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Microbial community successional patterns in beach sands impacted by the Deepwater Horizon oil spill

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Although petroleum hydrocarbons discharged from the Deepwater Horizon (DWH) blowout were shown to have a pronounced impact on indigenous microbial communities in the Gulf of Mexico, effects on nearshore or coastal ecosystems remain understudied. This study investigated the successional patterns of functional and taxonomic diversity for over 1 year after the DWH oil was deposited on Pensacola Beach sands (FL, USA), using metagenomic and 16S rRNA gene amplicon techniques. *Gamma*- and *Alphaproteobacteria* were enriched in oiled sediments, in corroboration of previous studies. In contrast to previous studies, we observed an increase in the functional diversity of the community in response to oil contamination and a functional transition from generalist populations within 4 months after oil came ashore to specialists a year later, when oil was undetectable. At the latter time point, a typical beach community had reestablished that showed little to no evidence of oil hydrocarbon degradation potential, was enriched in archaeal taxa known to be sensitive to xenobiotics, but differed significantly from the community before the oil spill. Further, a clear succession pattern was observed, where early responders to oil contamination, likely degrading aliphatic hydrocarbons, were replaced after 3 months by populations capable of aromatic hydrocarbon decomposition. Collectively, our results advance the understanding of how natural benthic microbial communities respond to crude oil perturbation, supporting the specialization-disturbance hypothesis; that is, the expectation that disturbance favors generalists, while providing (microbial) indicator species and genes for the chemical evolution of oil hydrocarbons during degradation and weathering.

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Introduction

The oil spill caused by the blowout of the Deepwater Horizon (DWH). Drilling rig in April 2010 constitutes the largest accidental release of oil into the marine environment in recorded history. Oil contamination from the DWH spill had a profound impact on indigenous microbial communities, and all available studies recognize shifts in the composition of microbial communities in direct contact with oiled seawater and sediments in comparison with pristine environments (Atlas and Hazen, 2011; Joye *et al.*, 2014; Kostka *et al.*, 2014; King *et al.*, 2015). Moreover, consistent patterns were observed in microbial communities exposed to DWH oil in the

Gulf of Mexico including an increase in the relative abundance of members of the *Gammaproteobacteria*, a prevalence of known hydrocarbon-degrading populations, and the enriched abundance and expression of genes related to hydrocarbon degradation (Joye *et al.*, 2014; Kostka *et al.*, 2014; King *et al.*, 2015). These patterns and microbial responses are also in accordance with observations from laboratory studies and previous accidental releases of oil in marine environments (Röling *et al.*, 2002; Head *et al.*, 2006; Yakimov *et al.*, 2007; Berthe-Corti and Nachtkamp, 2010; Greer, 2010; McGenity *et al.*, 2012).

The Unified Area Command estimated that approximately one-half of the ~4.9 million barrels of oil released from the DWH blowout reached the ocean surface (Lubchenco *et al.*, 2010), and a portion of this surfaced oil transported to nearshore and coastal ecosystems was buried in the sediments (Hayworth *et al.*, 2011; Wang and Roberts, 2013), impacting approximately 850 km of beaches from east Texas to west Florida (Michel *et al.*, 2013). Oil started depositing on the Pensacola Beach sands

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studied here on 22 June 2010. The input of large amounts of crude oil, including an array of potentially toxic compounds, posed a potential disturbance for benthic microbial communities (Valentine *et al.*, 2012). Available studies to date were primarily focused on the water column and/or deep sea ecosystems, and less is known about the response or adaptation of sedimentary communities to oiling (Huettel *et al.*, 2014). Studies characterizing the taxonomic shifts between contaminated and non-contaminated beach sediments recognized that the oil input strongly affected the beach sand microbial communities, which responded with increased bacterial cell densities (Kostka *et al.*, 2011), reduced taxonomic diversity, and a succession of microbial populations that paralleled the changes in abundance and composition of deposited hydrocarbons (Kostka *et al.*, 2011; Bik *et al.*, 2012; Lamendella *et al.*, 2014). Consistent responses have been observed across study sites, although other factors such as site heterogeneity and seasonal fluctuations in environmental parameters have been shown to somewhat confound assessments of the oil impact in certain beaches (Newton *et al.*, 2013), sometimes making them undetectable (Röling *et al.*, 2004). In general, an initial increase in the relative representation of known oil degraders, mostly of the *Gammaproteobacteria* class (most notably *Alcanivorax*), was observed together with a temporal succession characterized by an increase in relative abundance of *Bacillus*, *Microbacterium* and members of the *Alphaproteobacteria* class at later stages, when recalcitrant oil hydrocarbons predominate (Kostka *et al.*, 2011). Moreover, the increase in oil degraders was concomitant with an increased expression of polycyclic aromatic hydrocarbons, *n*-alkane and toluene degradation genes as assessed by metatranscriptomics (Lamendella *et al.*, 2014). Although these findings provided important insights into the effects of oil on benthic microbial community composition, the gene functions selected for and the genomic adaptations in response to the presence of oil remained mostly uncharacterized in the Gulf coast.

