Hydrobiologia (2019) 827:65–74
https://doi.org/10.1007/s10750-017-3461-3

COASTAL WETLANDS

The responses of indigenous oil-degrading bacteria to oil exposure in *Phragmites australis*-dominated marsh soil: a mesocosm study

Jiaoyue Wang · Chad Robert Judy · Aixin Hou

Received: 19 April 2017 / Revised: 20 November 2017 / Accepted: 1 December 2017 / Published online: 12 December 2017

Abstract This study examined impacts of Macondo MC252 oil from the *Deepwater Horizon* spill on oil-degrading bacteria in fresh and oligohaline marshes dominated by the common reed *Phragmites australis* (*Cav.*) Trin. ex Steud. and correlated microbial changes to soil variables. We hypothesized that indigenous oil-degrading bacteria and soil characteristics in the marshes effectively respond to Macondo oil. We tested this hypothesis with a greenhouse mesocosm study. Weathered and emulsified Macondo oil was applied to *P. australis* sods at different rates (0, 4, 8, 12, and 16 L m^{-2}). Populations of oil degrading bacteria containing alkane monooxygenase and PAH-ring hydroxylating dioxygenase alpha subunit (PAH-RHDα) genes, the expression of these genes, soil respiration rate, residual total petroleum hydrocarbon, redox potential (Eh), as well as dissolved organic carbon (DOC) and its aromaticity index of $SUVA_{254}$ were measured in soils 2 months after oil addition. Oil exposure generally increased oil-degrading bacterial populations, in particular Gram-positive (GP) PAH-degraders, soil respiration rate, DOC concentration, and aromaticity of DOC, but decreased Eh values. GP PAH-RHDα genes accounted for approximately 98% of total detected genes, and expression of these genes increased by a factor of 3 to 5 at various oil dosages. Both abundance and expression of GP PAH-RHDα genes significantly correlated to $SUVA_{254}$ ($P < 0.05$). The study revealed that indigenous oil-degrading bacteria effectively responded to weathered Macondo oil in the *P. australis* marsh soil wherein GP bacteria with PAH-RHDα genes played a major role in biodegradation of PAH-enriched petroleum hydrocarbons.

Keywords *Deepwater Horizon* oil spill · *Phragmites australis* · Alkane monooxygenase (alkB) · PAH-ring hydroxylating dioxygenase alpha subunit (PAH-RHDα) · Real-time PCR · Gene expression

Guest editors: Shuqing An, Jennifer Ann Davis & Dong Xie / Coastal Wetlands: Services, Uses and Conservation

J. Wang
Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, People's Republic of China

J. Wang
Key Laboratory of Pollution Ecology and Environmental Engineering, Chinese Academy of Sciences, Shenyang 110016, People's Republic of China

J. Wang · A. Hou
Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun 130102, People's Republic of China

C. R. Judy · A. Hou (✉)
Department of Environmental Sciences, College of the Coast and Environment, Louisiana State University, Baton Rouge, LA 70803, USA
e-mail: ahou@lsu.edu

Introduction

The blowout of the *Deepwater Horizon* (*DWH*) drilling rig on April 20, 2010 triggered the largest marine oil spill in United States history (Oil Spill Commission, 2011). Approximately 4.9 million barrels of light crude oil (Macondo MC252) was discharged into the Gulf of Mexico over a period of 87 days (Romero et al., 2015). The sheer volume of oil caused widespread impacts including the oiling of approximately 1040 km of Gulf Coast marshes (Oil Spill Commission, 2011). Due to the sensitivity of microbes to petroleum and their major roles in breakdown of petroleum hydrocarbons (Wasmund et al., 2009), a considerable number of studies have documented changes of the indigenous microbial communities in various Gulf Coast ecosystems in response to the *DWH* spill. Overall, the *DWH* spill had a profound impact on the abundance and community composition of microbes; *Colwellia*, *Gammaproteobacteria* (*Alcanivorax*, *Marinobacter*), *Alphaproteobacteria* (*Rhodobacteraceae*) and γ-*Proteobacteria* were identified as key players in oil degradation (e.g., Hazen et al., 2010; Kostka et al., 2011; Redmond &Valentine, 2012).

Alkanes and polycyclic aromatic hydrocarbons (PAHs) are the most common constitutes in crude oil. Alkanes are easier to degrade, and the initial stage of oil aerobic degradation is primarily catalyzed by alkane monooxygenases (alkB). The genetics of these enzymes have been characterized in *Pseudomonas* (Van Beilen et al., 1994, 2001), *Rhodococcus* (Whyte et al., 2002; Van Beilen et al., 2006), *Alcanivorax* (Hara et al., 2004), *Xanthobacter* (Van Beilen et al., 2003), and others (Powell et al., 2006; Yergeau et al., 2009). Degradation of PAHs is usually carried out by multicomponent aromatic-ring-hydroxylating dioxygenases (RHD), which is in part encoded by the PAH-RHDα genes (Yergeau et al., 2009; Muangchinda et al., 2013). The aerobic pathways of bacterial PAH-degradation that involve PAH-RHDα genes have been described in a wide range of Gram negative (GN) bacterial strains and some Gram positive (GP) strains (Habe & Omori, 2003; Cébron et al., 2008, 2009). The presence of different genes that encode similar enzymes in both GN and GP bacteria highlights the importance of paying attention to both of these groups to adequately evaluate the PAH-degradation potential of whole bacterial communities (Cébron et al., 2008).

