Microbial Dynamics Following the Macondo Oil Well Blowout across Gulf of Mexico Environments

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The Macondo blowout released roughly 5 million barrels of oil and up to 500,000 tons of natural gas into Gulf of Mexico at a depth of 1500 meters. Inarguably, the gas released from the Macondo blowout remained in the deep water. From 30% to 50% of the oil remained in the deepwater plume, whereas the remainder reached the surface, forming thick and expansive surface slicks. The hydrocarbon injection led to profound changes in microbial community composition and activity in the Gulf's waters and sediments and in nearshore benthic habitats. We provide a comprehensive literature review of the blowout's impacts on microbial community composition and on microbial activity across Gulf of Mexico ecosystems. Although microbiological research in the wake of the blowout led to many noteworthy discoveries, it also revealed an urgent need to develop a more comprehensive understanding of the environmental factors that regulate microbial hydrocarbon degradation in the environment.

Keywords: microbiology, oceanography, bioremediation

Hydrocarbons enter the marine environment naturally at cold seeps, which are distributed along continental shelves and slopes across the globe (NRC 2003). Hydrocarbons also enter the marine environment through anthropogenic mechanisms—namely, relatively small accidental discharges during petroleum extraction, transport, or consumption—and through large accidental discharges (e.g., tanker accidents, well blowouts; NRC 2003). At natural seeps, hydrocarbon inputs can be slow, diffuse, and sporadic, or they can be focused and intense (e.g., gas or oil vents, although those are equally sporadic; Joye et al. 2004, 2010).

The Gulf of Mexico (GOM) is a prolific hydrocarbon basin that encompasses an area of 1.6×10^6 square kilometers. At least 22,000 natural seeps (*http://1.usa.gov/1aKybyq*) exist in the GOM, and of those, about 1000 are intense and persistent enough to generate surface slicks that are detectable from space by satellite (MacDonald et al. 1993, MacDonald 1998). Estimates of natural seepage rates for oil from GOM natural seeps vary between 1500 and 3800 barrels of oil per day (NRC 2003) or up to 604,150 liters of oil per year (MacDonald 1998). Rates of natural gas (mainly methane) input from these seeps are poorly constrained, but estimates based on comigration of oil and gas generate fluxes of approximately 5×10^9 grams of carbon per year (MacDonald et al. 2002). Although the oil flux from the GOM can be estimated through satellite remote sensing (MacDonald et al. 1993), the technology required (e.g., instruments that can be deployed at depth and that have the appropriate sensitivity (subnanomolar to millimolar concentration range) and response time (less than 30-second equilibration time) to quantify methane fluxes is not widely available. However, recent technological advances (e.g., *in situ* membrane inlet mass spectrometry; Wankel et al. 2010) hold promise for improving the estimates of methane fluxes from the GOM seabed.

The largely diffuse and variable hydrocarbon inputs from natural seeps pale in comparison to the focused discharge generated through the 2010 Macondo blowout. During the 86-day period following the loss of well control on 20 April 2010, from 57,000 to 67,000 barrels of oil per day were injected from a discrete source at a depth of approximately 1500 meters (m; Camilli et al. 2011, Griffiths 2012), resulting in a total release of approximately 4.6 million barrels of oil (range = 4.6–6.2 million barrels; Griffiths 2012) into the GOM's waters. The input of natural gas—primarily methane—from the blowout was estimated to be up to 500,000 metric tons (Joye et al. 2011a). This intense, localized input of oil and gas was roughly 15 times the total input from natural seeps across the entire GOM ecosystem.

The Macondo blowout generated a number of unexpected features and events—namely, the formation of a deepwater plume enriched with water-soluble hydrocarbons, including

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benzene, ethylbenzene, toluene, xylene, polycyclic aromatic hydrocarbons (PAH), and low-molecular-weight alkanes (e.g., methane, ethane, propane; Diercks et al. 2010, Joye et al. 2011a, Reddy et al. 2012); immense surface oil slicks that stimulated blooms of microbial populations; and the subsequent formation of microbe-mediated marine snow (Passow et al. 2012), as well as the deep penetration of oil into the sands of the GOM's beaches. In the GOM's surface and deep waters, at the seabed, and along the GOM's beaches, microbial communities responded profoundly to the large hydrocarbon inputs in unexpected ways, which we articulate in the following pages.

Hydrocarbons and the microorganisms that consume them

Natural hydrocarbon reservoirs contain crude oil and low-molecular-weight gases (e.g., methane, ethane, propane, butane, pentane); the proportion of oil versus gas varies across reservoirs, as does the composition of crude oil. Crude oil is a complex mixture of saturated and aromatic hydrocarbons, resins, and asphaltenes. Light crude oils are predominately saturated (roughly 60%) and aromatic (roughly 30%) hydrocarbons, with lesser amounts of resins (7%) and asphaltenes (3%) (Head et al. 2006). Heavy crude oils contain a higher fraction of asphaltenes (10%) and resins (20%) while still being dominated by saturated (40%) and aromatic (30%) hydrocarbons (Head et al. 2006). The Macondo reservoir contained about 30% low-molecular-weight gases and light sweet Louisiana crude oil—specifically, 74% saturated hydrocarbons, 16% aromatics hydrocarbons, and 10% polar components that are highly resistant to biodegradation; methane gas accounted for 15% of the total released hydrocarbons (Reddy et al. 2012).

Microbial hydrocarbon degradation in the marine environment is mediated by diverse microbial assemblages operating in metabolic networks, in which the product of one oxidation fuels another. Although a particular microbial phylotype is capable of degrading a specific crude oil component (e.g., a PAH), crude oil degradation is more appropriately viewed as a cooperative biodegradation network. Primary oil degraders and other microbial groups produce surfactants that emulsify oil, increasing its bioavailability to the general microbial community. Degradation intermediates (e.g., alcohols) are degraded by secondary consumers. Oil-degrading microbial networks are regulated by bottom-up environmental factors (e.g., temperature and the availability of nutrients and electron acceptors) or top-down biological factors (e.g., viral-lysis and predation by protozoa or larger zooplankton; Head et al. 2006).