Previously identified shifts in microbial communities in response to DWH oil, both in the water column and sediments, indicated significant susceptibility of these communities; susceptibility defined as the degree to which community composition changes in response to disturbance (Shade *et al.*, 2012). These observations are in accordance with the majority of ecological studies addressing the effect of disturbances such as carbon inputs on microbial communities, which have found evidence of susceptibility (reviewed by Allison and Martiny, 2008). However, the magnitude, stability and stochasticity of functional responses, as well as the mechanisms driving the taxonomic and functional composition of the microbial community after disturbance are not well understood (Reed and Martiny, 2007). For example, it has been recognized

in plant and animal communities that generalist populations better withstand disturbances, whereas specialist populations tend to be favored in stable environments (specialization-disturbance hypothesis; Vázquez and Simberloff, 2002). According to the disturbance-specialization hypothesis, most specialist taxa are selected against when communities experience a severe disturbance, as they are adapted to relatively narrow niches in their natural ecosystem. In contrast, generalists are more resilient to disturbances altering the niches. In turn, the taxonomic diversity of the community is negatively impacted by a disturbance, but the functional diversity can increase as an effect of the disturbance. Although some previous studies applied ecological theory to describe the response and recovery of community dynamics to disturbance (*cf.* Prosser *et al.*, 2007; Shade *et al.*, 2012), the relationship of disturbance and specialization remains largely unexplored in microbial communities. Disturbed communities are typically observed to encompass reduced taxonomic and/or phylogenetic diversity compared with undisturbed controls, but whether this pattern translates to reduced functional diversity or increased specialization remains largely unknown. In this study, we aimed to characterize the response of sedimentary microbial communities from Pensacola Beach to the DWH oil spill, as an *in-situ* experiment of the effects of disturbance on functional and taxonomic diversity.

Materials and methods

Beach sands were collected at Pensacola Municipal Beach, FL, USA (30°19.57'N, 087°10.47'W) on 6, 10, 20 and 24 May 2010 (before arrival of the oil plume to the shoreline; hereafter, termed pre-oil communities/samples), 30 July 2010 (one month after the oil reached the beach; oiled), 20 October 2010 (when oil constituents were still present in the sand; weathered oiled), and 14 June 2011 (when oil was not visually detectable; recovered; Table 1 and Supporting methods). Samples were collected from aerobic beach sediments (oxygen concentrations between 210 and 230 $\mu\text{mol l}^{-1}$ down to 55 cm depth, which represents >50% of air saturation level) above groundwater level.

16S rRNA gene amplicons were sequenced, and the resulting sequences were analyzed as described recently (Poretsky *et al.*, 2014). Trimmed sequences were clustered into operational taxonomic units (OTUs) at 97% similarity using UCLUST (Edgar, 2010), OTUs that represented <0.005% of the total sequences were discarded (Bokulich *et al.*, 2013) and representative sequences of each OTU were classified using the RDP Classifier at 50% confidence (Wang *et al.*, 2007). Shotgun community DNA was sequenced, and the resulting metagenomic reads were quality checked, assembled and annotated as described in the supplementary online

Table 1 Samples used in this study

Designation	Reads after trimming ^a	Status ^b	Depth (cm)	Sampling date	Sediment temp. (°C) ^c
S1	2 937 972	Pre-oil	0	6 May 2010	29.96 ± 2.66 ^d
S2	7 951 456	Pre-oil	0	10 May 2010	
S3	7 837 390	Pre-oil	0	20 May 2010	
S4	6 710 972	Pre-oil	0	24 May 2010	
A	32 840 836	Oiled	30–40	30 Jul 2010	30.49 ± 2.72
B	32 392 430	Oiled	35		
C	25 024 134	Oiled	30–40		
D	21 469 632	Weathered oil	48–62		
E	26 279 070	Oiled	40–45	20 Oct 2010	23.73 ± 2.95
F	34 830 190	Oiled	25–47		
G	39 208 672	Oiled	24–36		
H	25 224 316	Weathered oil	50–55		
I600	33 188 686	Recovered	30–40	14 Jun 2011	31.02 ± 2.79
I606	31 477 910	Recovered	30–40		
J598	31 724 116	Recovered	50–65		
J604	28 119 496	Recovered	50–65		

^aReads after quality trimming with maximum probability of error of 1% and minimum length of 50 bp, and removal of contamination with adaptor sequences.

