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Impact of exposure of crude oil and dispersant (COREXIT[®] EC 9500A) on denitrification and organic matter mineralization in a Louisiana salt marsh sediment

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HIGHLIGHTS

- Crude oil slightly stimulated denitrification and organic matter mineralization.
- Dispersant significantly inhibited denitrification, but stimulated organic matter mineralization.
- Dispersant enhanced the development of more reducing conditions in the sediment.
- Dispersant may threaten wetland stability, and preserve the oil and dispersant for longer time.

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ABSTRACT

In response to the 2010 oil spill from the explosion of the Deepwater Horizon oil rig in the Gulf of Mexico, this experiment aims to study the ecological impact of the crude oil and dispersant (COREXIT[®] EC 9500A) in a coastal salt marsh ecosystem. The marsh sediment was incubated under an anaerobic condition with exposure to the crude oil or/and dispersant. The experiments were conducted in two continuous phases of nitrate addition to study denitrification potential using acetylene blockage technique and organic matter mineralization potential indicated by CO₂ production in the sediment. Results show that the oil slightly (with no statistical significance $p > 0.05$) increased both the denitrification and organic matter mineralization activities, likely due to oil components serving as additional organic matter. In contrast, the dispersant significantly ($p < 0.05$) inhibited denitrification, but stimulated organic matter mineralization activities in the sediment due to unknown mechanisms. As a consequence, redox potentials (Eh) were much lower in the dispersant treated systems. The ecological impacts from the dispersant exposure may come from two fronts. First, loss of organic matter from the coastal marsh will threaten the long-term stability of the ecosystem, and the decrease in denitrification activity will weaken the N removal efficiency. Secondly, more reducing conditions developed by the dispersant exposure will likely preserve the oil in the ecosystem for an extended period of time due to weaker oil biodegradation under anaerobic conditions.

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1. Introduction

In 2010 the explosion of the Deepwater Horizon oil rig became the worst environmental disaster in US history. This unprecedented incident is commonly referred as the BP (British Petroleum) oil spill, which released approximately 5 million barrels (780 million L) of crude oil into the Gulf of Mexico before the Macondo well

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was permanently sealed (BP, 2010; NOAA, 2010). This is equivalent to applying 0.5 mL crude oil m⁻² into entire area of the Gulf of Mexico (1.6 million km²). In addition, approximately 7 million L of dispersants (most COREXIT EC9500A and some COREXIT EC9527A) were applied in response to the oil spill, among which 3 million L were allocated to the oil wellhead at the sea floor and 4 million L on the Gulf surface (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2010). The environmental and human health impacts from this oil spill may take decades to unveil.

Some of the oil/dispersant have reached coastal wetlands in the northern Gulf coast. The oil spill poses additional stress to the

historic wetland losses along the Gulf region due to subsidence, drainage, and coastal erosion associated with tropical storms and rising sea levels (Conner and Day, 1988). Coastal wetlands, on the other hand, play a critical role in intercepting the nutrients from runoff out of terrestrial ecosystems to prevent eutrophication in the marine ecosystems (NRC, 2000). To take advantage of the ecological services provided by the wetlands, and to provide nutrients and sediment to maintain better wetland growth, large scale Mississippi River diversion projects have been implemented (Addison, 1999). In principle, this is an ideal “win-win” scenario with wetlands serving as a sink of nutrients to promote their plant growth while reducing the nutrient levels discharged into the Gulf of Mexico from the Mississippi River. However, recent studies suggest that the mineralization rate of the organic matter in the marsh can be substantially increased by the elevated nitrate in the diverted Mississippi River water, posing a serious threat to the ecosystem integrity (VanZomerem et al., 2011). It raises an important question on the sustainability of such river diversion projects, and the potential loss of the long-term ecological services provided by the coastal wetlands due to depleting its organic matter (Darby and Turner, 2008; Turner et al., 2009). In addition, wetlands are known for being an important carbon sink, which is critical for attenuating global climate change (Yu et al., 2006). Such an integrated analysis challenges the classical view of the “free” services provided by the wetlands.

In salt marsh ecosystem, nitrogen (N) cycle involves several mechanisms, including marsh plant uptake, nitrification, denitrification, dissimilatory nitrate reduction to ammonium (DNRA), and anaerobic ammonium oxidation (Anammox) (VanZomerem et al., 2011). Most of the processes involve transformation of N from one form to another with N remaining in the system. Denitrification is the dominant process for N removal from the system (Valiela and Cole, 2002), because its end products are gaseous N (mainly N_2O and N_2). The role of anammox in removal of N from a salt marsh is minor, less than 3% in some cases (Koop-Jakobsen and Giblin, 2009).

