The Potential Dire Consequences of Oiling for Carbon Sequestration and Restoration of Louisiana's Coastal Wetlands

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Abstract

Coastal Louisiana experiences hydrocarbon exposure in the environment through accidental releases through drilling operations, distressed vessels, leaking storage tanks and other anthropogenic activities. The influx of crude oil in salt marsh sediments of coastal Louisiana have been shown to increase the population of resident sulfate reducing bacteria (Natter et al., 2012). Crude oil, a mixture of various hydrocarbons, act as a novel energy source for the bacteria (Natter et al., 2012). Recent research by Land et al. (2011) shows that the addition of an artificial organic polymer amended to Louisiana coastal soils causes an increase in microbial metabolism and subsequent soil carbon loss. We sought to determine whether the addition of crude oil to Louisiana salt marsh sediment would change the resident microbial respiration which would in turn suggest the possibility of ecosystem impacts from oil spills. Crude oil contains a lower ratio of C^{13} to C^{12} compared to organic material found in Louisiana salt marsh sediments (Natter et al., 2012; Chmura et al., 1987). The selectivity of microorganisms for crude oil or indigenous organic material decomposition can be determined by isotope analysis of evolved CO_2. Our preliminary experiment monitored the amount of evolved CO_2 from Louisiana salt marsh sediments loaded with both fresh and weathered crude oil as well as a series of unoiled controls. Gas samples for CO_2 analysis were collected over a period of 12 days. Our findings show that the addition of both fresh and weathered crude oil to the salt marsh soil actually hinders the metabolic ability of the microbial community. There were no significant difference in evolved CO_2 rates between fresh oil and weathered oil additions.

Introduction

Wetlands are essential for the proper transformations of elements in the biotic and abiotic realms. The duality of aerobic and anaerobic conditions characteristic of wetlands provide the means for driving reversible oxidation-reduction reactions which are essential for biogeochemical cycling. On a larger scale, wetlands act as a physical buffer of hurricane surge, nurseries for fish development, can provide for wastewater treatment, and act as a source and sink of organic material. These valuable services are important for maintaining the health of our coastal system and any damage, active or potential, to wetlands are worth investigating.

Between May 15 and July 12, 2010, approximately 5 million barrels of oil were released into the Gulf of Mexico from the Deepwater Horizon oil spill (Crone and Tolstoy, 2010). Coastal Louisiana marshes were impacted by the spilled oil, especially in heavily contaminated sites such Bay Jimmy and Bay Batiste (Figure 1) (Natter et al., 2012). Spilled oil tend to stick to sediment and plant surfaces and is notably associated with neutral, nonpolar organic matter which is in greater content in marsh sediments than in quartz-rich beach sediments (Nyman et al., 1990). Marsh sediments contaminated with the spilled oil are

reported to contain 10-28% total organic carbon (TOC) while uncontaminated sediments contain below 3% TOC (Natter et al., 2012). In addition, contaminated pore-waters contain levels of dissolved organic carbon (DOC) that are 1 to 2 orders of magnitude greater than DOC levels in uncontaminated pore-waters suggesting that the elevated carbon content in contaminated sediments is due to the effect of spilled oil (Natter et al., 2012).