^bSamples with oiled and weathered oil status were distinguished based on visual assessment of oil presence. Recovered status was defined based on undetectable levels of hydrocarbons at depths similar to (previously) oiled samples.

^cSediment temperature between 0 and 50 cm depth presented as mean ± one standard deviation.

^dData for May 2010 not available, presented values were measured in June 2010. Cf. temperatures in May 2011: 25.21 ± 2.07.

material. The level of coverage of the community achieved by each metagenomic dataset was estimated and projected using Nonpareil with default parameters (Rodríguez-R and Konstantinidis, 2014b). Assembled contigs were taxonomically annotated using MyTaxa (Luo *et al.*, 2014). 18S rRNA gene-encoding reads were identified by Metaxa (Bengtsson *et al.*, 2011) with e-value <0.1 and taxonomically annotated using pplacer and taxtastic (Matsen *et al.*, 2010). Read mapping to estimate the relative abundance of genes and taxa was performed using BLAT with default parameters (Kent, 2002), considering only the best match with alignment length ≥80 bp and identity ≥97%. Annotation terms and taxa with significantly different abundance between groups of samples were identified using the negative binomial test as implemented in DESeq2 (Anders and Huber, 2010).

To measure the average number of genes per cell with a given functional annotation (genome equivalents), a set of universally conserved single-copy genes were identified among the assembled gene sequences from the metagenomes. All genes were compared against a collection of 101 HMMs (Dupont *et al.*, 2012), using HMMER3 (<http://hmmer.janelia.org/>) with default settings and trusted cutoff, excluding genes for which more than one model represented the same gene family. The median sequencing depth (in reads/bp) of the remaining 91 models was used as the normalizing factor for each dataset. The sequencing depth of genes with a given annotation (see below) was estimated for each dataset (in reads/bp), added up and divided by the normalizing factor of the corresponding dataset.

To identify genes related to oil degradation, gene-specific databases were compiled and manually curated. Sequences for AlkB (alkane hydroxylase)

and CYP153 (cytochrome P450 family) were derived from the annotated datasets by Wang *et al.* (2010); sequences for NahA (naphthalene 1,2-dioxygenase) were derived from the set compiled by Lu *et al.*, 2012; and sequences for ArhA (polycyclic aromatic hydrocarbon dioxygenase) and BBS (benzylsuccinyl-CoA dehydrogenase) were derived from UniRef50 clusters (Suzek *et al.*, 2007). Putative proteins of the assembled metagenomes were functionally identified using blastp (Altschul *et al.*, 1990) against each reference dataset, with a 250 bit-score threshold. The resulting dataset for AlkB was aligned using Muscle v3.8.31 with default parameters (Edgar, 2004), and the gene phylogeny was reconstructed using RAXML v7.7.2 with GTR model (proteins), gamma parameter optimization, and 'f a' algorithm (Stamatakis, 2006). Putative coding fragments predicted with FragGeneScan (Rho *et al.*, 2010) on sequence reads were subsequently placed onto the reconstructed tree based on a sequence-to-profile alignment built with Clustal Omega v1.1.0 (Sievers *et al.*, 2011), using the evolutionary placement algorithm (Berger *et al.*, 2011). The same placement strategy was independently applied to the partial sequences of AlkB reported in the study by Smith *et al.* (2013) (GenBank entries KF613175-KF613575).

Diversity was calculated as the true diversity of order one (¹D; equivalent to the exponential of Shannon index). The α and γ components were estimated from the abundance of categories in a sample and in all samples, respectively, and adjusted for unobserved fractions using the Chao-Shen correction (Chao and Shen, 2003) as implemented in the R package entropy (Hausser and Strimer, 2013). Richness was estimated using the Chao1 index (Chao, 1984), and evenness was calculated as the corrected true diversity of order

one (number of equivalent groups) divided by the estimated richness (number of groups).

Crude oil hydrocarbons in the sediment samples were identified by gas chromatography-mass spectrometry using an Agilent 7890A Series GC (Santa Clara, CA, USA), coupled to an Agilent 7000 triple quadrupole MS system, as described previously (Zuijdgeest and Huettel, 2012). The supplementary online material provides further information about procedures and analytical techniques.

