispersant stimulation of oil biodegradation, questioning the utility of dispersant application to pelagic ocean ecosystems. Different results could be expected in pelagic environments that are not characterized by natural oil seepage. 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 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 GC600 (18)). In fact, the presence of dispersant selected against the most effective hydrocarbon degrading microorganisms (*Marinobacter*). This multi-disciplinary data set strongly suggests that dispersants negatively influenced microbial hydrocarbon-degradation rates, with maximal oil-degradation rates occurring in WAF treatments. Though we quantified degradation

rates of only two hydrocarbons, hexadecane and naphthalene, biodegradation of other *n*-alkanes and PAHs may be similarly decreased or inhibited by dispersants. Quantification of the total crude oil showed that the highest levels of oil biodegradation occurred in treatments without dispersants. While microbial activities in CEWAF (±nutrients) microcosms were comparable for 1 week, rates were stimulated by nutrients in the later time points (e.g. hydrocarbon oxidation rates after 4 and 6 weeks), suggesting progressive nutrient limitation. Clearly, there was no need to chemically jump-start oil biodegradation through dispersant application in deep Gulf waters. Therefore, caution is advised when considering dispersant applications as a primary response for future oil spills in deep-water 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 oceanic and terrestrial habitats.

Material and Methods

Microcosm setup and sampling

Seawater (160 L) was sampled from 1178 m at an active natural hydrocarbon seep in the northern Gulf on 7th of March 2013 (site GC600, latitude 27.3614, longitude -90.6018; Fig. S1). After sampling, seawater was transferred to 20 L carboys and stored at 4°C onboard the ship for 3 days. The carboys were transported at 4°C to the laboratory at UGA where the experiment and sampling was conducted in an 8°C cold room. Setup and sampling of microcosms are described in detail in the *SI Appendix* and *Supplementary Material and Methods*. In brief, we incubated 72 2-L glass bottles (1.6 L sample per bottle) on a roller table (Fig. S2). Treatments (WAF, dispersant-only, and CEWAF±nutrients) and controls (abiotic, biotic) were set up in triplicate for each time point. Sampling (except for the CEWAF+nutrients treatment) was performed after 0

days (T_0), 1 week (T_1), 2.5 weeks (16 days; T_2), 4 weeks (T_3), and 6 weeks (T_4); CEWAF+nutrients treatments were sampled at T_0, T_1 and T_4. Water accommodated fractions (WAFs) were prepared by mixing pasteurized seawater with oil and/or dispersants for 48 h at room temperature and subsequently sub-sampling WAFs, excluding contamination by oil or dispersants phases; see also *SI Appendix*.

Molecular, microbiological and geochemical analyses

Nutrients (nitrate, nitrite, phosphate, and ammonium), DIC and oxygen as well as hydrocarbons (48) and dispersants concentrations were monitored during the course of the experiment (see *SI Appendix*). Microbial community evolution and cell numbers were investigated for each sample using 16S rRNA amplicon Illumina sequencing (Bioproject accession PRJNA253405), computational oligotyping analysis (27), and total cell counts (see also *SI Appendix*). Activity measurements were performed using enzyme assays (peptidase, glucosidase, lipase) (49), ^{3}H-leucine incorporation analysis (50), as well as a newly developed method for the analysis of ^{14}C-hexadecane and ^{14}C-naphthalene oxidation (see *SI Appendix*). TEP analyses were carried out for controls and oil-only treatments (51) and CARD-FISH analysis (52) 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 hexane:dichloromethane (1:1, v/v). After concentration, hydrocarbon compounds were identified and quantified by Gas Chromatography/Mass Selective Detector (GC/MSD) using conditions described previously (53) (see *SI Appendix*). Analysis of the surfactant components of the dispersant Corexit was performed as described elsewhere (13), with minor modification (see *SI Appendix*). Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) was carried out to analyze