Accurately quantifying functional genes in the environment via real-time PCR (qPCR) has become an effective approach to understanding ecological processes (Powell et al., 2006). Researchers have successfully quantified copy numbers of alkB, and GP and GN PAH-RHDα genes from soil samples by qPCR (Baldwin et al., 2003; Powell et al., 2006; Cébron et al., 2008), yet expression of these genes has not been measured even though it has a closer association with contaminant degradation (Lu et al., 2011). Additionally, in contrast to the documentation of microbial responses in marine and salt mash habitats, there has been little published research evaluating the impacts of the *DWH* spill on microbes in fresh and oligohaline marsh. Although the spill mainly contaminated salt marsh dominated by *Spartinaalterniflora*, *Juncusroemerianus*, and *Avicenniagerminans*, it oiled a sizable area of fresh and oligohaline marsh dominated by the common reed, *Phragmites australis* (*P. australis* (*Cav.*) Trin. ex Steud.) in the Birdfoot Delta at the mouth of the Mississippi River. An understanding of how indigenous oil-degrading bacteria respond to oil exposure is important for the remediation and management of oil-contaminated *P. australis* marshes. Our companion study previously reported the impacts of weathered and emulsified Macondo oil on *Phragmites australis* growth (Judy et al., 2014). The objectives of this study were to (1) quantify the abundance of oil-degrading bacterial populations and their functional gene expression in response to different dosages of oil in *P. australis* marsh soil and (2) determine the correlation of microbial changes to soil variables. We tested two hypotheses: the first was that indigenous oil-degrading bacteria can sensitively respond to Macondo oil addition, and the second was that indigenous oil-degrading bacteria have close relationships with soil characteristics affected by different oil dosages.

Materials and methods

Experimental design and procedures

In our companion paper (Judy et al., 2014), we presented the impacts of Macondo oil on the growth response of the common reed *P. australis*. In this work, we adopted the soils from the experiment 2 of that study (Judy et al., 2014) to be used for soil variable

and bacterial analyses, with our focus on the responses of indigenous oil-degrading bacteria to oil exposure. The details of the experimental settings employed have been described previously (Judy et al., 2014). Briefly, *P. australis* sods with intact vegetation and soil (28 cm in diameter and approx. 30 cm deep) were collected from an unoiled marsh site (30°23.205′N, 90°09.551′W) in Madisonville, LA, USA in October 2011. Soil pH was 6.4 ± 1.1. Soil salinity was 1.6 ± 0.5. Soil texture was classified as loam with 27.9 ± 3.6% sand, 45.3 ± 3.3% silt, 26.8 ± 2.9% clay, and 22.4 ± 2.8% organic matter. At the completion of the first experiment (only with shoot oiling) on effects of common oiling scenarios on *P. australis* growth response, the aboveground biomass was harvested. After regrowing for 2 months, the weathered MC252 oil (see the method of *DWH* oil weathering process, Judy et al., 2014) was applied to the marsh sods at rates of 0 (control), 4, 8, 12, and 16 L m^{-2}, respectively, to investigate the effects of oil added to the soil. To evenly disperse oil into soil, weathered and emulsified oil was applied over standing water in each sod, and the water was then drained through the soil column allowing the oil to contact the soil and encouraging penetration into the sediment. The experimental design was a randomized block containing five treatment levels replicated in five blocks, resulting in a total of 25 sods.

Sample collection and analysis

Two months after the oil application, soil respiration rate, expressed as g CO_2 m^{-2} h^{-1}, was measured using a portable infrared gas analyzer (IRGA). The portable IRGA system consisted of the PP Systems Soil Respiration System, with an EGM-4 Environmental Gas Monitor attached to an SRC-1 Soil Respiration Chamber (PP Systems, Hertfordshire, UK). Measurements were taken from 25 PVC couplings (10 cm in diameter) installed to 3-cm depth in the marsh soil. The CO_2 flux from each collar was measured for a 60-s period (Jackson et al., 2009). Soil redox potential (Eh) was measured at 10 cm into the soil column with an Accument AP71 pH/mV/°C m (Fisher Scientific, Waltham, Ma) connected to a Corning Hi-stab calomel reference electrode and bright platinum electrodes. Measurements were taken from each sod with three platinum electrodes. The electrodes were left in place for 24 h before data were collected to assure stable readings (Judy et al., 2014). After soil respiration and Eh measurements, surface soil (about 10 cm deep) was collected from the sod. Some fresh soil without sieve was used for DOC, aromaticity of DOC and residual total petroleum hydrocarbon (TPH) analyses. The rest was stored at −80°C for DNA and RNA extraction.

DNA from soils was extracted by the MoBioPowerSoil® DNA Isolation kit, whereas RNA was isolated with the MoBio RNA PowerSoil™ Total RNA Isolation kit. The quality of the DNA and RNA was confirmed by spectrophotometric analysis (NanoDrop2000C, Thermo Scientific). Meanwhile, 1.5% agrose gel was run to confirm the acceptable DNA quality. RNA was treated (15 min at 20°C) with Rnase-free DNase (Qiagen) and subsequently purified using RNA easy mini Kit (Qiagen) before reverse–transcription (Louvel et al., 2011). Soil DOC was measured by the methods of Dong et al. (2009). The aromaticity composition in dissolved carbonaceous substrates index of $SUVA_{254}$ was defined as the UV absorbance at 254 nm divided by the DOC concentration (mg L^{-1}), and expressed in units of L mg^{-1} m^{-1} (Weishaar et al., 2003; Peichl et al., 2007). Soil residual TPH was determined by the method of Lin & Mendelssohn (1996).