All sequencing datasets were deposited in the NCBI Sequence Read Archive under project PRJNA260285 and additional material is available at <http://enve-omics.ce.gatech.edu/data/oilspill>.

Results

Description of samples and their metagenomes

Concentrations of total petroleum hydrocarbons quantified by gas chromatography-mass spectrometry and visible oil stains monotonically decreased between sampling dates (P -values ≤ 0.05 , one-sided t -test; Figure 1a). Specifically, the depth-integrated sedimentary inventories of small molecular weight aliphatic and aromatic compounds decreased rapidly from 6 and 1 mg kg⁻¹, respectively, in July to less than 0.5 mg kg⁻¹ in October. In contrast,

gas chromatography-mass spectrometry profiles revealed that sedimentary inventories of aromatic compounds greater than C8 remained unchanged during this same time frame, whereas aliphatic compounds greater than C6 displayed only a marginal reduction.

A total of 16 metagenomic samples, ranging in size from 3 to 78 million reads after trimming (paired-end reads with average length of 90–190 bp per dataset), were recovered from each of the four sampling time points, with at least three replicates per time point (Table 1). The metagenomes from pre-oil samples had an estimated abundance-weighted average coverage (Rodriguez-R and Konstantinidis, 2014b) of 18–39%, the oiled samples a coverage of 35–60% and the samples from recovered communities an average coverage of 20–25%. Nonpareil curves indicated that the communities in the recovered samples had a higher sequence complexity than both pre-oil and oiled communities, with pre-oiled communities displaying a slightly lower sequence complexity (Supplementary Figure S1A). The described trend in sequence complexity corresponded to the estimated richness of these communities based on OTUs from 16S rRNA gene amplicon data (Supplementary Figure S1B). In general, all metagenomes showed lower sequence complexity than previously determined metagenomes from

Figure 1 Shifts in taxonomic and functional profiles in relation to oil concentration. (a) The concentration of total petroleum hydrocarbons was significantly higher in July samples (A, B, C) relative to October 2010 (E, F, G), and June 2011 samples (I600, I606, J598, J604). The comparisons between groups (July to October 2010, and October 2010 to June 2011) were performed using one-sided t -tests (P -values in grey boxes), and the average per group is indicated as horizontal lines. The non-metric multidimensional scaling of (b) genera and (c) subsystems reveals non-overlapping regions between pre-oiled (green), oiled (shadowed grey) and recovered (olive) samples. The two-dimensional stresses for genera and subsystems are 3.361% and 3.358%, respectively, and the origins are indicated with grey lines. Distance matrices were generated using Bray-Curtis dissimilarities of normalized read counts and ordination was selected by minimizing stress on two dimensions. Note that the heavily oiled samples also form non-overlapping areas by sampling date (dark green and brown), and are distinguishable from weathered oil samples (pink and dark red).

clayey or silty soils such as rain forest and permafrost but were more complex than freshwater or ocean planktonic metagenomes (Supplementary Figure S1A; cf. Rodriguez-R and Konstantinidis, 2014a). The July and October 2010 samples (oiled and weathered oil) were assembled into ~56 000 contigs per sample with N50 of ~1400 bp; while those from recovered samples resulted in ~12 000 contigs per sample with N50 of ~745 bp (Supplementary File S1). These results further supported the Nonpareil estimates of higher sequence complexity in the latter samples. In total, ~670 000 contigs were obtained with an overall N50 of 1101 bp (723 Mbp in total, from 37 Gbp of sequencing reads), on which ~1.2 million genes were predicted, resulting in an average coding density of 87% (Supplementary File S1).

Microbial community specialization in response to oiling

To assess the temporal effects of the oil spill on the microbial community composition and its recovery, the functional and taxonomic profiles at different time points were compared. Four main groups were identified which significantly differed in both taxonomic and functional distributions (P -values ≤ 0.003 , ANOSIM based on Bray-Curtis dissimilarity; Figures 1b and c) and were consistent with the oil concentrations measured *in-situ*: Pre-oil (S1, S2, S3, and S4), Oiled July 2010 (A, B, C), Oiled October 2010 (E, F, G) and Recovered (I600, I606, J598, J604). 16S rRNA gene amplicon data also demonstrated that sample depth played a limited role in structuring microbial communities (Supplementary Figure S2; ADONIS: 3% variance explained by depth vs 75% explained by oiling status and collection date), which was consistent with the facts that the beach sands studied here are subjected to high levels of erosion, and high levels of oxygen (>50% of air saturation level) were detectable at all sampling depths. Hence, our pre-oiled datasets, even though originated from different depths (surficial) compared to oiled datasets (30–65 cm), represented reliable controls for assessing the oiled and recovered microbial communities.

