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Spatial heterogeneity and oil pollution structured the soil microbial community in salt marshes in Barataria Bay, Louisiana, USA, eight years after the *Deepwater Horizon* oil spill

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ABSTRACT

The *Deepwater Horizon* (DWH) oil spill significantly impacted salt marsh ecosystems, with numerous repercussions observed in the subsequent years. However, the long-term effects of chronic oil exposure on soil microbial communities remain unexplored. This study, conducted in 2018, aimed to identify how the legacy of heavy oiling from the DWH spill in 2010 influenced the soil microbial community. We surveyed vegetation and soil variables while simultaneously employing high-throughput 16s rRNA gene sequencing to examine microbial communities in soils across 21 marsh sites that exhibited varying degrees of initial oil contamination following the DWH incident. The effects of these variables, including geographic location, on taxonomic and phylogenetic metrics of community composition were disentangled using variance partitioning analysis and permutational ANOVA. Results showed a strong and significant longitudinal trend in microbial community composition that was partially confounded with degree of initial oil exposure and contemporary vegetation biomass. However, significant indicator species for heavily oiled sites included oil-degrading bacteria, and there were significant differences in microbial community composition among marshes that received variable levels of initial oiling after controlling for this spatial effect. The level of soil petroleum residues that remained in 2018 had significant explanatory power for community composition after controlling for location, whereas the effects of vegetation biomass were largely confounded with longitude. Collectively, our results indicate that persistent oil pollution as well as unidentified spatial processes, possibly associated with spill-induced alterations in erosion or wave dynamics, played a role in structuring soil microbial communities within this ecosystem.

1. Introduction

Coastal wetlands are globally distributed systems that provide critical ecosystem services (Costanza et al., 2008). They now face a burgeoning threat worldwide from anthropogenic oil pollution, as ocean oil slick occurrences have surged tenfold over natural levels and are predominantly located near shore (Dong et al., 2022). One example followed the explosion of *the Deepwater Horizon* (DWH) platform in the northern Gulf of Mexico on 20 April 2010, which led to the release of an estimated (judge-decreed) 3.19 million barrels of oil over 87 days from the Macondo well (United States v. BP, 2014). Nearly 800 km of coastal wetland habitat in Louisiana, USA, was contaminated during the spill (Nixon et al., 2016). The fate of the oil landing in coastal wetlands is a major concern because the fine-grained sediment, waterlogged soils, and sedimentary accretion can result in the burial of oil where decomposition is very slow, and it has the potential to resurface during storm events. Indeed, elevated total petroleum hydrocarbons (TPH) were detected consistently in DWH-impacted sites for over eight years following the spill (Deis et al., 2020; Turner et al., 2019), as has been documented in other petroleum-impacted coastal salt marshes (Bergen et al., 2000; Culbertson et al., 2008; Reddy et al., 2002). Soil microorganisms, here defined as prokaryotic organisms, provide critical ecosystem services in salt marshes including supporting the benthic food web through the decomposition of organic matter (Howarth and Hobbie,

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1982), and reducing aquatic pollution through removal of nitrate from the water column (Rivera-Monroy et al., 2011). Yet, the long-term repercussions of chronic petroleum residues on salt marsh soil microbial communities in the aftermath of the DWH spill remain uncertain.

Vascular plants in DWH-impacted coastal salt marshes experienced a multiyear decline (Zengel et al., 2022b) that could modify the soil environment and therefore alter microbial community dynamics. Vegetation regulates the chemical and physical properties of salt marsh soils through the input of organic matter, transport of gases, and shading of the soil surface (Hodson et al., 1984; Mendelssohn et al., 1981; Whitcraft and Levin, 2007). The presence and abundance of microorganisms are tightly linked with the biogeochemistry and physical properties of soil, and therefore also vegetation dynamics, in wetlands (Reddy and DeLaune, 2008). *Sporobolus alterniflorus* (previously *Spartina alterniflora*), the dominant plant in Louisiana salt marshes, can exert strong control over the soil microbial community during recovery from an oil spill (Cagle et al., 2020). However, changes in vegetation associated with oiling have yet to be linked to shifts in the soil microbial community.