Among the known aerobic hydrocarbon degraders, members of the Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, and Deltaproteobacteria and the Firmicutes and fungi (Head et al. 2006) degrade crude oil components. Many aerobic microorganisms use saturated hydrocarbons exclusively as their carbon source (e.g., *Alcanivorax* spp., *Marinobacter* spp., *Oleiphilus* spp., *Oleispira* spp., or *Planomicrobium* spp.), whereas others (e.g., *Cycloclasticus* spp., *Neptumonas* spp.) use PAHs exclusively (Head et al. 2006). The ubiquitous nature of hydrocarbon-degrading bacteria in northern GOM pelagic waters was revealed in a study of alkane hydroxylase genes conducted in March 2010, just prior to the Macondo discharge (Smith et al. 2013). The results from *alkB* gene sequence analyses indicated that the community structure of alkane-degrading bacteria in the northern GOM varies among sites independently of depth and location. The absence of vertical structure contrasted with distinct patterns observed for overall bacterioplankton communities (Tolar et al. 2013), but was consistent with the distribution of alkane-degrading genera identified by small subunit (SSU) ribosomal RNA (rRNA) gene sequences. Sequences most closely affiliated with known alkane degraders *Alcanivorax* and *Marinobacter* accounted for nearly two-thirds of all sequences.

Anaerobic degradation of hydrocarbon gases (e.g., methane, butane, propane) is coupled primarily to sulfate reduction but could also be coupled to denitrification in surface cold-seep sediments (Bowles et al. 2011, Adams et al. 2013, Bose et al. 2013, Jaekel et al. 2013). Evidence for hydrocarbon degradation in deep petroleum reservoirs also exists (Orphan et al. 2000, Aitken et al. 2004). However, there is a shocking lack of rate measurements of crude oil degradation rates in marine sediments or waters.

The large-scale environmental perturbation induced by the Macondo blowout generated intense interest in understanding the microbiology of hydrocarbon biodegradation of the GOM ecosystem. Below, we describe Macondo-related microbial discoveries in the GOM's deep water, surface waters and sediments, and along its coastline.

Microbial dynamics in deep pelagic waters

The water column microbial community responded first to the Macondo blowout hydrocarbon injection. Although natural hydrocarbon inputs to the GOM are diffuse and patchy, a substantial fraction (roughly 7%; Yang et al. 2014) of the water column population possesses the metabolic machinery necessary to degrade hydrocarbons. Native microorganisms responded rapidly to the hydrocarbon infusion (Yang et al. 2014), but their activity appears to have been limited ultimately by environmental factors (Crespo-Medina et al. 2014), nutrients (Edwards et al. 2011), temperature (Redmond and Valentine 2011), fluid dynamics (Valentine et al. 2012), or other factors (Camilli et al. 2010) and may have been further affected by the addition of chemical dispersants (Macías-Zamora et al. 2013, Passow 2014), which were used as a primary response action at the seabed and at the sea surface (Lubchenco et al. 2012).

Initial microbial surveys of the Macondo-affected water column were focused on the deep hydrocarbon plume, which was discovered, documented, and tracked beginning in mid-May 2010 (Camilli et al. 2010, Diercks et al. 2010, Hazen et al. 2010, Joye et al. 2011a). Detailed time-series studies of water column microbial dynamics after the Macondo blowout

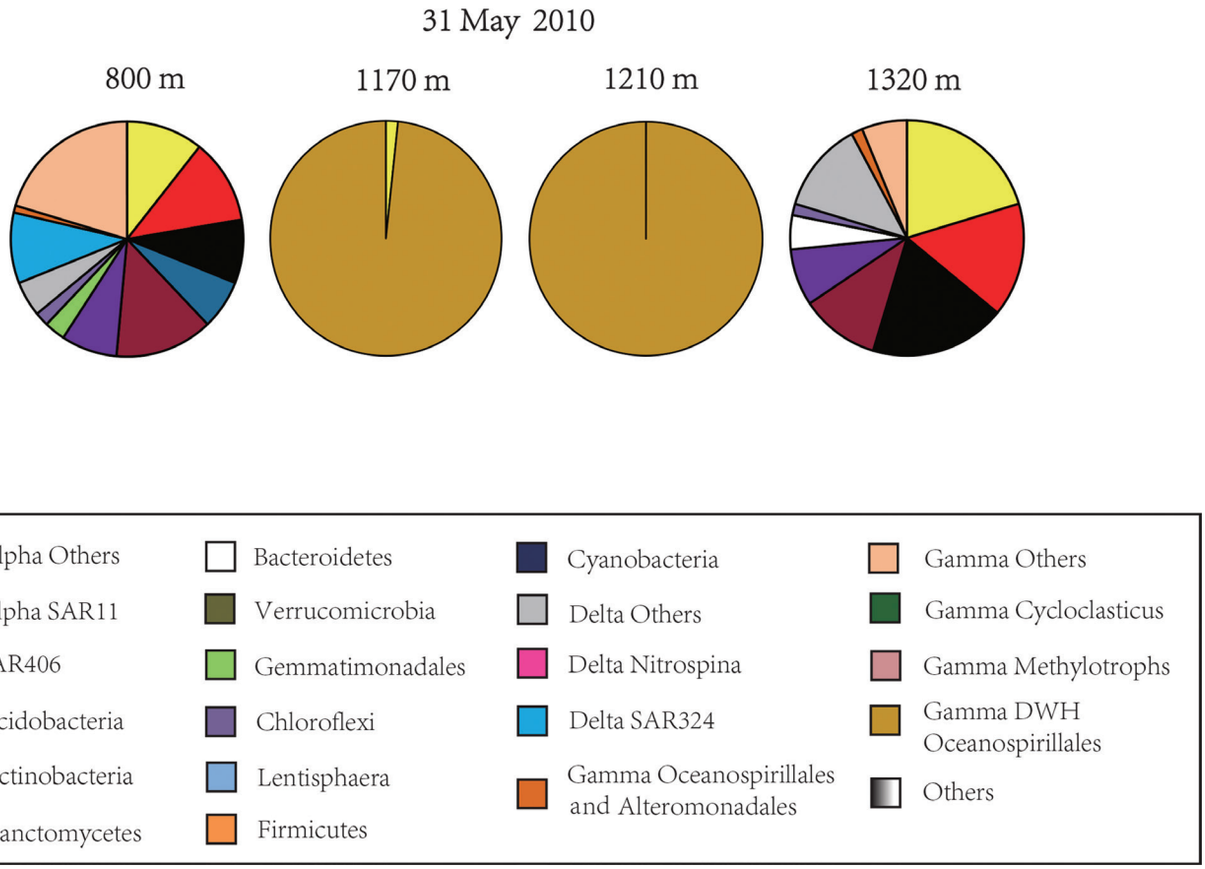

Figure 1. The water column microbial fingerprint (via 454 pyrosequencing using five bar-coded bacterial 16S-targeted primers) as a function of depth at a site approximately 8 kilometers from the Macondo wellhead on 31 May 2010. The abundance of Gammaproteobacteria, particularly Macondo Oceanospirillales and Alteromonadales and* Cycoclasticus *is clear. Abbreviations: DWH,* Deepwater Horizon*; m, meters. Source: Adapted from Yang and colleagues (2014).