Exposure of oil/dispersant and their potential degradation products provides additional stresses to coastal wetlands, and its impact on such an ecosystem is unknown. In this study, mineralization of organic matter (CO_2 production) and denitrification (N_2O production with acetylene blockage) in salt marsh sediment is quantified under oil and dispersant exposure. To our knowledge, this is the first study to investigate the impact of oil spills on the coupling interactions between N and C cycle in coastal wetlands.

2. Materials and methods

2.1. Sediment sample

A sediment sample (top 30 cm) was taken from a salt (saline) marsh near Lake Pontchartrain, Louisiana (N30° 08.78', W89° 44.67'). The sampling site was dominantly covered by salt tolerant plant species of *Spartina alterniflora* and *Spartina patens*. The sediment sample was immediately transported to the laboratory with ice, and stored in a refrigerator (4 °C) before the experiment. The sediment texture was classified as silty clay loam according to USDA taxonomy with 11% sand, 57% silt and 32% clay. Salinity of the sediment pore water was close to 14‰ (ppt) with concentration (in $mg\ L^{-1}$) of chloride 7947, nitrate 11.4, nitrite 21.7, sulfate 109, and total phosphorus 2.4. Water content of the sediment was determined to be 76%. In dry weight basis, the sediment had 7.2% total carbon, 0.43% total nitrogen, and contents of other major elements (in $g\ kg^{-1}$) including iron (Fe) 19.91, manganese (Mn) 0.12, magnesium (Mg) 5.56, potassium (K) 2.36, sulfur (S) 5.30, and phosphorus (P) 0.61.

2.2. Crude oil and dispersant

A surrogate Macondo 252 well oil sample, sweet petroleum crude oil, was obtained through BP America Production Company (Houston, TX). The oil sample is a brown to black liquid with a density between 0.74 and 1.03 $kg\ L^{-1}$. Major compositions of the crude oil include various petroleum hydrocarbons, butane, pentane, BTEX (benzene, toluene, ethylbenzene and xylene), *n*-hexane and hydrogen sulfide. Naphthalene is the dominant PAH (polycyclic aromatic hydrocarbon) in the oil sample. A “topped” oil sample was prepared for this experiment by leaving the original oil in an open glass container to allow the volatile components naturally evaporate at room temperature (23 °C). It took approximately one month to reach a steady oil weight with about 25% oil mass loss. A dispersant sample, COREXIT® EC 9500A, was obtained from Nalco Environmental Solution LLC (Sugar Land, TX). Major ingredients of the dispersant include distillates, petroleum, hydrotreated light (10–30%), propylene glycol (1–5%), and organic sulfonic acid salt (10–30%). The dispersant has a pH of 6.2 and a density of 0.95 $kg\ L^{-1}$ at 15.6 °C.

2.3. Experimental setup

To maintain salinity of the sediment as the sampling site, sediment slurry was prepared by adding 30 g (wet weight) sediment with 80 mL 14 ppt water into a 237-mL wide-mouth glass bottle. Artificial ocean water (36 ppt, ‰) was prepared by dissolving nitrate free sea salt (Instant Ocean, Spectrum Brands Inc.) into D.I. water, and was diluted to a final salinity of 14 ppt. In total, 12 sediment slurries were prepared for 4 treatments with 3 replicates. The treatments include control (no oil and no dispersant), oil (1 mL topped oil), dispersant (0.1 mL COREXIT® EC 9500A), and oil + dispersant (1 mL topped oil + 0.1 mL COREXIT® EC 9500A). The sediment slurries with the 4 treatments were incubated in two phases by adding 50 ppm (1.5 mL 0.01 M KNO_3) and 100 ppm (3.0 mL 0.01 M KNO_3) nitrate (wet sediment basis), respectively. In the first phase of the experiment with 50 ppm nitrate, all bottles were capped with Teflon tape to prevent gas leakage. Gas samples can be taken through a rubber stopper in the center of the cap. Anaerobic condition was created by flushing the bottle through the rubber stopper with pressurized nitrogen gas (ultra high purity grade) for 5 min to displace the headspace air. After that, 20 mL pure acetylene (C_2H_2) was injected into each bottle through the rubber stopper to replace 15% of its headspace volume. In most cases, 10% C_2H_2 in the headspace of an incubation system is sufficient to inhibit N_2O reduction to N_2 (Knowles, 1990). Extra C_2H_2 was introduced in this study in case of consumption of C_2H_2 in the studied system. To ensure efficacy of C_2H_2 for the denitrification assay, the incubation at each phase of the experiment lasted for only one day with 3 samplings for determining gas production rates by linear regression. At end of the first phase, all bottles were uncapped for overnight. Then the second phase of the experiment with the same 12 sediment slurries was conducted following the same procedure as in the first phase. Two levels of nitrate were added in two separate phases in this experiment, while oil and/or dispersant were added only once at the beginning of the experiment. Addition of nitrate simulates cases such as nitrate input from agricultural runoff and nitrate application for oil biodegradation (Tao and Yu, 2013). Acetylene blockage technique works better at higher concentrations of nitrate (Knowles, 1990).