Evidence has emerged for a chronic effect of oiling on the ecology of *Deepwater Horizon* (DWH)-impacted salt marshes (Beyer et al., 2016; Lumibao et al., 2018; Zengel et al., 2022b, 2016a). Density declines in meiofauna, including juvenile bivalves, large amphipods, ostracods, and juvenile gastropods, were observed in the 42 months after the spill in heavily oiled sites compared with minimally oiled sites (Fleeger et al., 2019, 2017; Lin et al., 2016; Zengel et al., 2022b), and populations of the periwinkle snail (*Littoraria irrorata*) were suppressed up to nine years after the spill (Deis et al., 2020). The impact of the spill on multiple species of benthic invertebrates, including the greenhead horse fly (*Tabanus nigrovittatus*; Husseneder et al., 2018), amphipods (Fleeger et al., 2022), and fiddler crabs (*Uca* spp.; Zengel et al., 2016b), as well as the reduced trophic niche width observed in seaside sparrows at oiled sites (Moyo et al., 2021) are suggestive of alterations to ecosystem function. Early work predicted that soil microbes would rapidly attenuate Macondo oil in salt marsh sediments (Mahmoudi et al., 2013), but compositional shifts associated with the availability of organic C sources three years after the spill suggested that impacts could be longer-lasting (Engel et al., 2017). Given the ecological impacts reported in the salt marshes, further information regarding the effects of chronic oiling on soil microbial communities in DWH-impacted salt marshes over longer time scales is needed.

Here, we considered if vegetation-related changes, chronic exposure to petroleum residues, or both could be linked to shifts in the soil microbial community. The objectives of this study were to determine 1) if microbial communities differed among sites that received variable amounts of oil during the 2010 spill, and 2) if differences in vegetation biomass, soil bulk density, or remnant soil petroleum hydrocarbon content explained this variation independently of a spatial gradient. We hypothesized that the structure of the soil microbial community would vary based on the level of initial oiling a marsh received during the DWH spill and that the differences would be associated with the oiling-associated vegetation changes described above. It is possible that the microbial species and environmental variables obeyed the same spatial structure and thus may be spuriously correlated (Legendre and Troussellier, 1988). We therefore considered the alternative hypothesis that both the species and the environment were spatially structured, and the species distribution was not directly dependent upon the environment.

2. Methods

2.1. Study design and data collection

The study was conducted in in the vegetated intertidal zone of salt marshes in Barataria Bay, Louisiana, USA, across an area of 40 km^2 (Fig. 1; N 29.44060°–29.47459°, W 89.88492°–89.94647°). The sites were established in January 2011 by randomly selecting seven locations from areas designated as “Reference”, “Moderately oiled” or “Heavily oiled” based on field observations and “Shoreline Cleanup Assessment Technique” data (Lin and Mendelssohn, 2012). The sites were sampled for vegetation, soil, and microbial parameters in May 2018 as part of an ongoing study following procedures that have been previously published (Deis et al., 2020, 2017; Fleeger et al., 2017, 2015; Lin et al., 2016; Lin and Mendelssohn, 2012). It was not a blinded experiment due to the investigators’ familiarity with the region.

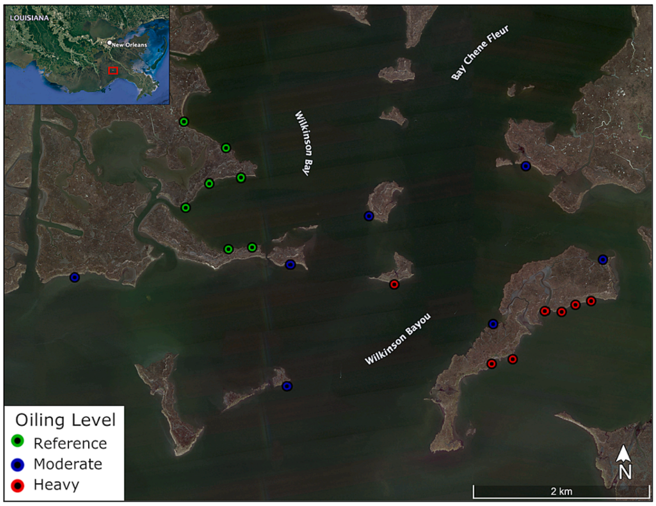

Fig. 1. The map shows the location of the marshes that were sampled in the study. Oiling level refers to the designation that was assigned in 2010 based on the amount of oil the marsh received during the DWH oil spill. N = 7 for each oiling level.

