

EFFECT OF THE BP DEEPWATER HORIZON OIL SPILL ON
CRITICAL MARSH SOIL MICROBIAL FUNCTIONS

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ABSTRACT

On April 20, 2010, the BP Deep-Water Horizon (DWH) offshore oil platform exploded releasing ~ 795 million L of southern Louisiana (LA) light sweet crude oil into the Gulf of Mexico. Approximately 7.9 million liters of dispersant, Corexit EC9500A, were applied for remediation. The effect of BP DWH crude oil and Corexit EC9500A on two marsh soil microbial processes, (mineralizable nitrogen and denitrification), were examined in the laboratory. Surface soil samples were collected from an unimpacted salt marsh site proximal to areas that suffered light to heavily oiling in Barataria Bay, LA. Additions of oil were at a ratio of 1:10 (crude oil:wet soil), mimicking a heavy oiling scenario. Carbon and nitrate based compounds were added to investigate the toxicology of oil and dispersant on denitrifier activity. Potential denitrification rates at the 1:10 weathered crude oil:wet soil ratio were $46 \pm 18.4\%$ of the control immediately after exposure and $62 \pm 8.0\%$ of the control following a two-week pre-incubation period. Potential denitrification rates of soil oiled with fresh crude oil were $51.5 \pm 5.3\%$ of the control after immediate exposure and significantly lower at $10.9 \pm 1.1\%$ after two-week exposure. Potential denitrification rates (acetylene blockage) after immediate exposure to Corexit:wet soil at ratios of 0:10 (control), 1:10, 1:100, 1:1,000, and 1:10,000 were below detection for the 1:10 treatment while the 1:100 was $7.6 \pm 2.7\%$ of the control and the 1:1,000 was $33 \pm 4.3\%$ of the control. The 1:10,000 treatment was not significantly different from the control. Denitrification rates measured after two-week pre-incubation were below the detection limit for the 1:10 treatment and the 1:100 treatment was $12 \pm 2.6\%$ of the control. Both fresh and weathered crude oil and Corexit can significantly impact activity of denitrification in the short-term. Corexit also

negatively affected other microbial measures. Microbial biomass nitrogen (N) values were below detection for the 1:10, 1:100 and 1:1,000 Corexit:wet soil treatments. Potentially mineralizable N rates were significantly lower for 1:10 and 1:100 Corexit:wet soil treatments. Future research should include additional oiling levels and extended exposure periods to determine the recovery of key wetland soil microbial processes.

CHAPTER 1: REVIEW OF LITERATURE

1.1 Crude Oil Spills

Crude oil spills are a result of leakage from storage tanks, onshore and offshore petroleum wells, or accidental releases during transportation (Bonin et al., 1990). While occasional crude oil spills are an unavoidable part of crude oil extraction, transportation, and refinement, attempts to mitigate damage are important to control effects on the aquatic environment. There are over 3,800 fixed structures and 6,500 producing wells connected to an integrated pipeline network more than 48,000 km in length in the Gulf of Mexico alone (Figure 1.1; Kaiser, 2009). The average daily production of crude oil from the federal waters of the Gulf of Mexico is 1.3 million barrels (Kaiser, 2009). The majority of crude oil is produced from deep-water fields in water depth greater than 305 meters and most of these structures are directly off the Louisiana (LA) coast (Figure 1; Kaiser, 2009). In LA alone there are approximately 1,500 oil spill notifications to the National Response Center with an average volume of 1.25 million L yr⁻¹ (Louisiana Oil Spill Coordinator's Office). Louisiana oil spills account for 20% of the United States total amount of spills and volume of oil spilled. Major oil spills in the Gulf of Mexico are rare, and experts predict that 80% of future Gulf spills will be less than 7949 liters (50 barrels), but if a spill occurs, LA coastal wetlands could receive the brunt of the impact because majority of oil rigs are located directly off the LA coast (Louisiana Oil Spill Coordinator's Office). Most shelf structures in the Gulf of Mexico are primarily gas producers, with average production rates averaging less than 1.6 million liters (10,000 barrels) of crude oil per day. In the deep water, there are only a few dozen (about 50 or

so) production facilities, but these structures generally produce 7.9 million liters (50,000 barrels) of crude oil per day (Kaiser, 2009).

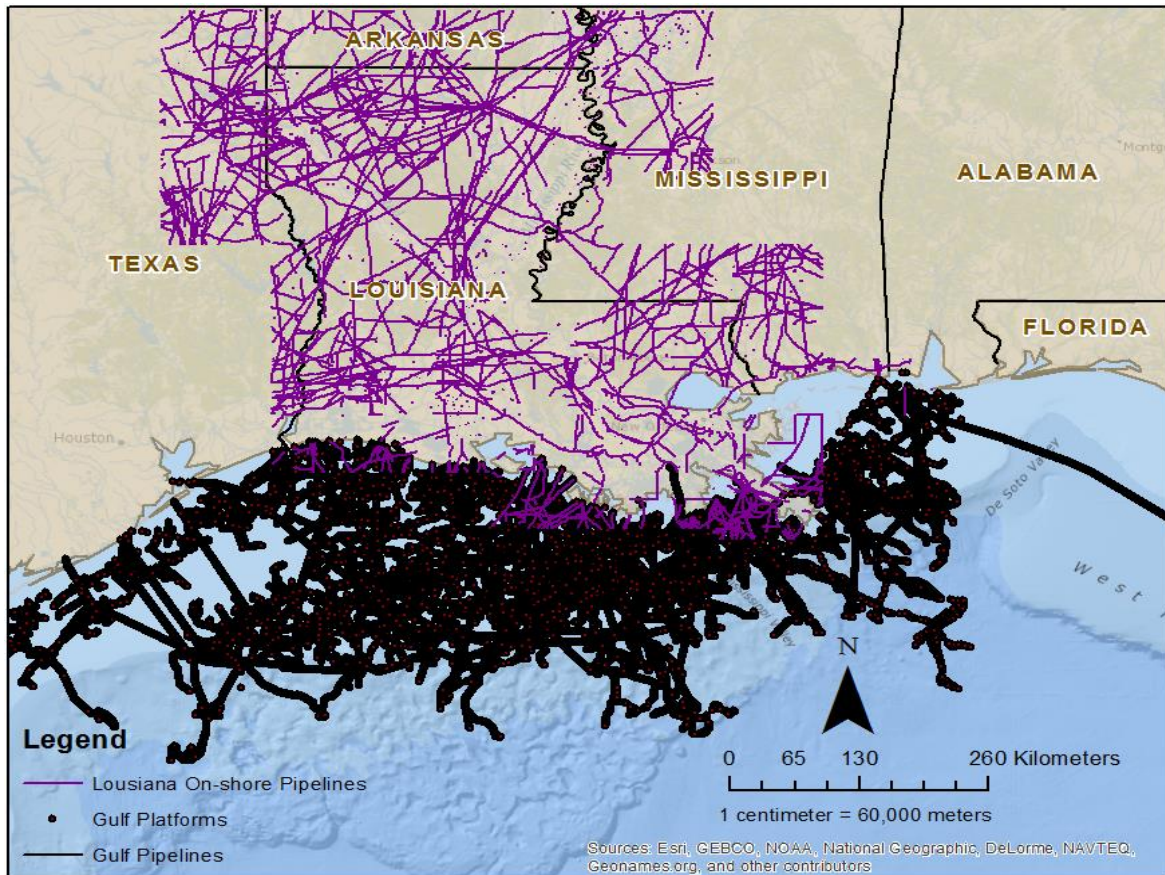


Figure 1.1 Oil production pipelines in the Gulf of Mexico and Louisiana, as well as offshore Gulf platforms.

1.1.1 Crude Oil Spill Effects on Salt Marsh Ecosystems

Brackish and saline marshes are among the environments most likely to be adversely affected by crude oil spills in LA (Gundlach and Hayes, 1978). Marshes are among the most productive of all aquatic environments. A vast variety of organisms and plants live in a delicate balance with the environment. It is spawning ground for a large number of sport and commercial fish. Detritus from the marsh provides an important food source for many marine organisms. If oiled, contamination may persist

with detrimental effects for years (Gundlach and Hayes, 1978). According to Gundlach and Hayes, (1978), Salt marshes should be designated as the primary environment to receive protection upon the occurrence of an crude oil spill. Booms or sorbent material should be applied to prevent crude oil from entering these areas (Gundlach and Hayes, 1978). In extreme cases, such as the Urquiola spill, booms may be utilized to trap oil within one area to prevent it from spreading to other previously unspoiled shorelines (Gundlach and Hayes, 1978). Once a salt marsh is severely contaminated, burning or cutting has been suggested, but only as a last resort (Gundlach and Hayes, 1978). Some studies indicate that cutting has been used effectively in a number of instances, though study of plant regeneration in untreated areas was not made (Gundlach and Hayes, 1978). Flushing of the oiled marsh with water under low pressure is a good option, since burning or cutting will result in almost total destruction of the resident benthic community (Gundlach and Hayes, 1978). Often the greatest long-term damage to the marsh is inflicted by heavy machinery and the large numbers of untrained personnel brought into the marsh to clean it. In most cases, and particularly where tidal action and seasonal plant growth is great, physical marine processes should be allowed to naturally cleanse the marsh (DeLaune and Wright, 2011; Gundlach and Hayes, 1978).

1.1.2 Crude Oil Spill Effects on Soil Microbial Populations

Microbes facilitate many biogeochemical processes in coastal wetlands and estuaries, including carbon sequestration (DeLaune and Wright, 2011), water quality improvements and serve as the essential component that regulates the base of the detrital food chain (Dosskey et al., 2010). Heterotrophic microbes are present in the

environment and some species are also important for the breakdown of hydrocarbons released as a consequence of natural crude oil seeps, as well as anthropogenic hydrocarbon releases into the marine environment (Hamdan and Fulmer, 2011; Mahmoudi et al., 2013). Microbial degradation of crude oil is a particularly important process for the recovery of wetland systems because most components of crude oil are biodegradable (DeLaune and Wright, 2011). Previous studies on spilled crude oil in coastal wetlands, including the LA Gulf coast, have focused on crude oil effects on wetland plants and benthic organisms (Delaune et al., 1979; Delaune et al., 1984; Patterson et al., 1993). Little effort has been directed at understanding the microbial response to oiling, especially in southern LA coastal marshes (DeLaune and Wright, 2011; Hamdan and Fulmer, 2011). Some mesocosm studies in Texas have focused on the microbial response to crude oil additions (Wright et al., 1996; Wright et al., 1997). Other studies have documented changes in microbial populations in wetlands in response to crude oil exposure (Hood et al., 1975; Oppenheich et al., 1971). These responses showed general increases in total microbial populations and increases in the ratio of hydrocarbon degraders to total heterotrophs (DeLaune and Wright, 2011). Biodegradation of crude oil components in wetlands have been demonstrated in greenhouse studies (Delaune et al., 1990), but the rates of degradation are strongly dependent on nutrient availability and other environmental conditions. The processes affecting spilled crude oil in open waters are dominated by volatilization, photolysis, and dispersion (Prince, 1997), while the processes in wetland soils and sediments are dominated by transport and biodegradation.

There have also been studies conducted on the effects of crude oil and chemical additives on mixed microbial populations in fresh marsh soils (Nyman, 1999). Soil respiration and reduction potential were compared for 6 months among microcosms containing freshwater marsh soils using Arabian and LA crude oil. None of the treatments slowed microbial activity. Instead, LA crude oil with fertilizer additives accelerated activity. This shows that there may be a difference in the effects of crude oil on freshwater marsh soil microbes compared to those of saltwater marshes.

1.1.3 Past Research on the Effects of Crude Oil Spills on Denitrification

Past studies of the effects of crude oil on denitrification in marine salt marsh sediments include the study conducted by Bonin et al., 1990, which looked at the denitrification activity in different types of sediment from the Mediterranean coast of France before and after a massive contamination (30-100 g kg⁻¹ sediment) of hydrocarbons. They used a closed system in order to maintain anoxic conditions and to control substrates and gaseous product concentrations. All analytical assays to determine the physical, chemical, and bacterial characteristics of the sediment were performed before and after the enrichment with petroleum (Bonin et al., 1990). The crude oil used was Arabian light crude. Within the 6 locations analyzed, the sediment characteristics ranged from 95% fine sandy sediment to 95% muddy clay sediment. This study only collected the top two centimeters of several cores of sediment and homogenized them. The concentrations of crude oil to sediment ranged from 5 to 10%. This study concluded that denitrification decreases 4 to 7 weeks in total inhibition between the crude oil contaminated samples and non-crude oil contaminated samples (Bonin et al., 1990).

Other studies conducted on denitrification potential found similar results of decreased rates with crude oil contamination (Griffiths et al., 1982; Haines et al. 1981). Haines et al., (1981) found that in surface sediment samples collected from the Alaskan continental shelf (Beaufort Sea), crude oil inhibited the natural denitrification, but there was no effect on potential denitrification measurements with added nitrate. Griffiths et al., (1982), performed a similar study collecting sediments from the Kasitsna Bay on the Alaskan shelf showing that both natural and potential denitrification was negatively affected by exposure to crude oil. The experiment conducted is not completely comparable to the reports of these previous studies as ours was only conducted for short time periods as compared to Haines et al., (1981), and Griffiths et al., (1982), whose studies lasted up to 18 months. The author did not determine the concentrations of crude oil actually mixed into the sediment. It has been concluded by past experiments that the percentage of crude oil insertion is dependent on the nature of the sediment. Although the exact mechanism involved in the denitrification inhibition of crude oil is not known, it was suggested that the perturbation of natural environmental conditions by petroleum could explain changes and decrease of the denitrification (Griffiths et al., 1982).

1.1.4 2010 BP Deepwater Horizon Oil Spill

The BP Deepwater Horizon (DWH) spill released a U.S. Government estimated 4.9 million barrels of crude oil in to the Gulf of Mexico over an 87-day period, from April 20 to July 15, 2010 (Michel et al., 2013). The BP DWH spill oiled several Gulf Coast ecosystems including LA barrier islands and marshes (DeLaune and Wright, 2011). The degree of impact on coastal wetlands and their recovery is variable depending on

the amount of oiling, type of oil, weathering state of the oil, climate, ecosystem type, environmental conditions, and scope of response. During the spill there were conflicting reports about the extent of shoreline that was impacted by the BP DWH Oil Spill. Shoreline assessment team's documented stranded crude oil along the shoreline of the Gulf of Mexico concluding that 1,773 km of shoreline was oiled (Michel, et al., 2013). Studies conducted from municipal Pensacola Beach, FL, revealed weathered crude oil petroleum hydrocarbon (C₈ to C₄₀) concentrations ranged from 3.1 to 4,500 mg kg⁻¹ in beach sands (Kostka et al., 2011). The documented range of oiling varied between locations. Of the shoreline impacted, beaches comprised 50.8%, marshes 44.9%, and other shoreline types 4.3%. Shoreline cleanup activities were authorized on 660km, or 73.3%, of oiled beaches and up to 71 km, or 8.9%, of oiled marshes and associated habitats (Michel et al., 2013). One year after the spill began, crude oil remained on 847 km; two years later, crude oil remained on 687 km, though much lesser degrees of crude oil (Michel et al., 2013). Crude oil is still present even four years after the spill in heavily impacted areas. Shorelines characterized as heavily oiled went from approximately 360 km, to 22.4 km after one year, and to 6.4 km two years later (Michel et al., 2013). Understanding the short and long term response of Gulf wetland ecosystems to the spill is critical. Natural recovery of crude oil impacted ecosystems involves a complex series of processes including crude oil volatilization, dispersion, and biodegradation, all of which serve to remove crude oil, given enough time and proper environmental conditions (DeLaune and Wright, 2011). Studies have shown that the abundance of hydrocarbon degrading bacteria was 10 times greater in oiled beach sands and were confirmed as oil degrading microorganisms (Kostka et al., 2011).