PCR amplification of specific genes

cDNA was synthesized by DNase-treated RNA through reverse transcription using Thermo Scientific Maxima First Strand cDNA Synthesis kit. The synthetic cDNA and the extracted DNA were used for qPCR amplification. The abundance and expression of 16S rRNA, alkB, and GN and GP PAH-RHDα genes were determined by a CFX Connect ™ Real-time system (Bio-RAD). Sequences for these specific gene fragments were acquired from the Genbank database (https://www.ncbi.nlm.nih.gov/genbank/) with accession numbers of KC207085 (16Sr RNA, *Pseudomonas putida*), M83949 (GN PAH-RHD, *Pseudomonas putida*), DQ846881 (GP PAH-RHD, *Rhodococcus opacus*), and AJ233397 (alkB, *Pseudomonas putida*). Standards for qPCR were generated by serial dilution of template DNA, and the corresponding standard curves over the dilution range and a detection limit of 10^1 copies were obtained. The target gene copy numbers were quantified relative to a standard curve of positive control (Powell et al., 2006).

The reaction mixtures for PCR amplification contained 5.0 µl of Bio-Rad SsoAdvanced™ SYBR Green Supermix (including necessary buffer, hot start Sso7d-fusion polymerase, SYBR® green dye, dNTPs, $MgCl_2$, enhancers, and stabilizers), 500 nM of each forward and reverse primer, 4.0 µl of DNA template diluted to 10^{-1} or 10^{-2} concentration of the original DNA extraction or the synthetic cDNA, and nuclease free water adjusted to a final volume of 10 µl. Primers and thermal cycling conditions were all derived from the published research (Table 1) but with some modifications. For the genes of 16S rRNA and GP PAH-RHDα, thermal cycling conditions were as follows: 95°C for 5 min, forty cycles of denaturing temperature of 95°C for 30 s; annealing temperature of 56°C for 30 s; and 72°C for 40 s, and 72°C for 7 min. For the GN PAH-RHDα, the thermal cycling conditions were consistent with above genes but the annealing temperature was 57°C. For the alkB, thermal cycling conditions were as follows: 94°C for 15 min, forty cycles of denaturing temperature of 84°C for 20 s; annealing temperature of 50°C for 30 s; and 72°C for 45 s, and 45°C for 1 min.

Statistical analysis

Statistical analyses were done with IBM SPSS 18 for Windows. One-way ANOVA analysis (LSD) was performed to compare the differences in soil oil degrading bacteria containing alkB and PAH-RADα genes, soil respiration rate, DOC, Eh, and residual TPH at various oil dosages. Spearman's rank correlation tests were used to describe the relationships among target genes, residual TPH, soil respiration rate, and DOC, and the analysis was done after a natural logarithm transformation of the data to get a better normal distribution. All measures of significance were identified at $P < 0.05$ unless otherwise stated.

Results

Soil residual TPH significantly increased with oil dosages ($P < 0.01$). The DOC (one expression of labile organic carbon) and its aromaticity index of $SUVA_{254}$ increased by 11.62–51.52% and 20.63–86.25%, respectively, after oil addition (Table 2). Oil addition also increased soil respiration

Table 1 Primers and conditions used for real-time PCR assays

Primers	Target gene	Sequence: 5′ to 3′	Amplicon size	Annealing temperature	References
1401 F	16S rRNA	CGGTGTGTACAAGACCC	435	56	Cébron et al. (2008)
968 R		AACGCGAAGAACCTTAC			
AlkBFd	Alkane monooxygenase (alkB)	AACTACMTCGARCAYTACGG	100	50	Powell et al. (2006)
AlkBRd		TGAAGATGTGGTTGCTGTTCC			
RHDα GN F	Gram negative PAH-RHDα	GAGATGCATACCACGTKGGTTGGA	306	57	Cébron et al. (2008)
RHDα GN R		AGCTGTTGTTCGGGAAGAYWGTGCMGTT			
RHDα GP F	Gram positive PAH-RHDα	CGGCGCCGACAAYTTYGTNGG	292	56	Cébron et al. (2008)
RHDα GP R		GGGGAACACGGTGCCRTGDATRAA			

Table 2 The effects of oil on soil respiration, residual TPH, Eh, and DOC and its aromaticity index with various oil dosages in *Phragmites australis* marsh soil

	Treatment				
	Control	4 L m^{-2}	8 L m^{-2}	12 L m^{-2}	16 L m^{-2}
TPH (mg g^{-1} dry soil)	0.96 ± 0.12^{e}	9.86 ± 2.76^{d}	28.40 ± 4.88^{c}	48.84 ± 2.40^{b}	70.20 ± 16.18^{a}
DOC (mg kg^{-1} dry soil)	973.61 ± 43.30^{c}	1177.33 ± 38.86^{b}	1107.39 ± 48.53^{b}	1068.66 ± 22.43^{bc}	1475.22 ± 118.10^{a}
$SUVA_{254}$ (L mg^{-1} m^{-1})	0.53 ± 0.02^{d}	0.99 ± 0.04^{a}	0.82 ± 0.04^{b}	0.87 ± 0.02^{b}	0.64 ± 0.05^{c}
Soil respiration rate (g CO_2 m^{-2} h^{-1})	0.76 ± 0.45^{b}	1.57 ± 0.79^{ab}	1.40 ± 0.36^{ab}	1.82 ± 0.57^{ab}	2.41 ± 1.48^{a}
Eh (mV)	-68.5 ± 15.3^{b}	-111.9 ± 12.2^{a}	-125.1 ± 10.5^{a}	-110.2 ± 14.5^{ab}	-145.7 ± 13.2^{a}

