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Response of microbial populations regulating nutrient biogeochemical cycles to oiling of coastal saltmarshes from the Deepwater Horizon oil spill☆

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ABSTRACT

Microbial communities play vital roles in the biogeochemistry of nutrients in coastal saltmarshes, ultimately controlling water quality, nutrient cycling, and detoxification. We determined the structure of microbial populations inhabiting coastal saltmarsh sediments from northern Barataria Bay, Louisiana, USA to gain insight into impacts on the biogeochemical cycles affected by Macondo oil from the 2010 Deepwater Horizon well blowout two years after the accident. Quantitative PCR directed toward specific functional genes revealed that oiled marshes were greatly diminished in the population sizes of diazotrophs, denitrifiers, nitrate-reducers to ammonia, methanogens, sulfate-reducers and anaerobic aromatic degraders, and harbored elevated numbers of alkane-degraders. Illumina 16S rRNA gene sequencing indicated that oiling greatly changed the structure of the microbial communities, including significant decreases in diversity. Oil-driven changes were also demonstrated in the structure of two functional populations, denitrifying and sulfate reducing prokaryotes, using *nirS* and *dsrB* as biomarkers, respectively. Collectively, the results from 16S rRNA and functional genes indicated that oiling not only markedly altered the microbial community structures, but also the sizes and structures of populations involved in (or regulating) a number of important nutrient biogeochemical cycles in the saltmarshes. Alterations such as these are associated with potential deterioration of ecological services, and further studies are necessary to assess the trajectory of recovery of microbial-mediated ecosystem functions over time in oiled saltmarsh sediment.

1. Introduction

In April 2010, the Macondo 252 wellhead blowout on the seafloor below the Deepwater Horizon (DWH) oil-drilling rig discharged over a million barrels of crude oil into the northern Gulf of Mexico (nGoM) (Crone and Tolstoy, 2010). The Macondo oil rose 1–1.5 km up to the water's surface in forms of buoyant droplets that included dispersant, and formed a thin oil slick on the water's surface (Camilli et al., 2010). The oil eventually landed on shorelines, contaminating ~1773 km of nGoM shorelines, of which approximately 44.9% were saltmarshes (Michel et al., 2013). Oil in the coastal marshes was highly weathered, such that lower molecular weight hydrocarbons were lost through dispersion, dissolution, evaporation, and biological degradation (Liu et al., 2012; Turner et al., 2014). The impacts of oiling on coastal marshes are of great concern because of the potential degradation of ecological services, such as provision of habitats for wild life, regulation of water and air quality, and carbon sequestration (UK National Ecosystem Assessment [http://eprints.lancs.ac.uk/id/eprint/49673]).

Intensive efforts have been made to assess the impacts of oil on marsh ecosystems, specifically on vegetation that provides the foundation of wetland ecology and ecological services. Damage and recovery of vegetation is largely dependent on the toxicity of the oil, the amounts of the oil in contact with the sediments, the plant

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species, and the frequency of oiling (Pezeshki et al., 2000; Silliman et al., 2012). Surprisingly, much less attention has been paid to the impacts of oil on biogeochemical cycles, even though biogeochemical processes are critical for regulating water quality, nutrient cycling and detoxifying chemicals in wetlands (Engle, 2011). Regarding biogeochemical cycles, laboratory incubation studies have shown that crude or weathered oil exhibits potential inhibitory effects on ammonia oxidation, denitrification and N_2-fixation (Urakawa et al., 2012; Horel et al., 2014; Pietroski et al., 2015; Levine et al., 2017b). Natter et al. (2012) reported that oiling marsh sediments enhanced sulfate reduction and increased the numbers of sulfate-reducing prokaryotes. Nevertheless, much remains unknown regarding the deterioration and recovery of biogeochemical cycles in wetlands with long-term exposure to crude oil.

Microbes are powerful determinants of oil-weathering in contaminated environments. Many studies have focused on microbial communities in oiled environments to evaluate the weathering (decomposition) rate of oil. Great progress has been made in assessing the distribution of oil-degrading bacteria and in monitoring the succession in microbial community structures with time during oil weathering in deep-sea oil plumes, sea surface waters and sediments, and shorelines including saltmarshes (Joye et al., 2014; Kimes et al., 2014; Rodriguez-R et al., 2015; Looper et al., 2013; Mahmoudi et al., 2013; Atlas et al., 2015).

Microbial community structures are known to be related to geochemical cycles impacted by oiling in saltmarshes. A few studies have investigated the response of functional populations to oiling, most of which are focused on a few processes in the nitrogen cycle (N-cycle). The structure of ammonia oxidizers (i.e., nitrifiers) responding to oiling has been characterized in detail from oiled and nonoiled marshes (Newell et al., 2014; Marton et al., 2015; Bernhard et al., 2016). More recently, Hinshaw et al. (2017) determined the abundance of denitrifiers and their activities for assessing the ecological roles of saltmarshes in a DWH oiled nGoM shoreline. However, other populations involved in the N-cycle, such as anaerobic ammonium oxidation (ANAMMOX) (Penton et al., 2006) and dissimilatory nitrate reduction to ammonium (DNRA) (An and Gardner, 2002) are largely unstudied even though those processes exceed denitrification rates in some environments, such as marine and coastal ecosystems (Jetten et al., 2009; Giblin et al., 2013). The N-cycle is composed of an unusually diverse set of redox reactions, and these reactions are tightly coupled one another (Falkowski et al., 2008; Thamdrup, 2012). Therefore, it would be desirable to examine N-cycling populations from a comprehensive viewpoint.