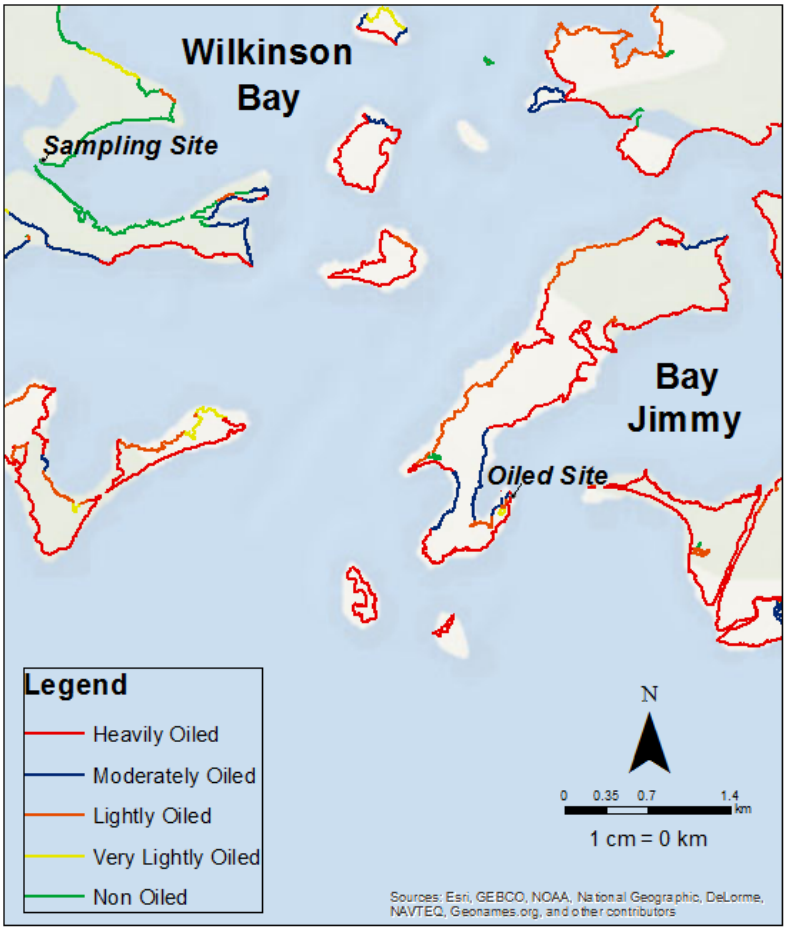

Figure 1. Distribution of the extent of oiling from the Deepwater Horizon oil spill in Wilkinson Bay and Bay Jimmy, observations are compiled by NOAA (Arc GIS Base Map and National Oceanic and Atmospheric Administration, 2013)

Organic material has varying degrees of isotopic Carbon-13 content. The amount of Carbon-13 relative to Carbon-12 can be described by δ^{13} C signature that is, a more positive δ^{13} C signature indicates a greater Carbon-13 content. Generally, crude oil has a more negative δ^{13} C value than wetlands plants and marsh sediments (Natter et al., 2012; Chmura et al., 1987). The Carbon-13 content in marsh sediments derives largely from local vegetation and thus shares similar δ^{13} C values with the overlying plants (Chmura et al., 1987). Plants can fix CO_2 to generate energy via two different forms of photosynthesis: C3 and C4. C4 plants have greater affinity for Carbon-13 than C3 plants and thus a more positive δ^{13} C signature (O'Leary, 1988). *Spartina patens* and *Spartina alterniflora,* C4 plants

that are dominant in Louisiana salt marshes, have δ^{13} C values ranging from -12 to -14% whereas sedimentary organic matter in Louisiana salt marshes have δ^{13} C values ranging from -14.4 to -17.7% (Chmura et al., 1987). The discrepancy in δ^{13} C values between plant and sediment in Louisiana salt marshes is attributed to contributions by C3 plants (Chmura and Aharon, 1995).

Oil taken from the surface of plants exposed to the spilled oil has a δ^{13} C value of -26.7% which is close to the δ^{13} C value of non-weathered BP crude oil (-27%) (Natter et al., 2012). This similarity in δ^{13} C between weathered and non-weathered oil suggests that the C^{13} signature in crude oil is not readily modified by environmental factors (Natter et al., 2012). The persistence of C^{13} in weathered crude oil provides a valuable opportunity to assess the presence and extent of crude oil contamination in marsh sediments (Natter et al., 2012). For example, the δ^{13} value for salt marsh sediments in Bay Jimmy, a site heavily affected by spilled oil, is around -20%, a far cry from the δ^{13} values normally associated with salt marsh sediments in Louisiana (Natter et al., 2012). This discrepancy in δ^{13} values is attributed to the contribution of crude oil in lowering the relative amount of C^{13} to C^{12} in salt marsh sediments (Natter et al., 2012).