1.2 Chemical Dispersants

Dispersant solutions are a combination of hydrocarbon based solvents and surfactants, with the purpose of lowering the interfacial tension between crude oil and water to prevent formation of large, surface oil slicks (Kujawinski et al., 2011).

Dispersant use during oil spills has both prevented and enhanced environmental damages in the past (Etkin, 1998). The dispersant effectiveness depends on the type of oil, the amount of oil spilled, proximity of the oil to the shore and sensitive ecosystems, the weather and sea conditions at the time of the spill and during response, the type of chemical dispersant available at the time of the spill, and the potential efficiency of other cleanup methodologies available (Etkin, 1998).

The first recorded use of chemical dispersants on an oil spill was for the 1966 tanker oil spill off the coast of Germany, which had favorable results and minimal environmental impact (Lewis and Aurand, 1997). The successful use of dispersants during this scenario encouraged use for future oil spill events. Of the 408 oil spills in the Oil Spill Intelligence Report-International Oil Spill Database, 90% of major spills during 1966-1969 used chemical dispersants as the main clean up method (Etkin, 1998).

The completely positive view of chemical dispersant use disappeared after the 1967 Torrey Canyon oil spill where 420,000 gallons of various chemical dispersants were applied directly on the shoreline to treat the spill of 38.2 million gallons of crude oil (Etkin, 1998; Linden et al., 1987). The ratio of chemical dispersant:oil was extremely high compared to present day applications and should not have been applied directly to the shoreline (Etkin, 1998). The dispersants had devastating effects on the coast that became apparent in later environmental evaluations (Smith, 1968). The first generation

of chemical dispersants used highly toxic aromatic hydrocarbons as the carrier solvent and was later discovered to result in environmental damages that far exceeded the damages from crude oil alone (Etkin, 1998). Much of the negative views towards chemical dispersants stem from the high toxicity of the formulations and improper application techniques used in response to the Torrey Canyon spill. Dispersant usage dropped significantly during the 1970's, and is currently used in only 52.2% of all oil spills as a cleanup response (Etkin, 1998). The environmental impact concerns regarding dispersants led to newer, safer formulas which diminished the content of aromatic hydrocarbons as the carrier solvent, replacing it with surfactants.

1.2.1 2010 BP Deepwater Horizon Crude Oil Spill Chemical Dispersant Use

One of the strategies used during the 2010 BP Deepwater Horizon (DWH) oil spill to mitigate the environmental impacts and safety concerns of the oil was to spray the dispersant Corexit EC9500A (Hamdan and Fulmer, 2011). Corexit EC9500A is a blend of oxyalkylate polymers, organic sulfonic acid salt, substituted fatty ester, glycol ether, and aliphatic hydrocarbons (Utleay, 1998). There is also the potential for reaction byproducts of Corexit EC9500A to form that are not listed in the dispersant ingredients (Place et al., 2010). A total of ~ 7.9 million liters of the dispersant, Corexit EC9500A, was used during the BP DWH Oil Spill between May 15th and July 12th 2010 (Kujawinski et al., 2011). The applications were split with ~ 5.3 million liters applied to the surface and 2.9 million liters dispensed at the wellhead (Kujawinski et al., 2011). The dispersant was applied by sprayer jet at the wellhead, and aerially by small vessels at the surface (Kujawinski et al., 2011). The rationale for surfactant application at the surface was to break up large surface oil slicks, while the use at the wellhead, located

1522 m underwater, was to prevent the crude oil from reaching the surface and becoming a slick (Paris et al., 2012).

1.3 Louisiana Coastal Wetland Loss

Wetland loss along the LA coastal zone has long been recognized as one of the state's most pressing environmental problems (Morton et al., 2006). Of the numerous factors contributing to this loss, perhaps the leveeing of the Mississippi River for flood control has had the most far-reaching impact, blocking the river's historic spring overflows and thus impeding the rush of marsh-supporting fresh water, nutrients and sediment to the coastal zone (Day et al., 2000).

Of the areas within the LA coast, the Barataria Basin has one of the greatest wetland loss rates at $16.9 \text{ km}^2 \text{ yr}^{-1}$ (FitzGerald et al., 2007) and is an area in need of sedimentation. The Barataria bay complex is an interdistributary estuary bordered by the Mississippi River and by Bayou Lafourche. The estuary system is shallow and turbid with an average depth of about 2 meters (Happ, et al., 1977).

1.4 Mississippi River Diversions

Mississippi River diversions are the use of new channels and/or structures to divert sediment and nutrient rich water from the river into adjacent basins and are the main restoration technique for deltaic sedimentation (Peyronnin et al., 2013). An added benefit to diversions is the ability of coastal marsh and wetlands to remove excess nutrients. In particular the nitrate load can be removed by burial, assimilation into plant or algal biomass, returned to the atmosphere by denitrification, or reduction to ammonia (Reddy and DeLaune, 2008).

The Davis Pond diversion was the first diversion built to combat erosion rates in the Barataria Basin by imitating historic spring floods, providing a controlled flow of fresh water, sediment, and nutrients from the Mississippi River to the estuary (U.S. Army Corps of Engineers). Located on the west bank of St. Charles Parish and completed in December 2000, the diversion is made up of four iron-gated 14 foot by 14 foot box culverts built into the Mississippi River levee. An inflow channel 535 feet long by 85 feet wide directs river water into the structure, while an outflow channel more than 11,000 feet long by 120 feet wide connects the structure to the ponding area and ultimately diverts fresh water into the estuary (U.S. ACE). The total project area is comprised of 10,084 acres, including the 9,300-acre ponding area. The Davis Pond diversion is able to divert up to 10,650 cubic feet per second (cfs) of fresh water and was opened to maximum capacity during the BP oil spill to divert oil away from the coastal marsh (U.S. ACE).

The Louisiana Coastal Protection and Restoration Authority have contracted two new sediment diversions to be built into both the mid and lower Barataria Basin (CPRA, 2012). The Mid and Lower Barataria diversion max flow rates are 250,000 cfs and 50,000 cfs. The Mid-Barataria Diversion is much larger than the Lower Barataria Diversion and both are part of the first implementation period of the Master Plan taking place from 2012-2031 (CPRA, 2012). Both projects will cost a combined total of approximately 478 million dollars (Peyronnin et al., 2013). These diversions are significantly larger than the Davis Pond Diversion.

1.5 Nitrogen Cycle

Nitrogen is an important nutrient in biologically active processes and is often the limiting nutrient in coastal systems for primary production. Plants and algae utilize either ammonium or nitrate as the source of nitrogen for growth. The nitrogen cycle is dynamically important in biological processes because of its five naturally occurring oxidation states, ranging from +5 to -3 (Reddy and DeLaune, 2008). Dinitrogen (N_2) gas completes several conversions from inorganic to organic and back to inorganic nitrogen with the release of N_2 gas into the atmosphere. Nitrogen fixation occurs only in few bacterial species where elemental nitrogen (N_2) is converted to ammonium (NH_4^+) (Reddy and DeLaune, 2008). This conversion makes N_2 gas available for use by assimilation into plant biomass. Mineralization is the breakdown of organic nitrogen to inorganic nitrogen by the conversion of NH_4^+ to nitrate (NO_3^-), with the midway conversion into nitrite (NO_2^-), to break down organic matter (Reddy and DeLaune, 2008). Denitrification is the conversion of NO_3^- to NO_2^- to N_2O and finally N_2 gas, where it is released back into the atmosphere. Ammonium is assimilated directly, while nitrate is reduced to ammonium in plant cells before use. Nitrate is reduced to ammonia through the nitrate reductase enzyme. Ammonia is hydrolyzed to ammonium and incorporated into plants and algae as amino acids and proteins (Reddy and DeLaune, 2008). The nitrogen cycle in wetlands includes inputs, storage, and outputs.

1.5.1 Denitrification

Denitrification is mediated by denitrifying microbes and occurs only in anaerobic conditions, where nitrate is used as the alternative electron donor in place of oxygen to oxidize organic matter (Lane, et al., 2003; Reddy and DeLaune, 2008). The pathway in

which this occurs is NO_3^- to NO_2^- to N_2O and finally to N_2 gas. Denitrification is an important component of the nitrogen cycle by removing excess nitrogen from the system and releasing N_2 gas back into the atmosphere. The rate of denitrification is limited by the nitrate concentration and presence of organic carbon (Dodla et al., 2008). Denitrification is a primary controlling factor in primary productivity by removing excess nitrate that would otherwise be available for assimilation by plant and algal growth. Nitrous Oxide (N_2O) is an obligate intermediate in the process of reducing NO_3^- to N_2 gas by facultative microbes (Blackmer and Bremner, 1978). A ratio of N_2O to N_2 gas indicates that about 94% of N_2O is reduced to N_2 during denitrification (Blackmer and Bremner, 1978).

1.6 Nitrogen Input into the Louisiana Coast

Inputs of nitrogen are derived from both natural and anthropogenic sources. Natural inputs include biologically fixed nitrogen, precipitation, and particulate matter, which are abundant in the environment (Reddy and DeLaune, 2008). Natural sources of nitrogen are usually low because the environment is at equilibrium between production and use of nitrogen (Antweiler et al., 1995). Ammonia is a readily available form of nitrogen for the use in biological processes of plant and algae species. The Haber-Bosch industrial process has increased the production of ammonia for use in the agricultural fields (Roy and White, 2012). The Haber-Bosch process has increased the bioavailable nitrogen pool by up to 50% and has changed the global nitrogen balance.

Over the past 80 years, nitrate concentration in the Mississippi River has steadily increased. Nitrate concentrations before 1940 were between 0.2 to 0.4 mg N L⁻¹. Since 1940, the nitrate concentration has increased to between 1.0 and 2 mg L⁻¹ (Lane et al.,

1999; Antweiler et al., 1995). It is estimated that seventy five percent of nitrate entering the Gulf of Mexico from the Mississippi River since 1940 is from anthropogenic sources (Antweiler et al., 1995). Nitrate concentrations are high during the late winter, spring, and early summer, while nitrate concentrations are lower during the mid to late summer, fall, and early winter (Antweiler et al., 1995). Anthropogenic nitrate inputs are both point source and non-point source to coastal wetlands. Point source inputs of nitrate include waste-water discharge and industrial wastes (Antweiler et al., 1995). Non point sources of nitrate are primarily from urban area runoff (Reddy and DeLaune, 2008), agricultural fields (Antweiler et al., 1995; Reddy and DeLaune, 2008), and animal wastes (Antweiler et al., 1995). Nitrogen storage in wetlands is a combination of plant and microbial biomass, soil organic nitrogen, pore water nitrogen, and exchangeable nitrogen. Outputs are the loss of nitrogen from storage in the wetland and are lost from the system from outflow of water, loss of dissolved gasses, and loss of plant biomass (Reddy and DeLaune, 2008).

The Mississippi River watershed basin is ~3.1 million km² and has an average flow rate at the mouth of the river of 600,000 cubic feet per second (National Park Service). Of those nutrients, nitrogen has significantly negative effects on the Mississippi delta and Gulf Coast, producing extensive seasonal hypoxia events (Rabalais et al., 2002). Though an overabundance of nitrogen can cause hypoxia events, nitrogen availability is often the limiting factor for crude oil degradation in coastal marshes and nitrogen additions may facilitate crude oil biodegradation in the case of an crude oil spill (DeLaune and Wright, 2011). The availability of nitrogen for crude oil biodegradation depends on many processes such as dinitrogen fixation, denitrification,

organic matter decomposition, plant uptake and release, and tidal action (DeLaune and Wright, 2011).

Nitrate is the predominant form of nitrogen present in the Mississippi River, therefore Mississippi River water diverted into the Barataria Basin is high in nitrate. Ammonia runoff from agricultural fields within the drainage basin is oxidized to nitrate under aerobic conditions in the Mississippi River before reaching diversion sites such as the Barataria Basin. As water from southern LA diversions flows through the estuary, nitrate concentrations decrease (Lane et al., 2003). Possible reductions in nitrate are from denitrification; dilution by exchange with the Gulf of Mexico; assimilation by plants, algae, or bacteria; and burial (Lane et al., 2003).

1.6.1 Nitrate Removal

Nitrate removal depends on several factors. Residence time (Reddy and DeLaune, 2008) and loading rate (Lane et al., 2003) are the main factors in the effectiveness of nitrate removal in wetlands. Residence time is related to discharge rate, where maximum removal rate occurs at low flow from the Davis Pond diversion. A study on residence time in the Davis Pond Diversion found high discharge rates into the Barataria Bay marshes, resulting in nitrate reaching the Gulf of Mexico (DeLaune et al., 2005). Also, low nitrate loading results in maximum removal efficiency. The soil water interface is important for nitrate removal to occur. Removal of nitrate occurs by assimilation into plant or algal biomass, denitrification, or burial (Lane et al., 2003).

1.6.2 Hypoxia

Hypoxia in the Gulf of Mexico occurs when dissolved oxygen concentration declines below $2 \text{ mg O}_2 \text{ L}^{-1}$ and is a common estuarine phenomenon (Turner et al.,

2008). Louisiana coastal marshes experience excess nitrate in coastal waters, promoting large phytoplankton blooms, leading to annual coastal hypoxia and anoxia (Turner et al., 2008). The Northern Gulf of Mexico near the mouth of the Mississippi River has the second largest zone of coastal hypoxia in the world (Rabalais et al., 2002). The combination of high freshwater discharge, wind mixing, regional circulation, and summer warming conditions are controlling factors for the strength of stratification that goes through a seasonal cycle and is necessary for hypoxic conditions. The formation of the large hypoxic mass is also a result of enhanced primary production from the physical structure of the water column and high nutrient loads (Rabalais et al., 2002).