353 DOM (54) (see *SI Appendix*). Statistical analyses were used to unravel factors that drive
354 microbial community evolution and microbial activities (see *SI Appendix*).
355
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367
368
369 **Author Contributions** S.K. and S.B.J designed the experiments and wrote the manuscript with
370 input from all authors. S.K. and M. Seidel setup and sampled the microcosms. S.K. and S.G.
371 accomplished DNA extraction, sequencing, oligotyping and phylogenetic analyses. S.K.
372 performed bacterial production and ^{14}C-hydrocarbon oxidation rate assays, total cell counts and
373 CARD-FISH analyses. K.Z. performed enzyme assays. U.P generated TEP data and S.K. and
374 U.P. described micro-aggregate formations M.P. and J.F. conducted Corexit surfactant analyses.
375 M.Seidel, P.M.M. and T.D. carried out FT-ICR-MS analyses. P.M.M., M.Seidel and K.M.L.

conducted hydrocarbon analyses (P.M. and M.S. *via* GC-MS and K.M.L. *via* EEMS). M. Sogin, S.G., S.K. and M.P. carried out statistical analyses. All authors discussed the results and their interpretation.

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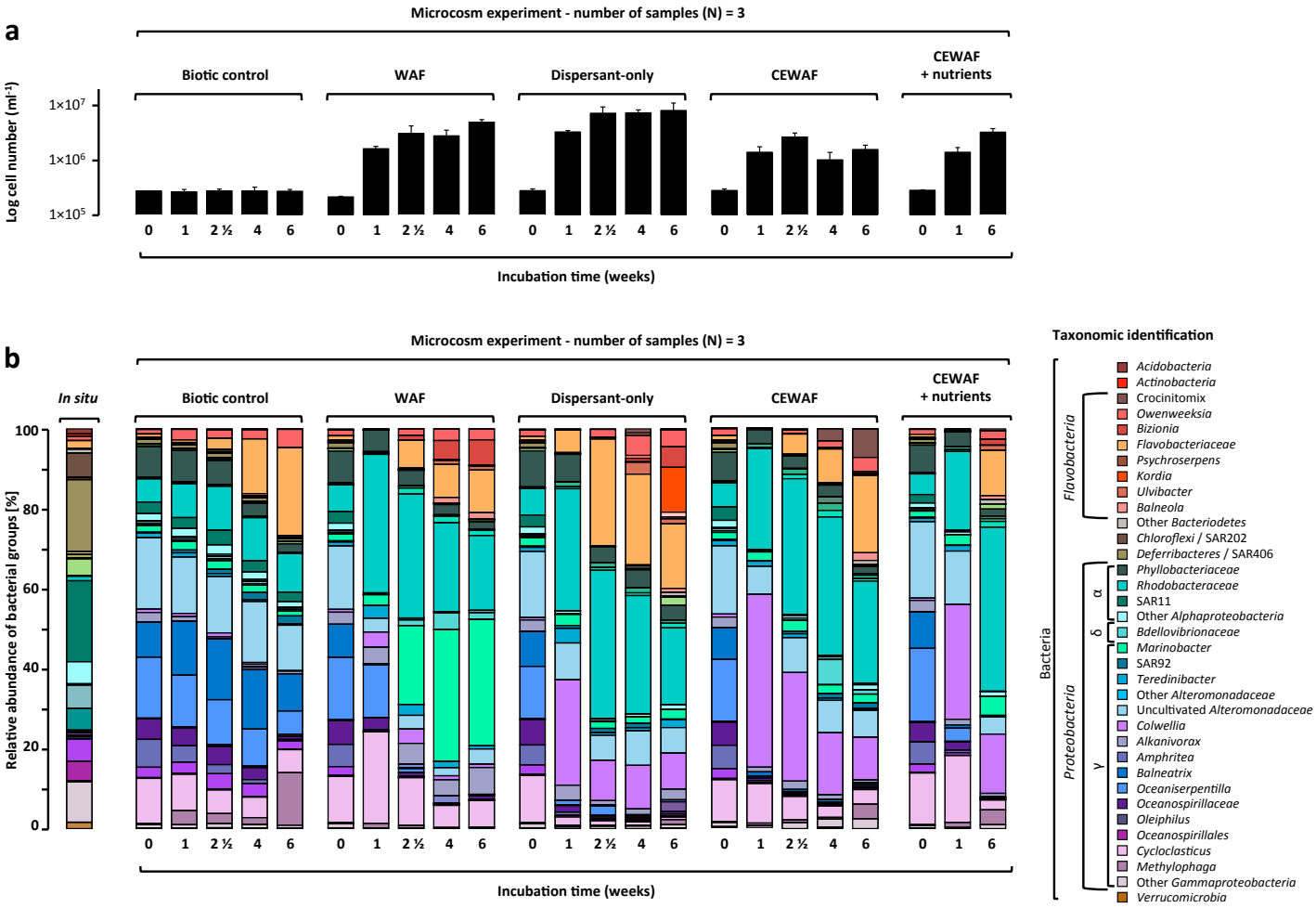
Fig. 1. Dispersants affect the evolution of oil-degrading microbial populations a, Average and standard deviation of cell numbers from sample triplicates (log scale) monitored for 6 weeks in microcosms. **b**, Relative abundance of bacterial groups in *in situ* Gulf of Mexico deep-water and in the microcosm (average of triplicate samples). Reads of the V4V5 regions of the 16S rRNA gene were clustered into OTUs and taxonomy was assigned with GAST.

