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Long-term effect of crude oil and dispersant on denitrification and organic matter mineralization in a salt marsh sediment

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HIGHLIGHTS

- Inhibition effect of dispersant on denitrification could recover over time.
- Dispersant stimulated organic matter mineralization, decreasing redox conditions.
- Mineralization of organic matter mobilized the preserved N in the sediment.
- Oil/dispersant should be prevented from moving into deeper layers of the sediment.

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ABSTRACT

The 2010 BP oil spill has an unprecedented impact on coastal wetland ecosystem along the northern Gulf of Mexico. A two-dimensional analysis (dispersant concentration and duration of exposure) was conducted by pre-incubation of a salt marsh sediment under an open or closed condition. Denitrification activity was characterized by N_2O production using an acetylene (C_2H_2) blockage technique, and organic matter (OM) mineralization by CO_2 production. The results show that even trace amount of the dispersant could significantly inhibit the denitrification activity by 20% ($p < 0.05$). However, the sediment was resilient to the oil/dispersant contamination, likely due to shift of its microbial communities, by recovering the denitrification activity within 46 days in the open incubation. Inhibitory effect of the oil/dispersant on denitrification persisted beyond 46 days in the closed incubation, and the recovery could take up to 137 days depending on the dispersant concentration. The dispersant continuously stimulated OM mineralization that lowered the sediment redox status. Mobilization of N in the sediment from the OM mineralization forms a positive feedback loop, leading to deterioration of the coastal ecosystem. The study concludes that minimum dispersant should be applied for oil spill remediation, and oil cleanup operations should avoid moving the oil/dispersant from surface into deeper layers of the sediment. Synergistic interactions between the crude oil and dispersant and their biodegradation products deserves future examinations.

1. Introduction

Despite only 6% of the global land surface, wetlands play a significant role in providing fundamental ecological services, and producing economically valuable products for human settlement (Reddy and DeLaune, 2008; Valiela et al., 2001; Zak et al., 2011). Louisiana alone accounts for 40% of the total wetlands in mainland US (Richardson and Pahl, 2006), and generates 30% of the nation's seafood production (Day et al., 2005). High vegetation productivity and high organic matter (OM) content make this ecosystem more important to the global carbon (C) budget than its area alone suggests. Hydrological conditions in wetlands favor C sequestration to mitigate global climate change (Yu and Patrick, 2004). Meanwhile, excess nitrogen (N) from various inland sources, frequently causing eutrophication in adjacent coastal regions (Turner and Rabalais, 1994; Yu et al., 2006), can be effectively removed by denitrification activities in wetlands.

In addition to natural events, human activities pose a serious threat to the integrity and sustainability of coastal wetland. The 2010 BP (British Petroleum) oil spill, also commonly called Deepwater Horizon oil spill (DHOS), in the Gulf of Mexico (GoM) was unprecedented with approximately 4.9 million barrels of

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petroleum hydrocarbons released into the ocean (BP, 2010; NOAA, 2010). This is equivalent to spilling 0.5 mL crude oil on every m^2 of the GoM (1.6 million km^2) that is larger than all five Gulf States combined (1.3 million km^2). Initial survey shows that 1773 km of GoM shoreline was significantly oiled, of which 45% was coastal marsh (Michel et al., 2013). Recent field monitoring indicates that most of the relative non-toxic alkanes are on their trajectories to reach background levels in 2015, but the most toxic polycyclic aromatic hydrocarbons (PAHs) show little sign of degradation (Turner et al., 2014). To respond to the 2010 DHOS, application of a large quantities (7 million L) of dispersants (NOAA, 2010) was a controversial decision because little is known about their fate and impact in the environment. There are evidence showing that dispersants could emulsify crude oil with sea water into smaller droplets (Schrope, 2013). This raises a query about the potential effects of the oil/dispersant mixture on coastal wetlands.

Hydrological fluctuations in wetland ecosystem make the wetland sediments experience wide range of oxidation-reduction (redox) conditions that can be determined by redox potential (Eh). In wetland sediments, a series of redox reactions can occur with OM (including petroleum hydrocarbons) as electron donors and various electron acceptors. In their thermodynamic order, the redox reactions proceed with reduction of oxygen, nitrate, manganese (IV), iron (III) and sulfate (Patrick and Jugsujinda, 1992). Carbon is a major driver for all biogeochemical activities in wetland habitats, since it serves as a primary energy source for microbial biogeochemical processes. Denitrification is favorable at Eh of +300 to +200 mV and near neutral conditions (Reddy and DeLaune, 2008), which is the dominant mechanism for N removal in wetlands. Mineralization of OM and denitrification are coupled processes taking place simultaneously. Major end product for OM mineralization is carbon dioxide (CO_2). In denitrification, nitrate is reduced by facultative bacteria to nitrogen gas (N_2), with nitrite (NO_2^-) and nitrous oxide (N_2O) as intermediate products (Groffman et al., 2006; Reddy and DeLaune, 2008).