With time, the DWH oil is likely to act as a selective pressure on marsh microbial communities, successively altering their activities and structures, as was shown in earlier studies. Such alterations likely include changes in populations functioning as regulators of biogeochemical cycles that are linked to ecological services of saltmarshes. We employed several culture-independent approaches; i.e., Illumina high throughput sequencing of 16S rRNA genes, cloning and sequencing of specific functional genes, and quantitative PCR (qPCR) of functional genes. The Illumina sequencing produces a large number of 16S rRNA gene reads that are necessary for the determination of phylogenetic structure of microbiota, but with a limitation in inferring functional traits. This limitation is compensated by using a gene marker that detect and quantify a specific target gene through PCR from marsh samples. We used 10 gene markers for N-cycling populations (i.e., nitrifiers [two genes], denitrifers [two genes], DNRA bacteria, and N_2 fixers), oil-degrading populations (aliphatic and aromatic degraders), methanogens, and sulfate reducers. To our best knowledge, except for nitrifiers (Bernhard et al., 2016; Marton et al., 2015), this is the first study to be conducted for determining the structure of these populations in the oiled marshes of saltmarshes of nGoM shorelines. The results from these approaches provide fundamental knowledge necessary for better understanding how the key populations regulating biogeochemical cycles changed in response to oiling of saltmarshes.

2. Materials and methods

2.1. Marsh sediment collection and processes

On June 19th, 2012, a total of eight cores (5 cm in diameter and 20 cm in length) were collected from salt marsh sediments: two cores from a heavily oiled site (H.O: N29°26.668'; W89°53.986′) in Bay Jimmy, and two cores from a lightly oiled site (L.O: N29°26.291'; W089°54.637′) in the upper Barataria Bay (Fig. S1). Two pairs of cores were taken from a non-oiled site (N.O: N29°27.977'; W089°56.051′) in Wilkinson Bay (Fig. S1), which were referred to N.O-I and N.O-II. The N.O soil collection site were one of the few areas deemed non-oiled from the BP DWH oil spill by the NOAA office of Response and Restoration (ERMA) mapping efforts (NOAA, 2013). Nitrogen (N), phosphorus (P), and carbon © contents for H.O and N.O sites are reported in Levine et al. (2017b).

Following sample collection, the sediment cores were kept on ice until transported to the lab in Baton Rouge, LA, where the intact cores were frozen at −20 °C in the Wetland Biogeochemistry Lab, Louisiana State University. Frozen cores were transported to the Soil Microbial Ecology Lab at University of Florida, Gainesville, FL on dry ice. Upon arrival in Gainesville, the cores were immediately sectioned into 2 cm intervals for DNA isolation and oil analysis. The oil composition and gene copies were determined in three of sections from depth 0–2 cm, 4–6 cm, and 8–10 cm.

2.2. Oil analysis

Total petroleum hydrocarbons (TPH) in the marsh soil were extracted using soxhlet extraction procedure according to EPA method 3540C (USA EPA Method, 1996). Briefly, 20 g of fresh marsh soil were weighed into cellulose thimbles and added sufficient amounts of Na_2SO_4 to remove water. Then, sample thimbles were placed in a soxhlet extraction chamber and extracted overnight using 200 mL of dichloromethane (DCM) solvent. A known amount of surrogate standard was added to the sample before starting the soxhlet extraction. After the extraction, the excess solvent was initially removed by rotary evaporator followed by further condensation using hot water bath and stream of N_2 gas. The condensed extractants were passed through Na_2SO_4 salt to remove any traces of water. The concentrated samples were spiked with internal standard followed by GC analysis for TPH.

TPH analysis was carried out using Shimadzu 2100 (Shimadzu, USA) gas chromatography equipped with flame ionization detector. An Rxi-5Sil MS (Restek, PA) capillary column that was 30 m long with 0.25 mm id and has 0.1 μm thick stationary phase with helium as a carrier gas was used for separation of analytical compounds. The GC column temperature program used was 50 °C for 2 min followed by ramping at 6 °C min^{-1} to a maximum temperature of 300 °C and held for 8 min. A flame ionization detector kept at 325 °C was used for analyte detection. The GC was calibrated with crude oil standards prepared in DCM solvent while 44-compound oil analysis standard (Absolute Standards Inc., USA) was used to identify the retention window of various C range groups and response factors. TPH in the soil was estimated using the formula Cs= (Cc x Vt x D)/Ws as given by TNRCC-1005 method (TNRCC, 2001), where Cs: concentration of TPH in sample in mg kg-1, Cc: concentration of TPH in extract in mg L^{-1}, Vt: volume of final extract in L, D: dilution or concentration factor, and Ws: weight of