All sediment slurries were placed on a rotary shaker at room temperature (23 °C) during the study. At end of the second phase of the experiments, all bottles were uncovered for pH and redox potential (Eh) measurements. Nitrate, nitrite and ammonium were not monitored in this study due to presence of oil, and the difficulty in interpreting the nitrate levels due to the complex nat-

ure of the N cycle (for example nitrate consumption by DNRA process).

2.4. Sample analysis

Texture of the sediment was determined by the hydrometer method according to USDA particle size classification (sand 2–0.05 mm, silt 0.05–0.002 mm, and clay <0.002 mm). EPA methods were used for analysis of anions (method 300.0), metals (method 200.7), and total phosphorus (method 365.3), respectively. Sediment pore water was obtained through centrifugation of the wet sediment. Salinity (chlorinity) was calculated by multiplying chloride content with a constant of 0.0018066. Concentrations of N₂O, CO₂ and C₂H₂ were analyzed by using a Shimadzu GC-2014 gas chromatograph with dual channels equipped with electron capture detector (ECD) for N₂O, and a flame ionization detector (FID) for CO₂ and C₂H₂. Carbon dioxide was reduced through a nickel (Ni) reduction column to methane (CH₄) before detection by the FID. Gas sample was injected into the GC through a 1-mL sample loop for analysis by both channels simultaneously. Helium (ultra high purity grade) was used as the carrier gas with a pressure of 132.2 kPa and a total flow rate of 4.0 mL min⁻¹. The oven, injector, and detector temperatures were 80 °C, 380 °C, and 250 °C for the FID and 325 °C for the ECD, respectively. The pH and Eh values of the sediment slurries were measured at the end of the study with careful cleaning up the electrodes after exposure to the oil. A pH/mV meter (Accumet AP62, Fisher Scientific Inc.) was used for the pH and Eh measurements with a combination pH electrode (Fisher Scientific, Inc.) and with an oxidation–reduction potential (ORP) electrode (SPER Scientific, Inc.), respectively. The pH and Eh data were recorded after the readings stabilized in about 5 min.

2.5. Calculations and statistical analysis

Production rates of N₂O and CO₂ are determined by linear regression of their concentrations with time. Gases dissolved in the liquid phase were considered by taking mole fraction solubility of CO₂ (6.5×10^{-4}) and N₂O (4.7×10^{-4}) in water at 23 °C (Lide, 1991). The effect of pH and salinity on the solubility of CO₂ and N₂O in water was not considered. All data are reported based on dry weight of the sediment. The redox potentials were calibrated to the standard H₂ electrode by adding the correction factor (+245 mV at 23 °C) for the calomel reference to the observed instrument reading. The effect of pH on Eh was calculated according to the inverse relationship of Eh and pH as described by the Nernst equation (Bohn, 1971). All statistical analyses was conducted using SAS 9.3 (SAS institute Inc., Cary, NC). Difference among the means of different treatments was determined by a two-way ANOVA (significance level $\alpha = 0.05$).