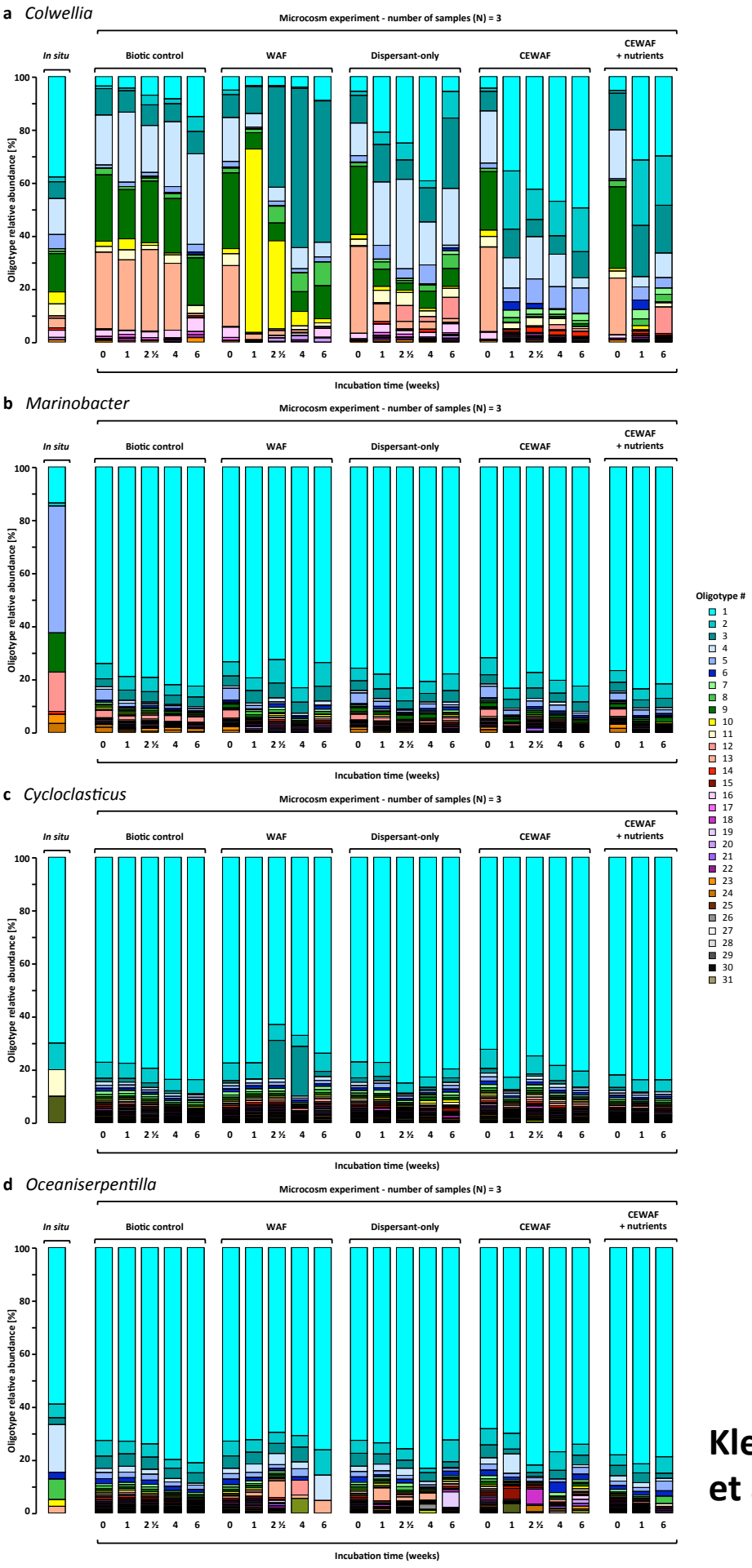
Fig. 2. Different microbial oligotypes respond to dispersants or oil (WAF). a-d, Oligotyping enabled the interpretation of 16S rRNA gene sequence diversity at the level of specific oligotypes. Relative abundance averaged across biological triplicates of **a,** *Colwellia,* **b,** *Marinobacter,* **c,** *Cycloclasticus* and **d,** *Oceanisperpentilla* oligotypes in microcosms, simulating DWH spill-like plumes (biotic control, dispersant-only, CEWAF, WAF, CEWAF+nutrients) monitored for 6 weeks.