The values are means and standard errors ($n = 5$). Different letters indicate significant differences between treatment-level means ($P < 0.05$)

TPH, total petroleum hydrocarbons; DOC, dissolved organic carbon; Eh, soil redox; $SUVA_{254}$, the UV absorbance at 254 nm

rate, but only at the highest oil level it reached to the significant level (Table 2). Oil exposure significantly decreased soil Eh (Table 2). Residual TPH was significantly correlated with DOC content and soil respiration rate, and there was a significant relationship between DOC content and soil respiration rate (Table 3).

Oil exposure numerically increased the abundance and expression of the 16S rRNA gene, and the oil-degrading bacterial populations containing alkB and PAH-RADα genes increased significantly ($P < 0.05$), approximately two or three times higher than the control (Fig. 1). The relative abundance of oil-degrading genes (i.e., relative to 16S rRNA gene) also increased with increasing oil dosages (Table 4). The percentage of GP PAH-RHDα gene was approximately 97 (gene abundance) and 226 (gene expression) times higher than the GN PAH-RHDα gene in the control treatment, and these numbers reached to the highest values of 152 (abundance) and 795 (expression) times in the oil treatment (Table 4). The expression of oil-degrading genes was positively correlated to residual TPH concentration (Table 3).

Table 3 The correlations among target genes, TPH, soil respiration rate, and dissolved organic carbon, $N = 25$

	TPH	DOC	$SUVA_{254}$	Soil respiration rate
DNA				
16S rRNA	0.192	−0.030	0.244	0.121
alkB	−0.230	0.102	0.260	−0.031
GP PAH-RHDα	0.215	0.018	0.527*	0.255
GN PAH-RHDα	0.617**	0.674**	0.355	0.304
Sum of oil-degrading genes	0.215	0.018	0.527*	0.255
RNA				
16S rRNA	0.122	0.381	−0.048	0.025
alkB	−0.258	−0.315	−0.113	−0.062
GP PAH-RHDα	0.504*	0.306	0.554*	0.173
GN PAH-RHDα	0.478*	−0.009	−0.045	0.157
Sum of oil-degrading genes	0.495*	0.273	0.569*	0.175
TPH	1			
DOC	0.602*	1		
$SUVA_{254}$	0.097	0.242	1	
Respiration	0.644**	0.570*	0.120	1

Spearman's rank correlation coefficients (range from −1 to +1) are followed by * values

TPH, total petroleum hydrocarbons; DOC, dissolved organic carbon; $SUVA_{254}$, UV absorbance at 254 nm

