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Effect of Macondo Prospect 252 Oil on Microbiota Associated with Pelagic *Sargassum* in the Northern Gulf of Mexico

Manolito G. Torralba[1] · James S. Franks[2] · Andres Gomez[1] · Shibu Yooseph[1] ·
Karen E. Nelson[1] · D. Jay Grimes[3]

Abstract The environmental impact of major oil spills on marine microorganisms has yet to be thoroughly investigated using molecular biology techniques. The Deepwater Horizon (DWH) drilling rig explosion of 2010 affected an approximately 176,000 km^2 surface area of the Gulf of Mexico (GOM) when an estimated 210 million gallons of oil from the Macondo Prospect spilled into the environment. Pelagic *Sargassum*, a complex of two surface drifting species (*Sargassum natans* and *Sargassum fluitans*) of marine brown macroalgae and a critically important habitat in the GOM ecosystem, was suffused by Macondo Prospect 252 oil released during the DWH event. Using 16S rRNA PCR and Roche 454 pyrosequencing, the effect of the oil on the bacterial population associated with pelagic *Sargassum* and contiguous waters was examined by comparing sequence data generated from samples collected from oiled and non-oiled locations in the northern GOM. Sequence data showed similar microbial composition in *Sargassum* regardless of exposure to oil primarily dominated by five phyla; Proteobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia, and unclassified bacteria. The microbial composition in water samples was significantly less diverse than for *Sargassum* and consisted primarily of *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*. Due to the evenly distributed abundance of microbial species on oiled and non-oiled pelagic *Sargassum*, study findings indicate that DWH spilled oil had minimal effect on the composition and diversity of the microbial community associated with *Sargassum* and contiguous waters. However, higher abundances of *Sulfitobacter* and one species of *Psychrobacter* were found in oiled water samples when compared to non-oiled water samples indicating some effect of DHW oil in the microbial composition of seawater. Though there are a number of marine studies using molecular biology approaches, this is the first molecular examination of the impact of the DWH oil spill on bacterial communities associated with pelagic *Sargassum* and contiguous waters from the GOM.

Keywords *Sargassum* · Deepwater · Gulf of Mexico · Oil

Introduction

Marine algae, or seaweed, refer to many different species of multicellular alga, including red, green, and brown algae. These algae are widely distributed organisms that have many different applications, including food, herbal supplements, drugs, and natural products [1–4]. Due to their prevalence in the world's oceans, marine algae have a large influence on marine ecosystems as they are capable of carbon fixation through photosynthesis and are adept at shaping the structure of habitats [5]. *Phaeophyceae*, or brown algae, is a large class of marine algae comprised of about 2000 species distributed worldwide [6]. Pelagic *Sargassum* (Family *Sargassaceae*), a genus of brown algae and commonly known as "gulf-weed," is distributed throughout tropical and subtropical waters and is a well-studied neustonic organism [4].

Pelagic *Sargassum* is a complex of two co-occurring species (*Sargassum natans* and *Sargassum fluitans*) of surface-drifting brown algae found exclusively in the Atlantic Ocean, including

✉ D. Jay Grimes
jay.grimes@usm.edu

1 J. Craig Venter Institute, 4120 Capricorn Lane, La Jolla, CA 92037, USA

2 The University of Southern Mississippi, Gulf Coast Research Laboratory, Center for Fisheries Research and Development, 703 East Beach Drive, Ocean Springs, MS 39564, USA

3 Division of Coastal Sciences, The University of Southern Mississippi, Gulf Coast Research Laboratory, 703 East Beach Drive, Ocean Springs, MS 39564, USA

the Gulf of Mexico (GOM) [7]. These are the only holopelagic species among the greater than 200 species of *Sargassum* found worldwide. Though the two species slightly differ morphologically, both employ small gas-filled vesicles to maintain positive buoyancy within sunlit ocean surface waters (Fig. 1). Winds and ocean currents aggregate these surface drifters into configurations that range from widely dispersed clumps to large neustonic rafts tens of meters wide and windrows that extend across the ocean surface for tens of kilometers [8, 9] (Fig. 2). Pelagic *Sargassum* contributes to primary productivity in ocean surface waters, whereas associated epiphytic filamentous cyanobacteria contribute to overall primary production and nutrient recycling within the pelagic *Sargassum* complex [10, 11]. This association was previously reported by Carpenter [10] and further investigated and reviewed by Phlips and Zeman [11].