1.7 Summary

Oil spills affecting the LA coast is an occurrence that will continue in the future due to the large amount of oil extraction, transport, and refinement that takes place in and around the LA coast. The location of a spill plays a big role in controlling the degree of weathering which takes place from the time the oil is released until it comes in contact with the coast. The effects of weathered oil compared the non-weathered (fresh) oil to LA coastal marsh is unknown. Though many studies have been conducted on the effects of oil and dispersants on coastal plant and animals, very few studies have been conducted on the effects of marsh soil microbial processes. Denitrification and nitrogen mineralization are two important processes within the nitrogen cycle which have important ecosystem function roles, including water quality and coastal marsh primary productivity. The findings of effects on microbial processes are compared to the effects on microbial presence through biomass measurements as well.

The research presented in this thesis seeks to compare the effects of weathered crude oil to that of fresh crude oil on denitrification rates, as well as effects following immediate exposure compared to 2 week pre exposure to see if effects vary over time. Studies focus on the effects of oil compared to that of dispersants on denitrification rates to determine if using dispersants is more harmful than the crude oil in the case of this important microbial process. The author hypothesizes that biogeochemical functions are affected by dispersant and oil additions.

1.9 Synopsis of Chapters

In Chapter 2, the impact of fresh and weathered crude oil are compared to determine if an oil spill that occurs on or near shore is more detrimental than an oil spill off shore. The reason being, on shore oil spills allow the crude oil to go through weathering processes, through the atmospheric interaction that changes the crude oils chemical and physical properties, before coming into contact with the coast. Additionally, explore if longer term (2 week) exposure, for both fresh and weathered crude oil, have a greater impact on denitrification rates than immediate exposure.

Having established the impacts of fresh and weathered crude oil on denitrification in Chapter 2, Chapter 3 determines the effects of dispersant additions on denitrification rates, nitrogen mineralization rates, and soil microbial biomass. Determining the impact of both crude oil and dispersant on the important biogeochemical processes, which regulate water quality functions and productivity functions. The implications of all research chapters are discussed in Chapter 4.

CHAPTER 2: THE EFFECT OF FRESH AND WEATHERED SOUTH LOUISIANA CRUDE OIL ON POTENTIAL DENITRIFICATION RATES OF COASTAL MARSH SOIL

2.1 Introduction

Crude oil platforms and associated infrastructure are located within the coastal zone all around the globe. Crude oil spills can be the result of leakage from storage tanks, distressed vessels, onshore and offshore petroleum wells, or accidental releases during transportation. While infrequent crude oil spills are likely an unavoidable consequence of crude oil extraction, transportation, and refinement, it is critical to understand the extent of damage to ecosystem functions, once exposed. In the Gulf of Mexico, there are over 3,800 fixed structures and 6,500 producing wells connected to an integrated pipeline network more than 48,000 km in length (Kaiser 2009). The average daily production of crude oil from the federal waters of the Gulf of Mexico is 1.3 million barrels (Kaiser 2009). The majority of crude oil is produced from deepwater fields in water depth greater than 305 meters and the majority of these structures are directly off the Louisiana (LA) coast (Kaiser 2009). In LA alone, there are approximately 1,500 oil spill notifications to the National Response Center each year with an annual mean volume of 1.25 million L yr⁻¹ (LOSCO). Oil spills in LA account for 20% of the United States total amount of spills and volume of oil spilled. Major oil spills in the Gulf of Mexico are rare, but if a spill occurs, LA's coastal wetlands can receive the brunt of the impact. The location of an crude oil spill (on-shore vs off-shore) can determine the characteristics of the crude oil that impacts the coastal system. The amount of time the crude oil is in contact with the atmosphere can change both its physical and chemical composition through an initial fractionation process. Fractionation, which is a major part of the

weathering process, results in the loss of the lighter, more volatile, smaller carbon chain fractions to the atmosphere (Wang and Fingas, 1995). The most recent and notable example of an offshore crude oil spill affecting the U.S. with primarily weathered crude oil was the 2010 BP Deepwater Horizon (DWH) oil spill which occurred 66 km off the LA coast. This spill released ~ 795 million liters of crude oil over the 87 day event (McNutt et al., 2012; Paris et al., 2012). Once the crude oil was released from the wellhead, 1.5 km below the surface, the crude oil rose through the water column and began impacting shorelines after about a week (Paris et al. 2012). The south LA light sweet crude oil came ashore in the states of Louisiana, Alabama, Mississippi, Texas, and Florida driven by wind, waves and currents (Levy and Gopalakrishnan, 2010). However, due to differences in travel time, the crude oil that reached the coastline exhibited variable levels of fractionation.

There are also other causes of major crude oil spills in the LA coastal zone. For example, storm surges and waves associated with Hurricanes Katrina and Rita in 2005 led to the destruction of 46 crude oil platforms, while disabling 20 others. In addition, over 50 oil spills were reported within the near shore environments due to damage of storage tanks and leaks from disabled vessels during the storms (Pine, 2006) which impacted the coastline with fresh (non-weathered) crude oil.

The characteristics of the spilled crude oil and the extent of the weathering process are potentially important in determining the extent of the impacts to coastal wetland ecosystems, including important biogeochemical processes. Highly weathered crude oil is dense, forming tar-ball masses and has a tendency to sink, affecting benthic communities. Fresh crude oil has components of both heavy fractions that sink and

lighter fractions which more effectively cover the plants and surface marsh soils due to high buoyancy related to hydrophobicity. The hydrocarbons in crude oil have variable size ranges of carbon chains. Lighter fraction compounds are with 16 carbon chains or less, and the heavy fraction comprised of >16 carbon chains. The significance of the number of carbons in the chain is that smaller chains are most susceptible to volatilization. In addition, the lighter fractions are not only the more volatile but tend to also be the more toxic and include decane, undecane, and naphthalene (Van Hamme et al., 2003).

The effects of spilled crude oil on benthic communities, invertebrates, and marsh vegetation have been widely documented (Delaune et al., 1979; Elmgren et al., 1983; Gesteira and Dauvin 2000; Hester and Mendelsohn 2000). Research conducted during the 1978 Amoco Cadiz oil spill and the 1992 Aegean Sea oil spill concluded that soft-bottom benthic communities were negatively impacted through disappearance of some species and reduction in the numbers of others (Gesteira and Dauvin 2000) while evidence from the 1977 Tsesis oil spill in in the Baltic sea found reductions of up to 95% of benthic amphipods within 16 days of the spill (Elmgren et al., 1983). Various field and greenhouse studies have been conducted to evaluate crude oil effects on selected Gulf Coast salt marsh plants (DeLaune et al., 2003; Delaune et al., 1979). The extent of oiling tends to determine salt marsh vegetation responses, which show different levels of sensitivity to oiling. *Spartina patens* was the most sensitive to crude oil exposure with complete die off within 1 month, while *Panicum hemitomon* and *Spartina alterniflora* had only minimal decreases in aboveground biomass. Biomass of *Sagittaria lancifolia*, and

Typha latifolia were not affected by surface soil oiling as long as the leaves were not coated (DeLaune et al., 2003).

While there has been significant research on the effect of crude oil spills on various coastal ecosystem components, few studies have been conducted on crude oil impacts on the wetland microbial communities or microbial-driven ecosystem functions (DeLaune et al., 2011; Hamdan and Fulmer 2011). Microbes facilitate many biogeochemical processes in coastal wetlands and estuaries, including carbon sequestration (DeLaune and White, 2012), water quality improvements (Wang et al., 2006) and serve as the essential component that regulates the base of the detrital food chain through enzymatic degradation of soil organic matter (Vidon et al., 2010; DeLaune and White, 2012). Some species of heterotrophic microbes also play an active role in the breakdown of hydrocarbons, which can be released as a consequence of natural crude oil seeps as well as anthropogenic hydrocarbon releases into the coastal and marine environments (Hamdan and Fulmer, 2011; Mahmoudi et al., 2013). Denitrifying bacteria, a facultative functional microbial group, reduces nitrate through their respiratory pathway by converting nitrate (NO_3^-) to nitrous oxide (N_2O) and nitrogen gas (Tiedje et al., 1982). Therefore, denitrification is an important ecosystem function of coastal wetlands as it is an important N removal process in coastal marshes (Gardner and White, 2010; VanZomerem et al., 2012). This ecosystem function is particularly important in coastal LA as excess NO_3^- in coastal waters can promote significant springtime phytoplankton blooms followed by large expanses of coastal hypoxia and anoxia (Turner et al., 2008). Therefore, the goal of this study was to determine the effects of both immediate and longer term exposure of fresh and weathered crude oil on

potential denitrification rates in coastal LA marsh soil. The extent of exposure to crude oil is investigated to understand if there are associated effects to denitrification rates with continued crude oil exposure over time since crude oil following an oil spill can persist in an soil profile for many years. The effects of fresh and weathered oil are compared to determine if a spill that occurs on or near shore contaminating a coast with fresh crude oil has the same effect as an spill occurring offshore contamination the coast with weathered crude oil.

2.2 Materials and Methods

2.2.1 Study Site

The Barataria bay complex is an interdistributary estuary bordered by the Mississippi River and by Bayou Lafourche, containing large tracts of marshes along the coast dominated by *Spartina alterniflora* and interspersed with small bays. The estuary system is shallow and turbid with an average depth of about 2 meters (Happ et al., 1977). Several marshes in the Barataria Basin received light to heavy oiling when onshore winds coupled with tidal action pushed the BP DWH crude oil into the estuary (Lin and Mendelsohn 2012).

Marsh soil samples were collected on April 8, 2013 from a *Spartina alterniflora* marsh site within Wilkinson Bay in the Barataria Basin, LA (Figure 2.1; N29° 27,976 W89° 56,072). The Emergency Response Management Agency (ERMA) Deepwater Gulf Response mapping efforts documented this section of the Wilkinson Bay was deemed non-oiled from the BP DWH oil spill. The bay is located approximately 6.4 km northwest of Bay Jimmy, which was heavily oiled by the spill and contains identical plant

communities (Figure 2.2). This specific site was chosen since it was deemed non-oiled by NOAA, allowing exact concentrations of dispersant and oil additions to be made in an laboratory setting. Although no significant BP DWH crude oil is currently washing up on shorelines of the Gulf of Mexico, the author noted, while sampling 3 years after the spill, that crude oil is buried in some coastal marshes in Barataria Basin with oil layers a few cm below the surface. Because Barataria Basin has one of the highest wetland loss rates on the LA coast at $16.9 \text{ km}^2 \text{ yr}^{-1}$ (FitzGerald et al., 2007), the author observed that buried crude oil has been exhumed along the erosional edges of the marsh and re-deposited on the surface anew, suggesting a chronic oiling process.

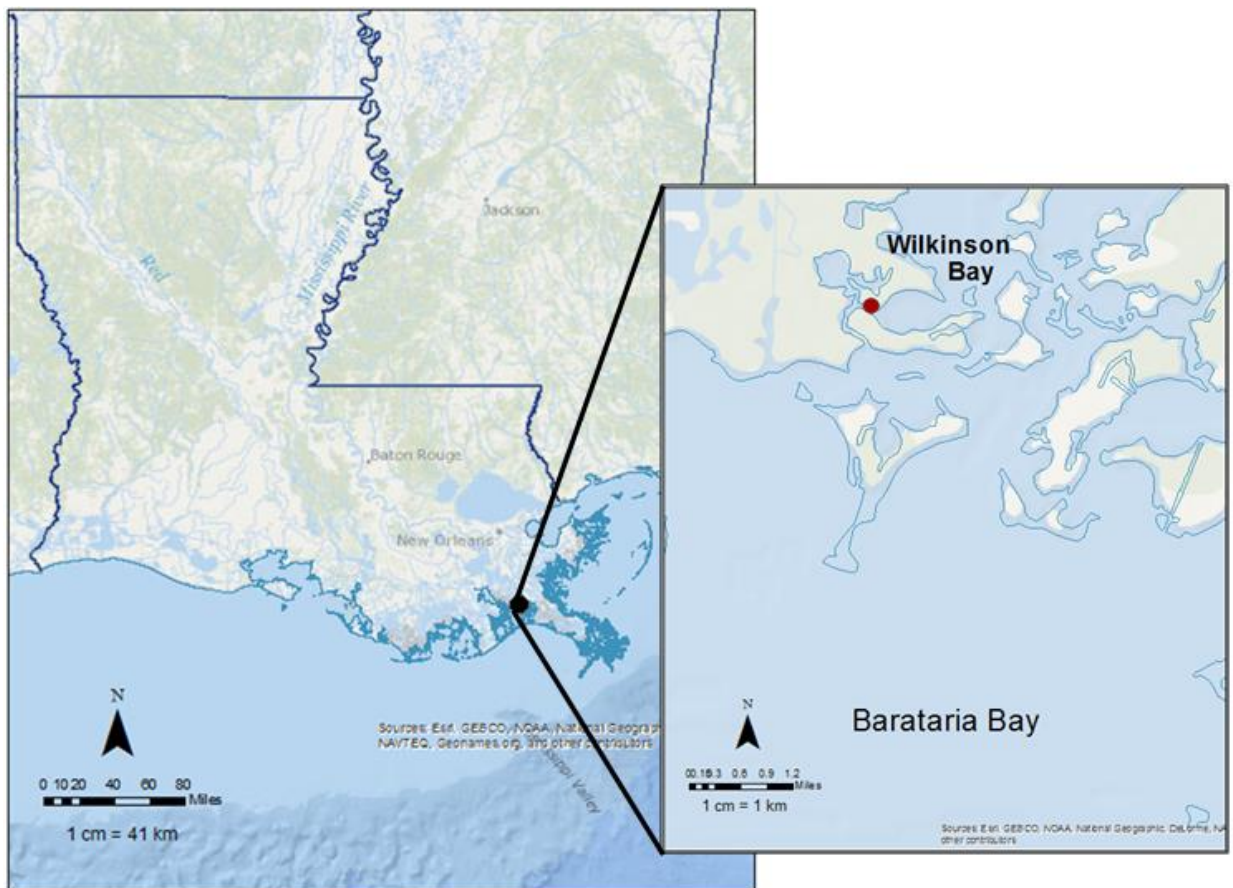


Figure 2.1 Location of the Barataria Bay complex within coastal Louisiana. Inset map denotes the Wilkinson Bay sampling site, located within Barataria Bay (Arc GIS Base Map and National Oceanic and Atmospheric Administration, 2013).