Crude oil contains a mixture of various hydrocarbons that could potentially serve as a source of energy for microorganisms. Recent research by Land et al. (2011) found that an artificial organic polymer, intended for use as an amendment to increase soil structural integrity, actually led to increased soil loss due to elevated indigenous microbial metabolism. Our null hypothesis was that the addition of crude oil to Louisiana salt marsh sediments would have no effect on microbial activity. Our investigation consisted of monitoring evolved CO_2 production from marsh sediments treated with crude oil vs unoiled controls over a period of 12 days. We used weathered and fresh crude oil to determine whether the presence of the more toxic, lighter compounds could engender a significant difference in microbial respiration.

Methods and Materials

Soil Sampling

We collected three 10 cm long (7cm diameter) sample cores by push method from the Wilkinson Bay marsh (Figure 2). Documentation by the Emergency Response Management Agency (ERMA) Deepwater Gulf Response shows this marsh to be uncontaminated with crude oil from the Deepwater Horizon Oil Spill. Each core was sampled approximately within a 3 m square area within the marsh site. The samples were stored on ice and returned to our lab where they were stored at 4° C until analysis.

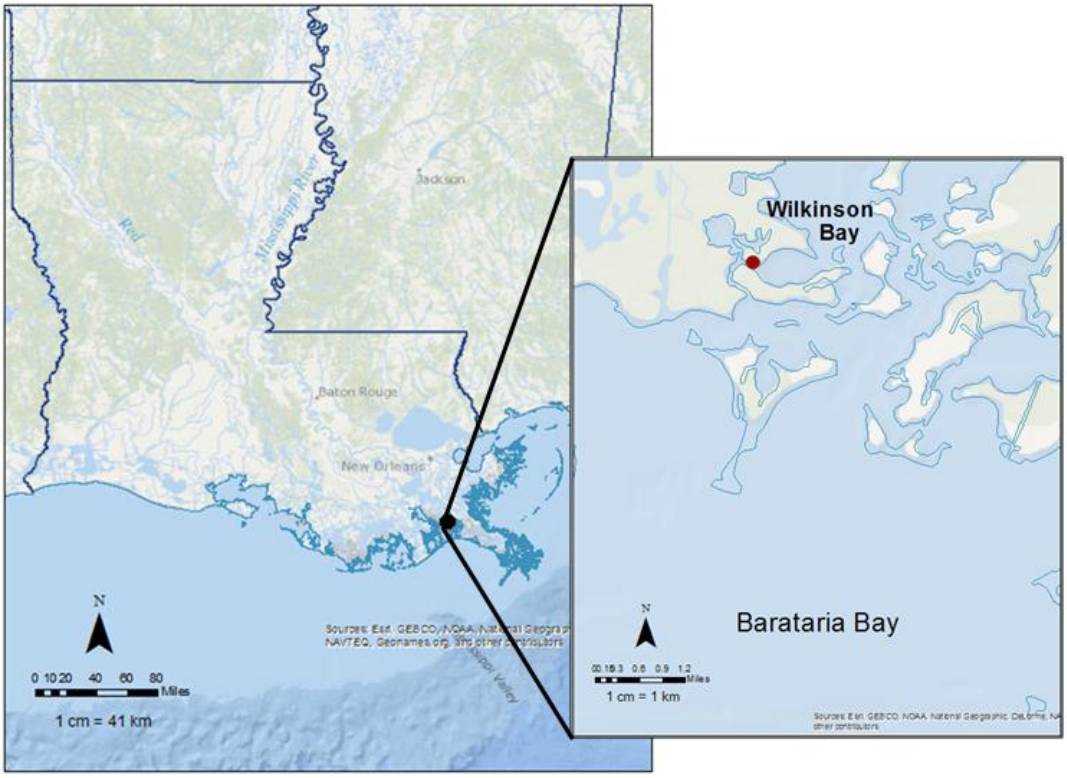

Figure 2. Red dot indicates sampling site in southeastern coastal Louisiana (Arc GIS Base Map and National Oceanic and Atmospheric Administration, 2013)

Moisture Content of Soil Samples

The moisture content for each sample was determined by collecting an approximately 32 g subsample of soil and placing it in a forced air drying oven at 70°C until constant weight. Moisture content was calculated to be the loss of water (in grams) divided by the wet soil weight.

Crude Oil Preparation

The weathered crude oil was prepared by placing fresh crude oil in a pan located in a greenhouse where the oil lost approximately 20% volume during a 72 hour period. The loss of volume was due to volatilization of light compounds in the oil.