provide a timeline of the bacterial community response and the changing microbial community composition over the lifetime of the deep hydrocarbon plume. Hazen and colleagues (2010) reported that uncultured members of the gammaproteobacterial order Oceanospirillales dominated 16S rRNA gene clone libraries in the deepwater plume between 1100 and 1220 m deep at the end of May 2010 (25 May–2 June). The Oceanospirillales group identified by Hazen and colleagues (2010; e.g., the Macondo Oceanospirillales) are considered active alkane degraders, because their closest cultured relatives, *Thalassolituus oleivorans* (Yakimov et al. 2004) and *Oleispira antarctica* (Yakimov et al. 2003), oxidize long-chain n-alkanes aerobically. As a caveat, alkane oxidation activity has not been documented in two other cultured relatives, *Oceaniserpentilla haliotis* (Schlösser et al. 2008) and *Spongiispira norvegica* (Kaesler et al. 2008). The Macondo Oceanospirillales appeared to dominate early in the spill before changing in mid-June into a community in which most clones grouped with the genera *Cycloclasticus*, obligate degraders of PAH, and *Colwellia*, a genus of psychrophilic marine heterotrophic generalists (figure 1; Yang et al. 2014). By early September, the original deepwater plume had moved far to the southwest of the wellhead and the bacterial community near the Macondo wellhead had diversified considerably and included different Alphaproteobacteria; multiple lineages within the Gammaproteobacteria; Flavobacteria; and several other phylum-level lineages such as the Actinobacteria, Planktomycetes, Chloroflexi; and the SAR406 cluster (Redmond and Valentine 2012, Yang et al. 2014).

The initial deepwater hydrocarbon plume was also strongly enriched in many heterotrophic marine Gammaproteobacterial genera, whose relative abundance, determined by the sequencing of SSU rRNA genes, increased by 100%–300% within the plume (Hazen et al. 2010, Mason et al. 2012). An initial microarray-based analysis of DNA extracted from the same oil plume samples showed increased normalized signal intensity for functional genes involved in hydrocarbon degradation, especially alkane-1 monooxygenase among the alkane, alkyne- and cycloalkane-degrading genes, and a wide spectrum of dehydrogenases, dioxygenases, and decarboxylases involved in aromatic carboxylic acid degradation (Hazen et al. 2010).

Subsequent probing of the deepwater microbial community using metagenomics, metatranscriptomics, and

GeoGhip 4.0 (Glomics, Norman, Oklahoma) hybridization assays showed that genes encoding for degradation of labile aliphatic hydrocarbons (e.g., alkanes, cycloalkanes, alcohols) were highly expressed, whereas those coding for biodegradation of recalcitrant compounds (e.g., benzene, ethylbenzene, toluene, PAHs) were present in the metagenome but were not actively transcribed (Mason et al. 2012, Lu et al. 2012). An interesting aspect of the microbial oil response is that 16S rRNA gene signatures of oil-degrading bacteria remain detectable in the water column—through high-throughput sequencing approaches—throughout the fall of 2010, independent of the presence of the deep hydrocarbon plume; these observations indicate that seed populations persist and could be maintained by small-scale accidental oil leakage and natural hydrocarbon seepage (Yang et al. 2014).

Absolute hydrocarbon degradation rates were poorly constrained and remain a subject of debate: Camilli and colleagues (2010) argued that hydrocarbons were degraded very slowly (over periods longer than months), whereas Hazen and colleagues (2010) suggested rapid turnover rates (days) of alkanes, which, when they are extrapolated to other hydrocarbon components (e.g., PAH), may or may not be appropriate because different classes of hydrocarbon compounds—alkanes versus simple aromatics (benzene) versus complex aromatics (PAH)—are degraded through distinct pathways and at different degradation rates (Wammer and Peters 2005). At this point, it is impossible to know with confidence how much of the released oil was degraded by microorganisms; however, some components were clearly biodegraded rapidly.

The fate of the gaseous hydrocarbons—namely, methane, released from the Macondo wellhead has also been the subject of debate. Ethane and propane degradation dominated oxygen consumption in the deepwater plume during mid-June 2010, and methane oxidation rates were much lower (Valentine et al. 2010). On the basis of SSU rRNA gene clone libraries, the genera *Cycloclasticus* and *Colwellia*, but not the Macondo Oceanospirillales, were argued to be the dominant short-chain alkane-degrading bacterial populations in the deep plume (Valentine et al. 2010). This interpretation contrasts with the known substrate spectrum of *Cycloclasticus*, a genus originally described as an obligate aerobic PAH degrader (Dyksterhouse et al. 1995); strains with this substrate preference were readily isolated from GOM sediments (Geiselbrecht et al. 1998).

Cycloclasticus and *Colwellia* constituted a large percentage of the environmental clones detected with bacterial SSU rRNA gene-targeted polymerase chain reaction primers in the mid-June plume samples (13–16 June 2010). The sole exception was the deepwater plume sample (15 June 2010) collected closest to the wellhead, where Macondo Oceanospirillales clones were detected; this group was not observed in aged plume samples west and down current of the wellhead (Valentine et al. 2010, Redmond and Valentine 2011). Clearly, the bacterial community changed within 2 weeks from a plume dominated by Macondo Oceanospirillales in late May (Hazen et al. 2010) to a plume dominated by *Colwellia* and *Cycloclasticus* in mid-June (Valentine et al. 2010).