Pelagic *Sargassum* is the only naturally occurring biogenic habitat in the open ocean and serves as an essential habitat for a large and diverse assemblage of marine organisms, including numerous species of fish (Fig. 3) [12–16]. Although marine life associated with this open ocean drift community is a focus of ongoing research, little is known of the annual biomass, growth, distribution, and microbial communities associated with pelagic *Sargassum* in the GOM. Bacterial interactions with a host such as pelagic *Sargassum* have yet to be fully studied, particularly using molecular and genomics approaches. As with other seaweeds, pelagic *Sargassum* is expected to release large amounts of organic compounds that have the potential to increase bacterial colonization as shown by other studies that investigated the microbial composition on the surfaces of other algae [17–20]. Studies of interactions between algae and their associated microbial community provide insight into host-microbe interactions, established core microbial communities, organic matter consumption, microbial influence on algal growth and morphology, detection of algal pathogens, and global carbon cycling [17–22]. However, the microbial communities associated with pelagic *Sargassum* have not been described using the next generation sequencing. More importantly, as pelagic *Sargassum* is an important component of tropical and sub-tropical waters, it is unknown how this host-microbe interaction responds to disastrous polluting events such as chemical or oil spills.

Fig. 2 Pelagic *Sargassum* windrow in the northern Gulf of Mexico. Photo credit: James Franks

The Deepwater Horizon (DWH) oil spill of 2010 resulted in an input of approximately 4.9 million barrels of crude oil from Macondo Prospect 252 (MP252) into the GOM [23, 24], affecting an estimated 176,000 km^2 of GOM surface area [25]. As pelagic *Sargassum* is an ubiquitous surface feature throughout the northern GOM, and considering the vast surface spatial extent of the DWH spill footprint, it was evident that there was overlap between pelagic *Sargassum* and oil during the DWH event [12]. During an offshore ichthyoplankton survey cruise in the northern GOM in May 2010, the University of Southern Mississippi Gulf Coast Research Laboratory (USM-GCRL) scientists observed vast surface oil sheens interspersed with amorphous oil globules (~0.3–12.0 cm dia.) and innumerable clumps of oil-infused pelagic *Sargassum* (Fig. 4a, b). These on-site observations (location, date, and time) were confirmed to be associated with the DWH spill via assessments of satellite imagery of ocean surface hydrography and oil spill surface movement in the northern GOM provided by Roffer's Ocean Fishing Forecasting Service, Inc. (Fig. 5) [26].

To investigate the microbial composition of pelagic *Sargassum* and contiguous waters observed to be impacted by the DWH oil spill, samples of each, both visibly oiled

Fig. 1 Pelagic *Sargassum*: *Sargassum natans* (*left*) and *Sargassum fluitans* (*right*). Photo credit: Hazel Oxenford

Fig. 3 Underwater photograph of fishes associated with pelagic *Sargassum* in the northern Gulf of Mexico. Photo credit: Ben Raines

Fig. 4 **a** Oiled pelagic *Sargassum* and surface water observed boat side at collection station 1A in the Gulf of Mexico, 23 May 2010. **b** Oiled *Sargassum* sample from station 1A. Photo credit: James Franks
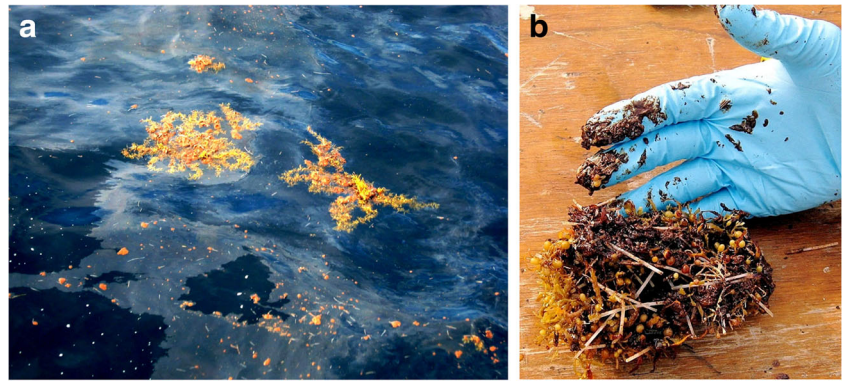
and non-oiled, were collected for assessment. We hypothesize that the spilled MP252 oil had an effect on the bacterial composition and diversity of the communities associated with *Sargassum* and surrounding waters.

Materials and Methods

Sample Collection

Samples of non-oiled and oiled pelagic *Sargassum* (hereafter also referred to as *Sargassum*) and contiguous surface waters were collected from northern GOM locations (stations) in 2010 on 19 and 24 May and 23, 24, and 25 May, respectively (Table 1, Fig. 5). The *Sargassum* and water samples were collected concurrently at individual locations during daytime hours using sampling gear deployed from the USM-GCRL

30.5-m research vessel R/V Tommy Munro. Non-oiled *Sargassum* was collected using a surface neuston net (1 m × 2 m × 4 m, 330-micron mesh; Sea-Gear, http://www.sea-gear. net) towed by the research vessel. Oiled *Sargassum* was collected from surface waters using a dip net (1.5-cm-mesh stretched) and surface neuston net (described above). Clumps of *Sargassum* were quickly removed from the sampling gear by hand using nitrile gloves and placed into sterile, individually labeled 50-mL polypropylene centrifuge tubes. Water samples were also placed into sterile, individually labeled 50-mL polypropylene centrifuge tubes. All samples were immediately frozen (−20 °C) onboard the research vessel. A total of eight *Sargassum* samples and eight water samples were collected.