Fig. 3. Microbial activity, hydrocarbon oxidation and enzymatic activities are not enhanced by dispersed oil (CEWAF ± nutrients). a, **b**, Oxidation rates of ^{14}C-hexadecane and ^{14}C-naphthalene as model compounds for alkanes and PAHs degradation, respectively (Table S1). **c**, Rates of bacterial production increased up to three orders of magnitude in the two weeks between the first and second sampling point (see also Table S1). **d-f**, Potential activities of peptidase, glucosidase and lipase measured using fluorogenic substrate analogs were up to one order of magnitude higher in the WAF and dispersant-only compared to the CEWAF ± nutrients

534 treatments. All data are illustrated as average of biological triplicates and error bars show
535 standard deviation of the mean (note that a lack of error bars means indicates standard deviations
536 too small to be shown on the plot scale).
537
538 **Fig. 4. Dispersants impact microbial turnover of dissolved organic matter.** Analysis of
539 molecular-level patterns in van-Krevelen diagrams (hydrogen-to-carbon, H/C, and oxygen-to-
540 carbon, O/C ratios; each circle represents a molecular formula). **a**, **b**, Molecular formulae present
541 in all treatments (n = 1205) and that significantly changed ($p \leq 0.01$, determined on triplicates
542 using Student's t-test) relative signal intensities between the initial and last time points. The
543 color scales represent changes in relative intensities (open circles, no significant change), **c**, **d**,
544 Van-Krevelen diagrams showing nitrogen-containing formulae (color scale depicts N/C ratios;
545 open circles, formula contained no nitrogen). **e-g**, Van-Krevelen diagrams presenting changes in
546 the presence or absences of sulfur-containing compounds (red circles, produced compounds, i.e.,
547 absent at T_0 but present at T_4; blue circles, degraded compounds , i.e. absent at T_4 but present at
548 T_0, open circles, common compounds present at T_0 and T_4). DOSS (molecular formula
549 $C_{20}H_{38}O_7S$, marked by arrow) was present at T_0 and T_4. Several sulfur-containing compounds
550 were exclusively produced in the dispersant-amended treatments (molecular formulae marked by
551 an ellipse).