The vegetation and soil variables collected included *S. alterniflorus* live aboveground biomass, *Juncus roemerianus* live aboveground biomass, total live aboveground vegetation biomass, live belowground vegetation biomass, soil total petroleum hydrocarbon concentration (TPH), and soil bulk density (Table 1). Aboveground biomass was collected by clipping to the ground surface all plants rooted within a 0.25 m^2 quadrat placed haphazardly in a location approximately 3 m from the shoreline and avoiding oyster beds, inlets, and debris. The stems were separated by species and into live and dead components and dried to a constant mass at 60 °C. Belowground biomass was determined from the top 0–6 cm of soil collected with a core 7.62 cm in diameter by washing over a 2-mm mesh sieve to remove sediment and particulate organic matter. The material was then separated as live and dead roots and rhizomes, dried to a constant mass at 60 °C, and weighed. Bulk density was determined from the top 0–15 cm of soil core collected with a semi-cylinder peat corer 5 cm in diameter by drying the soil to a constant mass at 60 °C and dividing the weight by the core volume.

Soil samples for microbial and TPH concentration analyses consisted of a composite sample collected from the surface soil (approximately the top 0–2 cm) from five locations at each site. Samples were placed in sterile glass sampling jars, transported to the laboratory on ice, and stored at 4 °C until processing in the laboratory (within 24 h). Each

Table 1
The mean and standard deviation (shown in parentheses) of the environmental variables collected with the microbial samples in May 2018.

Environmental variable	Heavy	Moderate	Reference
Total petroleum hydrocarbons (mg g^{-1})	63.07 (82.64)	1.12 (0.81)	0.3 (0.11)
S. alterniflorus biomass (g m^{-2})	422 (179)	512(358)	580 (392)
J. roemarianus biomass (g m^{-2})	65 (95)	451 (440)	366 (432)
Live aboveground biomass (g m^{-2})	502 (205)	963 (235)	956 (82)
Live belowground biomass (g m^{-2})	401 (110)	915 (240)	828 (127)
Bulk density (g cm^{-3})	0.26 (0.05)	0.28 (0.08)	0.23 (0.06)

sample was homogenized, and roots, conspicuous plant matter, shells, and other visible non-soil matter were removed aseptically. A portion of the sample was frozen at −80 °C for DNA extraction. Soil TPH was extracted using dichloromethane, determined gravimetrically to the nearest 0.0001 g, and expressed per g dry soil (Lin and Mendelssohn, 2012).

DNA for microbial community analysis was extracted using the freeze-grind method (Zhou et al., 1996). Amplification of the 16S rRNA gene was done with a two-step PCR with unmodified 515F/806R primers in the first step and 515F/806R primers modified with barcoded phasing primers in the second step (Wu et al., 2015). The PCR amplicons were bead purified with an Agencourt® AMPure XP kit (Beckman Coulter, Beverly, MA, USA) and used as templates for a second PCR step. Reactions for each sample were prepared in triplicate and combined after each step. The final PCR products were checked on a gel for the expected band size and combined, then purified with a QIAquick Gel Extraction Kit (QIAGEN Sciences, Germantown, MD, USA). Sequencing was done using an Illumina MiSeq at the Institute of Environmental Genomics, University of Oklahoma.