*Indicates significant effects at $P < 0.05$

**Indicates significant effects at $P < 0.01$

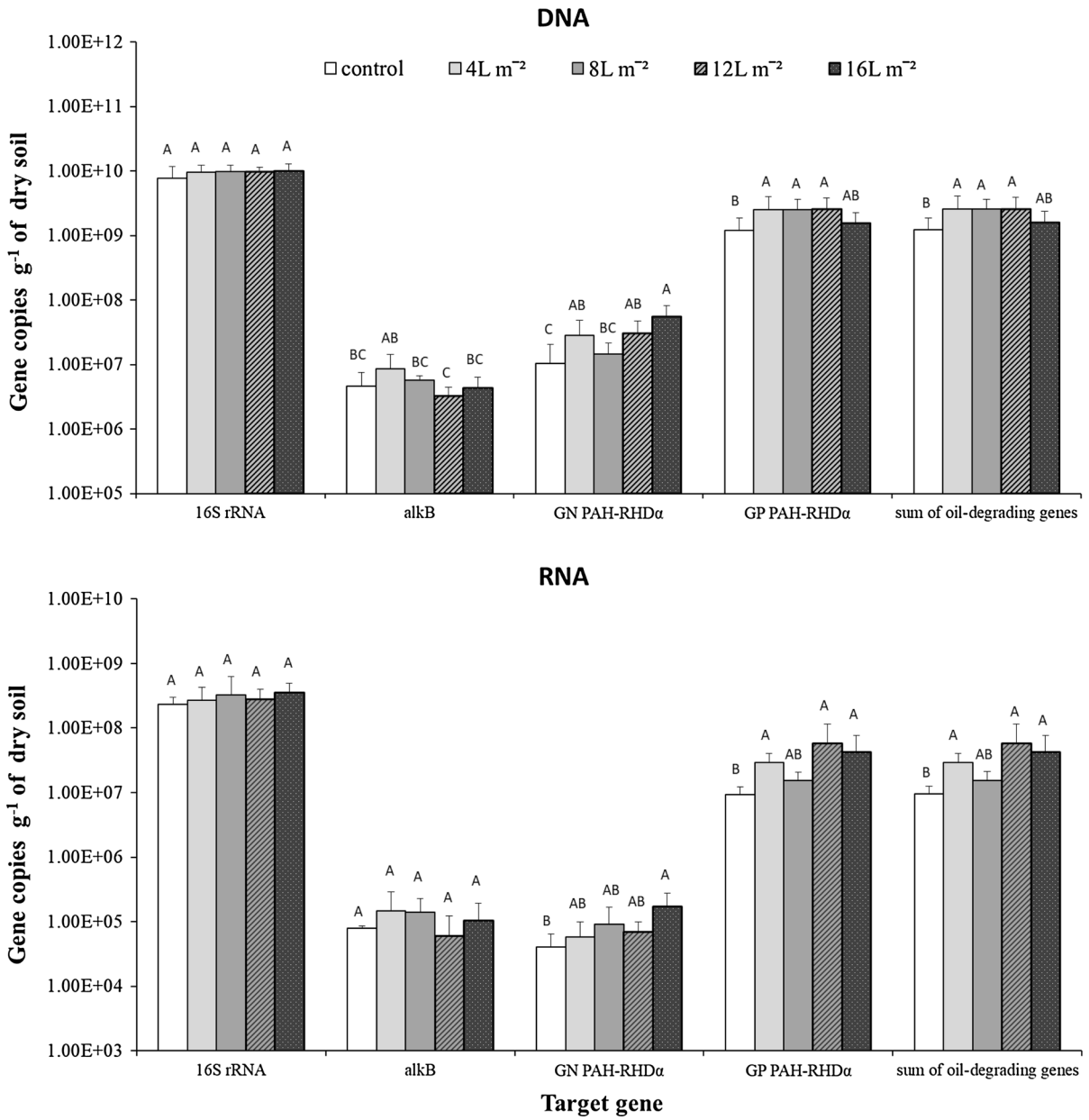

Fig. 1 The effects of oil on the abundance and expression of target genes with various oil dosages in *Phragmites australis* marsh soil. The values are means and standard errors ($n = 5$). Different letters indicate significant differences between treatment-level means ($P < 0.05$)

Discussion

Oil exposure significantly affected soil characteristics (Table 2). The significant correlations between residual TPH and DOC as well as the increased aromaticity index of $SUVA_{254}$ with increasing oil dosages (Table 3) were consistent with the findings of the rhizosphere of *P. australis* (common reed) and bulk soils in the Yellow River Delta when they were oil contaminated (Nie et al., 2009). The results indicated that petroleum hydrocarbons were an important source for the dissolved carbonaceous substrates when oil was added to soil, especially for the aromaticity constituents (Nie et al., 2009). Soil respiration rate, an

Table 4 The percentages of oil-degrading genes relative to 16S rRNA gene at various oil dosages in *Phragmites australis* marsh soil

Percentage (%)	Treatment				
	Control	4 L m^{-2}	8 L m^{-2}	12 L m^{-2}	16 L m^{-2}
DNA (target genes/16S rRNA)					
alkB	0.06 ± 0.03	0.09 ± 0.05	0.06 ± 0.03	0.03 ± 0.01	0.04 ± 0.02
GN PAH-RHDα	0.17 ± 0.20	0.28 ± 0.20	0.16 ± 0.12	0.32 ± 0.18	0.55 ± 0.24
GP PAH-RHDα	15.99 ± 3.52	28.25 ± 16.28	25.08 ± 6.52	25.96 ± 11.75	14.64 ± 3.94
Sum of oil-degrading genes	16.22 ± 3.75	28.61 ± 16.53	25.31 ± 6.67	26.31 ± 11.94	15.24 ± 4.21
RNA (target genes/16S rRNA)					
alkB	0.04 ± 0.01	0.11 ± 0.13	0.14 ± 0.15	0.03 ± 0.04	0.05 ± 0.06
GN PAH-RHDα	0.02 ± 0.01	0.04 ± 0.04	0.09 ± 0.09	0.03 ± 0.01	0.08 ± 0.10
GP PAH-RHDα	4.20 ± 1.52	15.02 ± 11.18	12.43 ± 10.48	21.91 ± 18.06	15.47 ± 14.93
Sum of oil-degrading genes	4.25 ± 1.55	15.17 ± 11.36	12.65 ± 10.72	21.97 ± 18.12	15.60 ± 15.09

The values are means and standard errors ($n = 5$)

indicator of microbial activity (Teepe et al., 2001), was significantly affected by oiling ($P < 0.05$). The soil respiration rate at the highest oil dosage (16 L m^{-2}) was about three times higher than the control (Table 2), which was in agreement with a previous study that found oil addition increased soil respiration in a tidal wetland (Qiao et al., 2012). The added oil provides a good amount of fresh organic carbon, stimulates the growth of heterotrophic microorganisms such as *Alkanexedens*, and thereby increases the strength of soil respiration of the rhizosphere (Wang et al., 2001; Li et al., 2007). The significant correlations between soil respiration rate and residual TPH and DOC (Table 3) in this study also support the above point. Meanwhile, the microbial metabolic activities reduced the level of oxygen and other electron acceptors in soil and thus decreased soil Eh with increased oil dosages (Table 2; Ellis & Adams, 1961; Llangovan & Vivekanandan, 1992; Nie et al., 2010).

