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Methane and microbial dynamics in the Gulf of Mexico water column

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In contrast to other oligotrophic water bodies the Gulf of Mexico (GOM) hosts an abundance of hydrocarbon seeps, which likely influences the microbial assemblages it hosts particularly regarding the availability of labile carbon in the aphotic GOM. The aphotic zone receives direct injection of seep methane (CH_4), but CH_4 from an unknown source has been reported at supersaturated concentrations relative to the atmosphere in the GOM photic zone. Here we used iTag sequencing of 16S rRNA genes to characterize GOM microbial communities and to relate changes in microbial community structure to the properties inherent to their oceanic province-seafloor to the photic zone, seep and non-seep. Along this trajectory water column communities were distinct in the euphotic zone compared to the mesopelagic and deep-sea. In the euphotic zone the relative abundance of a cyanobacterial species (*Prochlorococcus*) was significantly correlated with both CH_4 and chlorophyll a concentrations and was abundant in some deep-chlorophyll maximum (DCM) samples. The relative abundance of microorganisms related to known hydrocarbon degraders were also significantly correlated with CH_4 in the euphotic zone, but no canonical methanotrophs were observed. In the mesopelagic to the seafloor canonical methanotrophs were identified, but only a Marine Group II Euryarchaeota was significantly correlated with CH_4. Overall, depth and the associated environmental conditions were the primary drivers in structuring microbial communities over the GOM water column. Further, CH_4 concentrations and relative microbial abundances covaried significantly from the seafloor to the photic zone in the GOM. The lack of a significant relationship between canonical methanotrophs and CH_4 in the aphotic zone, even when sampling at seep sites, may suggest methane-oxidation by unknown microorganisms. Similarly their absence in the CH_4 maximum and DCM suggested that CH_4 is either oxidized by unrecognized methanotrophs or escapes the CH_4 biofilter and fluxes to the atmosphere.

Keywords: ITag, microbial community structure, Gulf of Mexico, methane seep, methanotroph, methane photic zone

Introduction

Analyses of spatial patterns of marine microbial plankton revealed that in several marine provinces dominant microbial players are stratified in the vertical water column (Giovannoni and Stingl, 2005 and references therein). This observed stratified vertical distribution was extended to resolve global scale microbial distributions and revealed disparate communities from surface to deep-water, with different environmental drivers influencing the structure of these communities (Zinger et al., 2011; Sunagawa et al., 2015). One of the primary organizing principles determining microbial plankton structure is the availability of sunlight throughout the water column, particularly for photosynthetic Cyanobacteria (Giovannoni and Stingl, 2005; Delong et al., 2006; Zinger et al., 2011). It would seem that microbial plankton in the oligotrophic Gulf of Mexico (GOM) are bounded by these very same organizing principles. However, the GOM has numerous natural hydrocarbon seeps that provide a carbon source, and in particular CH_4, for heterotrophic microbes near seep sites. Additionally, well over 30 years ago CH_4 at supersaturated concentrations relative to the atmosphere were reported in the near-surface water column in the GOM (Swinnerton and Lamontagne, 1974; Brooks et al., 1981) and more recently by Finke et al. (2011). The source of this CH_4 remains elusive. Thus, both the deep-sea and the near-surface photic zone have labile fixed carbon in the form of CH_4 that likely influences microbial structure.

There are few studies that have described microbial community stucuture and the environmental factors that influence this structure in the GOM. Recently, Gillies et al. (2015) found that dissolved oxygen concentrations played a significant role in influencing microbial abundances in the northern GOM coastal hypoxic zone. King et al. (2013) also analyzed bacterioplankton in the coastal northern GOM, but their study extended to the mesopelagic. They reported that depth, temperature and, similar to Gillies et al. (2015), oxygen were the primary drivers influencing microbial abundances in the GOM. Together these studies suggest that similar organizing principles as those discussed above shape microbal communities in the GOM, but hydrocarbon concentrations were not considered in these studies. Yet, several reports revealed the profound influence that oil and gas input during the Deepwater Horizon oil spill can have on microbial abundances from the photic zone to the deep-sea in the GOM (Hazen et al., 2010; Valentine et al., 2010; Mason et al., 2012; Redmond and Valentine, 2012; Dubinsky et al., 2013). In particular, CH_4 concentrations reported in the deep-sea plume were 10–1000 times higher than what is typically observed at GOM hydrocarbon seeps (Joye et al., 2011) which ranges from 4 nM to 20 μM (Solomon et al., 2009; Wankel et al., 2010). During the oil spill CH_4 oxidation rates were some of the highest reported in the pelagic ocean (Crespo-Medina et al., 2014). Active CH_4 oxidation was occurring soon after the spill began (Mason et al., 2012; Rivers et al., 2013), but canonical methanotrophs were not abundant in 16S rRNA gene data (Mason et al., 2012). Collectively, these studies illustrated how hydrocarbons, and in particular, CH_4 can shape microbial community structure in the GOM.