Sequence data were quality-checked and processed by the following steps. The data were quality-checked using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc) and uploaded into the QIIME2 interface (Bolyen et al., 2019). Primers were removed, and the sequences were filtered for chimeras using DADA2 (Callahan et al., 2016). The sequences were clustered in operational taxonomic units (OTUs) at the 97 % similarity threshold using vsearch (Rognes et al., 2016). Dereplicated sequences were aligned with mafft (Katoh, 2002), and a phylogeny was built using Fasttree2 (Price et al., 2010). Taxonomy was assigned based on the Greengenes version 13_8 reference database (DeSantis et al., 2006) with the program classify-sklearn (Bokulich et al., 2018) using a naïve Bayes taxonomy classifier downloaded from the QIIME 2 website (https://docs.qiime2.org). The resulting OTU count matrix was exported in biom format (McDonald et al., 2012) along with the representative sequence file, and all subsequent analyses of the data were done in R (R Core Team, 2021) facilitated by the phyloseq library (McMurdie and Holmes, 2013). Sequences labeled as “order Chloroplast” or “family mitochondria” were removed while retaining those with unassigned order or family (e.g., “order NA”). OTUs unassigned at the phylum level were removed. Species present at least two times in at least two samples were retained for analyses of community composition. Differences in sequencing depth were normalized by rarefying to an even depth of the sample with the lowest count (17,448).

2.2. Redundancy analysis of species composition

The hypothesis (1) that microbial community composition differed among oiling levels was assessed using redundancy analysis (RDA) of Hellinger-transformed OTU counts (Legendre et al., 2005). The effect of the environment and the spatial arrangement of the sites on the microbial community composition were also tested using two separate RDAs with environmental and spatial variables as predictors. We first identified the environmental and spatial variables most strongly associated with the observed communities in a forward selection procedure in which variables were retained at $p < 0.01$ and the adjusted R^2 of the model with all terms was the ceiling. The environmental variables considered in the selection procedure included *S. alterniflorus* biomass, *J. roemerianus* biomass, total aboveground biomass, live belowground biomass, and bulk density (Table 1). The spatial predictor variables were identified using a trend surface analysis consisting of orthogonal, third-degree polynomials computed from the centered coordinates of the sites (Blanchet et al., 2008). The parsimonious models were tested for significance using 999 permutations and used for further analysis.

Based on the results of RDA, we sought to determine if the oiling level effect was associated with TPH or vegetation-based metrics (hypothesis 2) when controlling for a significant spatial effect we identified. We used variance partitioning analysis (Borcard et al., 1992) to examine the variance in the species composition data associated with oiling level, environmental variables, and spatial location. This three-way partition allowed us to identify the variation shared by oiling level and environment that was independent of the spatial effect. Variance components were identified using the function ‘varpart’ and tested for significance with RDA using 9999 permutations in the ’vegan’ package.

2.3. Phylogenetic analysis of microbial communities

We further investigated the microbial communities in terms of phylogenetic diversity based on the UniFrac dissimilarity metric. To identify potential relationships between the environmental and spatial variables and phylogenetic diversity at the sites, dissimilarity (Lozupone and Knight, 2005) was visualized with non-metric multidimensional scaling (NMDS) and correlations were identified using permutational tests of vectors fitted to the NMDS ordination. The environmental variables with significant correlations were further considered in testing of hypothesis 2. Tests for statistical significance were done with permutational analysis of variance (PerMANOVA) of phylogenetic distance with 9999 permutations using the ‘vegan’ package (Anderson, 2001; Oksanen et al., 2020). The spatial component was first tested for significance, then included as a covariate in the tests of the environmental variables and oiling level.

2.4. Indicator species analysis

Indicator taxa are those which can be used as indicators of habitat conditions based on their niche preferences. Associations between species patterns and combinations of groups of sites were identified for each of the designated oiling levels using indicator species analysis as described by De Cáceres et al. (2010). Briefly, a multi-level pattern analysis is used to calculate an indicator value for each species in association to the oiling level, and the oiling level with the highest association to each species is selected. The statistical significance of the associations is tested using the indicator value as a test statistic in a permutation test. The test was implemented with the function ‘multipatt’ in the package picante with 999 permutations and the options “IndVal.g” and “duleg = F”. The inclusion of site combinations (e.g., indicator taxa, which are representative of both the heavy and moderate environments compared with the reference environment) has been shown to improve the accuracy of the analysis (De Cáceres et al., 2010). Taxa occurring at least five times in at least five samples were included to focus the analysis on the most prevalent taxa and reduce noise. The top indicator taxa were plotted in a heatmap with their relative abundance using the ComplexHeatmap package (Gu et al., 2016). An alpha value of 0.05 was selected to indicate statistical significance.