Kleindienst et al. Fig. 1



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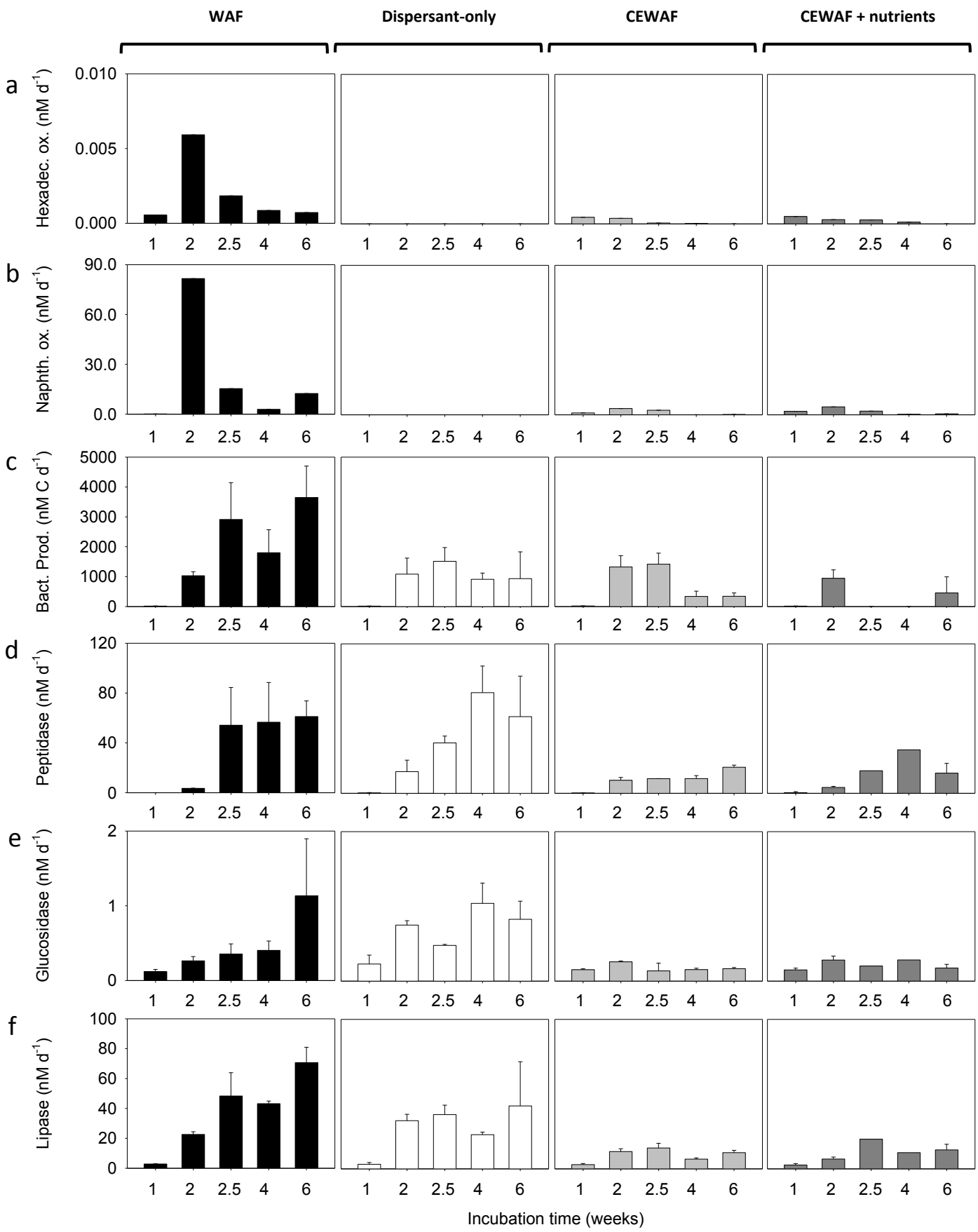