Given that methane is a potent greenhouse gas absorbing infrared radiation 25 times more effectively than CO_2 on a molecule per molecule basis (Lelieveld et al., 1998; Petit et al., 1999) there is an impetus to understand both the sources and sinks for this gas. In fact, the most significant perturbations to climate forcing, or radiative forcing, are changes related to greenhouse gases such as CH_4 (Forster et al., 2007). Methane has made a significant contribution to global warming over the past few 100 years. The ocean is a CH_4 source contributing 1–4% of annual emissions to the atmosphere (Karl et al., 2008). Yet, to our knowledge there are no studies that present a characterization of the bacterioplankton along with CH_4 data in the water column at natural seeps in the GOM. Further, what microorganisms are associated with the methane maxima in the GOM is not currently known. To fill this knowledge gap we sought to examine the correlations between CH_4 concentrations and other environmental variables and relative microbial abundances over the entire GOM water column, from the photic zone to the deep-sea, at hydrocarbon seep and non-seep sites. We hypothesized that this carbon source may play an important role in structuring pelagic microbial communities. Further, we sought to evaluate the relative abundances of canonical methanotrophs where CH_4 concentrations were highest—above seeps and in the chlorophyll/CH_4 maxima in the photic zone. Specifically, our research aim was to better understand the relationship between microbial distributions as they covary with CH_4 concentrations. Here we used iTag sequencing of bacterial and archaeal 16 rRNA genes in 58 samples using Illumina's MiSeq platform. We determined CH_4, oxygen, nitrate, nitrite, ammonium and phosphorus concentrations. We then evaluated the relationships between these environmental variables and relative microbial abundances.

Materials and Methods

Sample Collection

Eight sites in the GOM were sampled during expeditions in September and October 2012 (R/V Weatherbird II) and in May and June 2013 (R/V Pelican) (**Figure 1**). Sites were located in the Northeastern GOM continental shelf and varied from gas seep sites (SeepC and SeepC2), a gas and asphalt volcanism site (PeanutSeep), a prospective seep site with observed intermittent oil sheens (Oscar Garcia-Pineda, personal communication) (SeepA), an active oil seep with visible oil at the surface (GC600 and SeepS4) and non-seep sites (SiteAC5 and SiteP4). Collection depths spanned the near-surface to the seafloor at these eight sites. In the euphotic zone the deep-chlorophyll maximum was targeted for collection (Supplemental Table 1).

Oxygen, Chlorophyll a and nutrients

Oxygen concentrations were determined using the Winkler method (Environmental Protection Agency's Standard Operating Procedure of Dissolved Oxygen Micro Method, Winkler Titration, LG501). Chlorophyll a samples were concentrated on 25 mm Whatman GF/F filters from 1 to 2 L of seawater and stored at −20°C. Chlorophyll a was extracted using the methods described in the Environmental Protection Agency

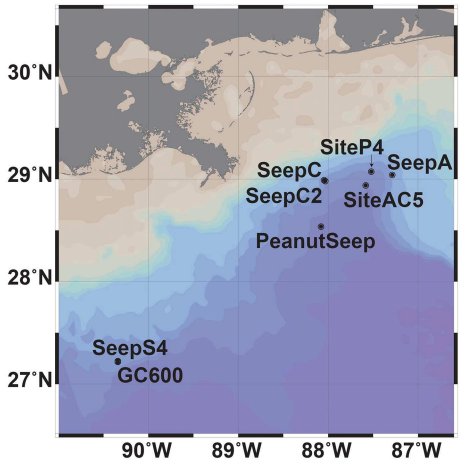


FIGURE 1 | Map showing the eight stations sampled in the Gulf of Mexico.

Method 445.0, "*In Vivo* Determination of Chlorophyll a in Marine and Freshwater Algae by Fluorescence"; however, no mechanical tissue grinder or HCl were used. Chlorophyll a concentrations were determined using a fluorometer with a chlorophyll a standard (*Anacystis nidulans* chlorophyll a). For nutrients, 60 ml of seawater was filtered through a Whatman GF/D filter (EMD Millipore, Billerica, MA) into two 30 ml Nalgene bottles using a swinnex filter holder and syringe and stored at −20°C. Nutrient concentrations were determined by the marine chemistry lab at the University of Washington following the WOCE Hydrographic Program using a Technicon AAII system (http://www.ocean.washington.edu/story/Marine+Chemistry+Laboratory).

Methane

Methane samples were collected from niskin bottles as soon as the CTD rosette was shipboard. 150 ml volume glass vials were filled with sample water until a reverse meniscus was present. To preserve samples vials were then injected with 0.5 ml of a 8 M KOH solution, using a technique to prevent injection of air into the sample. Finally, 10 ml of helium gas was injected into the vial as headspace. Vials were stored inverted until shorebased analysis via gas chromatography. A complete description of our analytical techniques can be found in Magen et al. (2014).

Microbial Sample Collection and DNA Extractions

From each station, up to 10 L of seawater were collected and filtered with a peristaltic pump. A 2.7 μM Whatman GF/D pre-filter was used and samples were concentrated on 0.22 μM Sterivex filters (EMD Millipore). Sterivex filters were sparged and filled with RNAlater. DNA was extracted directly off of the filter by placing half of the Sterivex filter in a Lysing matrix E (LME) glass/zirconia/silica beads Tube (MP Biomedicals, Santa Ana, CA) using the protocol described in Gillies et al. (2015) which combines phenol:chloroform:isoamyalcohol (25:24:1) and bead beating. Genomic DNA was stored at −80°C until purified. DNA was purified using a QIAGEN (Valencia, CA) AllPrep DNA/RNA Kit. DNA quantity was determined using a Qubit 2.0 Fluorometer (Life Technologies, Grand Island, NY).