3. Results

The individual and shared effects of environment, oiling level, and spatial location on microbial community composition were identified using variance partitioning analysis. The model selection procedure identified total live aboveground biomass as the parsimonious model for the vegetation and soil data, and it was included with TPH in environmental model. Longitude was identified for the spatial model. The results of RDAs showed that composition significantly differed among oiling levels, as well as by environmental conditions and spatial location (Table 2). The three components together explained 34 % of the variation in the microbial data (Fig. 2; adjusted $R^2 = 0.176$). The effects of environmental and oiling level were robust to controlling for spatial location using additional RDA models (Table 2). The significant pure oiling level component represents variance in community composition associated with initial oiling level that was not explained by the environmental model and spatial location (i.e., unmeasured effects of oiling level). The significant pure spatial component indicates that there were

Table 2

The results of permutation tests for RDA. The sample size was 21 for all tests. TPH: total petroleum hydrocarbons; LAB: live aboveground biomass.

Effect	Model	Df	F	*P*	Adj. R^2
Oiling level	Oiling level	2, 18	1.971	0.001	0.089
Environment	TPH + LAB	1, 19	2.024	0.001	0.093
Spatial trend	Longitude	1, 19	3.200	0.001	0.099
Oiling level controlling for spatial trend	Oiling level + Condition(Longitude)	2, 17	1.610	0.002	0.054
Environment controlling for spatial trend	TPH + LAB + Condition(Longitude)	2, 17	1.304	0.045	0.028
Spatial trend and Environment	Longitude + TPH + LAB	3, 17	1.970	0.001	0.127
Pure oiling level	Oiling level + Condition(Longitude + TPH + LAB)	2, 15	2.002	0.002	0.049
Pure spatial trend	Longitude + Condition (Oiling level + TPH + LAB)	1, 15	2.213	0.005	0.062

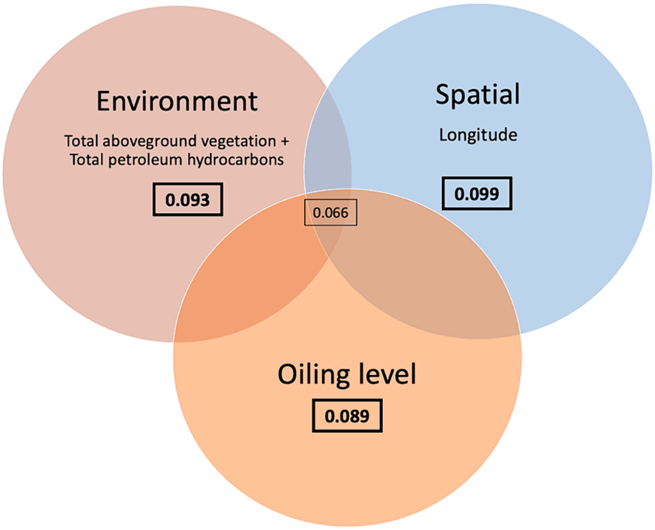

Fig. 2. A graphical representation of the components of variation in the microbial community composition based on Hellinger-transformed OTU abundances. The portions of variation explained by each component were determined using RDA and a permutational F test. The variables listed in the environmental and spatial components are those that were selected for the respective models (see the text). The adjusted R^2 for each component and the shared fraction is shown in a black box (values ≤ 0 not shown); see Table 2 for additional results. Total aboveground vegetation refers to biomass weight and total petroleum hydrocarbons refers to the gravimetric content from dichloromethane extraction.

effects of site's location on the microbial community beyond what was captured by the environmental model and oiling level.