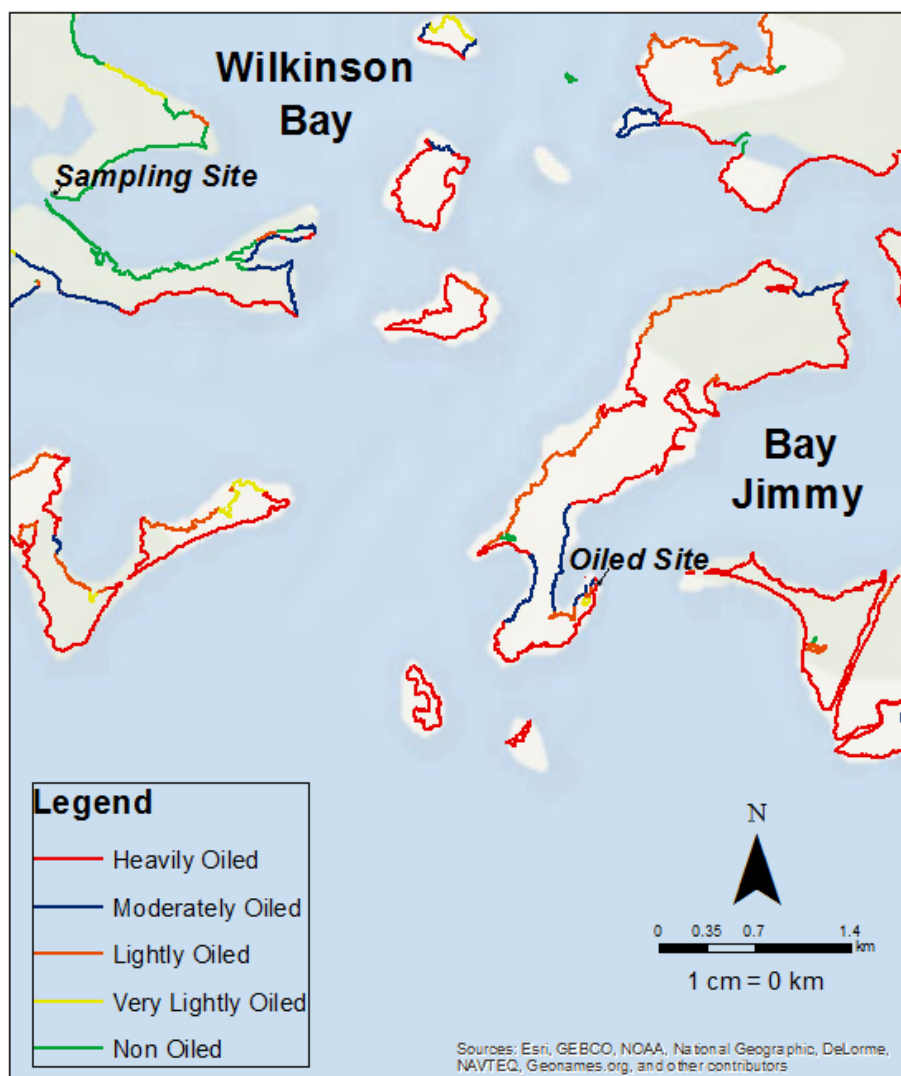


Figure 2.2 Distribution of observed maximum oiling of heavily, moderately, lightly, very lightly, and non-oiled coastal marshes in Wilkinson Bay and Bay Jimmy located within the Barataria Bay complex compiled by NOAA (Arc GIS Base Map and National Oceanic and Atmospheric Administration, 2013). The unoiled sampling site and an observed heavily oiled site are also labeled on the map.

2.2.2 Soil Sampling

Surface marsh soil was collected from three 3 x 3 meter quads randomly positioned within the location described in section 2.2.1 of this document. Four replicate 10 cm long (7cm diameter) cores were taken from each 3 x 3 meter quad areas by push-core and extruded in the field. The samples were stored on ice and upon return to

the lab, were refrigerated at 4° C until analysis. The top 10 cm of soil was the focus of this study since this top interval of soil contains the volume of soil most likely impacted by surface oiling and is the interval in which the majority of denitrification has been found in other LA coastal basin wetland sites (Gardner and White 2010; VanZomeren et al., 2012).

2.2.3 Soil Characteristics

The following soil characteristics were determined: moisture content, bulk density, weight % organic matter, particle size distribution, soil pore water salinity, total carbon (C), nitrogen (N), phosphorus (P), and microbial biomass N. Moisture content was determined by placing homogenized field moist soil subsamples into a drying oven at 70°C until constant weight. Bulk density was calculated for the collected soil intervals and expressed on a dry weight basis. Weight % organic matter content was determined as loss on ignition (LOI) using ash weight divided by pre-burned soil weight (White and Reddy, 1999). Particle size analysis was determined by Stoke's Law (Patrick, 1958). Salinity of the pore water was measured by first determining the moisture content of samples from the three replicate sampling quads and then combining the field moist samples with DI water at a 1:1 ratio in a beaker, mixing well (Putnam-Duhon et al, 2012). The solution was allowed to settle overnight and the conductivity was measured using a conductivity probe (Fisher Scientific Accumet Basic AB30). The conductivity, measured in $\mu\text{Siemens cm}^{-1}$, was corrected for dilution and converted to part per thousand (ppt) as total dissolved solids (TDS). Total C and N were measured on dried, ground subsamples of soil using an Elemental Combustion System with a detection limit of 0.005 g kg⁻¹ (Costech Analytical Technologies, Valencia, CA). Solid-phase total P

analysis involved combustion of oven-dried ground subsamples at 550°C for 4 hrs in a muffle furnace and subsequent dissolution of the ash in 6 M HCl on a hot plate (Mckerche and Anderson 1968). Concentrations were determined using a Seal Analytical AQ2 discrete analyzer (Method 365.1; USEPA, 1993). The chloroform fumigation extraction method was used to determine microbial biomass N by first extracting a set of controls with 25 mL of 0.5 M K₂SO₄ at a ratio of approximately 1:50 (g dry soil:extractant) on a longitudinal shaker for 1 hr. Chloroform fumigation was performed on a duplicate set of samples in a vacuum sealed glass desiccator containing a 100 mL beaker with chloroform over a 24 hour period. All 2 M KCL-extracted samples were centrifuged for 10 min and vacuum-filtered through Whatman #42 filter paper. Samples were preserved with HCl to a pH < 2 (Brookes et al., 1985) and analyzed using a Shimadzu TOC-V CSN Total Organic C and N Analyzer (Shimadzu Scientific Instruments, Columbia, MD).

2.2.4 Crude Oil Preparation and Analysis

The crude oil was weathered by placing the fresh crude oil in a shallow pan which was then placed in a greenhouse. The crude oil underwent weathering through the volatilization of light compounds which led to a ~20% loss by volume during the 72 hr period. The crude oil was then collected in an amber bottle and sealed for use in laboratory experiments. The crude oil was characterized by Jim Wang and Syam Dodla using a gas chromatography (GC) (Shimadzu Model 2010) equipped with a capillary column (Restek Rxi-5SilMS, 30 m, 0.25mm ID, 0.50 µm film thickness) and a flame ionization detector to detect the total petroleum hydrocarbons. The GC was standardized using oil standard purchased from Accustandard. The GC was operated

with a helium carrier gas flow rate of 7 mL min^{-1} , inlet temperature at 300°C , detector temperature at 330°C and an oven temperature program of 50°C (held 2 min) to 300°C (held 14 min) at $6^{\circ}\text{C min}^{-1}$ to 320°C (held 1.67 min) at $30^{\circ}\text{C min}^{-1}$.

2.2.5 Potential Denitrification

Potential denitrification was determined using the acetylene block method described by Smith et al., 1978; Yoshinari and Knowles, 1976. The 1:10 ratio of oil:wet soil weight was the chosen treatment level selected since it fully coated the soil surface and mimicked a heavy oiling scenario (Michel et al., 2013). Four replicates each of the 1) weathered crude oil, 2) non-weathered crude oil and 3) controls were used. Five grams of homogenized sample were placed in 160mL serum vials. The serum vials were sealed with Wheaton snap on style stoppers and aluminum crimps, and subjected to a vacuum until the vials were at -75 k Pascals of atmospheric pressure. The serum vials were then purged with 99.99% pure N_2 gas for 15 minutes to provide an anaerobic headspace. Saline solution was purged with 99.99% pure N_2 gas for 10 minutes to remove all oxygen from the water and then five mL was added to each serum vial to produce a slurry. Potassium nitrate (KNO_3) and glucose monohydrate ($\text{C}_6\text{H}_{12}\text{O}_6 \times \text{H}_2\text{O}$) was used to make a 100 mg L^{-1} nitrate-N and carbon solution to stimulate maximum denitrification rates. The solution was purged with 99.99% pure N_2 gas for 10 minutes and five mL were added to each serum vial. Ten percent of the total headspace of each vial was replaced with acetylene. The vials were agitated continuously on a longitudinal shaker in the dark at 25°C . Gas samples were extracted over time using 1mL BD disposable insulin syringes and were analyzed on a Shimadzu GC-8A equipped with an electron capture detector. The GC detection limit for gas samples was $0.006 \mu\text{g N}_2\text{O N}$

$\text{kg}^{-1} \text{hr}^{-1}$. Head space gas samples were taken at 0, 24, and 48 hours to determine the effects of immediate exposure of weathered and fresh crude oil on short-term denitrification rates. The longer term exposure experimental set up was identical to the immediate exposure except that the crude oil was added and allowed to incubate in the soil under anaerobic conditions on a longitudinal shaker in the dark at 25°C for 2 weeks. During the incubation, 1 mL of $10 \text{ mg NO}_3\text{-N L}^{-1}$ nitrate solution was added on days 1 and 7 to provide a substrate for the denitrifying microbial community. Following the 2 week pre-incubation period, potential denitrification was measured to determine the effects of longer-term exposure to fresh and weathered crude oil on this critical wetland microbial process. We repeated the experiment, pre-incubating the soil with the crude oil for 2 weeks prior to determine longer term effects on denitrification to expand further on these findings.

2.2.6 Data Analysis

Maximum denitrification rates were determined using regression analysis across the sampling time intervals, with mean and standard deviation calculated from the replicates. The Student t- tests ($\alpha=0.05$) was used to test significant differences ($P < 0.05$) for soil denitrification rates between the various treatments.

2.3 Results & Discussion

2.3.1 Soil Characterization

Soils were characterized by high weight percentage moisture contents averaging 79.8 ± 0.01 weight % and low dry weight bulk densities of $0.27 \pm 0.01 \text{ g cm}^{-3}$ for the 0-10 cm soil interval (Table 2.1). The average LOI was $26.1 \pm 0.01\%$ for organic matter content. Particle size analysis was dominated by the mud fraction (silt + clay) with low

percent sand (10.3% sand, 46.2% silt, and 43.6% clay) and classified as a silty clay (Gerrard, 2000). Pore water salinities averaged 13 ± 1.3 ppt TDS. Soil total C and N averaged 121 ± 5.7 g C kg⁻¹ and 6.7 ± 0.1 g N kg⁻¹, respectively, while the total P concentration averaged 553 ± 9.5 mg P kg⁻¹. These total nutrient values for coastal salt marsh soil are significantly lower than values seen in the more organic soil freshwater marshes of upper Barataria Basin (Kral et al., 2012). The microbial biomass N pool averaged 56.3 ± 1.7 mg N kg⁻¹. Measured soil properties were similar to those for adjacent marshes in the Barataria Basin (DeLaune et al., 1979; Dodla et al., 2012), suggesting they are representative of other LA coastal salt marshes in the basin.

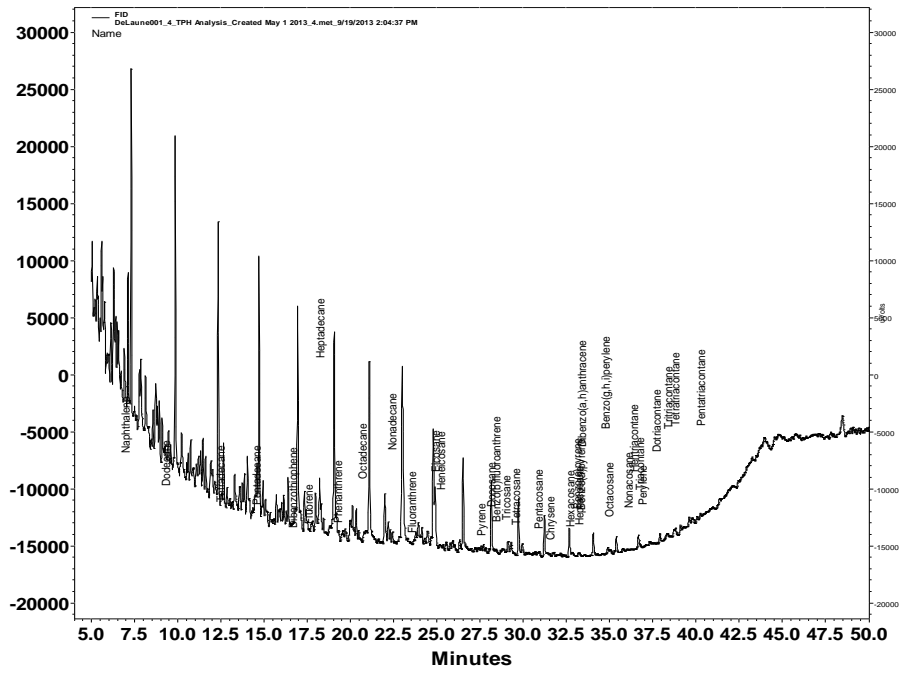
Table 2.1 Comparison of fresh and weathered crude oil peak areas and concentrations.

Light Fraction Compound	Fresh Crude Oil	Weathered Crude Oil
	Concentration (g L ⁻¹)	Concentration (g L ⁻¹)
Decane	3.29	1.83
Undecane	3.61	3.37
Naphthalene	0.69	0.56
Dodecane	2.93	3.33
Tridecane	3.08	3.65
Tetradecane	3.24	3.98
Pentadecane	3.18	3.91
Dibenzothiophene	1.15	0.96
Phenanthrene	0.91	0.60
Anthracene	0.52	0.38

2.3.2 Crude Oil Characterization

Both the fresh and weathered crude oil samples were analyzed for the concentration of lighter fractions, those compounds with C chains less than 16 and heavier hydrocarbon fractions (Figure 2.3). Results show that there was a relatively greater concentration of light compounds present in fresh crude oil at 50.03% compared to weathered crude oil at 45.83%. This change in concentration was a consequence of the loss of 20% of the volume of crude oil during the volatilization of compounds that were mostly smaller than 12 carbon chains. The light fraction compounds present in the fresh crude oil at higher concentrations include decane, naphthalene, and dodecane (Table 1).