16S rRNA Gene Sequencing and Analysis

16S rRNA genes were amplified from 10 ng of purified DNA in duplicate using primers 515F and 806R that amplify both bacteria and archaea, targeting the V4 region of *E. coli* in accordance with the protocol described by Caporaso et al. (2011, 2012) and used by the Earth Microbiome Project (http://www.earthmicrobiome.org/emp-standard-protocols/16s/), with a slight modification, specifically, the annealing temperature was modified to 60°C. PCR amplicons were purified using Agencourt AMPure XP PCR Purification beads (Beckman Coulter, Indianapolis, IN). Sequencing was carried out using the MiSeq (Illumina, San Diego, CA) platform. Sequences were analyzed using the QIIME version 1.7.0 (Caporaso et al., 2010a) pipeline. Raw sequences were demultiplexed and then quality filtered using the default parameters in QIIME. These sequences are available at: http://mason.eoas.fsu.edu and in the NCBI's sequence read archive (PRJNA294680). Sequences were then clustered into operational taxonomic units (OTU)s, which was defined as ≥97% 16S rRNA gene sequence similarity, using UCLUST (Edgar, 2010) using the open reference clustering protocol (http://qiime.org/tutorials/open_reference_illumina_processing.html). The resulting representative sequences were aligned using PyNAST (Caporaso et al., 2010b) and given a taxonomic classification using RDP (Wang et al., 2007), retrained with the Greengenes version 13.5 (McDonald et al., 2012). The resulting OTU table was filtered to keep only OTUs that had at least 10 observations, and then converted to relative abundance, by summing the total sequence count in a sample and then dividing each sample OTU count by this total. Those species that were significantly correlated with CH_4 concentrations (see below) were further classified using RDP (Wang et al., 2007), Silva (Pruesse et al., 2007) and Greengenes (McDonald et al., 2012).

Statistics

Nutrient, CH_4 and oxygen data were interpolated using Ocean Data View (Schlitzer, 2013). The differences in relativized OTU abundances in communities in samples that were categorized as either below or above 4 nM CH_4 (CH_4 concentrations in equilibrium with the atmosphere are >4 nM, Joye et al., 2011) were analyzed using nonparametric statistics (Mann-Whitney) to test for statistically significant differences using METAGENassist (Arndt et al., 2012) and the application of a False Discovery Rate (FDR) to account for multiple comparisons. Further, statistical analyses were carried out in R, including determination of Spearman correlation coefficients among all variables. The QIIME generated, normalized (by conversion to relative abundance), OTU abundances in the 58 different samples were then analyzed using non-metric multidimensional (NMDS) scaling in R using metaMDS in the Vegan package. *P*-values were derived from 999 permutations of the data.

Results and Discussion

Water Column Properties and Chemistry

Maximum CH_4 concentrations (24 and 45 nM) were observed in two samples collected at or near gas seep site GC600 (**Figures 1**, **2**, Supplementary Table 1). Of the remaining sites and depths, with one exception, sample SeepC2_025_1109 m, the highest CH_4 concentrations were observed in the photic zone (**Figure 2**, Supplementary Table 1), despite obtaining several additional samples directly above seeps. Specifically, CH_4 values ranged from 11 to 14 nM with an average depth of 84 m (**Figure 2**, Supplementary Table 1). These values were well above CH_4 concentrations in equilibrium with the atmosphere at <4 nM (Joye et al., 2011). Typical GOM seawater nutrient and oxygen profiles were observed (see for example Shiller and Joung, 2012; **Figure 1**) for nitrate, nitrite, ammonium and phosphate (**Figure 2**, Supplementary Table 1), although we acknowledge the dearth of profiles for the GOM. Chlorophyll a ranged from 0.92 µg/L to undetectable below the euphotic zone (**Figure 2**, Supplementary Table 1). Further, a deep-chlorophyll maximum (DCM) was observed at an average depth of 83 m (**Figure 2**). Spearman correlation analysis revealed that CH_4 concentrations were significantly correlated with salinity, nitrite, and chlorophyll a in the euphotic zone (**Figure 3**). A co-located CH_4/DCM in the GOM has been previously reported (for example, Finke et al., 2011). Below the euphotic zone CH_4 concentrations were inversely correlated with phosphate and nitrate, compared to ammonium

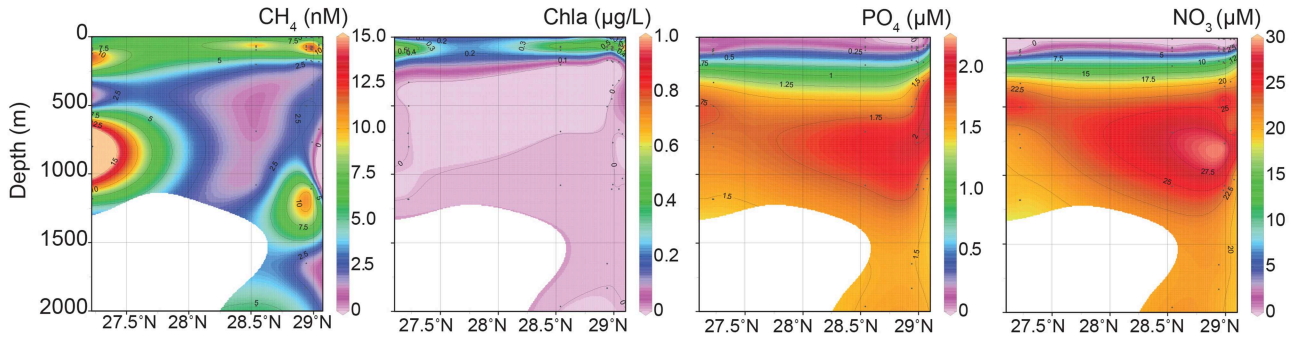


FIGURE 2 | Interpolation plots of methane, chlorophyll a, phosphate and nitrate data from eight stations sampled.