Preparation of Homogenized Soil Sample

Approximately 151-152 grams of soil from each core was extracted and added to a blender. 100 mL of a 10.73 ppt saline solution was added to the blender and the mixture was blended until a homogenized slurry was created. The moisture content of the homogenized slurry was calculated to be the total moisture from each soil fraction plus the 100 mL of saline divided by the total weight of the homogenized slurry.

Reactor Setup

Approximately 20 grams of homogenized soil slurry was placed on a tin tray. Approximately 2 grams of weathered crude oil was added and mixed into the slurry to create a 10% weathered crude oil- slurry mixture. Approximately 5 grams of this oiled mixture was added to a 162 mL serum bottle. Five mL of DI water purged with 99.99% pure N_2 gas for approximately 10 minutes was added to the serum bottle. The serum bottle was then capped with an impermeable butyl rubber stopper and sealed with an aluminum crimp. The reactor was then evacuated to a negative pressure. In order to create anaerobic conditions within the reactor, we purged the reactor with 99.99% pure N_2 gas for about 15 minutes at a moderate flow rate. We made sure to insert the needle of N_2 gas before the exhaust needle to prevent inflow on O_2 into the reactor. We placed the reactor in a small paper box on a longitudinal shaker to allow the reactor to incubate at 25°C in the dark with continual shaking. These steps were repeated for non-weathered crude oil and control sets. The non-weathered crude oil set had an addition of non-weathered crude oil instead of weathered crude oil. Control set had no addition of crude oil. Each experimental set consisted of 3 replicates.

Evolved CO_2 Analysis

Gas samples for CO_2 were measured every 24 hours for 12 days, with no gas collection on the third and eleventh day. Each gas sample collection day, right before we collect the gas samples, we measured and recorded the pressure of each reactor using a digital pressure gauge equipped with a small syringe. We withdrew approximately 600 µL of gas from each reactor by using a 1 mL BD disposable insulin syringe and used only 500 µL of the gas sample as our injection volume. The gas sample was analyzed for CO_2 using a Shimadzu GC-2014 fitted with a thermal conductivity detector. The Shimadzu GC-2014 operated at 160 °C with an oven temperature of 80°C and fitted with a packed Poropak N (6 ft; 80/100 mesh) column from Sigma-Aldrich.

Data Analysis and Statistics

The rate of evolved CO_2 production was determined using regression analysis. Standard deviation was calculated from replicates, and significant differences between the rates of evolved CO_2 were determined using a one-way ANOVA.

Results and Discussion

The rates of evolved CO_2 for the control samples were significantly greater than those of fresh oil and weathered oil additions (Table 1). There was no significant difference between respiration rates for the two different crude oil additions indicating that the

presence of hydrocarbon compounds in both the fresh and weathered oil has a negative effect on the microbial consortia in the wetland soil (Table 1). The decrease in respiration rate for oiled sediments suggests that the addition of crude oil spilled into coastal wetlands decreases the rate of the microbial mediated decomposition of the organic matter in salt marsh sediments. The exposure to crude oil may be toxic to some bacteria and thus reduce the metabolic ability of the microbial community. The results force us to reject our null hypothesis that crude oil additions will have no effect on the microbial activity of salt marsh sediments.

Table 1. **Evolved CO_2 Rates Expressed as mg-CO_2 day^{-1}/ kg-soil**

	Control	Fresh Oil	Weathered Oil
Replicate 1	0.315	0.260	0.239
Replicate 2	0.299	0.254	0.244
Replicate 3	0.341	0.230	0.273
Mean	0.318 ± 0.021	0.248 ± 0.015	0.252 ± 0.018

Future Work

An isotopic analysis of evolved CO_2 would be necessary to elucidate the contributions, if any, of crude oil versus the native organic matter to microbial respiration. In addition, an extended incubation period with regular, evolved CO_2 analysis would reveal whether the indigenous microbial community requires more time to adjust to the presence of a novel energy source.

Acknowledgements

We acknowledge the work done by Jason Pietroski in sample preparation and Dr. Jeremy Conkle for providing methods for CO_2 analysis

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