Fresh Crude Oil



Weathered Crude Oil

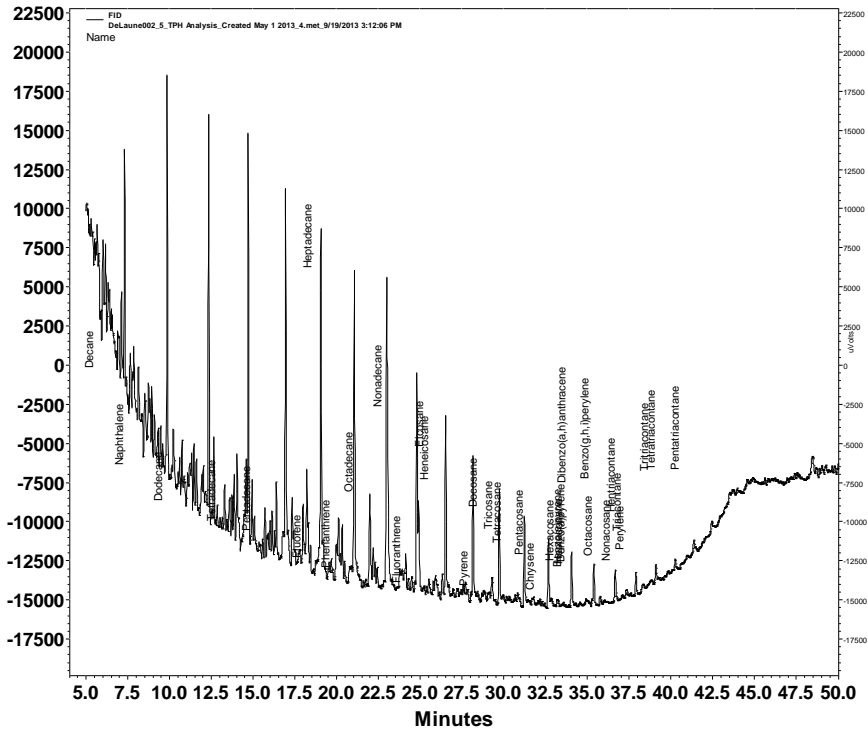


Figure 2.3 Mass spec analysis of light and heavy fraction peaks of fresh and weathered crude oil.

2.3.3 Fresh and Weathered Crude Oil Effects on Potential Denitrification - Immediate

The control denitrification rate averaged $10.5 \pm 0.3 \text{ mg N}_2\text{O kg}^{-1} \text{ hr}^{-1}$ (Figure 2.4). The 1:10 ratio of weathered crude oil:wet soil treatment denitrification rates averaged $4.8 \pm 1.2 \text{ mg N}_2\text{O kg}^{-1} \text{ hr}^{-1}$. The 1:10 ratio of fresh crude oil:wet soil weight treatment denitrification rate averaged $5.6 \pm 0.8 \text{ mg N}_2\text{O kg}^{-1} \text{ hr}^{-1}$. Consequently, there was no significant difference in denitrification rates of fresh and weathered crude oil. However, both the fresh and weathered crude oil additions led to a significant decrease at $46 \pm 18.4\%$ and $51.5 \pm 5.3\%$ of the control for the weathered and fresh crude oil, respectively (Figure 2.5). There was no significant difference between the two treatments for an immediate oiling scenario which was surprising considering that the fresh crude oil contained a higher concentration of the more volatile and potentially more toxic compounds.

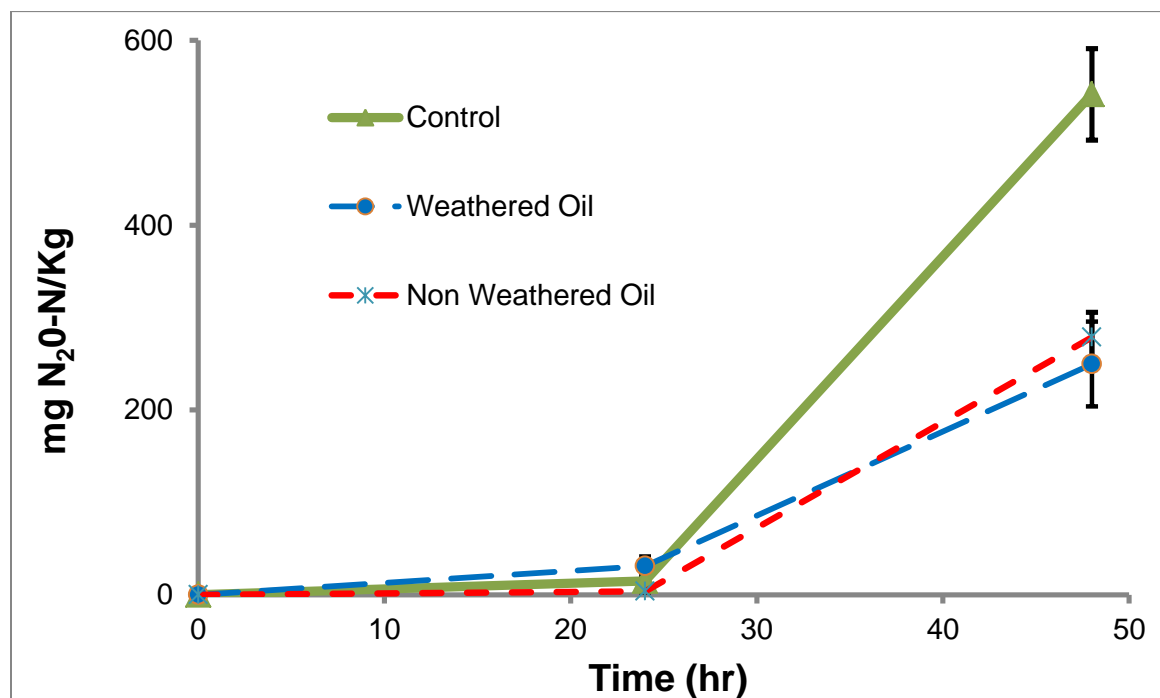


Figure 2.4 N₂O production potential of Louisiana coastal marsh soil N₂O production with 1:10 oil:wet soil ratio for both weathered and fresh crude oil additions. Data shown are mean values ± 1 standard error (n = 4).

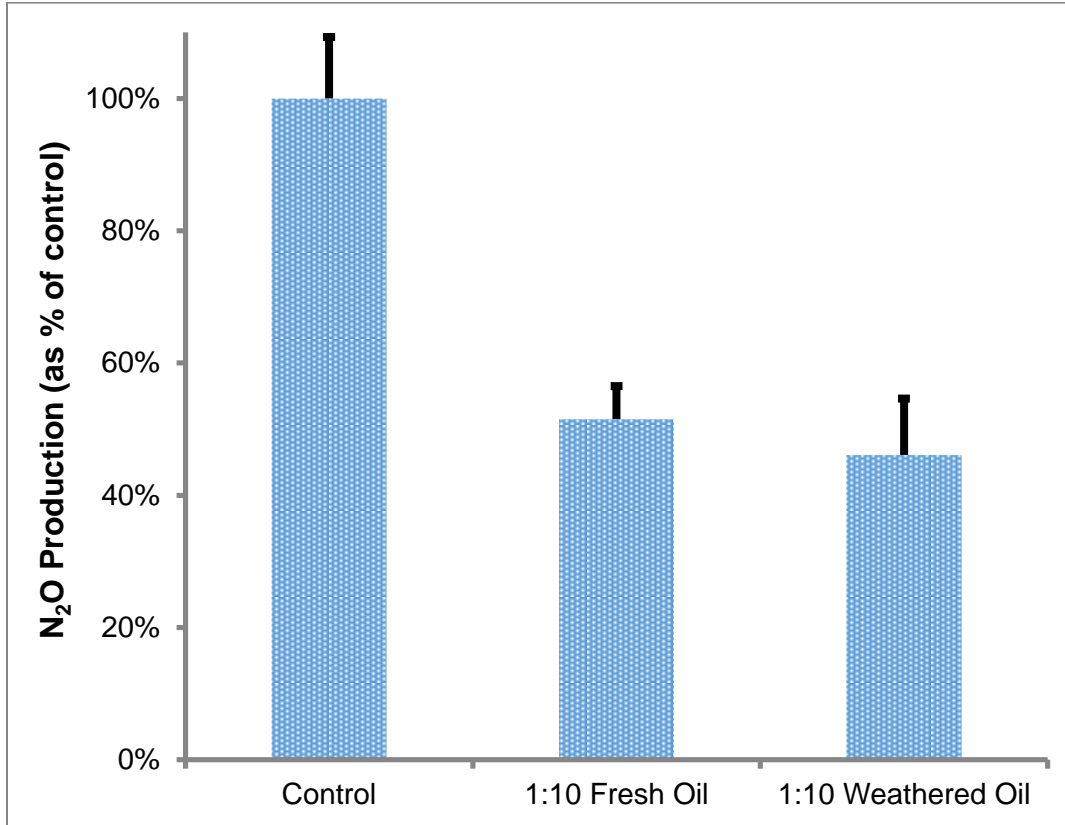


Figure 2.5 The N₂O production as percent of the control (+ 1 standard error) for weathered crude oil and non-weathered crude oil in 10% crude oil:wet soil, after initial exposure.

2.3.4 Fresh and Weathered Crude Oil Effects on Potential Denitrification – Longer term

The control samples denitrification rates averaged of $5.9 \pm 0.3 \text{ mg N}_2\text{O kg}^{-1} \text{ hr}^{-1}$. The 1:10 ratio of weathered crude oil:wet soil weight potential denitrification rate averaged $3.7 \pm 0.9 \text{ mg N}_2\text{O kg}^{-1} \text{ hr}^{-1}$ which was $62 \pm 8.0\%$ of the control. The 1:10 ratio of fresh crude oil:wet soil weight average denitrification rate was significantly lower at $0.6 \pm 0.01 \text{ mg N}_2\text{O kg}^{-1} \text{ hr}^{-1}$ and just $10.9 \pm 1.1\%$ of the control (Figure 2.6). Results suggest that the 2-week pre-incubation to higher concentrations of volatile and potentially more toxic compounds present in the fresh crude oil caused a dramatic

decrease in denitrification potential compared to both the control and weathered crude oil. In addition, the denitrification rate, as a percent of the control, for the weathered crude oil exposed for 2 weeks time suggests the microbial activity was possibly recovering when compared to the immediate exposure experiment (Figure 2.7). However, longer term experiments are needed in concern with molecular techniques to determine if there is a possible shift in microbial population, or is the same microbial community simply recovering.

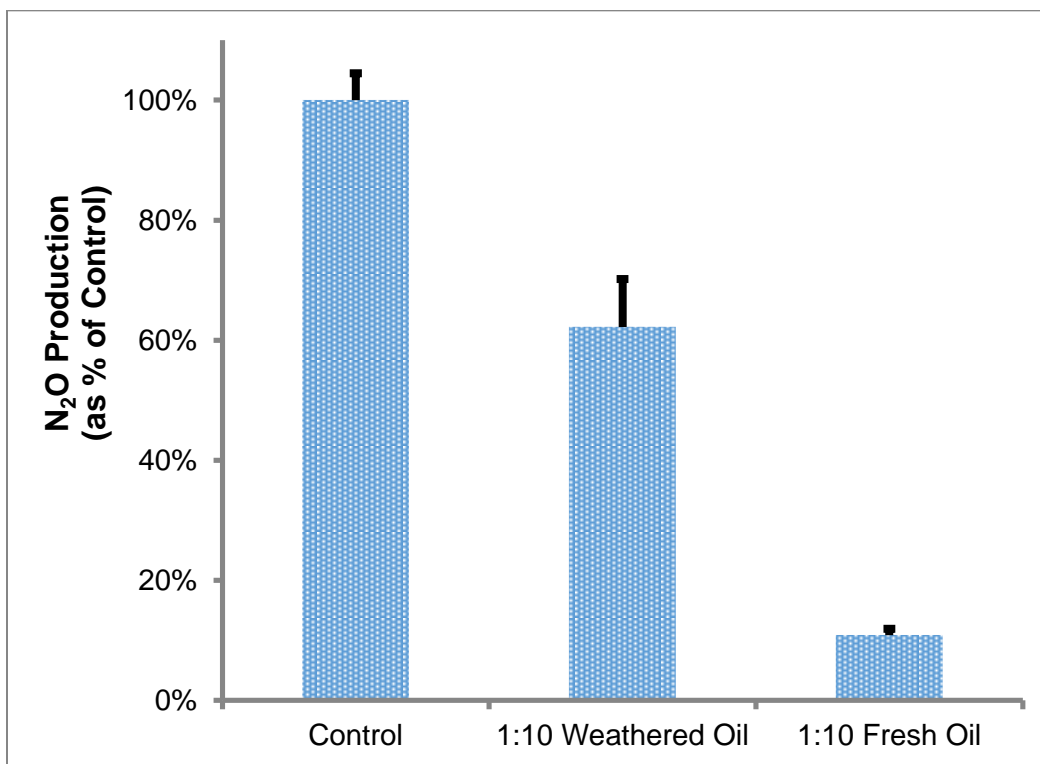


Figure 2.6 The N₂O production as percent of the control (+ 1 standard error) for weathered crude oil and non-weathered crude oil in 10% crude oil:wet soil, following 2 week incubation period.

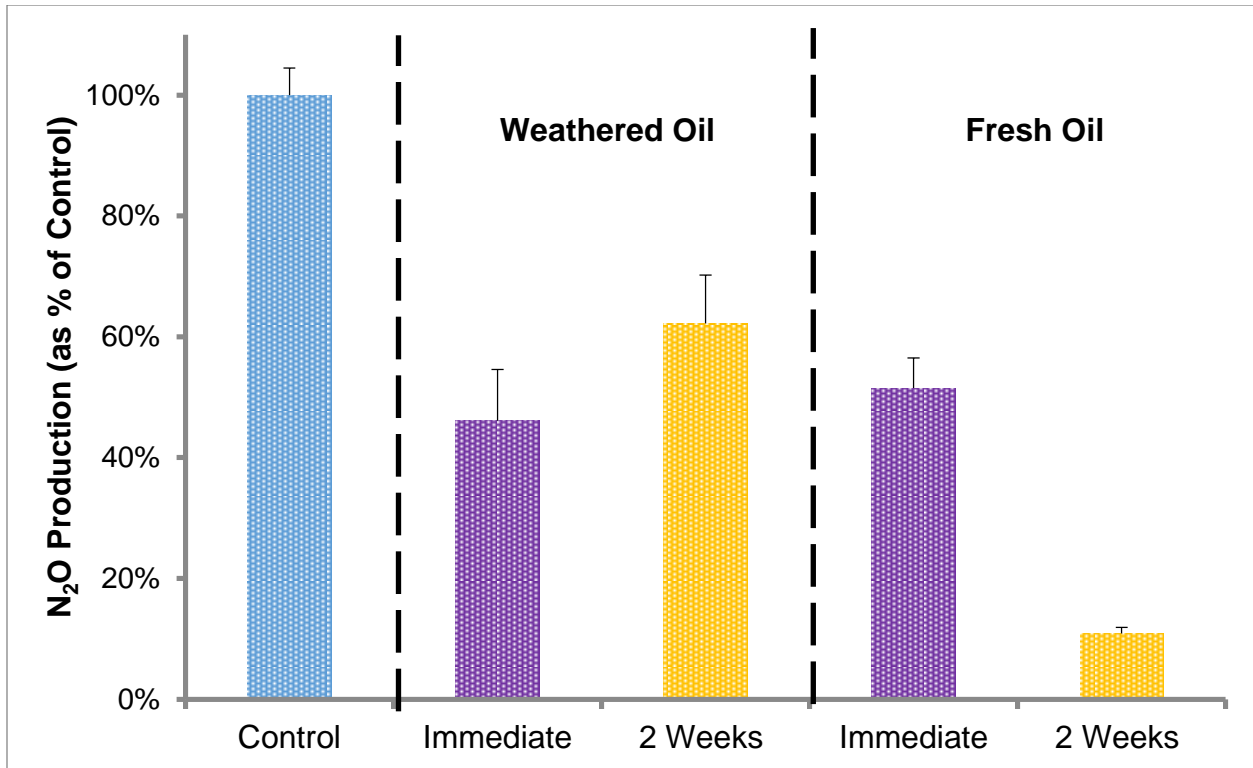


Figure 2.7 Comparison of N₂O production as percent of the control (+ 1 standard error) for weathered crude oil and non-weathered crude oil in 10% crude oil:wet soil, following immediate and 2 week incubation period.