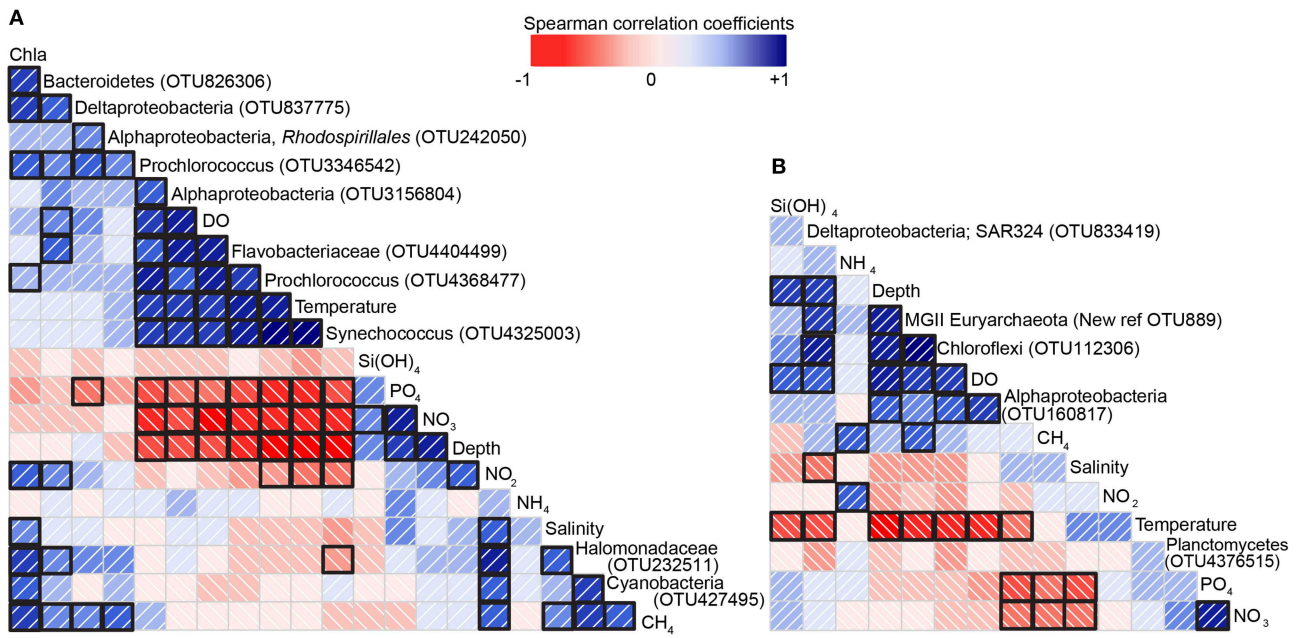


FIGURE 3 | Correlation matrix showing the sign and magnitude of the correlation (Spearman) coefficients between biological, chemical and physical data. (A) Correlation matrix for 0–200 m and **(B)** correlation matrix for 200–2000 m. Blue indicates a positive value and red a negative value (see scale bar). The diagonal lines also indicate the direction of the correlation. The intensity of each of these colors increases as the correlation value moves away from zero (see scale bar). Correlations that were statistically significant ($p \leq 0.05$) are shown by a black square around the borders (e.g., , has a black border indicating a significant correlation vs. , indicating a correlation that was not significant).

concentrations, which were positively correlated with CH_4 (**Figure 3**).

Surface to Seafloor Microbial Community Structure

Proteobacteria was the most dominant phyla over the entire water column, with an average relative abundance of 35% in the photic zone/43% below the photic zone (**Figure 4A**). Of the proteobacteria, gammaproteobacteria comprised 40.6% photic/42.8% aphotic, alphaproteobacteria were 40.0% photic/12.8% aphotic and deltaproteobacteria were 19.1% photic/42.0% aphotic (**Figure 4B**). King et al. (2013) analyzed the microbial communities in the Northern GOM, from the nearshore to offshore and from surface to deep with the same 16S rRNA gene primers we used here. They found a similar distribution of alpha and gammaproteobacteria, in that alphaproteobacteria abundances were higher in the photic zone and decreased with depth, while gammaproteobacteria showed an inverse trend. Cyanobacteria were also abundant in the photic zone (avg. 28%) compared to avg. 0.4% in the aphotic zone (**Figure 4**). Overall, these patterns were consistent with the global scale synthesis of pelagic verses benthic communities (Zinger et al., 2011). Thaumarchaeota increased with depth (avg. 5.6% photic/21.1% aphotic) (**Figure 4**). The increase in Thaumarchaeota (Crenarchaeota) abundances with depth was first reported more than a decade ago (Karner et al., 2001). In the GOM Tolar et al. (2013) reported that Thaumarchaeota abundance was strongly correlated with depth, consistent with our findings. Euryarchaeota showed a similar distribution over the water column (avg. 11.0% photic/11.6% aphotic) (**Figure 4**). This homogenous distribution with depth was also reported by Karner et al. (2001) in the North Pacific subtropical gyre. In contrast, Tolar et al. (2013) reported that Euryarchaeota were generally less abundant and decreased with depth in their GOM samples. Marine Group A (MGA; formerly referred to as SAR406) increased from 5.5% in the photic zone to 8.2% in the aphotic zone (**Figure 4**). A similar distribution, with an increase in relative abundance with depth of MGA, was recently shown by fluorescent *in situ* hybridization in the Northeast subarctic Pacific Ocean (Allers et al., 2013). The relative abundances of Actinobacteria, Bacteriodetes, Chloroflexi, Planctomycetes, and Acidobacteria changed with increasing depth in the water column but were never more than 3% of the overall microbial community.