3. Results

The experiments were conducted in two phases of nitrate addition (50 ppm and 100 ppm). Some nitrate residues from the first phase could be possibly carried over to the second phase of the experiment. This did not affect the overall setup of the experiment with two levels of nitrate treatments (low and high). All treatments showed a substantial decrease of pH after the two phases of anaerobic incubation (Table 1). The control bottles showed a slight increase of Eh due to addition of nitrate, meanwhile redox potentials were decreased in all oil or/and dispersant treatments despite of nitrate addition. The results clearly show that dispersant addition caused greater decrease in sediment Eh regardless of the presence of oil. This result is agreeable with the physical

Table 1

Redox potential (Eh) and pH \pm standard deviation at end of the experiment.

Treatment	pH ($n = 3$)	Eh (mV) at pH 7.0 ($n = 9$)
Control	5.3 \pm 0.1 ^a	+221 \pm 34 ^a
Oil	6.3 \pm 0.3 ^b	+123 \pm 18 ^b
Dispersant	6.4 \pm 0.1 ^b	-3 \pm 68 ^c
Oil + dispersant	5.5 \pm 0.3 ^a	+59 \pm 17 ^d

Before the experiment, initial pH and Eh of the sediment were 7.3 and +191 mV (at pH 7.0), respectively. Values labeled with different letters (a, b, c and d) represent significant difference ($p < 0.05$).

observation that the sediment slurries with dispersant addition were darker (due to formation of sulfide) than the other two treatments without the dispersant.

Three measurements were conducted in one day to capture the initial N₂O (with C₂H₂ blockage) and CO₂ productions, which represent denitrification potential and organic matter mineralization potential in the sediment, respectively. All N₂O production dynamics showed a typical pattern of a short lag period (first 12 h) before reaching the maximum production rate (Fig. 1). The lag period was more obvious for the two treatments with dispersant addition, especially in the phase with 50 ppm nitrate addition. Even when the same amount of nitrate was added for all the treatments in each phase of the incubation, accumulation of N₂O with C₂H₂ blockage was higher in the treatments without the dispersant. The inhibition effect of dispersant on denitrification was more obvious in the second phase with 100 ppm nitrate addition (Fig. 1). For CO₂ production, the results were the opposite. The presence of dispersant strongly stimulated CO₂ production during the study (Fig. 2). The results showed a rapid increase of CO₂ concentration in the first few hours of incubation, probably due to a decrease of pH in all treatments as seen in Table 1. Decreasing pH shifts the carbonate – bicarbonate – CO₂ buffer system toward CO₂ direction. Meanwhile accumulation of CO₂ during the incubation would also affect the equilibrium of the buffer system. Fluctuation of pH was possible during the incubation, which could partially explain the non-linear pattern of CO₂ accumulation (Fig. 2).

4. Discussion

The original sediment was under moderately reducing condition (Eh = 191 mV), and was ready for denitrification process to take place (Yu and Patrick, 2004). Production rates of N₂O (with C₂H₂ blockage) and CO₂ under different treatments are summarized in Figs. 3 and 4. Decrease of C₂H₂ concentration in headspace of the incubation system was found in the first 12 h, likely due to dissolving of C₂H₂ in the water phase. After that, C₂H₂ concentration in the headspace reached about 50–60% of the original concentration without changing for the remaining 13 h, indicating little C₂H₂ consumption by the system (data not shown). It is believed that the level of C₂H₂ was appropriate for quantifying denitrification potential in this study.

The results show that addition of the oil slightly ($p > 0.05$) increased N₂O production regardless of the dispersant treatments (Fig. 3). Hydrocarbons in the oil could serve as additional electron donors for the denitrification process. In contrast, addition of the dispersant inhibited denitrification activity as indicated by the N₂O production with C₂H₂ blockage, regardless of the oil treatments. The inhibitory effect was significant ($p < 0.05$) in the second phase of the experiment with addition of 100 ppm nitrate. There was no significant difference ($p > 0.05$) in N₂O production among the four treatments in the first phase of the experiment (50 ppm nitrate). However, N₂O production rates ($\mu\text{g N}_2\text{O g}^{-1} \text{ h}^{-1}$) in the first 12 h (Fig. 1 – nitrate addition 50 ppm) were significantly