Effects of oil, dispersant and combination of oil/dispersant on denitrification and OM mineralization processes were studied (Shi and Yu, 2014). The results showed little effect from the crude oil, but the dispersant (COREXIT® EC9500A) immediately inhibited denitrification and stimulated OM mineralization activities. In this follow-up study, both short-term (one and a half month) and long-term (four and a half month) effects of oil/dispersant exposure on denitrification and OM mineralization activities in a salt marsh sediment were investigated, with focus on concentrations of the dispersant. Two major hypotheses were tested: (1) the marsh sediment may recover overtime from the impact of oil/dispersant on denitrification and OM mineralization activities, and (2) the recovery may vary depending on concentrations of the dispersant.

2. Materials and methods

2.1. Sediment sample

A composite salt marsh sediment sample (top 30 cm, 5 locations) was taken in May 2014 near Lake Pontchartrain, Louisiana, USA (N30°08.78', W89°44.67'). The sediment sample was shipped to the laboratory in an ice cooler and was stored in a refrigerator (4 °C) less than a month before the experiment. The sediment from the same location has been used since the BP oil spill for several studies, including Tao et al. (2018), Shi and Yu (2014), Gupta et al. (2014), Tao and Yu (2013), and Yu et al. (2012). Salinity of the sediment determined using pore water was around 12‰. According to USDA taxonomy, the sediment was classified as silty clay loam with 57% silt, 32% clay, and 11% sand, respectively. The sediment had 7.2% total C, 0.43% total N, and 0.61 g kg^{-1} phosphorus. The sediment was rich in iron (19.91 g kg^{-1}) and sulfur (5.30 g kg^{-1}), but low manganese (0.12 g kg^{-1}). More physical and chemical characteristics of the sediment can be found in previous publications (Tao and Yu, 2013; Shi and Yu, 2014). By drying the sediment to a constant weight (12 h) at 105 °C, water content of the sediment was determined as 57% before this study.

2.2. Crude oil and dispersant samples

A crude oil sample was provided by the BP America Production Company with official agreement for this study. It is known as Louisiana sweet crude oil produced by the Macondo 252 well. It has a dark brown color and is insoluble in water with a density varying between 0.74 and 1.03 kg L^{-1}. Major ingredients in the oil are butane, pentane, n-hexane, BTEX (benzene, toluene, ethyl-benzene, and xylene), naphthalene, various PAHs and hydrogen sulfide (British Petroleum America Production Company, 2010). According to Jackson et al. (1996), sweet Louisiana crude oil is relatively nontoxic. For this experiment, the relatively more toxic components (generally with smaller molecular weight) were removed (Shi and Yu, 2014), and the residual oil, commonly named as "topped" oil (with larger molecular weight), was used as the crude oil sample.

Several products were used as oil dispersants in the 2010 DHOS. The most common one was COREXIT® EC9500A produced by the Nalco Environmental Solutions (NES) LLC. (Sugar Land, Texas). The dispersant is a clear hazy amber color liquid with pH of 6.2 and density of 0.95 kg L^{-1} at 15.6 °C (NES LLC, 2012). Major ingredients of the dispersant include distillates, petroleum, hydrotreated light (10–30%), organic sulfonic acid salt (10–30%), and propylene glycol (1–5%) (NES LLC, 2012). Therefore, some components accounting for more than 35% of the dispersant are unknown.

2.3. Experimental design

Redox conditions vary in the salt marsh environment due to hydrological fluctuations at the site, and at different depths of the sediment, which play a significant role in C and N cycles. Oil spills and associated application of dispersants serve as additional stressors to the already vulnerable coastal ecosystem. In this study, a series of incubation experiments were designed to imitate how denitrification and OM mineralization processes in the coastal sediment were influenced by oil/dispersant exposure under different redox conditions. The sediment was pre-incubated for different time with and without oil/dispersant to study the effect of oil/dispersant exposure. Different redox conditions were created by leaving the pre-incubation bottles open (aerobic conditions) or closed (anaerobic conditions). Three batches of experiment were conducted during five months. In the first batch, the original sediment without pre-incubation was used to study the immediate effects of oil/dispersant exposure on denitrification and mineralization of OM. Then the sediment was pre-incubated for 46 days (batch 2) and 137 days (batch 3), respectively, under either open bottle or closed bottle conditions to study the short-term (batch 2) and long-term (batch 3) effect of oil/dispersant exposure on denitrification and mineralization of OM under various redox conditions. For this study, denitrification rate was determined based on N_2O production using acetylene (C_2H_2) block method (Knowles, 1990; Groffman et al., 2006), while the mineralization of OM was represented by CO_2 production (Shi and Yu, 2014).