By September 2010 (7–17 September 2010), the deepwater bacterial community composition, based on SSU rRNA clone libraries, had diversified considerably and included a diverse range of Alpha- and Gammaproteobacteria, Flavobacteria, Chloroflexi, and Planctomycetales. The Gammaproteobacteria included *Cycloclasticus*, various Oceanospirillales (not the Macondo group), and members of the Methylophilaceae, Methylococcaceae, and the genus *Methylophaga* (Kessler et al. 2011). This presence of C1-oxidizing marine bacteria was taken as evidence for bacterial methane oxidation as a dominant hydrocarbon-degrading process in the water column, even though methane oxidation rates were low or below detection (Kessler et al. 2011). A closer inspection of the clone libraries and a comparison with substrate spectra of cultured C1-oxidizing bacteria suggested that methylotrophy is at least as likely as methanotrophy (Joye et al. 2011b). In fact, the C1-oxidizing clones form two sister lineages to the methylo- and methanotrophic genera *Methylobacterium*, *Methylosarcina*, *Methylobacter*, *Methylomonas*, and *Methylosphaera* and to the separately branching, obligately methylotrophic genus *Methylophaga* (Yang et al. 2014). On the basis of these molecular data, a model of proposed methane distribution over time, and calculated oxygen anomalies, Kessler and colleagues (2011) argued that methane was consumed quantitatively by the observed C1-metabolizing population during the summer of 2010.

If these uncultured bacteria from the Kessler and colleagues' (2011) September 2010 survey were, in fact, methylotrophs or methanotrophs, they would constitute new genera with potentially novel physiological properties. The presence of clones in waters with low methane concentrations and low methane oxidation potential was interpreted to reflect recent microbial oxidation and assimilation of methane from the deepwater hydrocarbon plume (Kessler et al. 2011); if that is correct, the sampling campaign captured the last signatures of a methanotrophic bacterial bloom that drove methane concentrations below those typical of GOM waters (Joye et al. 2011b).

However, alternative interpretations of these results are possible. Transcriptomics studies in which the impact of high-molecular-weight dissolved organic matter (DOM) on microbial community structure was explored showed a selective enrichment of marine heterotrophs within the Gammaproteobacteria and Alphaproteobacteria (*Alteromonas*, *Thalassobius*) and Gammaproteobacterial methylotrophs (*Methylophaga*) after only 27 hours of DOM amendment (McCarren et al. 2010). These results were consistent with a DOM-degrading heterotrophic cascade that releases naturally abundant methylated sugars from DOM and that leads to the frequently observed high abundance of methyltotrophic bacteria in clone libraries from DOM-rich coastal waters (McCarren et al. 2010) or liquid and gaseous

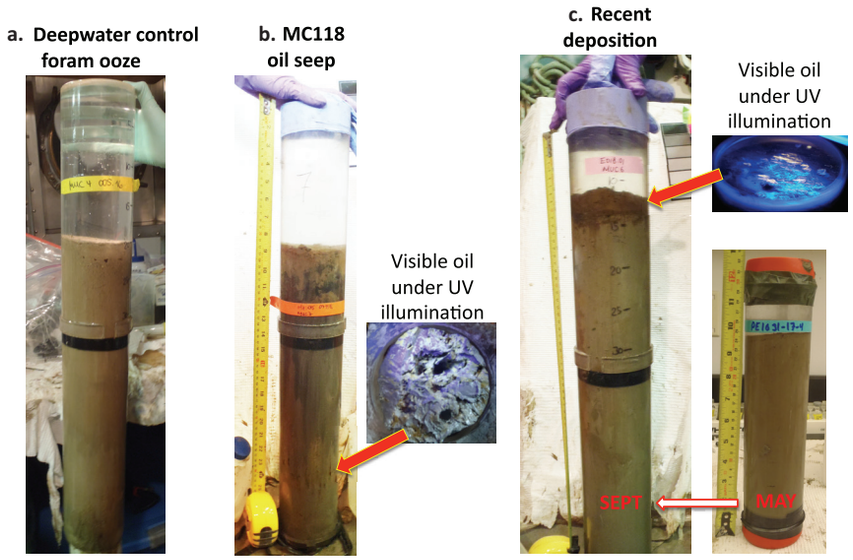

Figure 2. (a) Representative sediment cores from a deepwater foram ooze (control) site. (b) A natural oil seep showing darker coloration and clear oil staining (note the ultraviolet [UV] illumination of oil at depth in the core (the inset). (c) Cores from September and May 2010 showing a recently accumulated layer containing oil (note the green oil visible under UV illumination) that was not present at the same site in May 2010. Photographs: Samantha B. Joye.

petroleum (Dubinsky et al. 2013). In this interpretation, the combined presence of DOM-degrading heterotrophic Gammaproteobacteria, Alphaproteobacteria, and of methylotrophs marks the microbial degradation of a DOM pulse, not inconsistent with dissolved oxygen and fluorescence anomalies and the lack of methane and oil at the deep plume stations sampled by Kessler and colleagues (2011).

More recent studies (Rivers et al. 2013, Crespo-Medina et al. 2014) have advanced the understanding of methane cycling in the wake of the Macondo blowout. Using metatranscriptomic data, Rivers and colleagues (2013) showed a clear increase in methanotrophs (e.g., Methylococcales, *Methylobacter*) abundance in 16S rRNA gene clone libraries and in expression of genes involved in methane oxidation during late May 2010. Furthermore, multiple PmoA gene transcripts affiliated with known and potentially novel methanotrophs were observed, which suggests that methane oxidation was, in fact, occurring in late May 2010. Similarly, Crespo-Medina and colleagues (2014) observed high rates of methane oxidation in late May 2010 but low rates, similar to those observed by Valentine and colleagues (2010) and Kessler and colleagues (2011), in June and September 2010, respectively. These recent data suggest that methanotrophs in the deepwater plume responded rapidly to the methane infusion but that their activity was stymied by some presently unknown physiological or environmental factor after mid-June 2010, which suggests that, in fact, methane was not quantitatively consumed by the indigenous methanotroph population. Studies of nitrifying microorganisms, which are, in many ways, similar to methanotrophs, showed that crude oil exposure induced significant changes in nitrifying microbial populations (Urakawa et al. 2012, Newell et al. 2014). Following the Macondo blowout, the diversity of the archaeal nitrifier community increased, and the community composition shifted (Newell et al. 2014), which led Newell and colleagues (2014) to hypothesize that the oil exposure altered nitrifier community composition for at least a year. Urakawa and colleagues (2012) showed 20% inhibition of *Nitrosopumilus maritimus* at oil concentrations of 1 part per billion. The potential impacts of simultaneous exposure to crude oil and methane on methanotroph and nitrifier populations warrants further study.