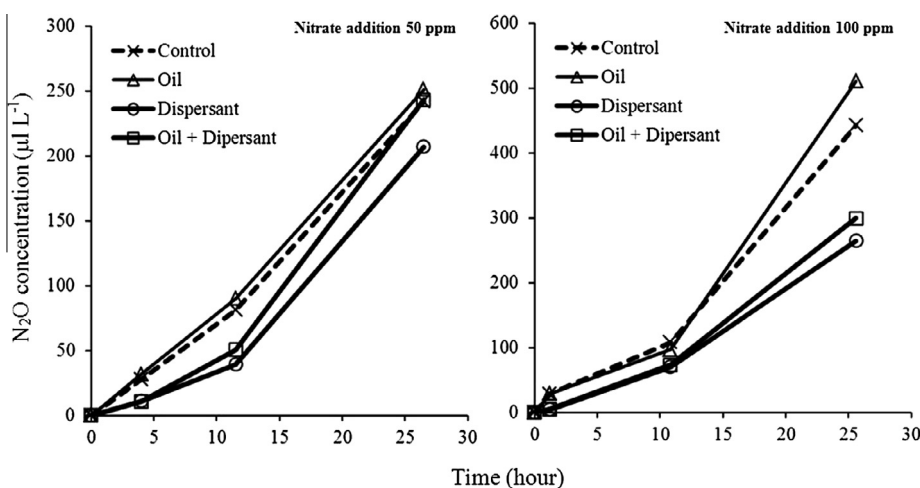


Fig. 1. Measurement of N₂O accumulation with C₂H₂ during the incubation. Standard deviations of the replicates (*n* = 3) are not denoted for clarity purpose.

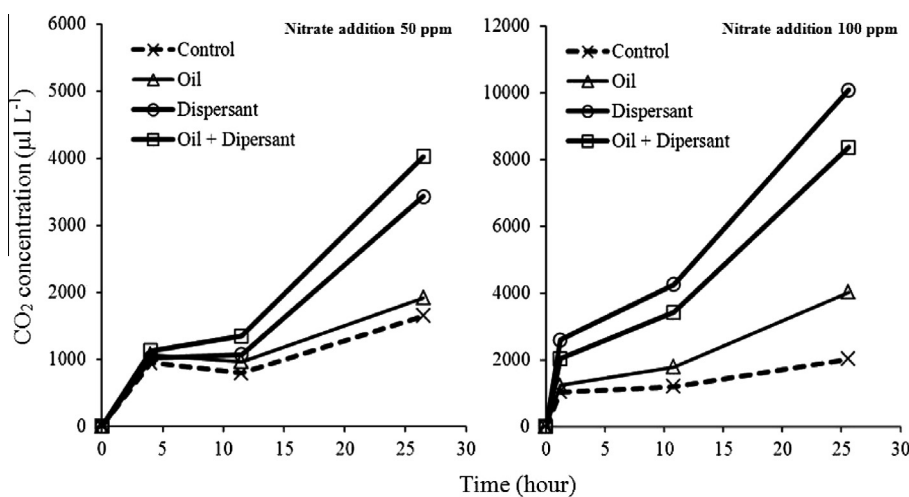


Fig. 2. Measurement of CO₂ accumulation with C₂H₂ during the incubation. Standard deviations of the replicates (*n* = 3) are not denoted for clarity purpose.

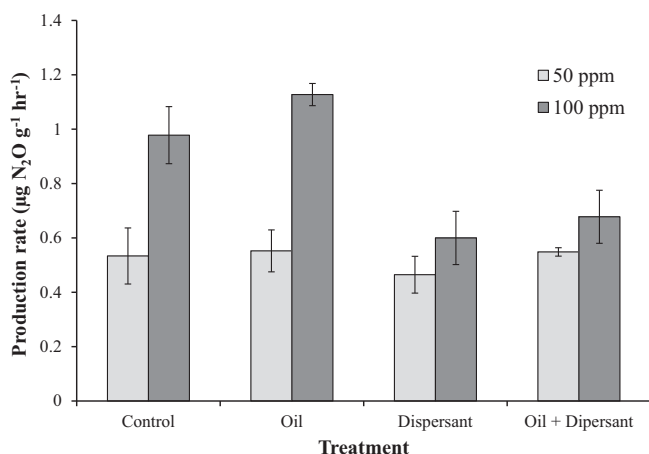


Fig. 3. Production rates of N₂O under different treatments. Error bars represents standard deviations of the replicates (*n* = 3).