2.4. Experimental setup

In each batch of the experiment, four treatments with three replicates were established, including control (CK) with no addition

of oil/dispersant, oil:dispersant = 1000:1 (O:D = 1000:1), oil:dispersant = 100:1 (O:D = 100:1), and oil:dispersant = 10:1 (O:D = 10:1). Therefore, the effects of different concentrations of the dispersant in the oil/dispersant mixtures on denitrification and OM mineralization activities can be referenced with the sediment with no oil/dispersant exposure under similar to field conditions (batch 1), and under short-term (batch 2) and long-term (batch 3) exposure with different redox status. The previous study (Shi and Yu, 2014) concluded that the crude oil had little effect on denitrification and OM mineralization activities in the studied sediment, while the dispersant showed a significant impact. Therefore, treatments with individual crude oil or dispersant were not included in this study, with attention on the effect of different concentrations of the dispersant in the oil/dispersant mixtures.

The experimental unit was a 140-mL wide-mouth glass bottle. For all treatments, 30 g of the wet sediment was well mixed and placed into each glass bottle. Following that, 50 mL of artificial ocean water (12‰) was added to create a sediment/water slurry with the same salinity as the original sediment (Shi and Yu, 2014). Artificial ocean water was prepared by dissolving nitrate free sea salt (Instant Ocean, Spectrum Brands Inc.) with deionized (DI) water. In total, 60 bottles of the sediment slurries were prepared for three batches (12 for batch 1, 24 for batch 2 and another 24 for batch 3). The control treatments were not provided with oil/dispersant, while the three levels of oil/dispersant treatments were added 1 mL mixture with the corresponding ratios of oil and dispersant. All sediment slurry treatments were prepared at room conditions of 23 °C. Batch 1 was used immediately for denitrification and mineralization of OM measurement. For batch 2 and 3, two sets of bottles (12 bottles for each set) were treated differently during the pre-incubation. One set of 12 bottles were kept open, while the other set of 12 bottles were tightly closed with caps lined with Teflon tape to avoid gas exchange. Both open/closed conditions of the bottles and length of the pre-incubation resulted in changes of the sediment redox status. The bottles were hand-shaken once a week during the pre-incubation period that was in the dark and at room temperature (23 °C) before conducting denitrification and OM mineralization measurement.

Redox potentials and pH values in all the sediment slurries were measured just before the denitrification and OM mineralization measurement. Then, the sediment slurries were prepared by air-tight sealing the bottle cap, and flushing the bottle with pressurized ultra-pure N_2 for 3 min to replace all gases in the headspace with N_2 and create an anaerobic environment. Then, 10 mL of the headspace volume was replaced with pure C_2H_2 (making up 15% of the headspace gas volume). For denitrification measurement, 1 mL KNO_3 solution (1000 mg N L^{-1}) was added to each bottle, serving as an additional substrate (Tao and Yu, 2013). No additional OM was added to the bottles. Gas samples (5 mL each time) from the bottle headspace were taken daily with a syringe for 4 days. Denitrification and OM mineralization activities were calculated by linear regression of the increase of N_2O and CO_2 concentration over time, respectively. After completing the gas analyses, all bottles were placed in a rotary shaker for 3 h after adding 2 mL 3.5 M KCl for extraction of nitrate, nitrite and ammonium in the sediment slurries. Then the extracted sample solutions were filtered (0.45 μm) and kept frozen at −20 °C before analysis.

2.5. Sample analysis

Gas concentrations were analyzed immediately after sampling using a Shimadzu GC-2014 gas chromatography with an electron capture detector (ECD) for N_2O and a flame ionization detector (FID) for CH_4, C_2H_2, and CO_2. Pure helium was used as a carrier gas with pressure of 116.4 kPa and a total flow rate of 25.0 mL min^{-1}. Temperature of the injector, oven, FID detector, and ECD detector was set up at 380 °C, 80 °C, 250 °C, and 325 °C, respectively. Nitrate and nitrite concentrations were determined by using the method 1686 of U.S. Environmental Protection Agency, while ammonium concentrations were determined based on a salicylate-hypochlorite method from Bower and Holm-Hanson (1980). A pH/mV meter (Fisher Scientific Accumet AP62) was used with a combination pH electrode (Fisher Scientific) to measure pH. Redox potential was determined by using a home-made Platinum (Pt) redox potential electrode (Faulkner et al., 1989) with a calomel reference electrode. All pH and redox values were taken after the instrumental readings became stable for 1 min.

2.6. Calculation and statistical analysis

Production rates of N_2O and CO_2 were calculated by using linear regression of their concentrations with time (Yu et al., 2006), and the amount of gases dissolved in the liquid phase of the sediment slurries were considered by taking mole fraction solubility of CO_2 (6.5×10^{-4}) and N_2O (4.7×10^{-4}) in water at 23 °C (Lide, 1991). The effects of salinity on N_2O and CO_2 dissolutions in water were not considered. The effect of pH on CO_2 dissolution and potential conversion to bicarbonate and carbonate was not considered, since all pH values were found in acidic range (Table 1). All calculations were based on dry weight of the sediment. The Eh values were converted to the standard H_2 electrode by using a correction factor for the calomel reference electrode to the observed readings. The correction factor at room temperature (23 °C) was +245 mV (Tao and Yu, 2013). Effects of pH on Eh values were evaluated based on the Nernst

Table 1
Status of pH and Eh in the sediment slurries before the denitrification and OM mineralization measurement.