Beta Diversity

Beta diversity analysis (nonmetric multidimensional scaling) of 10,912 OTUs revealed distinct microbial communities that were

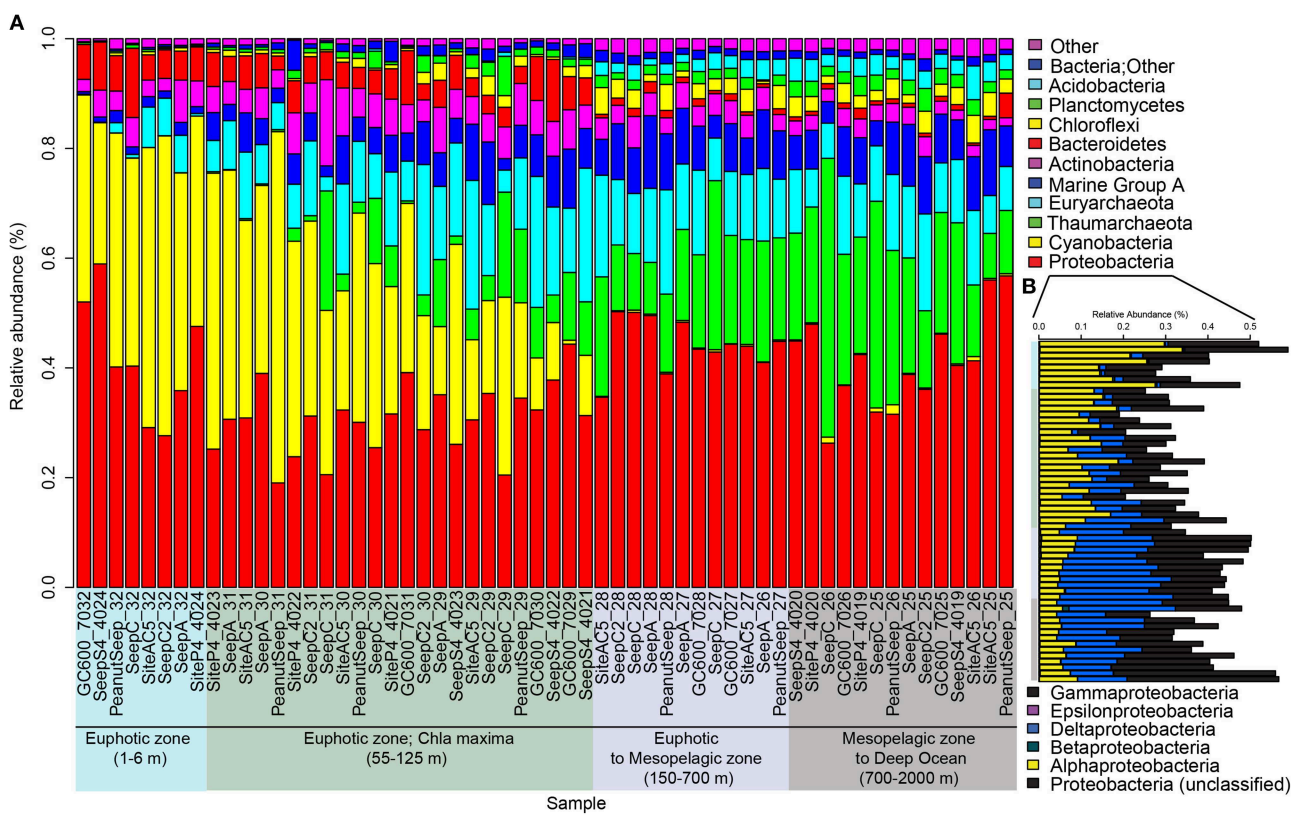


FIGURE 4 | Bar graphs of relativized 16S rRNA gene iTag sequence data. Graph **(A)** shows phyla level microbial community composition. Only the more abundant bacterial and archaeal groups are shown. Less abundant groups were summed under "Other." Graph **(B)** shows the relative abundances of sub-phyla in the Proteobacteria. Samples are sorted by depth on the x-axis in both **(A,B)**. Graph **(B)** shows the sample depths using the same color scheme shown in **(A)**.

structured by depth and the associated environmental conditions with these depths (**Figure 5**). The differences in microbial community structure with depth was previously reported by Zinger et al. (2011). No clustering by season was observed in the NMDS ordination. Given the disparate communities in the euphotic zone compared to the mesopelagic and deep-sea we elected to focus on these two environments independently in the following analyses.