2.4 Environmental Implications

Results from this experiment suggest that an crude oil spill at close proximity to the coastal wetlands can have a significantly greater impact, due to less weathering, on the wetland soil microbial processes over the longer term than an oil spill more distally located, which would incur greater weathering time. Regardless of the weathering status of the oil there appeared to be a similar impact immediately suppressing denitrification rates with negative impacts of fresh crude oil increasing over time. While any fresh crude oil spilled will undergo volatilization at the surface, crude oil spilled in the highly porous coastal marsh peat has the potential to infiltrate into the pore-space, adhering to the organic fabric of the soil. In this circumstance, the volatilization process

could potentially be retarded and some of alkanes and cyclic hydrocarbons in the light fractions of the fresh crude oil could exert toxicity (Van Hamme et al., 2003).

Additionally, crude oil impacting coastal marshes is also susceptible to being buried in the soil profile under the relatively high accretion rates found in these systems. The author found crude oil in the Barataria Basin buried several cm in the soil profile now 3 years after the BP DWH spill, which suggests the microbial communities active in the surface soil can potentially be exposed to crude oil compounds for years after oil spills occurred.

Previous research found that crude oil does affect soil microbial communities controlling nutrient mineralization and thereby can regulate vegetative productivity and energy flow through the food web (Pezeshki et al., 2000). These results stress potential implications with negative effects of oiling to soil microbial communities and processes. If toxic compounds found in crude oil inhibit bacterial decomposition of organic matter and associated nutrient remineralization, then plant growth might be retarded (Pezeshki et al., 2000).

This is the first study the author is aware of demonstrating that exposure to fresh crude oil can significantly affect denitrification in LA's coastal marshes, a function linked to the ecosystem service of water quality improvement. Barataria basin receives nitrate input from two primary sources. The first is the Davis Pond diversion from the north (Gardner and White, 2010). The Davis Pond diversion is intended to help restore LA's coastal wetlands by reintroducing Mississippi River water to Barataria Basin and lowering salinities. The river water contains high amounts of nitrate (up to 2 mg N L^{-1}) in the springtime which is reduced through the denitrification process in the coastal

wetlands (Gardner and White, 2010). The second source is high nitrate water within the Mississippi River coastal plume entering the Barataria estuary through coastal inlets driven by tidal and wind forcing (Li et al., 2009; Li et al., 2011). These same forces also transported the crude oil into the estuary leading to the consequent oiling of a number of coastal marshes (Figure 2.2). Future sediment diversions into Barataria Basin have been scheduled for completion by fall 2021 and would have the capacity to divert >5 times the amount of nitrate-rich freshwater into the basin than the Davis pond diversion. Therefore, the denitrification process in the coastal wetlands of the basin are an important ecosystem function for removing excess bioavailable N in the water column (Yu et al., 2006; Delaune et al., 2005) which can minimize potential algal blooms. A recent study in the Lake Pontchartrain estuary demonstrated that relatively little denitrification occurs in the open water bottom sediment (Roy et al., 2013). This finding was verified by VanZomeren et al, (2013) in the Breton Sound coastal marshes where the marsh soils were found to have a denitrification rate 18 times greater than the bottom sediments. Therefore, denitrification in marsh soils is important for removing nitrate in order to diminish the potential for expressions of eutrophication (Bargu et al., 2011).

2.5 Conclusion

Reduction of surface water concentrations of nitrate in coastal and marine environments depends on the ability of denitrifying bacteria in soils and sediment to reduce nitrate for respiration. Louisiana light sweet crude oil was shown to have a toxic effect on denitrification rates in southern LA coastal marsh soil, reducing denitrification to 50% of the control during initial exposure, and up to 90% of the control following 2

week exposure, the extent of the effects was dependent upon weathering of the crude oil. The immediate exposure of both fresh and weathered crude oil was not significantly different on wetland soil denitrification rates, but fresh crude oil effects were greater after a longer term (2 week) exposure. This result is most likely due to the higher concentration of light fraction carbon compounds found within fresh crude oil. Consequently, crude oil spills that take place proximal to or within the onshore region have a potential greater impact over time. These results demonstrate the potential for crude oil in both the short-term and longer-term to negatively affect the wetland soil microbial pool, and in this case, negatively impacting an important ecosystem function of water quality improvement through nitrate removal. Additional studies should be focused on investigating longer term exposure effects and changes in the microbial consortia as a result of oiling.

CHAPTER 3: EFFECT OF DISPERSANT USE FOR OIL SPILL REMEDIATION ON N CYCLING IN LOUISIANA COASTAL MARSH SOIL

3.1 Introduction

On April 20, 2010, the BP Deepwater Horizon (DWH) oil platform experienced a catastrophic explosion which triggered the largest marine crude oil spill in US history with an estimated average release rate of 60,000 barrels per day (McNutt et al., 2012) for the 87-day event (Paris et al., 2012). In total, approximately 795 million liters (5 million barrels) of crude oil were released from the seabed into the Gulf of Mexico (Natter et al., 2012), 90 km offshore of the Mississippi River Delta (Klemas, 2010). Much of the south Louisiana (LA) light crude oil rose to the surface and washed ashore in the states of Louisiana, Alabama, Mississippi, Texas, and Florida driven by wind, waves, tides, and currents (Levy and Gopalakrishnan, 2010).

One of the strategies used during the BP DWH oil spill to mitigate the environmental impacts and safety concerns of the crude oil was the application of dispersant, Corexit EC9500A. Dispersant solutions are a combination of hydrocarbon-based solvents and surfactants, with the purpose of lowering the interfacial tension between oil and water (Kujawinski et al., 2011). The rationale for using surfactants was to increase surface area of the oil in order to increase the degradation rate and to prevent surface oil slicks from reaching land (Baker, 1995). Corexit EC9500A is a blend of oxyalkylate polymers, organic sulfonic acid salt, substituted fatty ester, glycol ether, and aliphatic hydrocarbons (Utley, 1998). There is also the potential for reaction byproducts of Corexit EC9500A to form that are not listed in the dispersant ingredients (Place et al., 2010). A total of ~ 7.9 million liters of the dispersant, Corexit EC9500A, was used during the BP DWH oil spill between May 15th and July 12th 2010 (Kujawinski

et al., 2011). The applications were split with ~ 5.3 million liters applied to the surface and ~ 2.9 million liters dispensed at the wellhead (Kujawinski et al., 2011). The dispersant was applied aerially by small vessels at the surface and by sprayer jet at the well head (Kujawinski et al., 2011). The rationale for surfactant application at the surface was to break up large surface crude oil slicks, while the use at the well head, located 1,522 m underwater, was to prevent the crude oil from reaching the surface and becoming a slick (Paris et al., 2012).

The anionic surfactant present in Corexit EC9500A, dioctyl sodium sulfosuccinate (DOSS) (Place et al., 2010) was measured in the water column during the BP DWH oil spill and results showed that concentrations varied widely among sampling locations (Kujawinski et al., 2011). The applied volume of Corexit EC9500A also varied temporally from approximately 13,000 up to 80,000 L d⁻¹ (Kujawinski et al., 2011). There was an estimated average daily Corexit EC9500A application of 47,000 liters, based on the concentration of the anionic surfactant, dioctyl sodium sulfosuccinate, in surface waters (Kujawinski et al., 2011).

Dispersants are not likely to be deployed directly into coastal marshes since there is minimal water to aid in the dilution of the dispersed crude oil (Pezeshki et al., 2010). However, marsh plants and soil can be exposed to both dispersants and dispersed crude oil as a result of tidal exchange and storm driven surge (Li et al., 2011; Pezeshki et al., 2010). Use of dispersants in close proximity to coastal marshes occurs when an oil slick is approaching a shoreline and the only logistically practical response is often aerial dispersant spraying (Baker, 1995).

Toxicity tests of Corexit EC9500A have been conducted on invertebrates and adult fish showing no significant deleterious effects at environmental concentrations, but there has been a paucity of research on the effects of dispersants on wetland soil microbial communities (Hamdan and Fulmer, 2011). Microbes facilitate many biogeochemical processes in coastal wetlands and estuaries, including organic matter decomposition, water quality improvements, and are essential regulators of the base of the detrital food chain. The nitrogen cycle in wetland systems is controlled by the microbial consortia. Nitrogen mineralization is a process in which heterotrophic microbes play a key role in the oxidation of complex organic compounds and regeneration of available nitrogen. This process regulates primary productivity in wetlands and is therefore linked to the ecosystem services of providing critical habitat and contributing to carbon sequestration. A specialized functional microbial group, the denitrifiers, reduce bioavailable nitrate through their respiratory pathway by converting nitrate (NO_3^-) to nitrous oxide (N_2O) and nitrogen gas (N_2) (Reddy and DeLaune, 2008; Tiedje et al., 1982). Denitrification, therefore, provides an important ecosystem service to coastal wetlands by improving water quality by removing bioavailable N and returning it to the atmosphere. This excess nitrate in coastal waters can promote large phytoplankton blooms which contribute to coastal hypoxia and anoxia (Turner et al., 2008).

There have been few studies published on the effects of dispersants on microbial community profiles (Hamdan and Fulmer, 2011; Nyman et al., 2007) and to our knowledge, there are no published investigations on the effects of dispersant loading on denitrification or N mineralization rates of coastal marsh soils. Therefore, the goal of

our study was to determine the effects of a range of Corexit EC9500A additions on two important N cycling processes in coastal wetlands, N mineralization and denitrification. In addition, the author examined the effect of surfactant additions on the total microbial biomass of the soil as the microbial populations.

3.2 Materials and Methods

3.2.1 Study Site

Barataria Basin experiences one of the greatest coastal wetland loss rates on the Gulf of Mexico coastline at $16.9 \text{ km}^2 \text{ yr}^{-1}$ (FitzGerald et al., 2007). The Barataria bay complex is an interdistributary estuary bordered by the Mississippi River on the east and by Bayou Lafourche on the west. The estuarine system is shallow and turbid with an average depth of about 2 m (Happ et al., 1977). The estuary experiences a 30 cm diurnal lunar tide, although wind driven tides often cause greater water level changes (Happ et al., 1977). Marsh soil samples were collected on April 8, 2013 from a *Spartina alterniflora* marsh site within Wilkinson Bay in the Barataria Basin, LA (Figure 3.1; N29° 27,976 W89° 56,072). Several marshes in this basin received light to heavy oiling when coastal water circulation and onshore winds coupled with tidal action, pushed the BP DWH oil into the estuary (Lin and Mendelssohn, 2012). The marsh soil collection site was 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 and is located approximately 6.4 km northwest of Bay Jimmy, which was heavily oiled by the spill (Natter et al. 2012) and contains identical plant communities (Figure 3.2; National Oceanography and Atmospheric Administration, 2013). The marsh sites deemed oiled were not chosen for sampling due to the uncertainty of the time and amount of exposure

to dispersant concentrations. Soil from a non-oiled site allowed controlled additions of dispersant to be added in the lab.

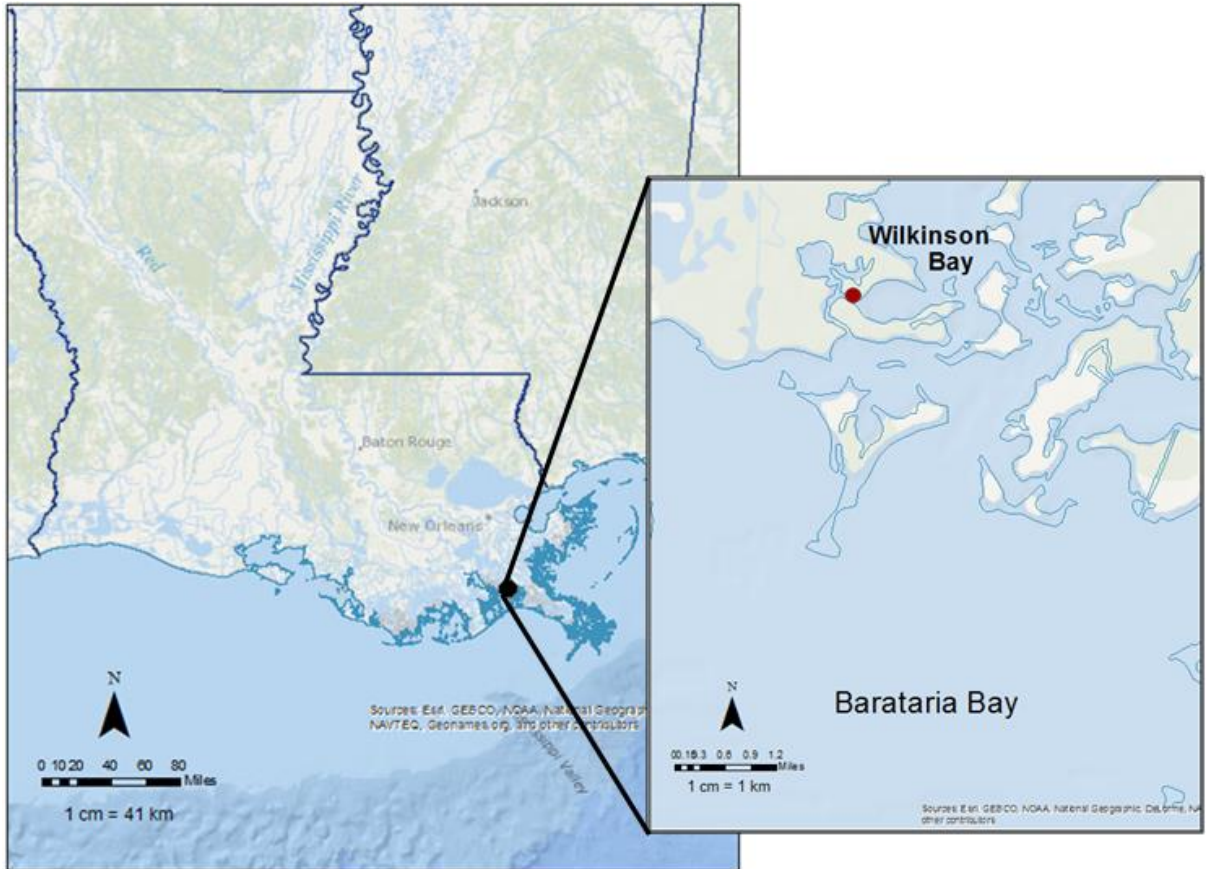


Figure 3.1 Location of the Barataria Bay complex within coastal Louisiana. Inset map denotes the Wilkinson Bay sampling site, located within Barataria Bay (Arc GIS Base Map and National Oceanic and Atmospheric Administration, 2013).