Treatment	Initial	Open pre-incubation		Closed pre-incubation	
		46 days	137 days	46 days	137 days
pH (n = 3)					
CK	5.6 ± 0.1 [a]	4.1 ± 0.0 [a]	3.8 ± 0.0 [a]	4.5 ± 0.1 [a]	4.3 ± 0.2 [a]
O:D = 1000:1	5.6 ± 0.0 [a]	4.7 ± 0.2 [b]	4.9 ± 0.2 [b]	5.2 ± 0.0 [d]	6.3 ± 0.0 [c]
O:D = 100:1	5.6 ± 0.1 [a]	5.2 ± 0.4 [c]	5.6 ± 0.2 [c]	5.6 ± 0.2 [c]	6.3 ± 0.1 [c]
O:D = 10:1	5.6 ± 0.0 [a]	4.7 ± 0.1 [b]	4.8 ± 0.1 [b]	5.8 ± 0.1 [b]	5.8 ± 0.2 [b]
Mean	5.6	4.7	4.8	5.3	5.7
Eh (mV) at pH 7.0 (n = 6)					
CK	+339 ± 12 [a]	+238 ± 7 [a]	+271 ± 20 [a]	+234 ± 6 [a]	+139 ± 39 [a]
O:D = 1000:1	+250 ± 8 [b]	+174 ± 5 [b]	+95 ± 17 [c]	+113 ± 14 [c]	−120 ± 12 [b]
O:D = 100:1	+277 ± 16 [b]	+114 ± 24 [c]	−199 ± 7 [b]	−66 ± 4 [b]	−110 ± 8 [b]
O:D = 10:1	+324 ± 6 [a]	+201 ± 6 [b]	−210 ± 6 [b]	−60 ± 7 [b]	−128 ± 24 [b]
Mean	+298	+182	−11	+55	−55

CK represents control with no addition of oil/dispersant; O:D = 1000:1, O:D = 100:1, and O:D = 10:1 represent addition of 1 mL mixture with oil:dispersant = 1000:1, oil:dispersant = 100:1, and oil:dispersant = 10:1, respectively. Different letters represent significant difference ($p < 0.05$).

equation, in which if pH decreases by one unit, the Eh value increases by 59 mV (Reddy and DeLaune, 2008). Independent sample *t*-test was used to determine the difference between the experimental treatments with a significance level chosen at 0.05 ($\alpha = 0.05$).

3. Results

The original sediment was close to neutral (pH = 7.3) and moderately reduced (Eh = +173 mV). Sediment handlings for setting up the experiment introduced more O_2 into the system, causing Eh increase and pH decrease (Table 1), which was consistent with the previous experience (Shi and Yu, 2014). Standing water in the sediment slurries limited O_2 intrusion to some extent during the open pre-incubation, resulting in a continuous decrease of Eh over the incubation period from short to long term. No significant CH_4 production was observed (data not shown). During the closed pre-incubation, O_2 supply from the ambient air was completely prevented after O_2 in the headspace of the bottles was depleted. As expected, the sediment slurries showed a lower Eh after the closed pre-incubation than after the open pre-incubation. The pH values showed further decline during the open pre-incubation, but remained almost steady during the closed pre-incubation (Table 1). Addition of oil/dispersant to the sediment slurries caused substantial Eh decrease and pH increase with statistical significance ($p < 0.05$). Changes in Eh and pH showed an inconsistent trend with the concentrations of dispersant, and the oil/dispersant = 100:1 treatment generally showed a higher pH and lower Eh than the other two oil/dispersant treatments (Table 1).

Results of the initial denitrification and OM mineralization rates in the sediment are presented in Fig. 1. Without the pre-incubation, the sediment characteristics, microbial communities and their activities were more close to actual field conditions. As observed in the previous study (Shi and Yu, 2014), the same results were confirmed in this study that oil/dispersant addition inhibited denitrification activity but stimulated OM mineralization activity in the marsh sediment. The denitrification rates (CK and OD = 10:1 treatment) in this study were lower than the previous study, likely due to higher Eh conditions. However, the CO_2 production rates were almost the same in these two studies using the sediment from the same location.

Effects of the pre-incubation and oil/dispersant exposure on denitrification and OM mineralization activities in the sediment are presented in Fig. 2. In absence of the oil/dispersant (CK), both denitrification and OM mineralization rates in the sediment were lower after the pre-incubations than the initial measurements (Fig. 1). However, presence of the oil/dispersant during the pre-incubation showed higher denitrification activity in the sediment than control, especially under the open pre-incubation conditions.