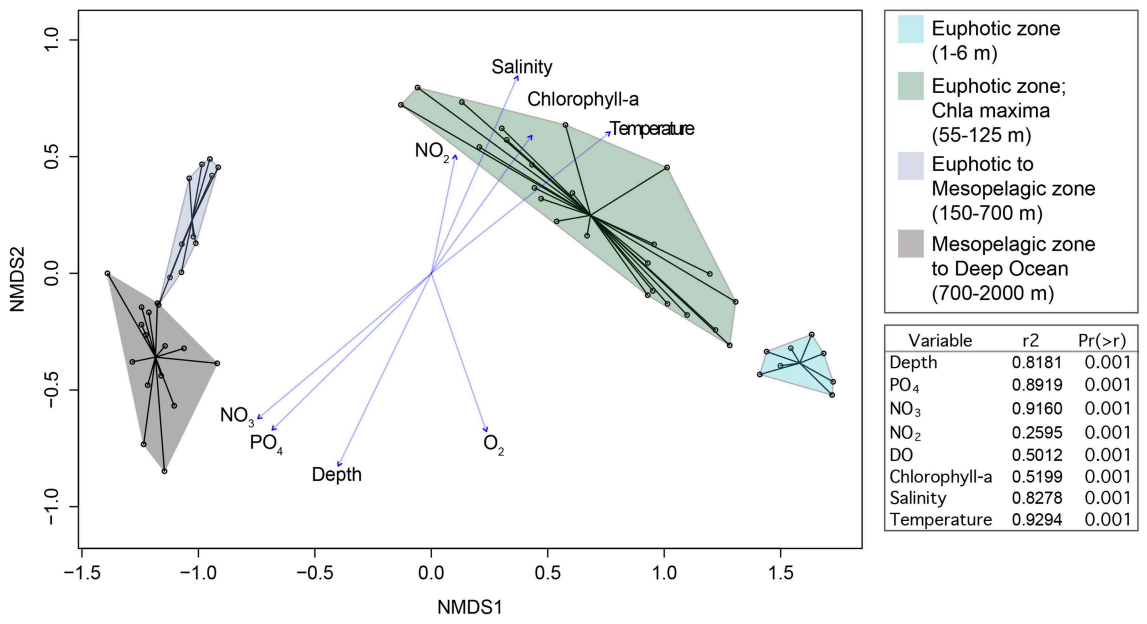
Euphotic Zone Bacterioplankton Structure and Diversity

Nonparametric statistics were used to determine which species were significantly different in samples with above or below background CH_4 concentrations (<4 nM, Joye et al., 2011) in photic zone samples (0–200 m). Specifically, samples were categorized as above or below background CH_4 concentrations and the relative abundances of 8156 OTUs were tested for significance using the Mann-Whitney test. The relative abundances of 10 microbial species were significantly different in samples that were above or below background. These species were members of the Cyanobacteria, Bacteroidetes, and Proteobacteria phyla (**Figure 3A**).

We next directly correlated relative abundances of these 10 species with CH_4 concentrations. Only those species that were significantly correlated with CH_4 at $\leq$0.05 p-value are discussed here. A Cyanobacteria (OTU427495) classified as a Family II, GPIIa [Ribosomal Database Project (RDP) (Cole et al., 2013)] was positively correlated with CH_4 (Spearman correlation coefficient = 0.53, p-value = 0.00) and chlorophyll a (**Figure 3A**). In fact, this species was highly abundant in the DCM, comprising up to 15% relative abundance in some samples. This species was 100% similar to bacterioplankton from the tropical Atlantic (Lekunberri et al., 2013), from 125 m North Pacific subtropical gyre seawater (Eiler et al., 2011) and from the Northern South China Sea (AC FJ598533).

Members of the cyanobacterial clade have been shown to evolve CH_4 when microcosms in which orthophosphate concentrations are low are supplemented with methylphosphonate (MPn) (Karl et al., 2008). These authors demonstrated that *Trichodesmium* from the North Pacific gyre evolves CH_4 when utilizing MPn. The mechanism suggested was MPn hydrolysis of phosphonate compounds by a carbon-phosphorus pathway. This pathway was actively expressed by the Cyanobacteria, *Trichodesmium erythreum* ISM101, when faced with phosphate-deficient conditions (Dyhrman et al., 2006). When comparing OTU427495 to cultured representatives it is most similar (99%) to *Prochlorococcus marinus* SS120, isolated from 120 m in the Sargasso Sea (Moore et al., 1995). *P. marinus* SS120 encodes an ABC-type transporter for potentially using phosphonates (Dufresne et al., 2003). To our knowledge however it is unknown whether this Cyanobacteria utilizes MPn and evolves CH_4 when orthophosphate is depleted.