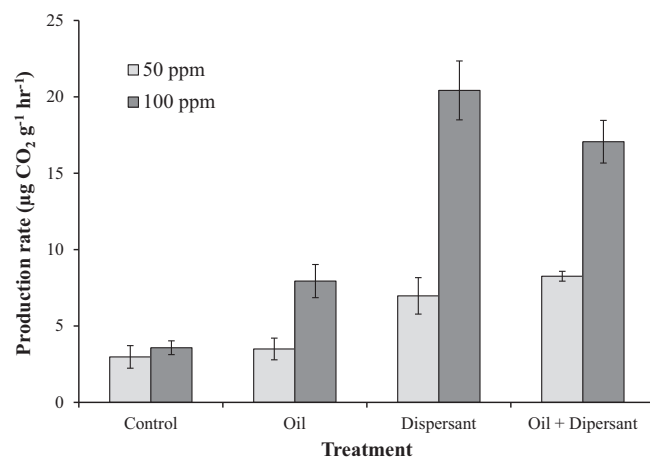


Fig. 4. Production rates of CO₂ under different treatments. Error bars represents standard deviations of the replicates (*n* = 3).

higher ($p < 0.05$) in the treatment of control (0.41) and oil (0.45) than in the two dispersant treatments (0.20 for the dispersant, and 0.26 for the oil + dispersant). The results exhibit a positive

correlation between N₂O production rates and Eh at end of the experiment in both 50 ppm ($R^2 = 0.35$) and 100 ppm ($R^2 = 0.59$) nitrate addition phases (Fig. 3 and Table 1). This is contradictory to

the general case where lower Eh normally leads to higher denitrification activity (Yu and Patrick, 2004). Validity of the C_2H_2 blockage technique could be a potential problem under lower Eh conditions, because of possible incomplete inhibition of N_2O reduction to N_2 by the C_2H_2 (Yu et al., 2010).

Higher levels of nitrate stimulated CO_2 production in all four treatments of the sediment by comparing the two phases of the experiment ($p < 0.05$). Excessive nutrients have caused concerns in organic matter loss in coastal marsh ecosystems (Turner et al., 2009). The sediment has a fairly low C/N ratio (16.7), thus ammonium generated from mineralization of the organic matter (ammonification) may continuously supply the nitrate pool through nitrification process (Ma and Aelion, 2005), forming a positive feedback loop toward deterioration of the marsh system. Higher CO_2 production in the oil treatment was partially due to introduction of additional organic matter from the oil components. However, additional organic matter from the dispersant components cannot explain the results of the increase of CO_2 production (Fig. 4). Higher CO_2 production in the oil or/and dispersant treatments corresponded to the observed results of lower redox potential well (Table 1). Strong negative correlations existed between the CO_2 production rates and Eh values at end of the experiment ($R^2 = 0.68$ and 0.95 for nitrate addition 50 ppm and 100 ppm, respectively). Generally less CO_2 production occurs in lower Eh conditions (positive correlation, where Eh is the controlling factor) than that in higher Eh conditions, as it is well-known that anaerobic conditions (low Eh) in wetlands favor more carbon sequestration due to less CO_2 production (Yu and Patrick, 2004; Yu et al., 2006). In this study, higher CO_2 production was the driver (controlling factor) for the observed lower redox potential. The mechanism of the stimulatory effect of dispersant on mineralization of organic matter in the sediment is not clear, but the ecological consequence can be serious considering of the small quantity of the dispersant applied to the treatment.

The results show a clear positive correlation between N_2O and CO_2 production (Table 2). Stronger correlation was found in the second phase with 100 ppm nitrate addition. Production of 1 mole N_2O from 2 moles nitrate reduction needs to obtain 8 moles of electrons from organic matters with 2 moles CO_2 production. If all CO_2 production comes from denitrification for N_2O production, theoretically the slope for the linear regression of CO_2 and N_2O production would be 2.0 (mole ratio is equivalent to concentration ratio for gases). In the second phase of the experiment (100 ppm nitrate), the control treatment showed a slope of 2.5 in the linear regression (Table 2), close to the theoretical value of 2.0, indicating denitrification was the dominant redox reaction during the incubation period. When nitrate concentration was low (as in the first phase with 50 ppm nitrate), the slope was 4.5 for the control treatment, indicating other redox reactions contributing more than half of the CO_2 production. Both Mn and Fe reduction reactions

largely overlap with nitrate/nitrite reduction (Patrick and Jugsujinda, 1992). The sediment was rich in Fe content (19.91 g kg^{-1}), and substantial portion of the Fe could be in form of ferric Fe (III) at initial redox condition before the experiment, which was ready for reduction to ferrous Fe (II) in the studied anaerobic system contributing to CO_2 production.