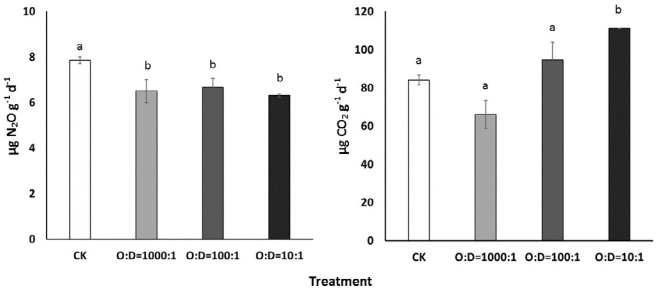

Fig. 1. Initial production of N_2O and CO_2 without pre-incubation. Note: Errors bars represent standard variation of the means. Different letters represent significant difference ($p < 0.05$). CK represents control with no addition of oil/dispersant; O:D = 1000:1, O:D = 100:1, and O:D = 10:1 represent addition of 1 mL mixture with oil:dispersant = 1000:1, oil:dispersant = 100:1, and oil:dispersant = 10:1, respectively.

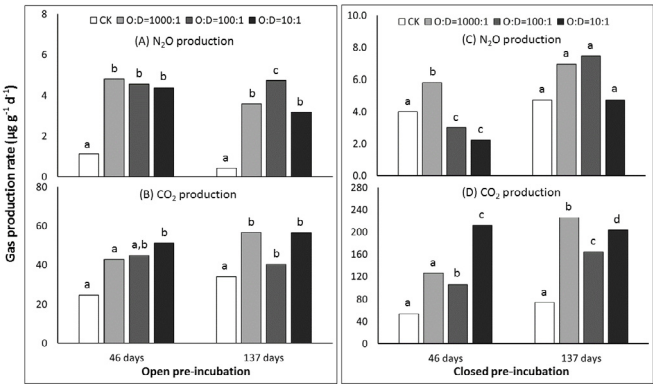

Fig. 2. Effect of pre-incubation on production of N_2O and CO_2. Note: Errors bars are not presented for clarity purpose. Different letters represent significant difference ($p < 0.05$). CK represents control with no addition of oil/dispersant; O:D = 1000:1, O:D = 100:1, and O:D = 10:1 represent addition of 1 mL mixture with oil:dispersant = 1000:1, oil:dispersant = 100:1, and oil:dispersant = 10:1, respectively.

After the closed pre-incubation, the inhibitory effect of oil/dispersant (especially for the treatments with higher dispersant concentration) on denitrification still existed in the short-term, but recovery of the denitrification activity prevailed in the long-term and even presented higher denitrification activity than control (Fig. 2 A and C). As seen in the initial measurement, CO_2 production rates remained higher in the oil/dispersant treatments than control after the pre-incubations, regardless of the open or closed conditions (Fig. 2 B and D).

Quantities of remaining total inorganic N (TIN, nitrate + nitrite + ammonium) in the sediment slurry system were determined after the denitrification and OM mineralization assay, and are summarized in Table 2. In most cases, the sediment

Table 2
Total inorganic N (TIN, μg N) at end of the denitrification and OM mineralization measurement.

Treatment	NO_3^--N	NO_2^--N	NH_4^+-N	Sum
Original (No nitrate addition)	132.1	4.6	232.0	368.7
Initial measurement				
CK	116.6	3.7	487.7	608.0
O:D = 1000:1	287.5	6.0	351.6	645.1
O:D = 100:1	244.8	8.2	337.3	590.3
O:D = 10:1	198.2	8.9	324.4	531.5
After open pre-incubation – 46 days				
CK	168.6	9.6	253.9	432.1
O:D = 1000:1	4.1	6.5	80.7	91.3
O:D = 100:1	0.6	4.4	186.8	191.8
O:D = 10:1	4.8	6.5	187.2	198.5
After open pre-incubation – 137 days				
CK	1022.5	7.7	676.3	1706.5
O:D = 1000:1	757.1	7.3	160.8	925.2
O:D = 100:1	7.8	21.3	435.1	464.2
O:D = 10:1	510.1	9.2	457.8	977.1
After closed pre-incubation – 46 days				
CK	82.1	11.8	379.3	473.2
O:D = 1000:1	2.0	3.9	327.0	332.9
O:D = 100:1	0.9	3.1	509.0	513.0
O:D = 10:1	4.7	3.9	461.8	470.4
After closed pre-incubation – 137 days				
CK	794.8	13.9	737.9	1546.6
O:D = 1000:1	1.7	9.7	510.5	521.9
O:D = 100:1	1.7	11.0	518.4	531.1
O:D = 10:1	7.5	11.6	915.8	934.9

Data represent means of three replicates (n = 3) without standard variations listed for clarity purpose. For all treatments, 1000 μg nitrate-N was added before the denitrification and OM mineralization assay. CK represents control with no addition of oil/dispersant; O:D = 1000:1, O:D = 100:1, and O:D = 10:1 represent addition of 1 mL mixture with oil:dispersant = 1000:1, oil:dispersant = 100:1, and oil:dispersant = 10:1, respectively.