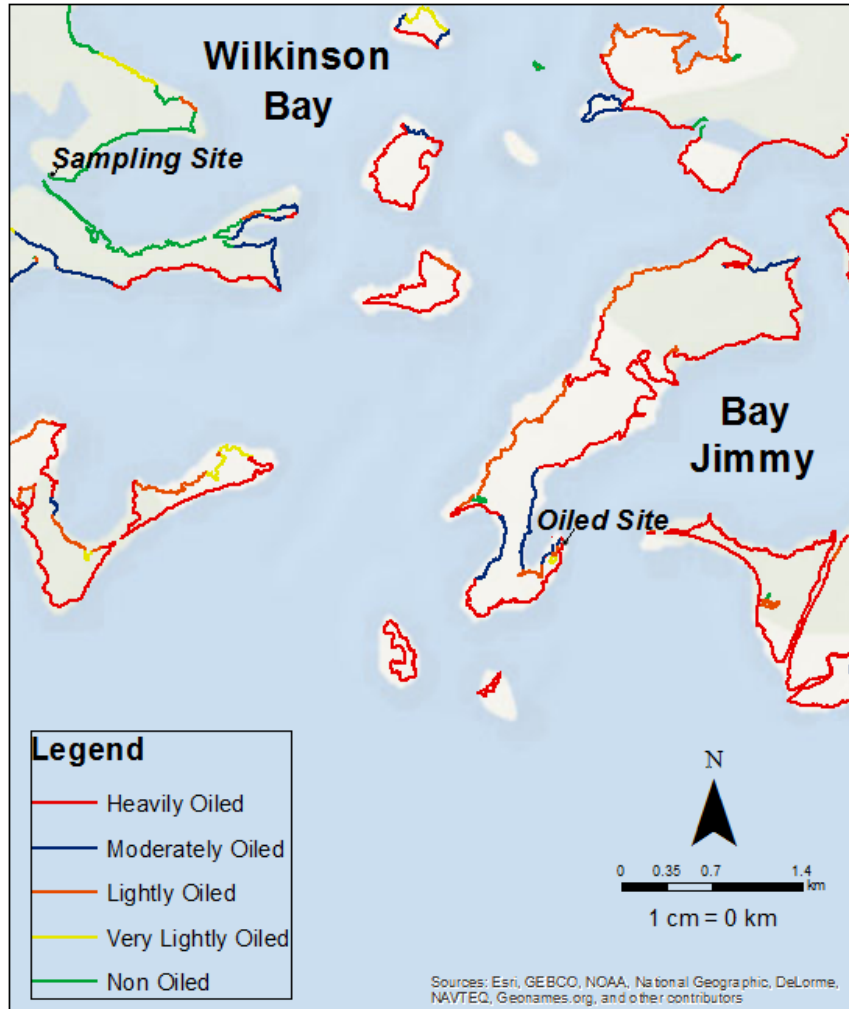


Figure 3.2 Distribution of observed maximum oiling of heavily, moderately, lightly, very lightly, and non-oiled coastal marshes in Wilkinson Bay and Bay Jimmy located within the Barataria Bay complex compiled by NOAA (Arc GIS Base Map and National Oceanic and Atmospheric Administration, 2013). The unoiled sampling site and an observed heavily oiled site are also labeled on the map.

3.2.2 Marsh Soil Sampling

Wetland soil was collected from three 3 x 3 m quads within the selected *Spartina alterniflora* salt marsh. Within each of the 3 x 3 m quads, four replicate 10 cm long (7 cm diameter) cores were taken by push-core. Samples were placed on ice until returned to the lab where they were stored at 4° C. The top 10 cm of marsh soil was collected since this top interval of soil is the volume of soil which is most likely to be

impacted by dispersant exposure and is the soil interval where the majority of denitrification and mineralization has been found in other LA coastal basin wetland sites (Gardner and White, 2010 and VanZomerem et al., 2013).

3.2.3 Soil Characteristics

Soil samples were analyzed for moisture content, bulk density, weight % organic matter, particle size distribution, soil pore water salinity, total carbon (C), nitrogen (N), phosphorus (P), and microbial biomass N. Gravimetric moisture content was determined by placing homogenized field moist marsh soil subsamples in a forced air oven at 70°C until constant weight was reached. Bulk density was calculated for the volume of the collected soil intervals and expressed on a dry weight basis. Weight % organic matter content was determined as loss of ignition (LOI) using ash weight divided by pre-burned marsh soil weight (White and Reddy, 1999). Particle size analysis was determined by Stoke's Law using the hydrometer method (Patrick, 1958). Salinity of the pore water was determined from 40 g field moist samples combined with DI water at a 1:1 ratio. The salinity was determined using a conductivity probe (Fisher Scientific Accumet Basic AB30), measured in $\mu\text{Siemens cm}^{-1}$, corrected for dilution and converted to part per thousand (ppt) as total dissolved solids (TDS). Total C and N were measured on dried, ground subsamples of soil using an elemental combustion system with a detection limit of 0.005 g kg^{-1} (Costech Analytical Technologies, Valencia, CA). Solid-phase total P analysis involved combustion of oven-dried, ground subsamples at 550°C for 4 hr in a muffle furnace and subsequent dissolution of the ash in 6 M HCl on a hot plate (Mckerche and Anderson, 1968). Concentrations of P were then measured

colorimetrically using a Seal Analytical AQ2 discrete analyzer (Method 351.2; U. S. EPA, 1983).

3.2.4 Microbial Biomass N (MBN)

Soils were exposed to the surfactant at Corexit:wet soil ratios of 0:10, 1:10, 1:100, 1:1,000 and 1:10,000 for 5 days under anaerobic conditions, after which Microbial Biomass N (MBN) was measured. The MBN was determined using the chloroform fumigation extraction method (Brookes et al. 1985) with modifications by White and Reddy (2000) by extracting a set of controls with 25 ml of 0.5 M K_2SO_4 at a ratio of approximately 1:50 (g dry soil:extractant). A duplicate set of samples underwent chloroform fumigation in a vacuum-sealed glass desiccator containing a 100 mL beaker with chloroform over a 24 hour period. Samples were centrifuged for 10 min at 4000 G, vacuum-filtered through Whatman #42 filters and analyzed using a Shimadzu TOC-V CSN Analyzer (White and Reddy, 2003).

3.2.5 Potentially Mineralizable Nitrogen (PMN)

The PMN assay provides a measure of the net anaerobic N mineralization (ammonification) rates. Soils were exposed to surfactant at Corexit:wet soil ratios (0:10, 1:10, 1:100, 1:1,000 and 1:10,000) and triplicates were incubated at 40° C under anaerobic conditions in the dark for both 1 and 5 day sample sets (White and Reddy, 2000). Following the incubation, 20 mL of 2.0 M KCL were added to each serum bottle and agitated on a reciprocating shaker for 1 hour. Samples were then centrifuged for 10 minutes at 4000 G followed by filtration of the supernatant through Whatman #41 filter paper, acidified to a pH < 2 and stored at 4°C until NH_4 -N analysis by AQ2

Automated Discrete Analyzer (SEAL Analytical Inc., Mequon, Wisconsin) (EPA Method 351.2; USEPA, 1983).

3.2.6 Potential Denitrification

Potential denitrification rates were determined using the acetylene block method (Yoshinari and Knowles, 1976). Five g of field moist soil were placed in 160 mL serum bottles. Four replicates were assigned to each of the 5 ratios of Corexit EC9500A to field moist soil weight (0:10, 1:10, 1:100, 1:1,000, 1:10,000). The serum bottles were sealed with aluminum crimps and the headspace air was replaced with 99.99 O₂-free N₂ gas. A saline solution, (12.7 ppt), matching the salinity of the site water, was prepared using Instant Ocean™ and purged with 99.99% pure N₂ gas to remove all oxygen from the water. Five mL of saline solution was then added to each serum bottle. Sixteen mL of head space was removed from each serum bottle representing 10% of the total headspace of the vial and replaced with acetylene. Potassium nitrate (KNO₃) and glucose monohydrate (C₆H₁₂O₆ x H₂O) were used to make a 100 mg NO₃-N L⁻¹ N and glucose-C solution. Five mL of N₂-purged solution was added to each serum vial to promote maximum denitrification potential. The bottles were agitated continuously on a longitudinal shaker in the dark at 25° C. Gas samples were extracted at 0, 24, and 48 hours and N₂O was measured on a Shimadzu GC-8A equipped with an electron capture detector. The GC detection limit was 0.006 µg N₂O-N kg⁻¹ hr⁻¹. The Bunsen adsorption coefficient was used to calculate N₂O dissolved in the aqueous phase. Denitrification rates were calculated by linear regression of N₂O concentrations over time with the maximum rates generally observed between 24 and 48 hrs, corresponding to an exponential increase in microbial activity.

A second set of samples were prepared for potential denitrification following the aforementioned procedure with the following modifications; a) there were only 3 Corexit treatment levels (0:10, 1:10, 1:100), b) the soil was pre-incubated with Corexit for 2 weeks, and c) during the incubation, 1 mL of 10 mg NO₃-N L⁻¹ nitrate solution was added on days 1 and 7 to provide a substrate for the denitrifying microbial community. Following the 2 week pre-incubation period, potential denitrification was measured over 48 hr to determine the effects of longer-term exposure of Corexit on this critical wetland microbial process.

3.2.7 Data Analysis

Potential denitrification and N mineralization rates were determined by linear regression between each time point, with mean and standard deviation calculated from the replicates. Student t- tests were performed to determine significant differences ($p < 0.05$) between Corexit additions for potential denitrification and PMN rates.

3.3 Results

3.3.1 Soil characterization

Soils were characterized by high weight percentage moisture content averaging $79.8 \pm 0.01\%$ and low dry weight bulk densities of $0.27 \pm 0.01 \text{ g cm}^{-3}$ for the 0-10 cm soil interval (Table 3.1). The average LOI (organic matter content) was $26.1 \pm 0.01\%$. Particle size was dominated by the silt + clay fraction with low weight % sand (10.3% sand, 46.2% silt, and 43.6% clay) and classified as a silty clay (Gerrard, 2000). Porewater salinity was $13 \pm 1.3 \text{ g kg}^{-1}$ total dissolved solids. Soil total C and N averaged $121 \pm 5.7 \text{ g C kg}^{-1}$ and $6.7 \pm 0.1 \text{ g N kg}^{-1}$, respectively, while the total P concentration averaged $553 \pm 9.5 \text{ mg P kg}^{-1}$. While the soil characteristics are

significantly different for values seen in the organic freshwater marshes of the upper Barataria basin (Kral et al. 2012), they are statistically similar to those measured in adjacent salt marshes of Barataria Bay (Delaune et al., 1979; Dodla et al., 2012), suggesting these soils are representative of other LA coastal salt marshes.

Table 3.1 Wilkinson Bay soil physiochemical properties. Data are means \pm 1 standard error, when n=4.

Parameter	Value
Moisture Content (weight %)	79 \pm 0.01
Bulk Density (g cm ⁻³)	0.27 \pm 0.01
Organic Matter (weight %)	26.1 \pm 0.01
Sand (weight of mineral fraction%)	10.3
Silt (weight of mineral fraction%)	46.2
Clay (weight of mineral fraction%)	43.5
Pore Water Salinity (g kg ⁻¹ TDS)	13 \pm 1.3
Total Carbon (g kg ⁻¹)	121 \pm 5.7
Total Phosphorus (mg kg ⁻¹)	553 \pm 9.5
Total Nitrogen (g kg ⁻¹)	6.7 \pm 0.1
Microbial Biomass N (mg kg ⁻¹)	56 \pm 1.7

TDS, total dissolved solids
n, number of replicates

3.3.2 Microbial Biomass Nitrogen

The average rate for the control soils of the longer term experiment were 13.7 \pm 5.5 mg N kg⁻¹ (Table 3.2). The exposure to Corexit after 5 days led to microbial biomass N values below detection limits for 1:10, 1:100, and 1:1,000 dispersant:wet soil additions suggesting the higher concentrations of dispersant led to significant cell

disruption of the microbial consortia. The 1:10,000 treatment was not significantly different from that of the control.

Table 3.2 Microbial biomass N and potentially mineralizable nitrogen production of Louisiana coastal marsh soil with a range of Corexit additions. Dispersant ratios range from 1:10 to 1:10,000 (Corexit : wet sediment weight). Data shown are mean values \pm 1 standard error (n=4)

Corexit Additions (Corexit:wet soil)	Microbial Biomass (mg N kg ⁻¹)	Potentially Mineralizable N (NH ₄ ⁺ mg kg ⁻¹ d ⁻¹)
1:10	b.d	-0.66 \pm 0.36 ^a
1:100	b.d	6.03 \pm 1.42 ^b
1:1,000	b.d	11.4 \pm 0.87 ^c
1:10,000	20 \pm 14.6 ^a	12.5 \pm 1.27 ^c
Control	13.7 \pm 5.5 ^a	11.6 \pm 1.89 ^c

b.d = below detection limits

a,b,c letters indicate significant differences between rates

3.3.3 Potentially Mineralizable Nitrogen

The mean PMN rate of the controls from days 1 to 5 was 11.63 \pm 1.9 NH₄⁺ mg kg⁻¹ d⁻¹ (Table 3.2). The 1:100 addition of Corexit:wet soil was significantly lower at 6.0 \pm 1.4 NH₄⁺ mg kg⁻¹ d⁻¹, and the 1:10 Corexit:wet soil addition had no detectable PMN rate. The 1:1,000 and 1:10,000 treatments were not significantly different from that of the controls. These results demonstrate that there is a reduction in N mineralization rates with increased Corexit additions, showing the negative impacts of Corexit on the soil microbial activity.

3.3.4 Potential Denitrification – Immediate Corexit additions

The mean (n=4) maximum denitrification rates of the control samples was 9.3 \pm 0.3 mg N₂O-N kg⁻¹ hr⁻¹ (Figure 3.3). The addition of dispersant, Corexit EC9500A,

significantly negatively impacted maximum denitrification rates for most treatments. The largest loading rate (1:10) produced no measureable denitrification over 48 h. The 1:100 treatments had a mean denitrification rate of $0.7 \pm 0.4 \text{ mg N}_2\text{O-N kg}^{-1} \text{ hr}^{-1}$ which was $7.6 \pm 2.7\%$ of the control (Figure 3.4). The 1:1,000 treatments had a mean denitrification rate of $3.1 \pm 0.6 \text{ mg N}_2\text{O-N kg}^{-1} \text{ hr}^{-1}$ which was $33 \pm 4.3\%$ of the denitrification rate of the controls. The smallest addition at 1:10,000 had denitrification rates not significantly different from the control at $9.0 \pm 0.7 \text{ mg N}_2\text{O-N kg}^{-1} \text{ hr}^{-1}$. Consequently, the critical loading of Corexit EC9500A on microbial denitrification for these Barataria Bay coastal marsh soils is between 1:1,000 and 1:10,000.