We further evaluated our hypotheses by considering microbial community composition in terms of phylogenetic dissimilarity. Consistent with the variance partitioning analysis of taxonomic composition, the results indicated a highly significant effect of longitude (PerMANOVA $F_{1,19} = 4.31$; $p < 0.001$). Taking this into account when testing hypothesis (1), the effect of oiling level was significant when controlling for longitude (PerMANOVA $F_{2,18} = 2.00$; $p = 0.036$). To identify the environmental variables potentially associated with phylogenetic diversity and address hypothesis (2), a correlation analysis was performed with an NMDS ordination (Fig. 3). The environmental variables with significant correlations to the ordination included total petroleum hydrocarbons (TPH; $R^2 = 0.535$; $p = 0.002$), and aboveground vegetation biomass ($R^2 = 0.375$; $p = 0.013$) and these were further evaluated to address hypothesis (2). Aboveground biomass did not significantly explain the variation in phylogenetic dissimilarity when controlling for longitude (PerMANOVA $F_{1,18} = 1.20$, $p = 0.235$), whereas TPH was significant when controlling for longitude (PerMANOVA $F_{1,18} = 2.07$; $p = 0.054$).

An indicator species analysis was conducted to further disentangle the processes structuring soil microbial communities (Fig. 4). Several indicator taxa for the heavily oiled sites included close relatives of known oil-degrading and petroleum-associated bacteria. For example, the most abundant indicator taxon for the heavily oiled sites was from the genus *KCM-B-112* (order Acidithiobacillales), a genus often found in chronically oil-polluted soils (Bodor et al., 2021; Marti et al., 2017). Immundisolibacterales, another indicator for the heavily oiled sites, is an order represented by a single taxon capable of degrading high-molecular-weight polycyclic aromatic hydrocarbons (Corteselli et al., 2017). Several species of myxobacteria (order Myxococcales) were indicators for the reference sites, including species from the genera *Phaselicystis*, *Nannocystis*, *Haliangium*, and uncultured members of the families BIrii41 and Sandaracinaceae. A complete list of the significant indicator taxa for each site and their taxonomy is provided in Supplementary Table 1.

4. Discussion

This investigation, conducted 8 years after the *Deepwater Horizon* spill, found significant differences in the composition of soil microbial communities among salt marshes that received varying degrees of initial impact. These long-term effects appeared to be related to petroleum residues and differences in aboveground vegetation biomass (hereafter, "vegetation"), which significantly explained variation in the microbial communities among sites. However, there was a significant spatial trend in microbial community structure in Barataria Bay associated with longitudinal positioning, reported here for the first time, that was at least partially confounded with these factors. We hypothesized that differences in environmental conditions we measured, which included residual petroleum content, would explain variation in the microbial communities among oiling levels. Our results showed that vegetation and petroleum residue content together explained a significant portion of the taxonomic variation among soil microbial communities after controlling for the spatial trend. Notably, these factors did not explain all the variation associated with initial oiling level, indicating the presence of an unmeasured variable associated with oiling level. Upon evaluating the community structure through phylogenetic composition, which reflects the evolutionary relatedness of taxa, soil TPH content

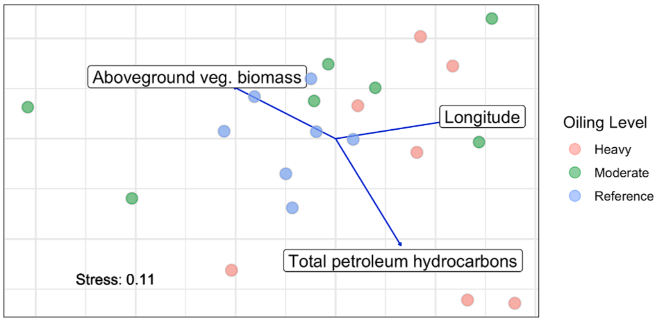

Fig. 3. NMDS plot of microbial community composition in salt marsh soils that received varying degrees of oil impact during the *Deepwater Horizon* oil spill based on phylogenetic dissimilarity. Arrows depict environmental variables with a significant correlation to the ordination.