treatments could denitrify all the added 1000 μg nitrate-N during the 4-day measurement. The amount of nitrate remaining after the denitrification assay followed the pattern with the observed N_2O production rates (Figs. 1 and 2). In the initial measurement, oil/dispersant treatments showed lower N_2O productions and resulted in more nitrate remaining in the system than control. In contrast, after the pre-incubations N_2O production rates were higher in the oil/dispersant treatments, and consequently with less nitrate left in the sediment slurries. As an intermediate product of denitrification, nitrite remained in low quantity in all treatments. In addition to CO_2 production, ammonium could be a by-product of OM mineralization process (ammonification of organic N). After the initial 4-day denitrification and OM mineralization measurement, all treatments showed higher amount of ammonium than the original 232.0 μg N. However, higher CO_2 production in presence of the oil/dispersant did not translate into more ammonium presented in the sediment slurries (Table 2). Generally, ammonium contents were higher after the closed than after the open pre-incubation, and after the long-term than after the short-term pre-incubation (Table 2).

4. Discussion

The northern Gulf ecosystem has been historically exposed to crude oil, due to natural seepage and human activities (Etkin, 2009; NRC, 2003). Previous studies reach the same conclusion that crude oil alone may not have much impact on the microbial communities (Al-Jawasim et al., 2015) and biogeochemical processes in the coastal ecosystem (Yu et al., 2012; Shi and Yu, 2014), at least in short term. However, the coastal ecosystem has no previous experience with the dispersant exposure. An immediate inhibitory effect of the dispersant on denitrification was reported in the previous study (Shi and Yu, 2014) and was verified in this study. A study reported that more than 80% of the bacteria were killed by addition of 1 mg mL^{-1} dispersant Corexit EC9500A, and when the dispersant concentration was increased to 10 mg mL^{-1} less than 5% of the bacteria could survive (Hamdan and Fulmer, 2011). This acute toxic effect of the dispersant on microbial communities is likely the major cause for the immediate inhibition of denitrification in the marsh sediment, even at very low concentration (Fig. 1).

The pre-incubation treatments in this study created not only different redox conditions in the sediment, but also different interactions between the oil/dispersant and sediment. During the pre-incubation, biodegradation of the oil/dispersant would release various more toxic intermediate products (Atlas, 1991) that would remain in the closed incubation bottles, but could escape from the open incubation bottles. In a natural wetland sediment profile, the surface sediment environment is similar to an open incubation in this study and the deeper sediment to a closed incubation condition. Meanwhile, the pre-incubations provided sufficient time for the microbial communities in the sediment to adapt the changing environment. Significant shift in microbial community was found in a previous study using the same sediment as in this study (Al-Jawasim et al., 2015). After a 30-day closed incubation at 30 °C with oil (2%), dispersant (0.2%) and combined oil/dispersant (10:1) treatments, hydrocarbon degrading bacteria (such as *Pseudomonas* sp. CZ5 for PAH) and denitrifying bacteria (*Rhodanobacter denitrificans* strain 2APBS1^T) became dominant. Even no obvious shift in microbial community was observed in the first 7 days of the treatments (Al-Jawasim et al., 2015), it is believed that intrinsic microbes would adjust their physiological characteristics to accommodate the oil and dispersant exposure before significant shift in their relative abundance. Biodegradation of oil/dispersant and their products were not monitored in this study. Growth of denitrifying bacteria (Al-Jawasim et al., 2015) in the sediment justified the higher denitrification activities than control observed in the oil/dispersant treatments after the open pre-incubations where oil/dispersant degradation products could escape from the system (Fig. 2 A). In contrast, following the short-term closed pre-incubation, the inhibitory effect of oil/dispersant (including potential degradation products) on denitrification still could be found in O:D = 100:1 and O:D = 10:1 treatments, while in long-term the denitrification activity could fully recover even for the treatment O:D = 10:1 (Fig. 2C). The transition from an immediate inhibition to later recovery of the impact of oil/dispersant on denitrification revealed in this study indicates the importance of time frame of the study (Pietroski et al., 2015; Zhang et al., 2015; Levine et al., 2017).

Redox status in the sediment plays an important role in denitrification activity (John, 1977; Tiedje et al., 1982). After the pre-incubations, the sediment denitrification activities of all treatments were lower than that in the initial measurement (Figs. 1 and 2), even the redox conditions became more favorable (Table 1). In this study, no additional OM, as an electron donor for denitrification process, was introduced into the sediment slurry system. With sufficient nitrate addition before the denitrification assay, lower denitrification activities were probably attributed to the OM depletion in the sediment during the pre-incubation. The 2 by 2 factorial design of the pre-incubation created 4 different redox conditions in the sediment slurry system. Linear correlation analysis indicates that redox status could explain 65% of the variations in denitrification activities of the sediment without oil/dispersant exposure (Table 3). When low concentration of the dispersant (OD = 1000:1) was added, the linear correlation coefficient between the denitrification activities and redox status decreased to 0.49. When the dispersant concentration was higher, toxic effect of the dispersant and shift of microbial communities played a more dominant role in denitrification activities of the sediment, and consequently presented poor correlation with the redox status (Table 3). The same mechanism could also interpret the inconsistent trends of changes of Eh and pH with the dispersant concentrations (Table 1).