Most notably, the communities exhibited an increase in the functional diversity in oiled samples with respect to pre-oil samples, and a reduction in functional diversity in recovered samples with respect to oiled samples (Supplementary Figure S3A), revealing a different state of lower functional diversity in the recovered communities (Supplementary Figure S3B; DECORANA analysis). Interestingly, this pattern was not observed in the taxonomic diversity, richness or evenness levels (Supplementary Figure S3C–E), indicating that it was primarily due to a decrease in functional specialization of the communities in the oiled samples. This interpretation is further supported by a concomitant decrease in the estimated

minimum doubling time in the oiled communities (Supplementary Figure S4A), as expected for bacteria with more generalist strategies (Dethlefsen and Schmidt, 2007). More generalist prokaryotes tend to have larger genomes (Konstantinidis and Tiedje, 2005), but no significant changes in the estimated average genome size were detected (Supplementary Figure S4B). Nevertheless, these results suggested that the oil disturbance caused community shifts characterized by a decrease in functional specialization and a consequent increase in functional diversity, which were reversed in the post-disturbance recovery process as the succession advanced.

Oil degradation and toxicity drives community phylogenetic composition

Differences in the composition of the communities from pre-oil, oiled and recovered sediments were detected at various levels of taxonomic resolution (Figure 2; Supplementary File S3). At the most general level (domain), recovered communities exhibited higher fractions of eukaryotic and archaeal members than oiled and pre-oiled communities (Figure 3a), although no differences in the taxonomic composition of the eukaryotic fraction were observed (Supplementary File S3). The higher fraction of eukaryotic sequences is also consistent with the lower coding potential of May, July and October 2010 metagenomes (~89% of total sequence length was protein-coding) vs the Recovered (June 2011) metagenomes (70%; Supplementary File S1). The higher representation of dominant taxa and lower evenness in communities from oiled samples was also evident at the class level, where *Gamma*- and *Alphaproteobacteria* increased in abundance, with a concomitant decrease of novel taxa (represented by the unclassified fraction; Figure 2b). The genera significantly more abundant in oiled than in pre-oiled and/or recovered samples were primarily well-known and suspected hydrocarbon degraders, including *Alcanivorax*, *Pseudomonas*, *Hyphomonas*, *Parvibaculum*, *Marinobacter* and *Micavibrio* (Figure 2c). In contrast, groups significantly enriched in recovered samples included taxa typically found in marine environments and known to be highly susceptible to xenobiotics such as the archaeal genera *Nitrosopumilus* and *Cenarchaeum*.

Functional gene content shift in response to oil

To further investigate the specific functional traits selected by oil presence and, presumably, accounted for the community compositional shifts observed, the abundances of genes associated with alkane and aromatic degradation pathways were compared between pre-oil, oiled and recovered samples. In all evaluated cases, oiled communities displayed a larger prevalence of gene annotations associated with aromatic and alkane hydrocarbon degradation

Figure 2 Taxonomic shifts in the microbial community in response to oil. The distribution of metagenomic reads in (a) domains and (b) classes is displayed for taxa that recruited more than 10% and 2% of the total reads, respectively (white numbers). (c) Genera with abundance above 0.1% and significantly different between pre-spill and oiled or between oiled and recovered samples (P -value adjusted ≤ 0.01) are also displayed. The minimum and maximum abundance of each genus is indicated with open and filled circles, respectively, and the class is indicated with superscripts.

as well as beta-oxidation than pre-oil and recovered communities (Figure 3). Interestingly, the relative abundance of most genes associated with aliphatics degradation dropped from July to October 2010, in particular those associated with rubredoxin-NAD⁺/NADP reduction and aldehyde oxidation (top panel

in Figure 3). In contrast, the abundance of genes associated with aromatics degradation was roughly maintained or, in some cases, increased from July to October 2010 (second panel in Figure 3). In addition, functions related to nutrient scavenging such as allantoicase and nitrogenase (low nitrogen response),

Figure 3 Microbial community functional shifts in response to oil. Selected molecular functions related to hydrocarbon degradation, nutrient scavenging and response, photosynthesis, and some house-keeping genes are listed (left) along with the mean genome equivalents per group of samples (middle) and the log₂ of Preoil/Oiled and Oiled/Recovered fold changes (right). The rightmost column indicates the GO ID of the terms. The abundance was assessed as average genome equivalents (mean copies per bacterial/archaeal cell) on each sampling time (downwards; see legend). The triangles indicate values below the plotted range. The log₂-fold-change was estimated as the log₂ of the ratio of normalized counts between pre-oiled samples (S1, S2, S3, S4) and oiled samples (A, B, C, E, F, G); and between oiled samples and recovered samples (I600, I606, J598, J604). *P*-values were estimated using a negative binomial test.