Surface microbial dynamics potentially create a fast path to the seafloor

Cold seeps are distributed in a patchy manner in the GOM and elsewhere; sediments influenced by hydrocarbon discharge have characteristic features; they are often oil stained, highly reducing, and support high rates of microbial activity (e.g., hydrocarbon degradation coupled to sulfate reduction; Joye et al. 2010). Nonseep sediments are much easier to find and collect and also have characteristic features; these sediments are bland (i.e., they have no color variation), oxidized, and have low rates of microbial activity. In the aftermath of the Macondo blowout, sediment cores collected from a large area around the wellhead exhibited very consistent features that were typical of cores ~~neither~~ from a cold seep nor from ~~a~~ control, nonseep area (figure 2).

The available evidence suggests a massive sedimentation pulse of oil-derived ~~fallout~~ settled in the seafloor around the Macondo wellhead following the oil spill (Passow et al. 2012, Passow 2014). Massive flocs of microbe-derived marine snow were observed in the upper water column and at the sea surface within 1–2 weeks after the Macondo blowout; these flocs were the direct precursors of the oil-derived material that subsequently settled on the seafloor during the summer and fall of 2010 (figure 3). Several mechanisms were potentially responsible for their formation: the production of mucus webs through the activities of bacterial oil degraders associated with the floating oil layer, the production of oily particulate matter through interactions of oil components with suspended matter and their coagulation, and the coagulation of phytoplankton with oil droplets incorporated into aggregates (Passow et al. 2012, Passow 2014).

Marine oil snow formation was induced in the laboratory in roller table bottle experiments where seawater was incubated with small amounts of weathered oil collected from the sea surface in May 2010 (see figure 3, central column);

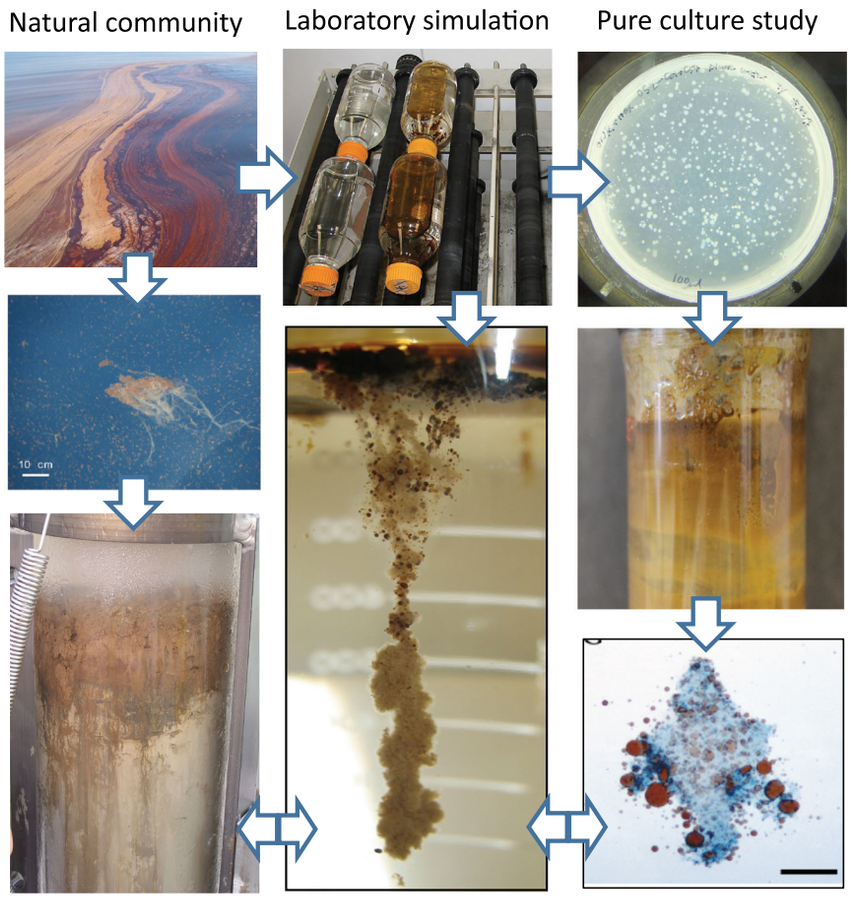

Figure 3. Microbiological investigation of the dirty blizzard hypothesis. Left column: In early May 2010, weathered oil on the sea surface near the Macondo site (photograph: Luke McKay) generates large marine snow aggregates (source: adapted from Passow et al. 2012) on the sea surface and in the upper water column—a "dirty blizzard"—that consecutively sink to the seafloor and form an approximately 3.8–5-centimeter-thick freshly deposited sediment layer (core taken on 30 November 2010; photograph: Andreas P. Teske). Central column: Small amounts of weathered surface oil incubated with Gulf of Mexico seawater in roller table experiments generate oily marine snow in the laboratory that is a hotspot of carbohydrate-degrading microbial activity (photograph: Kai Ziervogel); the close-up photo shows small oil droplets enclosed in the aggregate matrix (Ziervogel et al. 2012). Right column: Pure cultures of alkane- and aromatics-degrading bacteria obtained from surface oil, seawater, and laboratory bottle incubations (pictured: a plate of* Marinobacter *colonies; photograph: Tingting Yang) show activity in emulsifying weathered sea surface oil (middle image source: reprinted from Gutierrez et al. 2013a) and form extracellular polymeric substrates that entrap and emulsify oil. The extracellular polymeric substrates are stained blue, and the oil droplets are red–brown in the bottom image. The scale bar represents 10 micrometers (Gutierrez et al. 2013a). Isolates of the genus* Halomonas *were identified as efficient oil emulsifiers.

these experiments showed that marine snow formation in the laboratory model system proceeded on the same timescale (1–2 weeks) as field observations (Ziervogel et al. 2012). These oil-derived marine snow flocs showed increased cell densities and increased activities of carbohydrate-degrading enzymes; they also entrapped and emulsified small oil droplets in an extensive matrix of extracellular polysaccharides, representing microbial hotspots of enhanced hydrocarbon biodegradation. Such biological emulsifying agents are produced by either specialized aromatics-degrading bacteria (e.g., *Cycloclasticus*; Dyksterhouse et al. 1995) and alkane-degrading degrading bacteria (e.g., several Macondo isolates of the genera *Alcanivorax* and *Marinobacter*; Gutierrez et al. 2013a) or secondary hydrocarbon degraders (e.g., the genus *Halomonas*).