Substantial amount of OM in the sediment could be depleted during the pre-incubations, especially in an open environment with a continuous supply of O_2 (Yu and Patrick, 2004). The studied salt marsh sediment presented high content of iron and sulfur, but much lower manganese content. Incubation of the sediment under anaerobic conditions showed lasting duration at iron (earlier) and

Table 3
Correlation between denitrification and OM mineralization activities with Eh (mV at pH 7).

Treatment	Denitrification (μg N_2O g^{-1} d^{-1})		OM mineralization (μg CO_2 g^{-1} d^{-1})	
	Linear regression	R^2	Linear regression	R^2
CK	$N_2O = -21.6 \times Eh + 276.1$	0.65	$CO_2 = -2.2 \times Eh + 322.4$	0.72
O:D = 1000:1	$N_2O = -62.8 \times Eh + 397.6$	0.49	$CO_2 = -1.4 \times Eh + 223.4$	0.85
O:D = 100:1	$N_2O = -14.8 \times Eh + 7.8$	0.04	$CO_2 = -0.5 \times Eh - 23.8$	0.04
O:D = 10:1	$N_2O = 47.6 \times Eh - 222.1$	0.10	$CO_2 = -0.6 \times Eh + 30.9$	0.09

For all 4 pre-incubation treatments, each of the 3 replicates was used for the linear regression (n = 12). CK represents control with no addition of oil/dispersant; O:D = 1000:1, O:D = 100:1, and O:D = 10:1 represent addition of 1 mL mixture with oil:dispersant = 1000:1, oil:dispersant = 100:1, and oil:dispersant = 10:1, respectively.

sulfate (later) reduction phase, respectively (data not shown). Under anaerobic conditions, ferric iron (III) could serve as an electron acceptor for oxidation of OM. Thus, only a portion of the CO_2 production observed came from denitrification where nitrate served as an electron acceptor. Powers et al. (2007) reported that addition of oil supported the growth of some iron-oxidizing microorganisms. Besides the original OM in the sediment, addition of 1 mL of oil/dispersant for the experimental treatments could serve as a new electron donor to power all biogeochemical processes. Correlation analysis showed a negative relationship between CO_2 production and Eh, in contrast to a common case of a positive correlation that anaerobic respiration (lower Eh) produces less CO_2 (Yu and Patrick, 2004). Toxic effect of the dispersant stimulated rapid sediment respiration with higher CO_2 production, which in consequence lowered the sediment redox status. In other words, higher CO_2 production due to the dispersant exposure was the cause for the observed lower redox conditions in the sediment. As seen in N_2O production, the correlation between CO_2 production and Eh became weaker when the dispersant concentration became higher in the treatments (Table 3). Shift in microbial communities over time would affect the sediment respiration with OM mineralization. In the slightly acidic environment of this study (Table 1), fungi communities may play a more important role than bacteria in oil/dispersant degradation and CO_2 production (Blagodatskaya and Anderson, 1998; Harms et al., 2011).

Nitrogen transformation in the marsh sediment is a dynamic interacting process due to the complexity of N cycle and changing redox conditions (Dalsgaard and Thamdrup, 2002; Yu et al., 2010). Mass balance approach was not attempted in this study, because some gaseous N products were not monitored in this study (Petersen et al., 2003; Yu et al., 2008), such as nitric oxide (NO) and N_2. High percentage of C_2H_2 and nitrate addition as designed should be sufficient to prevent conversion of N_2O to N_2 during the denitrification assay (Groffman et al., 2006). There was no significant decline in the monitored C_2H_2 concentration during the 4-day denitrification assay (data not shown). Linear increase of N_2O accumulation observed during the denitrification assay provided direct evidence to confirm the validity of C_2H_2 blockage technique in this study (data not shown). In addition to the added nitrate, nitrification process during the pre-incubation would contribute to the nitrate pool before the denitrification assay. The impact of oil/dispersant on nitrification was not examined in this study, and more information can be found in a recent review by Urakawa et al. (2018). Remaining ammonium after the denitrification and OM mineralization assay showed a clear positive correlation with CO_2 production, indicating a potential source of ammonium from OM mineralization. Meanwhile, a clear negative correlation was observed between ammonium and Eh in the sediment slurry system (Table 4). The results suggest that nitrification process likely took place during the pre-incubations, especially in the open-bottle conditions with higher Eh, which could convert ammonium to nitrate in the sediment slurry system. The remaining ammonium reflected the dynamic balance between ammonification and nitrification processes. Nitrite, as an intermediate product of nitrification and denitrification, showed a negative correlation with Eh with an average $R^2 = 0.55$ among the 4 treatments of sediment slurries (Table 3). All treatments in this study received the same 1000 μg nitrate-N before the denitrification assay, thus the remaining TIN in the sediment slurry system represented the balance between input from the mineralization of organic N and output from the gaseous N productions. With the increase of dispersant concentration, the liner regression analysis showed a better correlation between the TIN and Eh (Table 5). The negative correlation indicates that more TIN would become available in the system when redox status in the sediment became more reducing (low Eh) driven by higher OM mineralization activity due to the dispersant exposure. Furthermore, higher content of mobile inorganic N in the sediment is known to stimulate more OM mineralization (Turner et al., 2009; Shi and Yu, 2014). Therefore, the dispersant application would lead to a positive feedback loop toward loss of OM in the marsh sediment (Davidson and Janssens, 2006), and the consequence would be more severe with the increase of dispersant concentration.