Figure 4 Phylogenetic reconstruction of AlkB protein sequences and putative sequences recovered from the metagenomes. The tree displays reference AlkB (alkane hydrolase) proteins (text colored by clusters, following the nomenclature of Wang *et al.*, 2010) along with variants assembled from the metagenomes (black text). Proteins with experimental evidence of activity (from heterologous expression or gene knockouts) are indicated by +. Reads mapping to different nodes of the tree are displayed as pie charts. The radius of the pie charts indicates the fraction of the metagenomes mapping to the node (expressed as reads per million, Reads mapping legend), and the different colors of the slices indicate the dataset of origin (Dataset legend). The terminal branch of the sequence A-87946 (cluster V) was shortened by 7.0 units, as indicated by a discontinuity. The right panel indicates the total abundance of each cluster averaged per group of datasets (in reads per million): Pre-oil samples (green), Oiled samples from July 2010 (mauve), Oiled samples from October 2010 (sea green) and recovered samples (olive). Reference sequences (including out-group sequence XylM from *Pseudomonas putida*) and clusters nomenclature (in squared parenthesis) are based on Wang *et al.* (2010), but the definition of the clusters (colored backgrounds) was broadened to include all sequences in the analysis, and two additional clusters were defined ('OS-I' and 'OS-II').

difference in abundance in the oiled communities returned to the pre-oil state in the recovered communities, in some cases exceeding their pre-oil levels (Figure 3 and Supplementary File S4).

To explore the phylogenetic diversity of genes related to oil degradation, we selected AlkB (alkane hydrolase) as a marker for alkane degradation and reconstructed a high-quality gene phylogeny based on 66 reference genes (mostly based on Wang *et al.*, 2010) and 43 genes recovered from metagenomic assemblies. In addition, individual metagenomic reads from all datasets were assigned to the most likely node in the tree to provide a quantitative picture of the shifts of AlkB variants over time (Figure 4). This dataset included sequences from 14 different genera in five different classes, hence spanning a large diversity of known alkane degraders. Additionally, this dataset covered the diversity of the partial AlkB sequences reported by Smith *et al.* (2013) for the northern Gulf of Mexico, most of which were assigned to clusters IV (73.5%) and II (20.9%). As expected, very few reads from recovered samples were placed in the tree, and most placed reads were derived from oiled or weathered oil samples. However, an intermediate abundance was detected in pre-oil samples (Figure 3, first row: alkane 1-monooxygenase). In fact, only cluster III was undetectable in pre-oil samples, whereas all other clusters followed the general trend observed for the entire gene abundance (*cf.* right panel on Figure 4 and first row on Figure 3). More importantly, the reads from different oiled and pre-oil samples were distributed across different clades, with larger concentrations in a few clades spanning the entire tree, that is, an uneven but phylogenetically unconstrained distribution. Notably, we identified a cluster formed exclusively by genes from this study (labeled 'OS-I' in Figure 4) most abundant in the pre-oil samples and negatively impacted by the oil spill.

Population successional patterns and community recovery

In addition to the large differences observed in community composition (both taxonomic and functional) between pre-oil, oiled and recovered samples, the microbial communities characterized in July 2010 differed from those in October 2010 (Figures 1b and c), concurrent with a significant reduction in total petroleum hydrocarbons (Figure 1a). Examination of the taxonomic distribution revealed that some populations responded rapidly, reaching high abundances in July 2010, with large reductions in abundance by October, and being barely detectable in the recovered samples of June 2011 (Figure 3c). These populations included members of the *Alcanivorax*, *Borrelia*, *Spirochaeta*, *Micavibrio* and *Bacteroides* genera. However, some populations were observed to peak in abundance in the October 2010 samples and significantly drop in the recovered samples, such as *Hyphomonas*,