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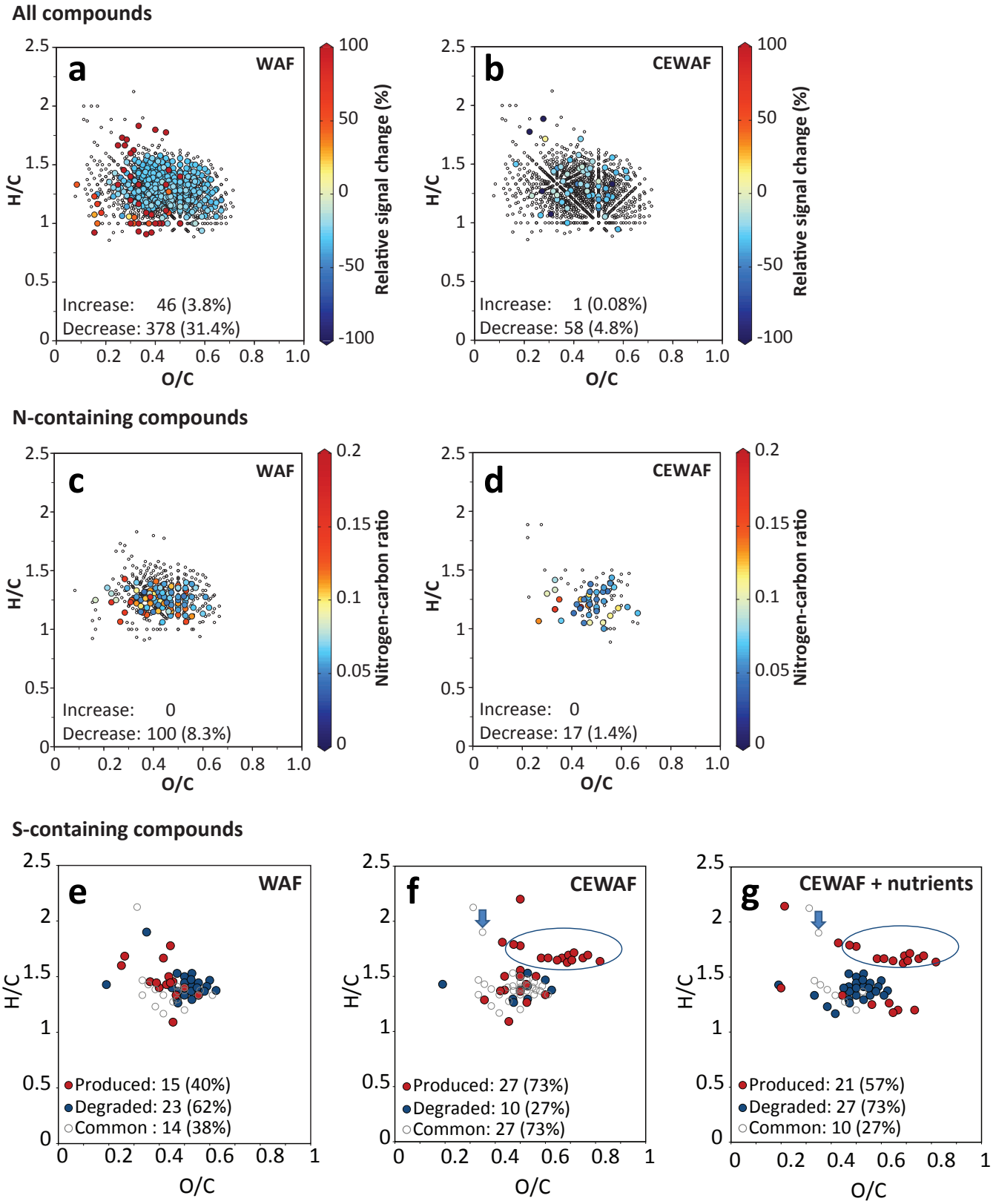

Kleindienst et al. Fig. 4