The oil addition slightly increased the slopes of linear regression equations between the CO_2 and N_2O production, while the dispersant addition dramatically increased the slopes in both phases of the experiment (Table 2). The results suggest that the dispersant could significantly stimulate organic matter mineralization in the sediment beyond the need for denitrification process, and this stimulatory effect was even stronger when excessive nitrate was available. This conclusion is supported by observing the intercepts of the regression equations, which represents CO_2 production when N_2O production stops. The intercepts values were much higher in the treatments with dispersant, indicating CO_2 production from processes other than denitrification (Table 2). Although pH plays an important role in CO_2 concentration in the incubation system, neither the slopes nor the intercepts of the regression equations showed a good correlation with the pH values measured at end of the experiment (Table 1). In each phase, the combined oil and dispersant treatment show a regression slope value between those in the treatment of oil alone and dispersant alone. When both oil and dispersant were added in the sediment, formation of oil-dispersant aggregates probably limited the contact of dispersant with the sediment, and consequently attenuated the effect of dispersant on biogeochemical processes in the sediment. However, the long-term effect of oil/dispersant is unknown.

5. Conclusions

This laboratory experiment with oil and dispersant treatments provides an initial insight on the impact of the oil and dispersant from the 2010 BP oil spill on coastal salt marsh ecosystems. Oil exposure slightly stimulated both N_2O and CO_2 production in the marsh sediment, which could be due to additional organic matter from the oil components. Significant impact from the BP oil spill may come from application of the dispersant COREXIT® EC 9500A. The dispersant significantly inhibited denitrification activity, the dominant N removal mechanism in the salt marsh ecosystem. Meanwhile, the sediment showed a significant increase in its organic matter mineralization following exposure of the dispersant. Longer retention of the nitrate in the sediment due to weaker denitrification under the dispersant exposure could also contribute to the increase of organic matter mineralization. The ecological service of N removal provided by the coastal marsh ecosystem may not be “free”, and in fact could be at the expense of deterioration of this ecosystem.

The mechanisms of the above feedback in the salt marsh sediment following the dispersant exposure remain unknown. Coastal marsh may well adapt to oil contamination due to historical exposure of oil spill or natural seepage (NRC, 2003; Yu et al., 2012), but may not adapt to the dispersant exposure. Foreign chemical components in the dispersant may cause severe toxicity to the microbial communities in the system, which may have various implications on the critical ecological function of the ecosystem beyond nitrogen and carbon cycle. It is not clear whether higher rate of organic matter mineralization due to dispersant exposure would reduce or enhance the long-term impact of the oil and dispersant on the function and services provided by the coastal ecosystem. Ecosystem recovery from the dispersant impact will largely depend on the dispersant degradation and adaptation of microbial communities to the dispersant exposure. More reducing conditions developed due to stronger organic matter

Table 2

Correlation between CO_2 and N_2O concentration ($\mu\text{L L}^{-1}$) during the incubation under different treatments.

Treatment	Linear regression equation	R^2 ($n = 9$)
<i>Addition of 50 ppm nitrate</i>		
Control	$CO_2 = 4.5 \times N_2O + 599$	0.78
Oil	$CO_2 = 5.1 \times N_2O + 700$	0.72
Dispersant	$CO_2 = 13.3 \times N_2O + 864$	0.96
Oil + dispersant	$CO_2 = 12.8 \times N_2O + 689$	0.95
<i>Addition of 100 ppm nitrate</i>		
Control	$CO_2 = 2.5 \times N_2O + 932$	0.94
Oil	$CO_2 = 5.7 \times N_2O + 2519$	0.86
Dispersant	$CO_2 = 27.6 \times N_2O + 2211$	0.98
Oil + dispersant	$CO_2 = 19.0 \times N_2O + 1136$	0.81

mineralization would likely preserve the oil (and probably dispersant) in the system for a longer time, since oil biodegradation rates are commonly lower in anaerobic environment (Atlas, 1981). Loss of organic matter due to stronger mineralization activity will certainly threaten the stability of the coastal marsh ecosystem, and will limit its potential for carbon sequestration in association with the challenge of global climate change (Yu et al., 2006).