Treponema, *Sphingopyxis* and *Hirschia*. Most oil-associated genera did not maintain their abundance in oiled samples of July and October 2010 with the notable exceptions of *Marinobacter* and *Parvibaculum*. The abundance profiles probably reflected organisms with different metabolic properties with respect to oil degradation such as fast responders to easily degradable oil constituents, organisms specialized in degradation of aromatic and more recalcitrant oil fractions, and oil degradation generalists. Finally, we identified a significant increase in minimum doubling time between oiled samples of July and October 2010 based on codon usage bias patterns (Vieira-Silva and Rocha, 2010) (difference of means: 3 h 9 m, P -value $< 10^{-16}$, two-sided t -test; Supplementary Figure S4A). The increase in doubling time observed from October 2010 to the recovered samples (June 2011) was much smaller and not statistically significant (difference of means: 27 m, P -value: 0.19, two-sided t -test). Altogether, these results indicate that the community response to the oil spill involved well-defined successional trends: a rapid response (from May to July 2010), with a peak growth rate in July 2010, followed by a continued decrease in taxonomic diversity (between May and October 2010) and, finally, a reduction in abundance of several known and suspected oil degraders, concomitant with the increase in abundance of several typical marine groups undetectable or rare in oiled samples, a large increase in taxonomic diversity and a decrease in functional diversity.

Discussion

The sands of the Pensacola Municipal Beach received repeated pulses of oil deposition for over a month, and, after about a year, oil was still detected in the beach sands, although it had concentrations below 5 mg kg^{-1} (Figure 1a). This indicates that the microbial community faced largely a press (long-term) disturbance given the time scale of microbial generation cycles and migration processes (Shade *et al.*, 2012). Press disturbances often result in community shifts driven by the response traits of individual populations to the disturbance, presumably sensitivity to toxic compounds and hydrocarbon degradation capabilities in the case of oil contamination. The diversity and abundance of indigenous alkane-degraders preceding the oil spill in the beach ecosystem, as well as the origin of the degraders observed after the spill, was not robustly assessed in previous studies mostly owing to the incomplete diversity recovered in cultures of alkane-degraders and lack of complete understanding of their ecophysiology. The observation of a large and phylogenetically unconstrained diversity of *alkB* genes in the oiled and pre-oil samples supports the hypothesis that the response to the oil spill was primarily caused by shifts in abundances of pre-existing populations, as previously

observed in the deep-sea oil plume (Hazen *et al.*, 2010). In other words, the *alkB* genes present in the oiled communities were not derived from a single or a few recent gene alleles but, instead, a large diversity of degraders was latent in the sand and/or surface waters seeping into the sands before the oil spill.

Initial responders (July 2010) included members of the genera *Alcanivorax*, *Borrelia*, *Spirochaeta*, *Micavibrio* and *Bacteroides*, all members of the abundant fraction ($\geq 1\%$ of the total community) in the oiled samples. *Alcanivorax* is a genus known for its hydrocarbonoclastic capabilities that can utilize alkanes but not aromatic hydrocarbons (Schneiker *et al.*, 2006); the metabolic capabilities of the other genera in oil hydrocarbon degradation remain speculative. Interestingly, we found putative *alkB* genes (alkane hydrolase) in contigs classified as *Alcanivorax*, *Borrelia* and *Bacteroides*, but no evidence of *arhA* (polycyclic aromatic hydrocarbon dioxygenase) in any of these genera, and putative *nahA* genes (naphthalene 1,2-dioxygenase) only in *Alcanivorax*. The former populations were replaced in the abundant fraction in October 2010 by members of the genus *Treponema* and the class *Alphaproteobacteria* (including *Hyphomonas*, *Sphingopyxis* and *Hirschia*), suggesting a successional dynamic as previously observed based on 16S rRNA gene amplicon data (Kostka *et al.*, 2011; Lamendella *et al.*, 2014). Members of the *Hyphomonas* genus have been reported as abundant members in consortia degrading aromatic compounds, which are typically more recalcitrant components of the crude oil than alkanes and hence, more prevalent in later post-spill stages (Maeda *et al.*, 2009, 2010). Similarly, *Sphingopyxis* is known to have aromatic hydrocarbon degradation capabilities (Kertesz and Kawasaki, 2010) and was previously detected as a dominant group in soil-derived oil-degrading consortia amended with natural organic matter (Hassan *et al.*, 2011).

Very few microbial groups, including members of the genera *Marinobacter* and *Parvibaculum*, were consistently enriched in the oiled samples with no noticeable change in abundance between July and October 2010. Putative *alkB* and *cyp153* (cytochrome P450 family) genes, associated with alkane degradation, were identified in assembled contigs assigned to both genera, and putative *nahA* genes, associated with aromatic hydrocarbon degradation, were identified in contigs classified as *Marinobacter*. Members of the *Marinobacter* genus are able to degrade a large variety of aliphatic and aromatic hydrocarbons (Gauthier *et al.*, 1992). Similarly, members of the *Parvibaculum* genus exhibit metabolic capabilities for both aliphatic and aromatic degradation (Schneiker *et al.*, 2006; Wang *et al.*, 2010; Lai *et al.*, 2011). In contrast to previous analyses based on 18S rRNA gene amplicons (Bik *et al.*, 2012), no consistent, statistically significant shifts in the taxonomic composition of the eukaryotic fraction were detected between sampling dates or degree of oiling (Supplementary File S3).