In experiments monitoring formation and oil-emulsifying properties of extracellular polymeric substrates (EPS), members of the marine genus *Halomonas*—some of them isolated directly from Macondo weathered oil samples collected in early May 2010—were found to be especially effective EPS producers and oil-emulsifying agents (Gutierrez et al. 2013b). Members of the genera *Halomonas*, *Marinobacter*, and *Cycloclasticus* were readily and consistently detected in clone library analyses of mature oil-derived marine snow flocs generated in the laboratory, in alkane-based enrichments from deep plume water (FISH assay focused on *Marinobacter*; McKay et al. 2014), in pyrosequencing surveys of surface oil samples collected in May 2010, and in pyrosequencing analyses of water column samples collected in September and October 2010 (Gutierrez et al. 2013a, Yang et al. 2014).

Experimentally determined sinking velocities for oil-derived marine snow particles collected in the GOM were in the range of 68–553 m per day (Passow et al. 2012) would support rapid export of surface oil-derived marine snow to the seafloor on the GOM continental slope. This "~~dirty blizzard~~" scenario (Schrope 2013) is now substantiated and has been fleshed out in detail by current work in the deep GOM (figures 2 and 3), starting with collections and analyses of freshly sedimented material on the GOM seafloor in late August and early September 2010 with the R/V *Oceanus* and continuing through seafloor sampling cruises in fall 2010 and throughout 2011–2013.

When deposited to the seabed, aerobic oil-degrading bacterial communities that were active in the water column are

replaced slowly by anaerobic bacteria that are active under oxygen depleted conditions at the seabed. For example, initial metagenome and functional gene surveys of two sediment cores within 16 kilometers of the Macondo wellhead and a distant control core sampled in September and October 2010 demonstrated an increased proportion of sulfate-reducing Deltaproteobacteria and their alkyl- and benzyl-succinate synthase genes—key genes of anaerobic alkane oxidation—near the wellhead (Kimes et al. 2013). A multicore survey of the seafloor near the Macondo wellhead between May 2010 to July 2011 shows that within the benthic microbial community, specific family- and genus-level groups within the Alphaproteobacteria, Bacteroidetes, Gammaproteobacteria, and Deltaproteobacteria react most strongly to the presence of oil-derived fallout on the seafloor and form transient blooms or population waves that dominate clone libraries at specific times (Yang et al. 2014).

The microbial origins of these bacterial blooms lie partly in the water column—sinking oil-derived marine snow entrains a large amount of phytoplankton debris and particle-associated bacteria—and on the seafloor itself, where the sedimentation pulse of oily marine snow provides carbon and energy sources to indigenous bacteria that colonize freshly sedimented material. Detailed phylogenetic analyses of bacterial clades with well-characterized water column and sediment populations—for example, within the *Roseobacter* cluster (Wagner-Döbler and Riedl 2006, Brinkhoff et al. 2008)—show promise for providing specific information on the balance between sedimentation input and indigenous benthic origin of the bacteria that dominate the sediment surface in the wake of the dirty blizzard (Thorston Brinkhoff, Institute for Chemistry and Biology of the Marine Environment, University of Oldenburg, Oldenburg, Germany, personal communication, 26 June 2014). The organic carbon and nitrogen content of the oil marine snow blizzard sedimentation pulse was significantly reduced; the upper 2 centimeters of this surface sediment layer (see figure 3, right column), sampled in late November 2010 near the Macondo site, contained approximately 10%–20% less total organic carbon and total nitrogen (by weight) than the underlying sediment (Ziervogel et al. 2014). Most likely, much of the biodegradable fraction of the oil-derived sedimenting material was consumed by microbes during its passage through the water column and also after deposition on the seafloor. Although bacterial cell numbers did not show a strong surface maximum, bacterial protein production in the upper 2 centimeters of recently deposited sediment near the wellhead (sampled in late November 2010) remained elevated by an order of magnitude over that of the underlying sediments; this trend leveled out in control sediments at increasing distance from the Macondo wellhead (Ziervogel et al. 2014). The tracking of microbial community dynamics and concurrent geochemical change in the seafloor sediments over time is a topic of ongoing investigation.

Microbial dynamics in nearshore and coastal sediments

Oil from the Macondo blowout affected 1773 kilometers of shoreline that includes beaches (50.8%), marshes (44.9%), and other shoreline types (4%) in the GOM (Michel et al. 2013). Oil hydrocarbons were substantially weathered during transit from offshore waters to the coast. However, potentially harmful concentrations of hydrocarbons reached coastal ecosystems, peaking in summer 2010 and persisting into 2011. Levels of total petroleum hydrocarbons and PAH were high with respect to human consumption and marine biota exposure, especially in coastal sediments (Allen et al. 2012, Sammarco et al. 2013).

Biodegradation is the ultimate sink for petroleum hydrocarbons released into the marine environment (Leahy and Colwell 1990, Prince 2010, Atlas and Hazen 2011), although some compounds, such as asphaltene and tar, are resistant to biodegradation (Leahy and Colwell 1990). Therefore, the response of coastal ecosystems was probably determined by physicochemical parameters, either directly through chemical and physical weathering or indirectly through effects on microbial distributions and microbial activity. During transit to the coast, much of the volatile hydrocarbon fraction evaporated, was degraded because of photochemical activity, or was microbially degraded. The supply of oxygen and major nutrients (nitrogen, phosphorus, and perhaps trace metals) probably controlled the fate of hydrocarbons that reached the coast. Beaches are dynamic systems in which water and sediments are rapidly mixed (Huettel et al. 2013), generating an ideal environment for hydrocarbon degradation (figure 4). However, in muddy salt marshes, oxygen is rapidly depleted within a few centimeters of the sediment surface (Furukawa et al. 2004), and oil hydrocarbons are likely to persist much longer because anaerobic hydrocarbon degradation is much slower than aerobic degradation.