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References

- Addison, J., 1999. Davis pond freshwater diversion structure. US Army Corps of Engineers. <<http://www.mvn.usace.army.mil/pao/dpond/davispond.htm>>.
- Atlas, R.M., 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiol. Rev.* 45, 180–209.
- Bohn, H.L., 1971. Redox potentials. *Soil Sci.* 112, 39–45.
- British Petroleum (BP), 2010. Deepwater Horizon accident. <<http://www.bp.com/sectiongenericarticle800.do?categoryId=9036575&contentId=7067541>>.
- Conner, W.H., Day, J.W., 1988. Rising water levels in coastal Louisiana: implications for two coastal forested wetland areas of Louisiana. *J. Coastal Res.* 10, 1045–1049.
- Darby, F.A., Turner, R.E., 2008. Below and aboveground biomass of *Spartina alterniflora*: response to nutrient addition in a Louisiana salt marsh. *Estuar. Coast* 31, 326–334.
- Knowles, R., 1990. Acetylene inhibition technique: development, advantages, and potential problems. In: Revsbech, N.P., Sørensen, J. (Eds.), *Denitrification in Soil and Sediment*. Plenum Press, London, pp. 151–166.
- Koop-Jakobsen, K., Giblin, A.E., 2009. Anammox in tidal marsh sediments: the role of salinity, nitrogen loading, and marsh vegetation. *Estuar. Coast* 32, 238–245.
- Lide, D.R., 1991. *Handbook of Chemistry and Physics*, 72nd ed. CRC Press.
- Ma, H.B., Aelion, C.M., 2005. Ammonium production during microbial nitrate removal in soil microcosms from a developing marsh estuary. *Soil Bio. Biochem.* 37, 1869–1878.
- National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2010. The use of surface and subsea dispersants during the BP Deepwater Horizon oil spill. (06.10.10).
- National Oceanic and Atmospheric Administration (NOAA), 2010. BP oil spill: NOAA Gulf spill restoration. <<http://www.gulfspillrestoration.noaa.gov/oil-spill/>>.
- National Research Council (NRC), 2000. *Clean Coastal Waters: Understanding and Reducing the Effects of Nutrient Pollution*. Ocean Studies Board and Water Science and Technology Board, Commission on Geosciences, Environment, and Resources. National Academy Press, Washington, D.C..
- National Research Council (NRC), 2003. *Oil in the Sea III, Inputs, Fates, and Effects*. National Academies Press, Washington, D.C..
- Patrick Jr., W.H., Jugsujinda, A., 1992. Sequential reduction and oxidation of inorganic nitrogen, manganese and iron in flooded soil. *Soil Sci. Soc. Am. J.* 56, 1071–1073.
- Tao, R., Yu, K.W., 2013. Nitrate addition has minimal effect on anaerobic biodegradation of benzene in coastal saline (salt), brackish and freshwater marsh sediments. *Wetlands* 33, 759–767.
- Turner, R.E., Howes, B.L., Teal, J.M., Milan, C.S., Swenson, E.M., Goehring-Toner, D.D., 2009. Salt marshes and eutrophication: an unsustainable outcome. *Limnol. Oceanogr.* 54, 1634–1642.
- Valiela, I., Cole, M.L., 2002. Comparative evidence that salt marshes and mangroves may protect seagrass meadows from land-derived nitrogen loads. *Ecosystems* 5, 92–102.
- VanZomeren, C.M., White, J.R., DeLaune, R.D., 2011. Fate of nitrate in vegetated brackish coastal marsh. *Soil Sci. Soc. Am. J.* 76, 1919–1927.
- Yu, J.O., Tao, R., Yu, K.W., 2012. Anaerobic biodegradation of benzene in salt marsh sediment of the Louisiana Gulf coast. *Ecol. Eng.* 40, 6–10.
- Yu, K.W., Faulkner, S.P., Patrick, W.H., 2006. Redox potential characterization and soil greenhouse gas concentration across a hydrological gradient in a Gulf coast forest. *Chemosphere* 62, 905–914.
- Yu, K.W., Patrick, W.H., 2004. Redox window with minimum global warming potential contribution from rice soils. *Soil Sci. Soc. Am. J.* 68, 2086–2091.
- Yu, K.W., Seo, D.C., DeLaune, R.D., 2010. Incomplete acetylene inhibition of nitrous oxide reduction in potential denitrification assay as revealed by using ¹⁵N-nitrate tracer. *Commun. Soil Sci. Plan.* 41, 2201–2210.