Finally, in June 2011, *Synechococcus*, *Pedococcus* and archaeal genera including *Nitrosopumilus*, *Cenarchaeum* and *Nitrosoarchaeum* dominated the abundant fraction (in contrast to oiled samples), and an overall increase in the eukaryotic fraction was observed. Many of the former microbial groups are abundant in oligotrophic or nutrient-poor marine ecosystems, indicating that they represent the sensitive fraction of the community to the oil spill, but to a large extent the community was resilient, as generally observed in microbial communities (Allison and Martiny, 2008). Notably, the observed succession process exhibited signs of community recovery, but the community in June 2011, 1 year after the oil reached the shoreline, significantly differed from that in May 2010, before oiling, similar to the results of previous microcosm experiments on oil amendment of beach sediment inocula (Röling *et al.*, 2002). The differences between the recovered community and its counterpart before the oil spill may be due to the long-term effects of the oil disturbance (for example, establishment of new taxa), stochastic events or other environmental factors such as organic matter input, nutrient input and salinity changes. Clearly, more samples and analyses would be required to obtain further insights into the latter issue. Nonetheless, our results also suggest that these sensitive marine groups could serve as indicator species of oil presence and toxicity in future oil spill studies, and thus, potentially provide useful information for guiding bioremediation efforts and decisions by site managers.

In general, microbial communities changed both taxonomically and functionally after exposure to a range of petroleum hydrocarbon concentrations. The community shifts caused a decrease in taxonomic diversity during May to October 2010, with a significant recovery by June 2011 (Supplementary Figure S3C). Interestingly, the functional diversity was observed to follow a contrasting trend: it increased between May and July 2010, was maintained between July and October 2010, and significantly decreased in June 2011 (Supplementary Figure S3A-B). We hypothesize that several oligotrophic (specialized) taxa were strongly outcompeted upon deposition of oil onshore. Growth arrest due to limited hydrocarbon degradation capabilities and/or sensitivity to toxic compounds from the continued presence of oil onshore would impact more severely oligotrophic and/or specialist than copiotrophic and/or generalist populations. Hence, a significant reduction in taxonomic diversity but not functional diversity was expected, as observed in these communities. Moreover, we provided evidence indicating that specific fast-growing organisms (typically assumed to be copiotrophic) thrived in the presence of relatively high concentrations of petroleum hydrocarbons. In other words, the disturbance favored generalist organisms in the communities, and the post-disturbance communities

were characterized by a narrower set of more specialized functions. This observation seems counterintuitive because a common expectation is that a press disturbance would exclusively select for few highly specialized organisms, in this case oil-degraders. Nevertheless, this trend is predicted by the disturbance-specialization hypothesis (Vázquez and Simberloff, 2002), is consistent with ecological succession theory and was previously observed in plant communities (for example, Bazzaz and Pickett, 1980). It should be mentioned, however, that the patterns observed here might be specific to disturbances from crude oil and sand beach ecosystems and not immediately generalizable to other, more selective disturbances (Röling and van Bodegom, 2014). Crude oil is composed of tens of thousands of different carbon sources that would favor generalists in early succession, as well as toxic compounds that would preferentially select against specialists. Sand beaches in the Gulf of Mexico are characterized by low carbon content and nutrient-poor conditions relative to marshes or other coastal sediments (Huettel *et al.*, 2014). This could explain the relative abundance of putative chemolithoautotrophic archaea (*Nitrosopumilus*, *Cenarchaeum*) in the recovered communities and suggests a suppression of a range of organisms that are adapted to carbon-limited conditions.

In summary, the community response was primarily characterized by two concomitant trends. First, most of the community is selected based on the ability to survive under disturbed conditions, that is, the response to the disturbance correlates negatively with the level of specialization. Second, few organisms with traits selected by the disturbance become highly abundant, as niche opportunity promotes invasion (Shea and Chesson, 2002; Pintor *et al.*, 2011). Overall, our results provide evidence of complex successional patterns in the studied communities, involving invasion promoted by capabilities for oil hydrocarbon degradation, as well as population survival generally hindered by specialization and susceptibility to oil toxicity, and a general recovery of diversity, specialization and sensitive marine groups a year after the disturbance.

Conflict of Interest

The authors declare no conflicts of interest.

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