Interestingly, in our samples CH_4 and chlorophyll a concentrations were significantly correlated; in contrast CH_4 and phosphate concentrations were not (**Figure 3A**). It has been previously reported that in the GOM the near surface CH_4 maximum is coincident with the deep-chlorophyll maximum (DCM) (Finke et al., 2011). These authors also reported that in samples from the GOM DCM addition of MPn (or other previously identified CH_4 precursors) did not influence CH_4 production, but that light exposure did. They suggest a hitherto unrecognized photosynthetic process in



Variable	r2	Pr(>r)
Depth	0.8181	0.001
PO_4	0.8919	0.001
NO_3	0.9160	0.001
NO_2	0.2595	0.001
DO	0.5012	0.001
Chlorophyll-a	0.5199	0.001
Salinity	0.8278	0.001
Temperature	0.9294	0.001

FIGURE 5 | NMDS ordination of relativized 16S rRNA gene iTag sequence data. Samples grouped by depth as shown in the ordination plot. Vectors are shown for environmental variables that were significantly correlated with NMDS ordination axes. Goodness of fit statistics (r2) and p-values are shown.

the DCM as responsible for the observed CH_4 maxima in the GOM. The correlations we reported here provided some evidence that indeed photosynthetic processes may be involved in CH_4 formation in the GOM DCM, but that the mechanism is unresolved. Although we acknowledge that 1) while statistically significant, the correlation between the Cyanobacteria (OTU427495) species discussed above and CH_4 concentrations at 0.53 is moderate and 2) the CH_4 source was not explicitly tested herein and that the relative abundance of a Cyanobacteria closely related to a *Prochlorococcus* sp. in the GOM DCM (and CH_4 maximum) may not be a CH_4 source.

Except for Bacteroidetes OTU826306, close relatives of the remaining species that were significantly correlated with CH_4 concentrations in the euphotic zone have been shown to degrade hydrocarbons. Specifically, in the Oceanospirillales a Halomonadaceae species (OTU232511) was positively correlated with CH_4 (0.70, $p = 0.00$) (**Figure 3A**). This species was 100% similar to bacteria from 300 m in the Challenger Deep, Mariana Trench (Acc. AB703798), 99% similar to a diffuse hydrothermal vent clone (Acc. HG819048), a clone from the eastern tropical South Pacific oxygen minimum zone (Stevens and Ulloa, 2008) and an oil sheen from the Deepwater Horizon wreckage in the GOM (Acc. KF786504). In the deep-sea plume that resulted from Deepwater Horizon oil spill Oceanospirillales was highly abundant (Hazen et al., 2010; Redmond and Valentine, 2012). A single cell genome sequence of this Oceanospirillales revealed a capacity for aliphatic hydrocarbon degradation (Mason et al., 2012). Further, the Oceanospirillales *Alcanivorax borkumensis* is a ubiquitous *n*-alkane degrading marine bacterium (Schneiker et al., 2006). Further, several Halomonadaceae are capable of growth on hydrocarbons (for example Gasperotti et al., 2015). However, whether the Halomonadaceae discussed here is capable of oxidizing CH_4 is unknown.

Alphaproteobacteria OTU242050 was also significantly correlated with CH_4 (0.46, $p = 0.01$). This microorganism was classified as a *Rhodospirillales* with 100% similarity to bacteria associated with a marine sponge (AC JQ240926), the South China Sea (Zhang et al., 2011) and a shallow CH_4 seep (Wasmund et al., 2009). Redmond and Valentine (2012) reported that surface samples with a visible oil sheen collected during the Deepwater Horizon oil spill, were dominated by *Rhodospirillales,* other Alphaproteobacteria and Cyanobacteria. Members of the *Rhodospirillales* clade have been shown to degrade hydrocarbons (Kodama et al., 2008; Zhao et al., 2008), but not, to our knowledge, short chain *n*-alkanes. Thus, the relationship between CH_4 and *Rhodospirillales* requires further investigation.

Deltaproteobacteria OTU837775 was significantly correlated with CH_4 (0.39, $p = 0.02$) (**Figure 3A**). This species was 100% similar to bacterioplankton SAR324 from an oxygen minimum zone of the Arabian Sea (AC KJ589823), an oil sheen from the Deepwater Horizon wreckage in the GOM (AC KF786619) and the Sargasso Sea (Sjöstedt et al., 2014). Further, deltaproteobacteria OTU837775 16S rRNA gene sequence was 100% similar to the uncultured SAR324 clone HF130_05G09 presented by Pham et al. (2008) and also discussed by Chitsaz et al. (2011). SAR324 have been reported in numerous marine habitats, for example in the North Pacific Subtropical Gyre (Delong et al., 2006) and in oxygen minimum zones (Wright et al., 2012). Swan et al. (2011) sequenced two SAR324 single cell genomes (SAGs), of which SAG AAA240-J09 was 93% similar to Deltaproteobacteria OTU837775. In this report they suggested that SAR324 have the potential to oxidize CH_4 and other C_1 compounds. Recently, Sheik et al. (2014) reported that deep-sea SAR324 possess an actively transcribed particulate hydrocarbon monooxygenase (pHMO). Whether this enzyme acts on CH_4 or C_2-C_4 gases only is currently unresolved. The significant relationship between CH_4 and the relative abundance of Deltaproteobacteria OTU837775 suggested that CH_4 may indeed be a substrate utilized by SAR324.

Finally, in the Bacteroidetes, Cytophagales OTU826306 (correlation coefficient with $CH_4 = 0.33$, $p = 0.05$), was most similar to microorganisms from a variety of marine environments including surface seawater (Li et al., 2014), seawater particles (D'Ambrosio et al., 2014) and corals (Sunagawa et al., 2010). As discussed above, the Cytophagales species described here is not closely related to a known hydrocarbon degrader, thus why its abundance is significantly correlated with CH_4 concentrations is unknown.