Global positioning system (GPS) coordinates were recorded for each collection, and hydrographic parameters, including sea surface temperature (°C), salinity (ppt), and dissolved oxygen (mg/L), were measured at each location using a
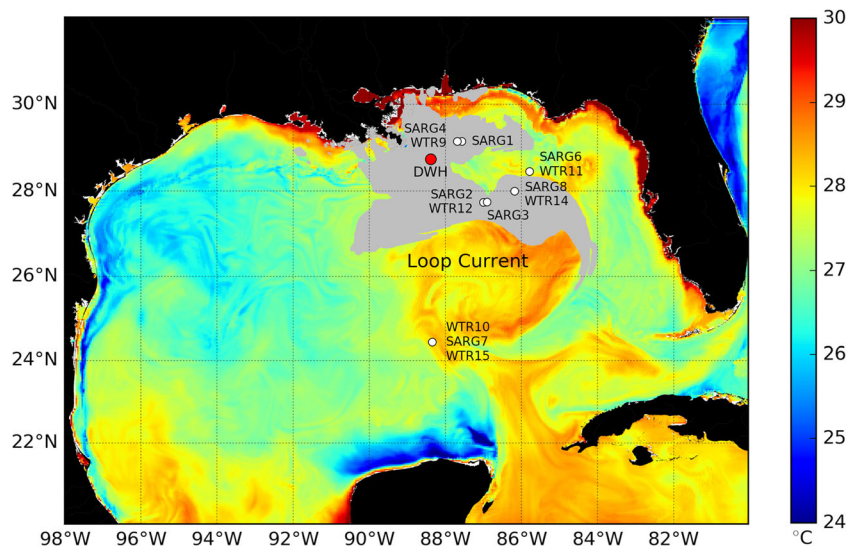

Fig. 5 Collection locations of pelagic *Sargassum* and contiguous water samples analyzed in this study are shown on a map of the Gulf of Mexico (source: http://matplotlib.org/basemap) as *white dots* with Station ID number (see Table 1). Surface spatial extent of the Deepwater Horizon oil spill for 23–30 May 2010 (cumulative coverage) is shown as a *gray overlay* (provided by Roffer's Ocean Fishing Forecasting Service, Inc.; http://www.roffs.com). The location of Station B01 was far removed from DWH surface oil and is shown on the map to provide perspective. Sea surface temperature data provided by the HYbrid Coordinate Ocean Model Consortium (https://hycom.org) are shown for 24 May 2010. The *red circle* indicates the location of the Deepwater Horizon well site (DWH)

Table 1 Field data for non-oiled and oiled pelagic *Sargassum* and contiguous water samples collected from the northern Gulf of Mexico in May 2005

Date	Sample ID	Sample type	Collection location		Distance/bearing	Collection gear	Sea surface		
			N lat.	W long.	(km)[a]		Temp. °C	Sal. ppt	D.O. mg/L
5/19/10	WTR10	Water non-oiled	24° 26.38′	−88° 20.84′	478 S	Bottle	27.4	36.6	5.86
	SARG7	Sargassum non-oiled				Neuston net			
	WTR15	Water non-oiled				Bottle			
5/23/10	SARG2	Sargassum oiled	27° 45.39′	−86° 59.95′	173 SE	Dip net	28.4	35.9	6.03
	WTR12	Water oiled				Bottle			
5/23/10	SARG3	Sargassum oiled	27° 44.65′	−86° 59.51′	171 NE	Dip net	28.4	35.9	6.03
5/24/10	SARG8	Sargassum oiled	27° 59.85′	−86° 10.96′	231 SE	Neuston net	28.5	35.9	5.92
	WTR14	Water oiled				Bottle			
5/24/10	SARG6	Sargassum oiled	28° 27.15′	−86° 47.56′	257 E	Neuston net	29.6	36.6	5.91
	WTR11	Water non-oiled				Bottle			
5/25/10	SARG4	Sargassum oiled	29° 09.03′	−87° 39.51′	84 NE	Dip net	27.5	36.2	6.08
	WTR9	Water oiled				Bottle			
5/25/10	SARG1	Sargassum oiled	29° 09.02′	−87° 41.39′	82 NE	Dip net	27.7	34.4	6.22

[a] Approximate distance and bearing from Deepwater Horizon well site

calibrated YSI Model 85 multi-parameter instrument (Table 1; YSI Incorporated, www.ysi.com). Macrofauna collected in association with *Sargassum* were removed from the sample and returned to the sea. At the conclusion of the cruise, the frozen *Sargassum* and water samples were transferred on ice to USM-GCRL and maintained at −20 °C until analyzed. No specific permissions were required for this work.