Despite the fact that offshore and deep sea ecosystems are more difficult to access, the response of microbial communities to the Macondo blowout was documented in far more detail in offshore environments compared to nearshore and coastal environments. A substantial number of studies investigated response efforts, chemical evolution of oil hydrocarbons, and impacts to microbial communities in beaches. Less information is available for salt marshes, and very little is known about the microbial response in coastal planktonic or subtidal benthic ecosystems affected by the Macondo blowout. This is likely due to the fact that oil is easier to detect visually in exposed intertidal ecosystems, such as beaches or marshes, than in murky subtidal environments. In addition, more attention was paid to beaches because of their high economic value (Wang and Roberts 2013).

Oil contamination from the Macondo blowout had a profound impact on the abundance, structure, and metabolic potential of beach microbial communities, in particular. At municipal Pensacola Beach, Florida, where total petroleum hydrocarbons reached 11,000 mg kg^{-1}, a time-series sampling campaign documented the microbial response (Kostka et al.

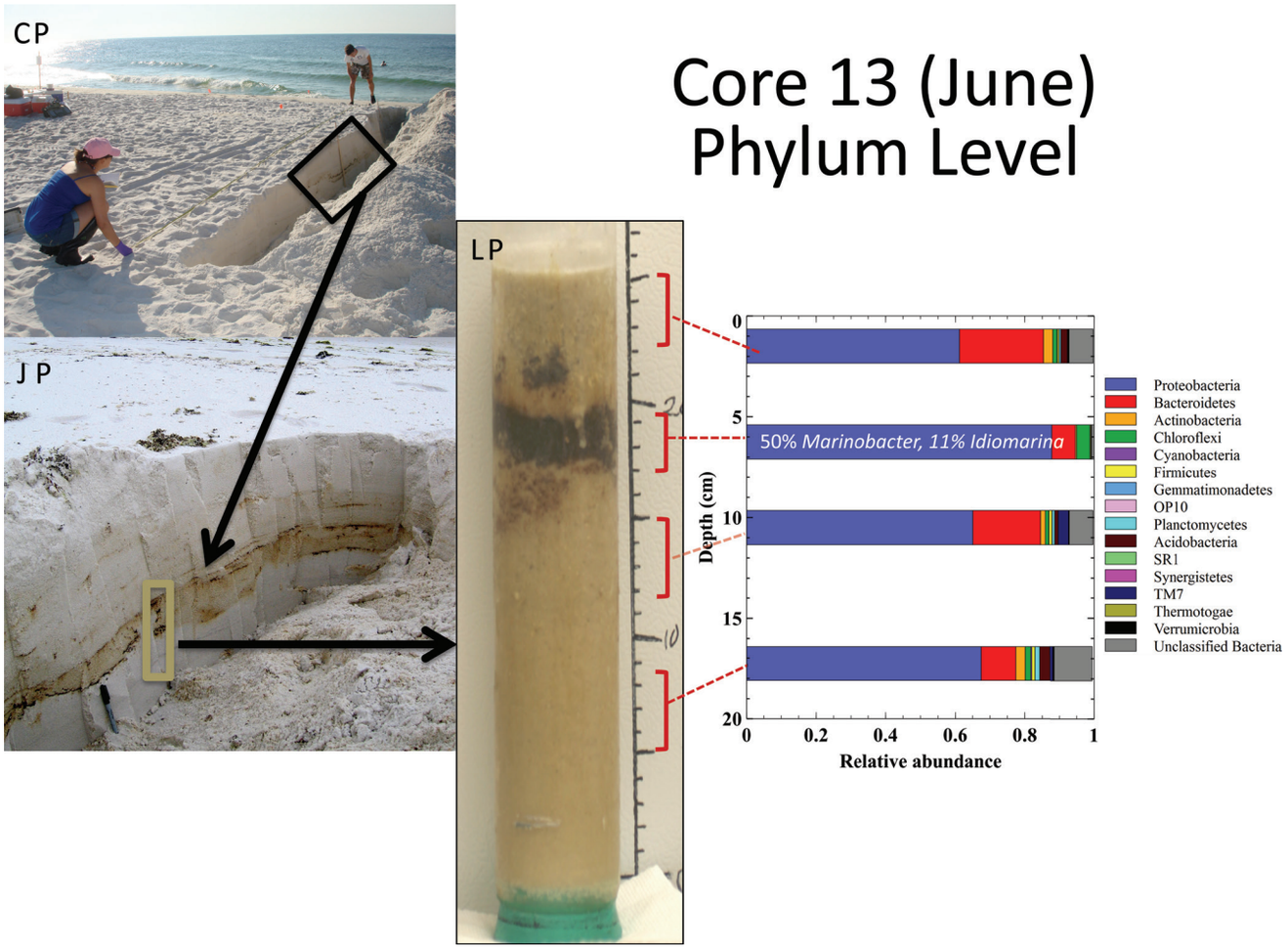

Figure 4. (a) Oil-contaminated beach sand from Pensacola Beach. (b) A clear layer of oil-stained sand (several centimeters thick) is present well below the surface. (c) Within the oily layer, the microbial community shifts show ingrowth and dominance by* Marinobacter *and* Idiomarina. *Photographs: Markus Huettel.

2011). A bloom of bacteria in Pensacola Beach sands during the first 4 months after oil came ashore was observed, with microbial abundance in oiled sands exceeding that of clean sands by 1 to 4 orders of magnitude. State-of-the-art analytical chemistry (e.g., petroleomics), confirmed that biodegradation of weathered oil was concomitant with the bacterial bloom (Appeli et al. 2012, Ruddy et al. 2014). A subset of the indigenous bacterial groups present in pristine sands, the Gammproteobacteria and Alphaproteobacteria, were stimulated by the presence of oil, including many bacterial groups that are known to degrade hydrocarbons. Members of the Oceanospiralles group, especially *Alcanivorax*, which degrades aliphatic compounds, increased dramatically in relative abundance during the early stages of hydrocarbon degradation at Pensacola Beach. Some evidence of the succession of microbial populations related to the use of different hydrocarbon compound classes was observed as Gram-positive members of the Rhodobacteraceae within the Alphaproteobacteria persisted after levels of *Alcanivorax* declined. In previous studies of heavily weathered oil in marine sediments, similar Gram-positive populations were noted as the dominant groups, suggesting that these organisms are involved in the degradation of more recalcitrant oil hydrocarbons, such as PAHs (Alonso-Gutierrez et al. 2009).