To further understand the process of oil biodegradation in fresh and oligohaline marsh soil, oil-degrading genes and their expression were quantified by qPCR. The results indicated that the relatively higher bacterial population after oil contamination was mostly attributed to the increase in oil-degrading bacteria as they can use petroleum as carbonaceous substrates for their growth (Hamamura et al., 2006; Paissé et al., 2008). The activities of oil-degrading bacteria were also correlated with the aromaticity composition in the dissolved carbonaceous substrates, especially for the PAH-RHDα gene expression (Table 3). The distribution of oil-degrading bacteria changes with the composition of the hydrocarbons (Sotsk et al., 1994). In this study, the oil applied had been weathered approximately 40% by weight (Judy et al., 2014), so the main petroleum constitutes would be PAHs, and therefore the oil-degrading bacteria containing PAH-RHDα genes played a more important role.

The oil-degrading bacteria containing alkB gene did not appear to show a significant increase 2 months after oil addition; in fact, they showed a decreasing trend at higher oil dosages (Fig. 1). The relationship between oil dosage and the alkB gene did not reach a significant level, though (Table 3). These results were consistent with the findings in chronically polluted coastal sediments (Paisse et al., 2011) and contaminated and pristine alpine soils (Margesin et al., 2003). On the one hand, the potential for alkane degradation and the use of alkanes as substrates by microorganisms is a widespread trait, not only restricted to the case of oil exposure (Kloos et al., 2006). On the other hand, much of the alkanes in soils may have disappeared two months after oil application as alkanes exhaust more quickly than PAHs (Atlas et al., 1991) as well as because the weathered oil was applied in this study. The significantly increased percentages of PAH-RHDα genes with oil addition (Table 4) and its significantly positive relationships with residual TPH

concentration and the aromaticity index of $SUVA_{254}$ (Table 3) suggest that primary oil-degraders have become PAH-degrading bacteria 2 months after oil application. This was consistent with the report that the compositions of bacterial populations following hydrocarbon contamination were dynamic and new community structures would form which are better suited to oil contamination (Powell et al., 2006; Paissé et al., 2008; Kostka et al., 2011).

For the oil-degrading bacteria containing PAH-RHDα gene, the percentage of GP PAH-RHDα gene was significantly higher than the relative abundance of GN PAH-RHDα gene (Table 4) in this study, especially for the gene expression that reflects the level of living bacteria. This suggests that bacteria containing GP PAH-RHDα gene played a major role in degradation of aromatic constitutes in the oil-contaminated soils, which corresponded to the significant correlation between GP PAH-RHDα gene and the aromaticity index of $SUVA_{254}$ (Table 3). The relatively large populations of GP PAH oil-degraders in this study differed from previous reports in which GN bacteria were identified as the main PAH degrading bacteria in mangrove sediments of Thailand (Muangchinda et al., 2013) and Antarctic soils (Ma et al., 2006; Panicker et al., 2010). Nonetheless, other studies have suggested that GP bacteria may be more important in the environmental biodegradation of high-molecular-weight PAHs than GN bacteria (Ceébron et al., 2008; Jurelevicius et al., 2012). It is likely that GP oil degraders are more resilient and more capable of degrading high molecular weight oil components (Cébron et al., 2008). The peptidoglycan cellular envelope of GP bacterial cells makes them better fit for survival than their GN counterparts in certain volatile environments (Zhuang et al., 2003; Alonso-Gutiérrez et al., 2010); Also, the anatomy of GP bacteria may provide extra protection against toxic agents and thus increase the capacity to breakdown recalcitrant hydrocarbons (Alonso-Gutiérrez et al., 2010). The results of this study indicate that GP bacteria may be used as an effective tool for bioremediation in oil-polluted *P. australis* marsh soils.

Conclusions

This study evaluated the responses of soil characteristics and indigenous bacterial populations to various oil dosages to the marsh soil dominated by *P. australis*. Oil exposure generally increased oil-degrading bacterial populations, soil respiration rate, DOC concentration, and aromaticity in dissolved carbonaceous substrates, but decreased soil Eh. The expression of oil-degrading genes was also significantly increased by oiling. When compared with alkB degrading bacteria, PAH degraders seemed to play a more important role in oil biodegradation possibly due to the depletion of alkanes and persistence of recalcitrant PAHs 2 months after oil addition. The abundance and expression of GP PAH-RHDα gene accounted for approximately 98% of the total detected genes and it significantly correlated to $SUVA_{254}$, which together indicate that GP bacteria were the primary drivers in PAH degradation. Future research on temporal changes of both oil-degrading genes and corresponding hydrocarbons compounds would help further identify the dynamic mechanisms involved in biodegradation of petroleum in marsh soils at different stages of oiling.

Acknowledgements This research was made possible by grants from The Gulf of Mexico Research Initiative (GoMRI), BP/GoMRI through the Northern Gulf Institute, and National Natural Science Foundation of China (No. 41671105; 41603068). We thank Kristopher Nolan Ackoury for laboratory assistance.

References

Alonso-Gutiérrez, J., A. Figueras, J. Albaigés, et al., 2010. Bacterial communities from shoreline environments (Costa daMorte, Northwestern Spain) affected by the prestige oil spill. Applied and Environmental Microbiology 75: 3407–3418.

Atlas, R. M., A. Horowitz, M. Krichevsky & K. Asim, 1991. Response of microbial populations to environmental disturbance. Microbial Ecology 22: 249–256.

Baldwin, B., C. H. Nakatsu & L. Nies, 2003. Detection and enumeration of aromatic oxygenase genes by multiplex and real-time PCR. Applied Environmental Microbiology 69: 3350–3358.