Mesopelagic to Deep-sea Bacterioplankton Structure and Diversity

Nonparametric statistics were used to determine which species in the mesopelagic to the seafloor were significantly different in samples with CH_4 above or below background concentrations. Specifically, samples from below 200 m were categorized as above or below and the relative abundances of 6928 OTUs were then tested for significance using the Mann-Whitney test. The relative abundances of five species were significantly different in samples that were either above or below background CH_4 concentrations. Of these five species, however, only one was significantly correlated with CH_4 (**Figure 3B**). This species was an unclassified Euryarchaeota (new reference OTU 889), that was 100% similar to archaea in the deep-sea hydrocarbon plume that resulted from the Deepwater Horizon oil spill (Redmond and Valentine, 2012) and to Euryarchaeota from 2940 m in the Japan Trench (Kawagucci et al., 2012). It was also 99% similar to a Marine Group II Euryarchaeota (MGII) from a Guaymas Basin hydrothermal plume (Dick and Tebo, 2010). This MGII species (new reference OTU 889) was also positively correlated with depth and dissolved oxygen and inversely correlated with temperature. The role of MGII in biogeochemical cycling is largely uncharacterized given that there are currently no cultured representatives described in the literature. To date, one MGII has been assembled from metagenomic data revealing a photo-heterotrophic metabolism (Iverson et al., 2012). The MGII species discussed above is not closely related (91% similar, 16S rRNA gene) to the MGII presented by Iverson et al. (2012). Given the photo-heterotrophic lifestyle of a surface ocean MGII, and the distant phylogenetic relationships between these MGII species there are few clues provided as to the ecological role of MGII in the deep-sea, particularly as it relates to CH_4. Thus, why the relative abundance of the MGII species described herein was significantly correlated with CH_4 is unknown.

It should be noted that a Methylococcales (OTU724366) was observed in several deep-sea samples, but was never more than 1% in relative abundance (Supplemental Figure 1). Methylococcales require CH_4 and other C_1 compounds for both carbon and energy (Bowman, 2005). Surprisingly, its relative abundance was not significantly correlated with CH_4. It is discussed here because it was the only canonical methanotroph observed in any of our GOM samples. This species was 100% similar to a microorganism from a remnant hydrocarbon plume sampled 2 months after the Deepwater Horizon oil spill ceased (Yang et al., 2014) and to microorganisms from hydrothermal vent fluids (Dick and Tebo, 2010; Yoshida-Takashima et al., 2012). There are several interpretations as to why canonical methanotrophs were low in relative abundance, one being that the primers used herein were biased against them. It should be noted, however, that in Mason et al. (2012) a different 16S rRNA gene primer set was used for pyrotag sequencing and 16S rRNA genes were annotated in metagenomes and metatranscriptomes from Deepwater Horizon oil spill deep-sea plumes and uncontaminated seawater from plume depth revealing relative abundances of canonical methanotrophs <1%, yet methane-oxidation was an active and abundant process. Together, the data suggests that unrecognized marine methanotrophs mediate methane-oxidation, rather than primer bias against canonical methanotrophs as an explanation for why their relative abundances were low in our study.

Conclusion

Unlike other large oligotrophic water bodies the GOM is rich in hydrocarbon seeps. These hydrocarbon seeps inject fixed carbon into the deep-sea water column. As these hydrocarbons migrate toward the surface they likely influence the microbial community structure. Given the paucity of information regarding the microbial community structure in the GOM water column outside of the Deepwater Horizon oil spill we characterized the microbial communities at seep and non-seep sites from the seafloor to the near surface in the water column. We found that depth and the inherent properties of those depths from the seafloor to the near-surface photic zone were the dominant organizing principles structuring water column microbial communities. In the photic zone we observed a coincident CH_4 and chlorophyll a maxima. At this maxima a Cyanobacteria putatively identified as *Prochlorococcus* was abundant and significantly correlated with both CH_4 and chlorophyll a. Whether this microorganism acts as a CH_4 source by an unidentified photosynthetic process is unknown, but warrants future research. No canonical methanotrophs were identified in the photic zone CH_4 maximum, yet several relatives of hydrocarbon degraders were present. In the mesopelagic to the deep-sea a MGII Euryarchaeota was significantly correlated with CH_4, but whether it can oxidize CH_4 aerobically is unknown. Canonical methanotrophs were identified in deep-sea samples but their relative abundances did not co-vary significantly with CH_4. Collectively our data revealed that similar to other oligotrophic water masses, disparate microbial communities comprised the euphotic zone compared to the mesopelagic and deep-sea in the GOM. Unlike other water bodies, however, our findings suggested that CH_4 input from the many natural GOM seeps to the deep-sea and from an unknown source in the euphotic zone did appear to significantly influence the relative abundance of some of the key microbial players. Future research efforts will be directed toward linking CH_4 production and consumption rates with specific microbial species.

Author Contributions

CVR participated in the research cruises to obtain samples, prepped samples and carried out sequencing, data analyses and helped write the manuscript. CM and SB determined methane concentrations in samples. SB, KLR, and LEG participated in cruises and collected samples. JPC provided methane data. OUM conceived of the experimental design, prepped samples, helped with sequencing, carried out data analyses and helped write the manuscript.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars.2015.00069

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