DNA Extraction, 16S PCR, and Sequencing

The frozen *Sargassum* and water samples were express shipped to the J. Craig Venter Institute (JCVI) in Rockville, Maryland. *Sargassum* samples were thawed at 4 °C and then re-suspended in 50 mL TE buffer and vortexed, and the bacterial fraction was collected from the supernatant using a filtration kit provided by MO BIO Laboratories (Water Filter kit cat # 14880-50-WF), following manufacturer's specifications. Water samples were filtered using the same method. Bacterial cells were lysed from each filter using lysozyme and proteinase digest, and DNA was extracted with phenol chloroform isoamyl alcohol and precipitated by ethanol precipitation. DNA was then quantified via NanoDrop (Thermo Scientific, http://www.nanodrop.com/) to determine concentration and overall quality using 260/280 ratio. Approximately 100 ng of DNA was used in each PCR reaction (5 min at 95 °C denaturing step followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 2 min) using barcoded and Roche 454 adaptor ligated V1-3 16S primers 27F and 534R targeting the V1–3 variable regions of the 16S rRNA gene [27–30]. 16S rDNA gene amplicons were purified using the Qiagen PCR purification kit (Qiagen, www.qiagen.com) according to

manufacturer's specifications, followed by quantification using Sybr Gold, normalization, and pooling in preparation for Roche 454 sequencing. Emulsion PCR and Roche 454 pyrosequencing was completed with titanium chemistry using standard manufacturer's protocols.

Quality Control and Processing of DNA Sequences

The DNA sequences were processed to ensure that only quality sequences were applied to the MOTHUR pipeline [31]. The SFF output files generated by the Roche 454 sequencer were first converted to fasta and qual files using sffinfo program included as part of the Roche 454 software package. The sequences were then demultiplexed according to barcode using the trim.seqs function in MOTHUR (version v.1.35.0) [31]. Stringent settings were kept to ensure no barcode mismatches among the demultiplexed reads. Additional sequence filtering was used by applying the screen.seqs function of mothur to remove all sequences shorter than 220 bp [31]. Additional QC steps were implemented and the sequences were aligned against the SILVA database to confirm the orientation of noise-filtered sequences along with ensuring the correct positioning of the reads with respect to which variable regions were amplified and sequenced [32]. The sequences passing QC were then checked for chimeras using chimera slayer in mothur [31]. Sequences were classified taxonomically using the RDP classifier (CITE) and hits matching mitochondria, chloroplast, archaea, unknown, and eukaryote were eliminated to avoid noise on the data. Sequence reads were then clustered at various taxonomic levels including 97 % rRNA sequence similarity (OTU), genus, and phyla.

Data Analysis

Species, genus and phyla count tables, and taxonomic classification of 16Sr RNA reads from the RDP Bayesian classifier (CITE) were used for all subsequent statistical analyses in the R statistical environment (CITE). The VEGAN and APE packages of R were used for all multivariate analyses, including Bray-Curtis dissimilarity, PCoA, PERMANOVA, ANOSIM, and sample clustering [31, 33, 34]. The mothur open-source software was also used to calculate Shannon, CHAO, and Jackknife diversity indices to calculate species richness and evenness. Kruskal-Wallis testing was used as a non-parametric approach to determine the statistical significance of the varying levels of abundance at the genus level between the samples. P values <0.05 were considered significant in these calculations.

Results

The extracted DNA from the majority of both *Sargassum* and water samples were amplified using standard 16S rRNA PCR conditions. However, one *Sargassum* sample (SARG5) and two water samples (WTR13 and WTR16) were unable to produce any amplicons after multiple rounds of PCR due to low-DNA yield. Post quality trimming and QC enabled analysis of 101,406 sequence reads with an average read length of 335 bp. Each sample averaged approximately 7800 sequence reads which were subsequently clustered at 97 % sequence identity to generate operational taxonomic units (OTUs).

Microbial Composition in Oiled and Non-Oiled *Sargassum* and Water Samples

Analysis of the 16S rRNA data demonstrated that the microbial population was overwhelmingly comprised of gram-negative species in pelagic *Sargassum* and water samples indicating that the oil had little effect on the overall gram-negative and gram-positive populations. Pelagic *Sargassum* collected at various distances from the DWH site was associated with a complex microbial community dominated mostly by various species of *Rhodobacteraceae* where the average among the *Sargassum* samples was 48.4 ± 17 %, followed by the second most abundant genera, Saprospiraceae 1.6 ± 0.81 %. Permutational analysis of variance and analysis of similarities indicated that there is a significant difference in microbial composition between *Sargassum* and water samples ($P = 0.005$ and $P = 0.002$ with R values, $R = 0.903$ and $R = 0.9696$, for PERMANOVA and ANOSIM, respectively). *Sargassum* and water, regardless of exposure to oil, were quite different in microbial diversity (Fig. 6a, b). The microbial composition in the water samples was less diverse compared to *Sargassum*, and consisted mostly of various species of *Psychrobacter*, *Sulfitobacter*, and *Salegentibacter* (Fig. 6b).