Cébron, A., M. P. Norini, T. Beguiristain & C. Leyval, 2008. Real-time PCR quantification of PAH-ring hydroxylating dioxygenase (PAH-RHDα) genes from Gram positive and Gram negative bacteria in soil and sediment samples. Journal of Microbiological Methods 73: 148–159.

Cébron, A., T. Beguiristain & P. Faure, 2009. Influence of vegetation on the in site bacterial community and polycyclic aromatic hydrocarbon (PAH) degraders in aged PAH-contaminated or thermal-desorption-treated soil. Applied and Environmental Microbiology 75: 6233–6330.

Dong, W. X., C. S. Hu, S. Y. Chen & Y. M. Zhang, 2009. Tillage and residue management effects on soil carbon and CO_2 emission in a wheat-corn double-cropping system. Nutrient Cycling Agroecosystems 83: 27–37.

Ellis, R. & R. J. Adams Jr., 1961. Contamination of soils by petroleum hydrodcarbons. Advanced Agronomy 13: 197–213.

Jackson, R. B., C. W. Cook, J. S. Pippen & S. M. Palmer, 2009. Increased belowground biomass and soil CO_2 fluxes after a decade of carbon dioxide enrichment in a warm-temperate forest. Ecology 90: 3352–3366.

Jurelevicius, D., V. M. Vlvarez, R. Peixoto, A. S. Rosado & L. Seldin, 2012. Bacterial polycyclic aromatic hydrocarbon ring-hydroxylating dioxygenases (PAH-RHD) encoding genes in different soils from King George Bay, Antarctic Peninsula. Applied Soil Ecology 55: 1–9.

Habe, H. & T. Omori, 2003. Genetics of polycyclic aromatic hydrocarbon metabolism in diverse aerobic bacteria. Bioscience Biotechnology and Biochemistry 67: 225–243.

Hara, A., S. Baik, K. Syutsubo, et al., 2004. Cloning and functional analysis for alkB genes in Alcanivorax borkumensis SK2. Environmental Microbiology 6: 191–197.

Hamamura, N., S. H. Olson, D. M. Ward, et al., 2006. Microbial population dynamics associated with crude-oil biodegradation in diverse soils. Applied and Environmental Microbiology 72: 6316–6324.

Hazen, T. C., E. A. Dubinsky, T. Z. DeSantis, et al., 2010. Deep-sea oil plume enriches indigenous oil-degrading bacteria. Science 330: 201. https://doi.org/10.1126/science.1195979.

Judy, C. R., S. A. Graham, Q. X. Lin, A. X. Hou & I. A. Mendelssohn, 2014. Impacts of Macondo oil from *Deepwater horizon* spill on the growth response of the common reed *Phragmitesaustralis*: a mesocosm study. Marine Pollution Bulletin 79: 69–76.

Kloos, K., J. C. Munch & M. Schloter, 2006. A new method for the detection of alkane-monooxygenase homologous genes (alkB) in soils based on PCR-hybridization. Journal of Microbiological Methods 66: 486–496.

Kostka, J. E., O. Prakash, W. A. Overholt, et al., 2011. Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico Beach Sands impacted by the deepwater horizon oil spill. Applied and Environmental Microbiology 77: 7962–7974.

Lin, Q. & I. A. Mendelssohn, 1996. A comparative investigation of the effects of Louisiana crude oil on the vegetation of fresh, brackish, and salt marsh. Marine Pollution Bulletin 32: 202–209.

Li, X. L., G. B. Liu, S. Xue & M. X. Xue, 2007. Effects of crude oil on growth of plant seedling and soil respiration in Loess Hilly region of North Shaanxi. Journal of Soil and Water Conservation 21(3): 95–98. **(in Chinese)**.

Llangovan, K. & M. Vivekanandan, 1992. Effect of oil pollution on soil respiration and growth of Vigna mungo (L.) Hepper. Science of the Total Environment 116: 187–194.

Louvel, B., A. Cébron & C. Leyval, 2011. Root excudates affect phenanthrene biodegradation, bacterial community and functional gene expression in sand microcosms. International Biodeterioration and Biodegradation 65: 947–953.

Lu, X. Y., T. Zhang, et al., 2011. Bacteria-mediated PAH degradation in soil and sediment. Applied Microbiology and Biotechnology 89: 1357–1371.

Ma, Y. F., L. Wang & Z. Z. Shao, 2006. Pseudomonas, the dominant polycyclic aromatic hydrocarbon-degrading bacteria isolated from Antarctic soils and the role of large plasmids in horizontal gene transfer. Environmental Microbiology 8: 455–465.

Margesin, R., D. Labbé, F. Schinner, et al., 2003. Characterization of hydrocarbon-degrading microbial populations in contaminated and pristine alpine soils. Applied and Environmental Microbilogy 69: 3085–3092.

Muangchinda, C., R. Pansri, W. Wongwongsee & O. Pinyakong, 2013. Assessment of polycyclic aromatic hydrocarbon biodegradation potential in mangrove sediment from Don Hoi Lot, Samut Songkram province, Thailand. Journal of Applied Microbiology 114: 1311–1324.

National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling. Deep Water: The Gulf Oil Disaster and the Future of Offshore Drilling, Report to the President, January 2011. Government Printing office: 392 pp. (http://www.oilspillcommission.gov).

Nie, M., X. D. Zhang & J. Q. Wang, 2009. Rhizosphere effects on soil bacterial abundance and diversity in the Yellow River Deltaic ecosystem as influenced by pertroleum contamination and soil salinization. Soil Biology and Biochemistry 41: 2535–2542.