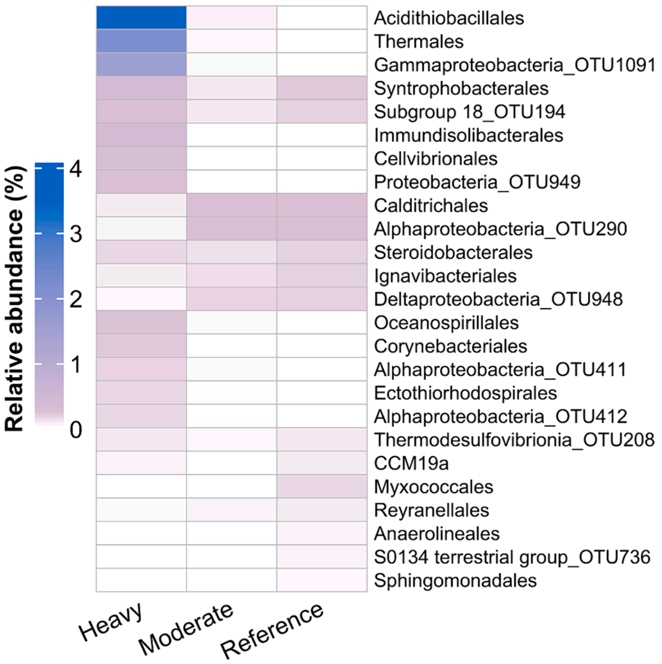

Fig. 4. A heatmap of the relative abundance of the 15 top significant indicator taxa in Heavy and Reference sites based on the indicator value statistic. Taxa are summarized at the order level and OTUs that were unidentified at the order level are shown with the highest level of taxonomic identification available and the OTU number. Taxa, which were indicators in either Heavy or Reference sites (or in combination with Moderate), are shown for brevity. The full list of the significant indicator taxa for each oiling level and combination of oiling levels, with associated taxonomy, is provided in Supplementary Table 1.

emerged as significant after controlling for the spatial trend, but vegetation was confounded with location to a large degree and was not significant. Furthermore, several significant indicator taxa associated with the heavily oiled sites were identified as oil-degrading bacteria. Our results suggest a potential role of chronic petroleum residues in shaping microbial communities in Barataria Bay salt marshes impacted by the DWH oil spill and highlight the presence of a spatial-environmental gradient in community structure.

The identification of several known oil degrading bacteria as indicator taxa for the heavily oiled sites, including some previously identified as responders to Macondo Well oil, provides compelling evidence their presence was likely in response to long-term petroleum contamination. For example, one of the indicators for heavily oiled sites was a *Mycobacterium* sp., a genus containing bacteria capable of degrading high-molecular-weight hydrocarbons (Kanaly and Harayama, 2010) that was identified in association with oil-impacted shoreline microbial communities (Atlas et al., 2015; Kappell et al., 2014). Another indicator for the heavily oiled sites was Oceanospirillales, which has previously been reported in salt marshes exposed to Macondo Well oil (Engel et al., 2017; Koo et al., 2014). Oceanospirillales and Cellvibrionales, also identified here as oil indicators, were enriched in sediments with chronic petroleum exposure in the Persian Gulf (Rezaei Somee et al., 2021). These results suggest that petroleum compounds can act as a selective force on the bacterial community in salt marshes, as has been demonstrated in ocean sediments (Mason et al., 2014).

It is somewhat surprising that variation due to oiling level was not fully explained by the variables we measured, which included residual petroleum content. One possible explanation for this unexplained variation could be increased tidal or storm induced inundation due to higher erosion rates that occurred as a result of oiling. Oiling that causes plant mortality also contributes to shoreline erosion as evidenced by decreased shear strength at heavily oiled sites (Lin et al., 2016; Zengel et al., 2022a). Oiling-induced erosion exposes nearshore locations such as those studied here to more frequent flooding (Zengel et al., 2022a), which can significantly affect bacterial composition and diversity in coastal marshes (McLain et al., 2020). Therefore, increased inundation due to oiling-induced erosion is a plausible explanation for the observed differences in microbial communities among oiling levels that was unexplained by the variables we measured.