These genera were significantly more abundant in water samples compared to non-oiled or oiled *Sargassum* (Kruskal-Wallis tests, $P = 0.0011$, 0.0044, and 0.0425, respectively). When comparing the two sample types and sample conditions, *Sargassum* samples, non-oiled or oiled, showed higher abundance of unclassified *Rhodobacteraceae*, unclassified *Saprospiraceae*, unclassified *Sphingobacteriales*, and *Bacteroidetes*, when compared to water samples (Kruskal-Wallis tests, $P =$ values of 0.0042, 0.0016, 0.0275, and 0.0016, respectively). The oiled water samples appeared to be considerably different from the non-oiled water samples (Fig. 7) and were distinguishable by the abundance of two different species of *Psychrobacter* (Fig. 6b). Simpson diversity indices along with Shannon diversity indices were calculated to show the varying species richness and diversity between *Sargassum* and water (Fig. 8). Results indicate that the surface of pelagic *Sargassum* is contributing to the increased variation and number of species present in *Sargassum* samples compared to water samples.

Lack of Changes in Microbial Community Associated with Pelagic *Sargassum*

Overall, the microbial composition between oiled and non-oiled *Sargassum* samples did not change with exposure to DHW oil. Though our sample size was low, the microbial composition in the *Sargassum* samples did not seem to be affected by the presence of DWH oil as the most abundant taxa were present in nearly all samples, oiled or non-oiled. However, SARG3 had a higher abundance of *Acidoverax*, *Aquabacterium*, *Ralstonia*, *Cloacibacterium*, and *Diaphorobacter* when compared to the other oiled samples (Fig. 6a). The minimal effect of the oil on the *Sargassum* samples was also confirmed by principle coordinates analysis, as both the oiled *Sargassum* and non-oiled *Sargassum* samples clustered similarly with the exception of one oiled sample SARG3 (Fig. 7). These results indicate that although SARG7 was collected from an unaffected area several hundred miles away from the oil spill and SARG3 was well within the areas affected by DHW oil (Fig. 5), the microbial community did not significantly vary between the oiled and non oiled sample types.

DWH Oil Effect on Microbial Composition in Water

The effect of DWH oil on the water samples was much more apparent than on the *Sargassum* samples. The bacterial community in the water samples was dominated by various species of *Psychrobacter* averaging approximately 75.4 ± 16.4 % for each sample. More importantly, the oiled water samples were considerably different from the non-oiled samples ($P = 0.0014$, $R^2 = 0.816$) due to variation between two species of *Psychrobacter* (Fig. 6b). Due to the limitations of taxonomic

identification using 16S sequencing, we were only able to differentiate between the two genera without identifying actual species names. The oiled water samples had significantly higher levels of what we designated as *Psychrobacter 2* whereas the non-oiled water samples were dominated with what we designated as *Psychrobacter 1*. Principle coordinates analysis also showed that the oiled water samples clustered much differently from the non-oiled water samples where the variation was apparent along PCoA2 (Fig. 7). Three out of the four oiled water samples also showed a higher abundance of one species of

Sulfitobacter when compared to non-oiled water samples (Fig. 6b). These striking differences clearly show that the DHW oil had an effect on the microbial population of the water.

Dissolved Oxygen Readings and Presence of Oil Metabolizing Bacteria in Oiled *Sargassum* and Water

Microbial community composition did not correlate with the amount of dissolved oxygen in the water. There was a lack of any specific trends with the number of species present in each