A time series of 12 metagenomes was constructed from oiled and clean sands sampled at Pensacola Beach between 2010 and 2011. In general, shifts in microbial community composition recorded in the metagenomes were consistent with those obtained by SSU rRNA gene amplicon sequencing (Kostka et al. 2011). For example, a large decrease in taxonomic diversity was observed in oiled sands and diversity rebounded 1 year after oil came ashore. A functional transition was observed from generalist populations within 4 months after oil came ashore to specialists a year later, when oil was undetectable, thereby supporting the specialization–disturbance hypothesis. Collectively, these results advance understanding of how native microbial communities respond to crude oil perturbation in near shore ecosystems and provide biomarkers for the chemical evolution of oil hydrocarbons during degradation and weathering.

Evidence from molecular-based approaches was corroborated by cultivation of hydrocarbon-degrading bacteria from oiled Pensacola Beach sands. Organisms from several known oil-degrading bacterial groups (e.g., *Alcanivorax*, *Marinobacter*, *Pseudomonas*, *Acinetobacter*, *Bacillus*) were isolated and found to be representative of the dominant microbial populations detected *in situ* by gene sequencing in the same contaminated sands (figure 4; Kostka et al. 2011). The oil degradation capability was confirmed for these strains from the quantification of residual oil and concomitant growth in the laboratory. Initial physiological characterization revealed contrasts between strains, which suggests niche specialization in carbon metabolism and nutrient acquisition.

Draft genomes of 10 oil-degrading isolates were assembled to illuminate the metabolic potential to degrade crude oil components at the strain level and to provide reference genomes in support of metagenomic work to characterize the dominant populations detected in beach sands (Overholt et al. 2013). Substantial variation in the assembly and number of hydrocarbon degradation genes between strains of the same genus (e.g., *Alcanivorax*, *Marinobacter*) suggests strain-specific differences in metabolic potential and hydrocarbon degradation potential. Furthermore, bacterial isolates from the genus *Labrenzia* (Alphaproteobacteria) represent the first genome of this group derived from growth on crude oil.

Studies conducted at other beaches generally corroborated the observations from Pensacola Beach. Newton and colleagues (2013) explored the feasibility of microbial indicators as a sensitive measure of ecosystem disturbance at seven beaches from Mississippi to Florida. Although community-specific patterns were not evident because of large degrees of variation, an increase in the relative abundance of known hydrocarbon degraders (e.g., *Alteromonas*, *Marinobacter*, *Alcanivorax*) was observed in oiled sands. In addition, an evaluation of microbial eukaryote communities in sediments collected from Louisiana and Alabama beaches revealed a dramatic change in community structure associated with oil contamination from May to September of 2010 (Bik et al. 2012). Fungal taxa, including known hydrocarbon-degrading members, appeared to thrive in oiled sands at the expense of metazoan groups.

Although high degrees of oil contamination were observed in Louisiana salt marsh sediments, oiling was generally restricted to the marsh periphery (within 10 m of the shoreline; Silliman et al. 2012). Rapid microbial degradation of lighter hydrocarbons was observed in marsh sediments, whereas heavier fractions persisted (Natter et al. 2012). A natural abundance ^{14}C analysis of microbial phospholipid fatty acids provided direct evidence for biodegradation and the incorporation of petrocarbon into microbial biomass at affected sites several months after oil intrusion, when the highest concentrations of oil were present (Mahmoudi et al. 2013). Although relatively minor shifts in overall bacterial community composition were observed in sediments from oiled sites in comparison to reference sites, a higher relative abundance of bacterial groups from known hydrocarbon-degrading taxa, Rhodobacterales and Sphingomonadales, was found in affected sediments.

Despite the relatively mild oil contamination observed in Alabama marshes, an increase in the relative abundance of bacteria and functional genes involved in hydrocarbon degradation was observed coincident with the presence of oil (Horel et al. 2012, Beazley et al. 2012). Elevated numbers of sulfate-reducing bacteria and sulfide concentrations in heavily oiled Louisiana marsh sediment suggested anaerobic hydrocarbon degradation (Natter et al. 2012).

Microbial communities in nearshore habitats provide critical ecosystem services, such as nutrient regeneration and pollutant removal at the land–sea boundary in the GOM region (Huettel et al. 2013). Future response efforts should include plans to document the impacts of oil deposition on microorganisms in these ecosystems, especially in the planktonic environments that were neglected during the response to the Macondo blowout.

Conclusions

The Macondo blowout provided a unique opportunity to study the response of indigenous microbial communities to a localized, large infusion of hydrocarbons into offshore pelagic waters and in deepwater and nearshore sediments. The available data show that a diverse array of microorganisms, ranging from alkane and PAH degraders to methane degraders and nitrifying microorganisms responded to the large, sustained hydrocarbon input generated from the Macondo blowout. However, the available data raise many more questions than they answer.

Microbial communities shifted rapidly in response to the hydrocarbon injection. However, actual rates of oil and gas degradation remain poorly constrained and actual rates, particularly of oil degradation, were rarely measured, making it impossible to constrain the actual amounts of oil and gas that were biologically degraded. The discovery of microbial formation of marine oil snow is a major accomplishment, and further research is required to understand nature of this process and to learn how best to regulate it. The formation of marine oil snow, which is clearly linked to microbial processes, represents a previously unrecognized fate for discharged oil and should be factored into the federal oil budget. Buried layers of oil along the GOM's beaches, which are invisible to the eye when walking along the beach, support unique microbial communities and activities that also require further research in order to document their long-term impacts. Studies in which the environmental and physiological controls on hydrocarbon degradation are explored under *in situ* conditions and isolation efforts to describe the novel hydrocarbon degraders that responded to the unprecedented hydrocarbon input will significantly advance the knowledge of hydrocarbon microbiology.

Finally, the impact of dispersants on microbial community structure and function remains highly debated, but the available data suggest that dispersants exerted a likely negative

impact on the GOM's microbial communities (Hamdan and Fulmer 2011, Paul et al. 2013) and on the microbe-based food web (Ortmann et al. 2012). The impact of dispersants on microorganisms has been a subject of debate for decades, but in the wake of the Macondo blowout, it is clear that additional studies of dispersant impacts on microbial communities are sorely needed.

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