The results of this study showed a strong effect of longitude on microbial community composition that was partly confounded with the degree of oiling sites received during the spill as well as with vegetation biomass and residual petroleum content. Oil from the DWH entered Barataria Bay from the south, and heavy oiling was most common along the eastern shoreline (Michel et al., 2013). Consequently, heavily oiled marshes were primarily located to the east and south relative to the reference and moderately oiled areas, which were protected by marsh islands. Deis et al. (2019) examined our study sites (Fig. 1) and found that wind and wave energy tended to be higher in the regions where the heavily oiled sites were located. Thus, the processes that led to the initial impact at our heavily oiled sites could also lead to differences in microbial community composition. Increased wave exposure also promotes a stress response in *S. alterniflorus* that can lead to reduced biomass (Shao et al., 2020). Our sampling design does not allow us to fully separate these influences. However, our results clearly show significant turnover in microbial community composition along a gradient of spatial-environmental heterogeneity over a relatively short distance (e.g., 40 km). This information will be useful for interpreting the impact of the oil spill on salt marsh microbial communities and highlights the need for research on how plant biomass influences microbial communities in coastal wetlands as suggested by Kim et al. (2022).

Although several studies have identified bacterial responders to petroleum, fewer have reported bacterial indicators of salt marsh health. The myxobacteria we identified as indicators for reference sites warrant further exploration in subsequent studies. Myxobacteria, ubiquitously found across soil, aquatic, and marine ecosystems (Wang et al., 2021), predominantly thrive on decaying plant matter (Shimkets et al., 2006). Their status as an indicator for the reference sites in this study might be linked to the reduced belowground biomass at heavily oiled sites (Table 1). The growth of myxobacteria is likely not inhibited by petroleum compounds as previous work has found myxobacteria were present in petroleum-contaminated coastal sediments (Acosta-González et al., 2013). However, the sensitivity level of myxobacteria to petroleum hydrocarbons remains uninvestigated. We identified several OTUs from different families within the Myxococcales, including BIrii41, Haliangiaceae, Nannocystaceae, Phaselicystidaceae, and Sandaracinaceae (Supplementary Table 1). Given their cosmopolitan distribution and apparent response to chronic oil pollution or reduced belowground biomass reported herein, myxobacteria could serve as potential indicators of coastal wetland habitat.

Our findings contradict a reported indicator group linked to petroleum contamination in another coastal deltaic ecosystem. Herein, several taxa within the Ectothiorhodospiralles (primarily represented by the family Ectothiorhodospiraceae) emerged as significant indicators of heavily oiled sites, contrasting with Zoppini et al. (2020) who reported a negative correlation between Ectothiorhodospiraceae and petroleum-derived pollutants in Po River delta lagoon sediments. This discrepancy might stem from salinity or soil type variations that co-varied with pollution in their study, as they targeted sites along a gradient of freshwater influence (Zoppini et al., 2020). These contrasting observations suggest that freshwater influence may be a confounding factor when considering microbial responders to petroleum pollution in the estuarine environment.

In conclusion, our study conducted 8 years after the DWH spill delineated significant differences in soil microbial community composition in marshes based on the level of oiling they initially received in 2010. These differences were robust to controlling for a longitudinal spatial gradient and were partially explained by variation in aboveground vegetation biomass and TPH content measured in 2018. TPH content had the strongest explanatory power with respect to phylogenetic community composition when controlling for spatial location. Moreover, several bacterial indicator taxa at heavily oiled sites were identified as oil-degrading bacteria, suggesting that residual petroleum was a factor governing microbial community structure over the long term. Further research is imperative to unravel the intricate relationships between oiling, vegetation, soil variables, and possibly erosion, which likely contributed to the observed differences in Barataria Bay soil microbial communities.

CRediT authorship contribution statement

Grace A. Cagle: Conceptualization, Writing - original draft, Writing - review & editing, Visualization, Investigation, Formal analysis, Methodology. **Huan Chen:** Conceptualization, Writing - review & editing, Methodology. **John W. Fleeger:** Conceptualization, Funding acquisition, Writing - review & editing. **Don Deis:** Conceptualization, Investigation, Writing - review & editing. **Qianxin Lin:** Conceptualization, Investigation, Project administration. **Aixin Hou:** Conceptualization, Funding acquisition, Writing - original draft, Writing - review & editing, Investigation, Supervision, Resources, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data is publicly available

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2024.111884.

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