Fig. 6 Comparison of the microbial community in oiled and non-oiled water samples. The top 20 genera in *Sargassum* (**a**) and water (**b**)
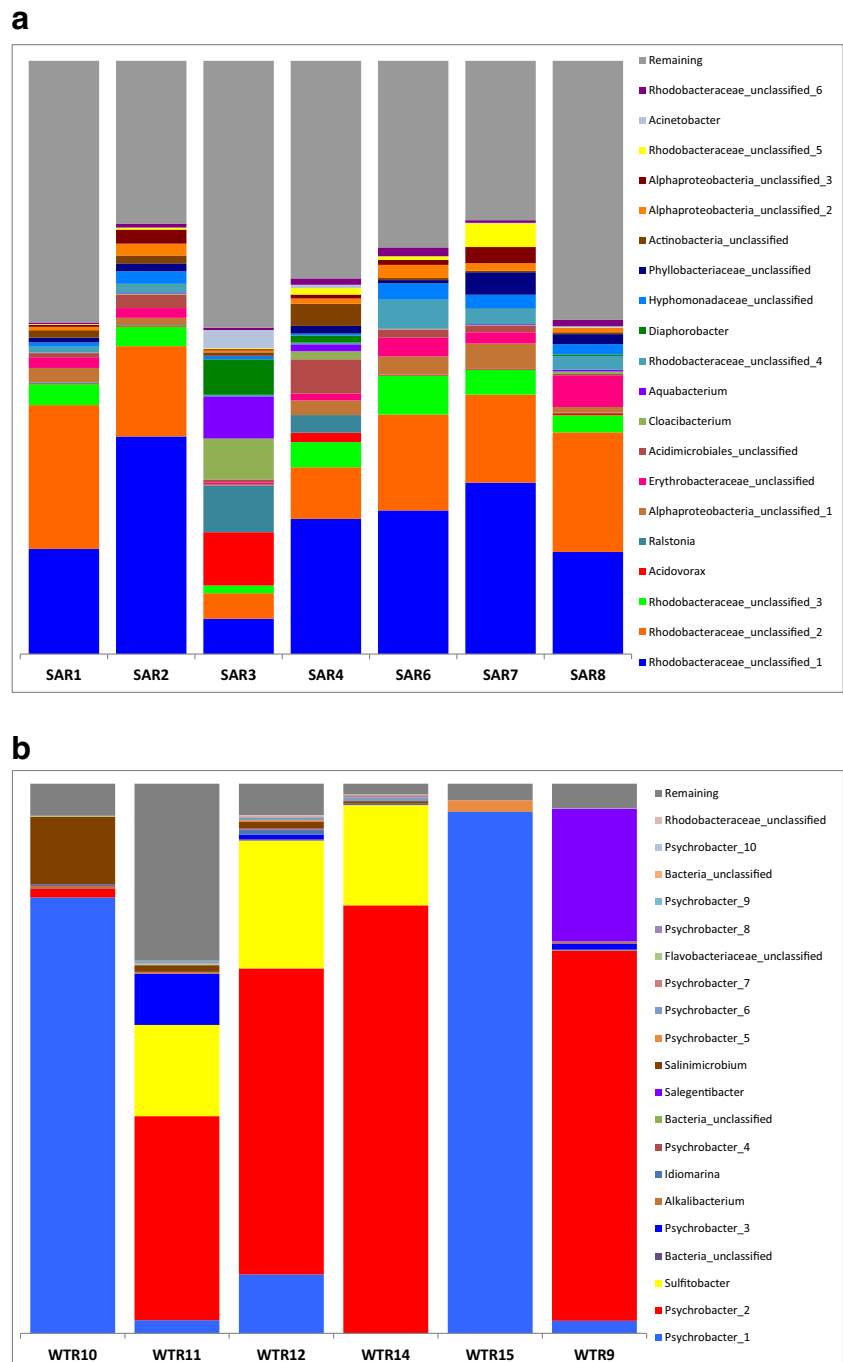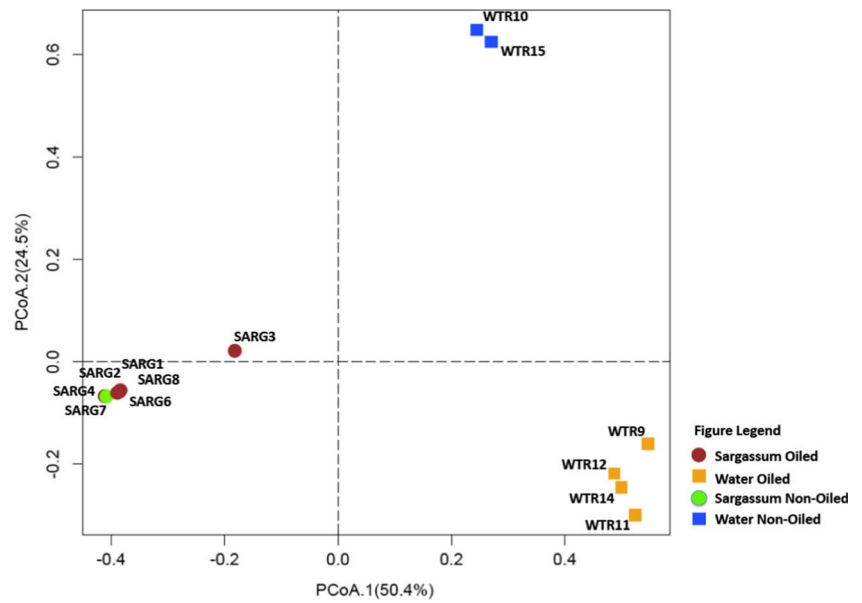
Fig. 7 Principal coordinates analysis (PCoA) of oiled and non-oiled *Sargassum* and oiled and non-oiled water samples. Each *data point* represents one sample, either *Sargassum* or water and oiled or non-oiled

sample and the exposure to MP252 oil in *Sargassum* and water samples. The oxygen concentration also did not show any specific trends based on sample type and MP252 oil exposure (Table 1). In addition to these readings, OTUs representing known oil-metabolizing species such as *Acinetobacter*, *Thalassospira*, and *Pseudomonas*, along with