5. Conclusion

The pre-incubation setup of this study provided a two-dimensional analysis of the effect of dispersant on major biogeochemical processes in the salt marsh sediment. Both concentration of the dispersion and its duration of exposure showed a vital impact on the C and N cycles in the coastal marsh sediment. In addition, open or closed incubation conditions provided an opportunity to

Table 4
Remaining ammonium (μg N) and its correlation with OM mineralization activity and Eh.

Treatment	CO_2 production (μg CO_2 g^{-1} d^{-1})		Redox (mV at pH 7)	
	Linear regression	R^2	Linear regression	R^2
CK	$NH_4^+ = 6.1 \times CO_2 + 227.0$	0.34	$NH_4^+ = -1.7 \times Eh + 885.9$	0.17
O:D = 1000:1	$NH_4^+ = 2.2 \times CO_2 + 18.1$	0.98	$NH_4^+ = -1.3 \times Eh + 357.0$	0.80
O:D = 100:1	$NH_4^+ = 1.8 \times CO_2 + 252.1$	0.46	$NH_4^+ = -0.9 \times Eh + 351.8$	0.62
O:D = 10:1	$NH_4^+ = 2.3 \times CO_2 + 202.2$	0.47	$NH_4^+ = -1.1 \times Eh + 451.7$	0.42

For all 4 pre-incubation treatments, means of the 3 replicates were used for the linear regression (n = 4). CK represents control with no addition of oil/dispersant; O:D = 1000:1, O:D = 100:1, and O:D = 10:1 represent addition of 1 mL mixture with oil:dispersant = 1000:1, oil:dispersant = 100:1, and oil:dispersant = 10:1, respectively.

Table 5
Remaining total inorganic N (TIN, μg N) and its correlation with Eh (mV at pH 7).

Treatment	TIN range	Eh range	Linear regression	R^2
CK	25.0 to 1706.5	+139 to +271	$TIN = -2.7 \times Eh + 1630.0$	0.05
O:D = 1000:1	91.3 to 325.2	−120 to +174	$TIN = -0.9 \times Eh + 526.0$	0.10
O:D = 100:1	191.8 to 531.1	−199 to +114	$TIN = -1.0 \times Eh + 360.0$	0.69
O:D = 10:1	198.5 to 934.9	−210 to +201	$TIN = -2.0 \times Eh + 548.5$	0.86

For all treatments, 1000 μg nitrate-N was added before the denitrification and OM mineralization assay. For all 4 pre-incubation treatments, means of the 3 replicates were used for the linear regression (n = 4). CK represents control with no addition of oil/dispersant; O:D = 1000:1, O:D = 100:1, and O:D = 10:1 represent addition of 1 mL mixture with oil:dispersant = 1000:1, oil:dispersant = 100:1, and oil:dispersant = 10:1, respectively.

examine the potential interactions between biodegradation products of the oil/dispersant and microbial communities in the sediment.

Crude oil alone may not have much impact on the coastal environment, at least for a surface contamination (as in an open incubation) over a short term. However, toxic biodegradation by-products of the crude oil may accumulate in deeper layers of the sediment (as in a closed incubation) over a long term. The dispersant application, even in trace amount, immediately inhibited the denitrification activity in the sediment by approximately 20%. Rapid recovery (< 46 days) of the denitrification activity occurred in an open incubation environment regardless of the dispersant concentration. In a closed incubation, the inhibitory effect of dispersant on denitrification persisted for short term, and recovery of the denitrification activity over time depended on the dispersant concentration. It is very encouraging to see the resilience of the marsh ecosystem upon oil spills due to the adaptation and shift of its microbial communities. The results clearly show that the dispersant could stimulate OM mineralization activity that lowered the sediment redox status, which meanwhile could release ammonium that could potentially be nitrified to nitrate. Mineralization of OM and mobilization of N in the sediment forms a positive feedback loop, leading to deterioration of the coastal ecosystem. It can be concluded that application of dispersant for oil spill remediation should be minimized if possible. Oil cleanup effort should avoid any operations to move the oil/dispersant into deeper layers of the sediment, where oil/dispersant degradation would be slow and their degradation products might buildup to toxic levels to affect the microbial biogeochemical processes and to damage plant roots in the coastal ecosystem.

Further studies are needed to examine the synergistic interactions between crude oil and dispersant and their biodegradation products, which could all contribute to the impact on ecosystem services in the coastal marsh ecosystem (Fuller et al., 2004; NRC, 2013).

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