Nie, M., Q. Yang, L. F. Jiang, C. M. Fang, J. K. Chen & B. Li, 2010. Do plants modulate biomass allocation in response to petroleum pollution? Biology Letters 6: 811–814.

Paissé, S., F. Coulon, et al., 2008. Structure of bacterial communities along a hydrocarbon contamination gradient in a coastal sediment. FEMS Microbial Ecology 66: 295–305.

Paisse, S., R. Duran & F. Coulon, 2011. Are alkane hydroxylase genes (alkB) relevant to assess petroleum bioremediation processes in charonically polluted coastal sdiments? Applied Microbiology and Biotechnology 92: 835–844.

Panicker, G., N. Mojib, J. Aislabie & A. K. Bej, 2010. Detection, expression and quantitation of the biodegradative genes in Antarctic microorganisms using PCR. Antonievan Leeuwenhoek 97: 275–287.

Peichl, M., T. Moore, M. Arain, et al., 2007. Concentrations and fluxes of dissolved organic carbon in an age-sequence of white pine forests in Southern Ontario, Canada. Biogeochemistry 86: 1–17.

Platts Oilgram News/OPR Extra. June 15, 2010. http://www.platts.com/IM.Platts.Content.InsightAnalysis/NewsFeature/2010/oilspill/20100615.Pdf.

Powell, S. M., S. H. Ferguson, J. P. Bowman & I. Snape, 2006. Using real-time pcr to assess changes in the hydrocarbon-degrading microbial community in Antarctic soil during bioremediation. Microbial Ecology 52: 523–532.

Qiao, J. L., L. Wang, Y. S. Tang, Y. H. Hu & J. W. Jia, 2012. Distribution and characteristics of total petroleum hydrocarbons in Jiuduansha tidal wetland and their potential impact on soil microbial respiration. Journal of Environmental Science and Health, Part A 47(2): 319–325.

Redmond, M. C. & D. L. Valentine, 2012. Natural gas and temperature structured a microbial community response to the deepwater horizon oil spill. PNAS 109: 20292–20297.

Romero, I. S., P. T. Schwing, G. R. Brooks, et al., 2015. Hydrocarbons in deep-sea sediments following the 2010 Deepwate Horizon Blowout in the Northeast Gulf of Mexico. PLoS ONE 10(5): e0128371. https://doi.org/10.1371/journal.pone.0128371.

Sotsk, Y. J. B., C. W. Greer & R. M. Atlas, 1994. Frequency of genes in aromatic and aliphatic hydrocarbon biodegradation pathways within bacterial populations from Alaskan sediments. Canadian Journal of Microbiology 40: 981–985.

Teepe, R., R. Brumme & F. Beese, 2001. Nitrous oxide emissions from soil during freezing and thawing periods. Soil Biology and Biochemistry 33: 1269–1275.

Van Beilen, J. B., M. G. Wubbolts & B. Witholt, 1994. Genetics of alkane oxidation by *Pseudomonas olevorans*. Biodegradation 5: 161–174.

Van Beilen, J. B., S. Panke, S. Lucchini, et al., 2001. Analysis of *Pseudomonas putida* alkane degradation gene clusters and flanking insertion sequences: evolution and regulation of the alk genes. Microbiology 147: 1621–1630.

Van Beilen, J. B., F. Mourlane, M. A. Seeger, et al., 2003. Cloning of Baeyer-Villigermonooxygenases from Comamonas, Xanthobacter and Rhodococcus using polymerase chain reaction with highly degenerate primers. Environmental Microbiology 5: 174–182.

Van Beilen, J. B., E. G. Funhoff, A. van Loon, et al., 2006. Cytochrome P450 alkane hydroxylases of the CYP153-family are common in alkane-degrading eubacteria lacking integral membrane alkane hydroxylases. Applied and Environmental Microbiology 72: 59–65.

Wang, Q. R., X. M. Liu, Y. S. Cui & Y. T. Dong, 2001. Concept and advances of applied bioremediation for organic pollutants in soil and water. Acta Ecologica Sinica 21(1): 159–163.

Wasmund, K., K. A. Burns, I. Kurtböke & D. G. Bourne, 2009. Novel alkane hydroxylase gene (alkB) diversity in sediments associated with hydrocarbon seeps in the Timor sea, Australia. Applied and Environmental Microbiology 75: 7391–7398.

Weishaar, J., G. Aiken, B. Bergamaschi, M. Fram, R. Fujii & K. Moppers, 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environmental Science and Technology 37: 4702–4708.

Whyte, L. G., T. H. M. Smits, D. Labbe, et al., 2002. Gene cloning and characterization of multiple alkane hydroxylase systems in Rhodococcus strains Q15 and NRRL B-16531. Applied and Environmental Microbiology 68: 5933–5942.

Yergeau, E., M. Arbour & R. Brousseau, 2009. Microarray and real-time PCR analyses of the response of high-arctic soil bacteria to hydrocarbon pollution and bioremediation treatments. Applied and Environmental Microbiology 75: 6258–6267.

Zhuang, W. Q., J. H. Tay, A. M. Maszenan, L. R. Krumholz & S. T. Tay, 2003. Importance of Gram-positive naphthalene-degrading bacteria in oil contaminated tropical marine sediments. Letters in Applied Microbiology 36: 251–257.