various genera belonging to the *Oceanospirillales* order [35, 36] were found in the oiled *Sargassum* and oiled water samples. Other known oil-degrading bacteria such as *Pseudoalteromonas* and *Halomonas*, along with unclassified genera belonging to the *Halomonadaceae* family [36], were detected in both oiled and non-oiled *Sargassum* and water samples showing no statistical difference in abundance between the sample types for these genera.
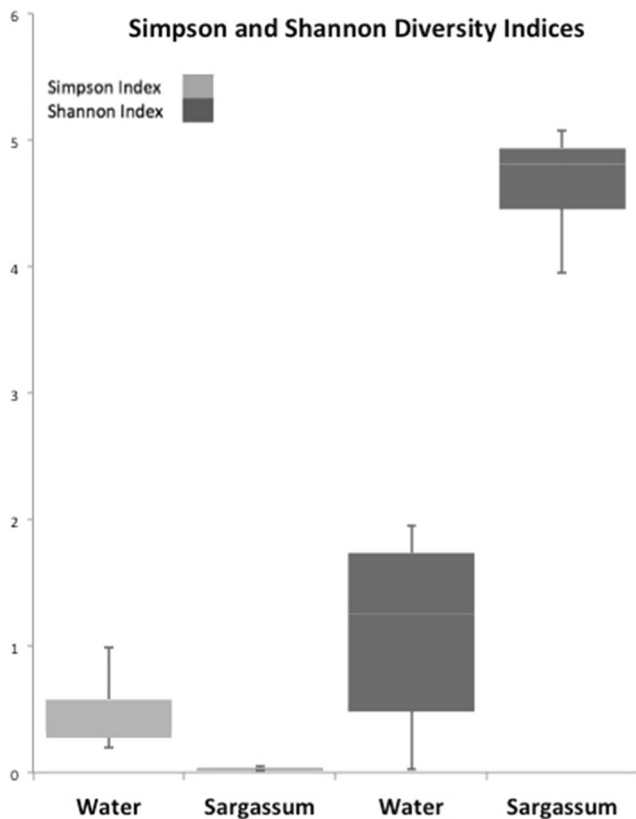
Discussion

Sargassum supports a dynamic GOM pelagic ecosystem as essential habitat and substrate for a multitude of motile and sessile organisms, including species of significant ecological and economic importance [6, 8]. The complexities of the pelagic *Sargassum* community and its multi-faceted contributions to ocean dynamics are poorly understood and require detailed investigation. The biomass of pelagic *Sargassum* impacted by MP252 oil is unknown; however, remotely sensed imagery of the areal surface extent of spilled oil included regions of the northern GOM where pelagic *Sargassum* habitat occurs [26, 37]. MP252 oil came into contact with numerous GOM *Sargassum* rafts, and aerial surveys conducted post- and pre-DWH spill provided evidence of both loss and subsequent recovery of *Sargassum* [6]. Although impacts to the microbial associates of *Sargassum* during the spill event are yet not well known, mesocosm experiments demonstrate potential pathways of MP252 oil injury to pelagic *Sargassum* and microbial associates [6].

Our results showed that there were clear differences between the two sample types through principal coordinates analysis and various statistical approaches. Though it was unusual to see one non-oiled *Sargassum* sample, SARG7, cluster with the other oiled

Fig. 8 Shannon diversity and Simpson diversity indices for *Sargassum* and water samples

Sargassum samples, we can only speculate the reasoning behind this result from what is known from previous studies in regards to other brown algae and the associated microbial population since our sample size is limited. Previous studies have shown that brown algae have associated bacteria that are responsible for growth and algal morphology, in addition to a "core" microbial population [17, 19, 21]. This core population in regards to our *Sargassum* study may be providing a protective effect from environmental contaminants and toxins such as MP252 oil. Currently, we can only speculate on this matter since our data shows that after contact with MP252 oil the microbial composition of oiled and non-oiled *Sargassum*, samples are relatively similar (Fig. 6a) in addition to the *Sargassum* samples clustering together regardless of exposure to MP252 oil (Fig. 7). Additional results also showed statistical significance in the abundance of several species between the two sample types as *Sargassum* hosts a more complex community of higher organisms. Pelagic *Sargassum* was observed to have a higher abundance of various species of unclassified *Rhodobacteraceae*, in addition to other common marine microbes belonging to the *Alphaproteobacteria* and *Betaproteobacteria* clades, when compared to the water samples. These results are somewhat anticipated as *Rhodobacteraceae* are metabolically versatile marine organisms and are capable of an extensive range of metabolic processes from nitrogen fixation and photosynthesis to oil degradation [35, 38]. It is also likely that the lack of changes in the microbiota associated with pelagic *Sargassum* was due to this high abundance of *Rhodobacteraceae* possibly providing a "protective" effect, as these families of bacteria are known hydrocarbon degraders. The significantly higher abundance of *Saprospiraceae* and *Sphingobacteriales* in *Sargassum* is likely due to the role that these two clades play in the breakdown of complex organic material in the environment [39, 40]. For instance, *Saprospiraceae* have been demonstrated to hydrolyze and utilize complex carbon sources such as sludge water, and *Sphingobacteriales* are often present in environmental samples that have come into contact with human waste matter [39, 40]. These previous findings are significant in that these two genera are absent in the water samples, yet present in pelagic *Sargassum*, an environment that serves as a nursery for juvenile fishes, where fish excrement is likely to be copious and has previously been demonstrated to influence the growth of *Sargassum* [41]. Though human and fish excrement vary in composition, the additional hydrocarbons and ammonia likely have a similar causative effect on the increased abundance of *Saprospiraceae* and *Sphingobacteriales*. Oiled and non-oiled water samples, on the other hand, were observed to have higher abundance of common marine microbes that are capable of surviving in harsh conditions with limited nutrients such as *Psychrobacter*, *Salinimicrobium*, and *Salegentibacter*, when compared to the *Sargassum* samples [42, 43].

Our sequence data also showed an unusually large proportion of *Psychrobacter* in all water samples regardless of exposure (or non-exposure) to oil. However, one species of *Psychrobacter* (designated as *Psychrobacter* 1) was present in larger abundance in non-oiled water samples when compared to another species of *Psychrobacter* (designated as *Psychrobacter* 2) in oiled samples (Fig. 6b). It would be interesting to see which species of *Psychrobacter* is more abundant in one sample type than the other; however, as our study only consisted of 16S rRNA sequencing, this would only be attained using metagenomics approaches or targeted PCR. As *Psychrobacter* are known oxidase positive, non-motile, and psychrotolerant organisms, it was surprising to see one species of *Psychrobacter* in higher abundance in oiled samples and a different *Psychrobacter* species in non-oiled samples, as this genus is known to be capable of surviving extreme conditions [44]. Since little is known on the effect of pollutants such as oil on the metabolic processes of various species of *Psychrobacter*, it would be interesting to see which species was able to tolerate the MP252 oil. Currently, we can only speculate on the striking differences between the two species of *Psychrobacter* without metagenomic and transcriptomic data.

Interestingly, it was also determined that the abundance of *Sulfitobacter* was much higher in oiled water samples, with the exception of WTR9. This is a noteworthy, yet expected, finding as this species of bacteria is capable of degrading crude oil in seawater [38]. However, Hazen et al. and Valentine et al. did not report *Sulfitobacter* from samples collected in the deep (ca. 1100–1200 m), dispersed GOM oil plumes, as this organism is known to be a photoheterotroph and would be less likely to survive deep waters where sunlight is virtually nonexistent [8, 45]. However, as these studies were completed using Phylochip microarray, it is likely that the molecular approaches in our study are able to detect other species of *Sulfitobacter* that were not detected using previous methods. The presence of other known obligate oil-degrading bacteria (OODB): *Acinetobacter*, *Thalassospira*, and *Pseudomonas*, along with various unclassified genera belonging to the *Oceanospirillales* order, on oiled *Sargassum* and in oiled water samples, was also quite significant as these organisms were not present in the non-oiled *Sargassum* and water samples. Though this is an important find in regards to *Sargassum* exposed to MP252 oil, it was also rather expected as these organisms have been either isolated from seawater exposed to oil or have been demonstrated to be associated with MP252 oil from the DHW oil spill [36, 46–48]. It was also noteworthy to see a few known OODB; *Pseudoalteromonas*, *Halomonas*, and unclassified *Halomonadaceae* present in both oiled and non-oiled *Sargassum* and water samples as previous studies have shown an increase in growth of *Pseudoalteromonas* and *Halomonas* when exposed to various types of hydrocarbons [47, 49]. Although 16S rRNA sequencing only provided us with a superficial interpretation of the effect of the DHW oil spill on the *Sargassum* associated bacterial communities, these results did

provide us with some insight on which species were present in what is likely a response to the DHW spill as specific OTUs belonging to OODB were not present in the non-oiled samples.

Our findings provide substantial insight into the microbial community associated with pelagic *Sargassum*; a prominent marine surface community, which has not been previously studied using molecular approaches. Though our results were based on a limited number of samples, a sample set such as this one (pre- and post-oil spill water and *Sargassum* samples) is rarely obtained. Additionally, our results provide novel insight concerning the effect of MP252 oil on the microbial communities associated with *Sargassum* in the GOM. Additional genomics approaches such as metagenomics and transcriptomics to characterize functional elements in the microbial community associated with *Sargassum* can provide useful information in determining which key species and bacterial strains, along with which genes are responsible for maintaining a balanced ecosystem. In this regard, these techniques could reveal whether the lack of changes in community composition in the *Sargassum* really mean no changes at the metabolic level (community redundancy as opposed to functional redundancy). Further genomic-level approaches would also shed light on to whether the *Sargassum* microecosystem is naturally adapted to hydrocarbon-associated disturbances and can also help in providing a complete picture of how marine microbial communities recover after an event such as the DHW oil spill. In addition to determining overall *Sargassum* and associated macro-fauna health, it would be interesting to characterize the microbial community associated with *Sargassum* during massive algal blooms, which have been of great interest, occurring in areas of the tropical Atlantic Ocean not commonly associated with this marine alga. Exploratory approaches using 'omics and next generation sequencing methods on pelagic *Sargassum* microbial communities are critical to understanding the role that these complex communities have in the health of the *Sargassum* and associated living marine organisms, as well as their recovery from devastating pollution events such as oil spills.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

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