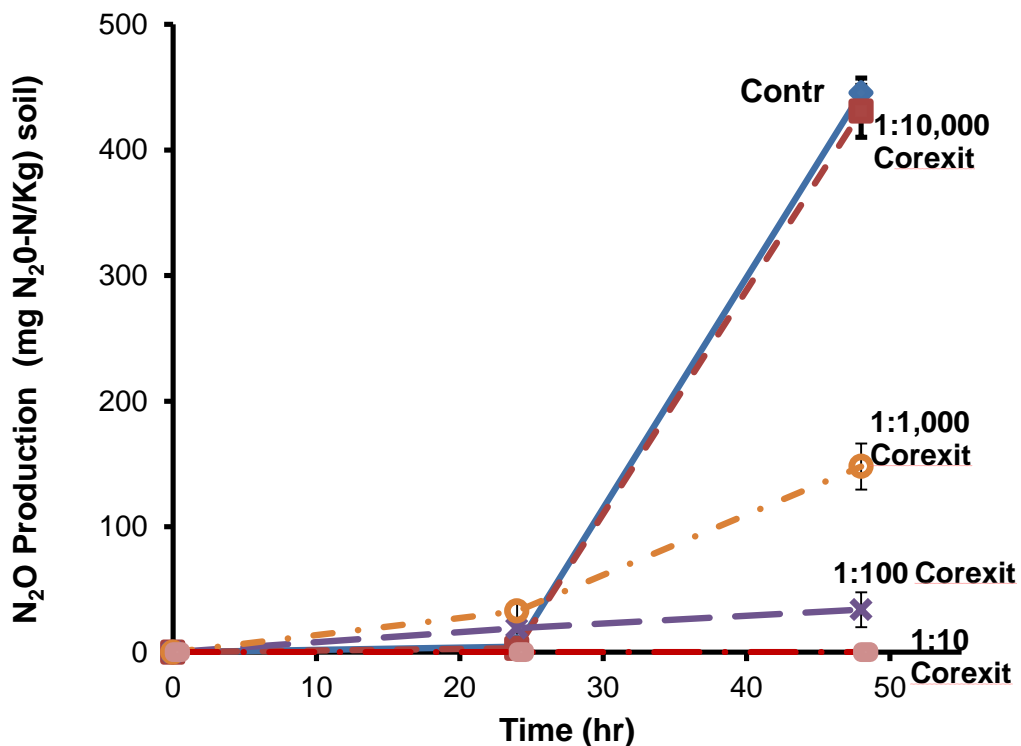


Figure 3.3 The N_2O production potential of Louisiana coastal marsh soil N_2O production with a range of Corexit additions. Dispersant ratios range from 1:10 to 1:10,000 (Corexit : wet sediment weight). Data shown are mean values ± 1 standard error ($n = 4$).

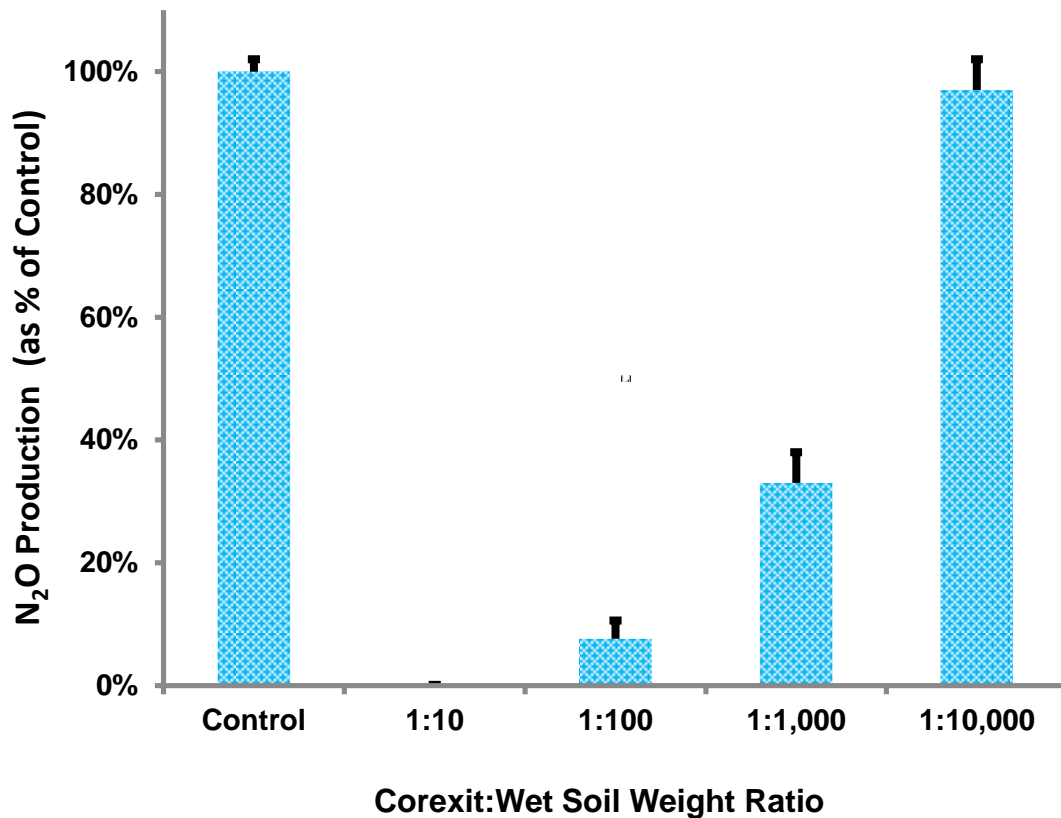


Figure 3.4 The N₂O Production as percent of the control (+ 1 standard error) for each range of Corexit EC9500A additions following immediate exposure.

3.3.5 Potential Denitrification Assay – Two week pre-incubation with Corexit

Following a 2 week exposure period, the 1:10 treatment still resulted in non-detectable denitrification (Figure 3.5). The 1:100 ratio of Corexit:wet soil weight potential denitrification rate was $12 \pm 2.6\%$ of the control. These results suggest that the denitrifiers are not able to adapt to exposure to the surfactant over the short term.

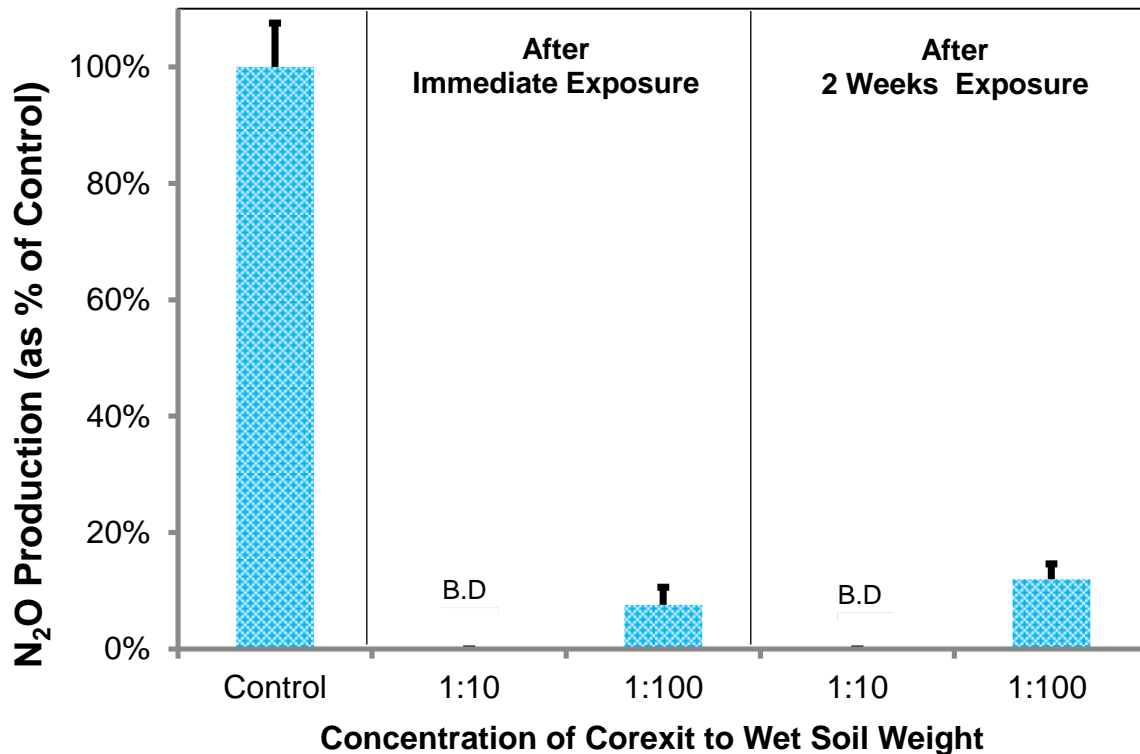


Figure 3.5 The N₂O production as percent of the control (+ 1 standard error) for concentration range of Corexit EC9500A observed during the BP DWH spill following immediate and 2 weeks exposure. (B.D = below detection limit)

3.4 Discussion

This is the first study the author is aware of which demonstrates that exposure to the dispersant, Corexit EC9500A, can significantly affect two important microbial-mediated N cycling processes in coastal marshes, N mineralization and denitrification. The N mineralization rates of the soil mediate primary production by increasing bioavailable N which stimulates plant growth. This process is therefore linked to important ecosystem functions including carbon sequestration, habitat value, and food web connection to higher trophic levels. The process of denitrification is linked to the ecosystem function of water quality improvement. Water with high nitrate concentration within the Mississippi River coastal plume has previously been documented entering the

Barataria Bay estuary through tidal and wind forcing (Li et al., 2009; Li et al., 2011). These same forces were also responsible for transporting crude oil and associated dispersant into the estuary from the BP DWH spill. Denitrification in the coastal wetlands of the bay is critical for removing excess bioavailable N in the water column, which could trigger algal blooms. A recent study in the Lake Pontchartrain estuary demonstrated that little denitrification occurs in the open water bottom sediment of the estuary (Roy et al. 2013). Therefore, the vegetated marsh denitrification is important for removing nitrate in order to diminish the potential for associated algal blooms (Bargu et al., 2011).

Corexit EC9500A has previously been reported to negatively affect microbial community populations (Hamdan and Fulmer, 2011) and our findings corroborate these results. The Hamdan and Fulmer (2011) study reported that likely Corexit:water concentrations encountered during the BP DWH spill ranged between 1:10 to 1:100 mg ml⁻¹ and that these levels resulted in near total cell death for all isolates in water ($\geq 99\%$). In our study, microbial biomass and N mineralization were below detection limits at those concentrations, while denitrification was diminished to $7.6 \pm 2.7\%$ of the control for the 1:100 treatment after immediate exposure and $12 \pm 2.6\%$ after 2 weeks exposure. A comparison of these results suggests that the soil may provide some protection to the native microbial denitrifiers due to sorption of the surfactant with the native organic matter at the 1:100 level. The corroboration between the results of our study and the study by Hamdan and Fulmer (2011) clearly demonstrate a similar trend of deleterious effects of Corexit EC9500A on ecologically important microbial communities as a consequence of oil spill remediation.

In LA there are ~ 1,500 oil spill notifications per year to the National Response Center with an average annual total volume of 1.25 million L (7862 barrels) yr⁻¹ (source: Louisiana Oil Spill Coordinator's Office). There are over 3,800 active production platforms in the Gulf of Mexico and the majority is located directly off the LA coast. Remediation of oil spills into the future will likely be required in coastal LA as long as oil extraction, transportation and refining activities continue. It is therefore important to quantify the longer term impact of surfactants used in remediation of spills on microbial mediated ecosystem services of coastal wetlands.

3.5 Conclusion

The N cycling processes of mineralization and denitrification are important for ecological and environmental quality, as the former stimulates primary productivity and the latter provides for water quality improvement by reducing bioavailable N concentrations. The exposure to the surfactant Corexit EC9500A, used for oil spill remediation, led to microbial biomass N values below detection limit for 1:10 and 1:100 Corexit:wet soil additions. The mean PMN rates for 1:100 addition of Corexit:wet soil were $52 \pm 12.2\%$ of the controls and the 1:10 Corexit:wet soil addition had no detectable rate suggesting Corexit is toxic to this heterotrophic microbial pool. Denitrification rates following immediate exposure to the 1:10 Corexit:wet soil additions resulted in no measureable denitrification and the 1:100 treatments had a mean denitrification rate of $0.7 \pm 0.4 \text{ mg N}_2\text{O-N kg}^{-1} \text{ hr}^{-1}$ which was $7.6 \pm 2.7\%$ of the control. Following a 2 week pre-incubation exposure, the 1:10 ratio of Corexit:wet soil weight still resulted in non-detectable denitrification and the 1:100 treatment was $12 \pm 2.6\%$ of the control. These results demonstrate the potential for dispersants, following immediate and 2 weeks

exposure, to negatively affect important wetland soil microbial processes linked to critical ecosystems functions. Future work should focus on the longer-term impacts of Corexit exposure on the microbial populations and processes as well as changes to the microbial consortia with time to determine if they will rebound, and if so when.

CHAPTER 4: CONCLUSION

The BP DWH oil spill was one of the most catastrophic crude oil spills in US history, not only taking the lives of nine men, but also releasing ~5 million barrels of Southern Louisiana (LA) Light Sweet crude oil into the Gulf of Mexico, ~90 km offshore from the Mississippi River Delta. The crude oil itself was not the only environmentally hazardous substance entering the Gulf of Mexico waters as 7.9 million liters of chemical dispersants were applied during restoration efforts. Hamdan and Falmer, (2011), reported likely Corexit:water concentrations to be encountered during the BP DWH spill ranged between 1:10 to 1:100 mg ml⁻¹ Corexit:water. The combination of crude oil and crude oil dispersant washed ashore in the states of Louisiana, Alabama, Mississippi, Texas, and Florida. The location of an crude oil spill (onshore vs offshore) can determine the characteristics of the oil that impacts the coastal system. The amount of time the crude oil is in contact with the atmosphere can change both its physical and chemical composition through the fractionation process. Fractionation, which is a major part of the weathering process, results in the loss of the lighter, more volatile, smaller carbon chain fractions to the atmosphere.

Even four years after the spill the extent of the environmental impact is not fully known. Though some of the effects are known, little is known about the impacts crude oil and dispersants have on marsh soil microbial processes. Microbial processes play a major role in the ecological functions of coastal marshes. Two important microbial processes to ecosystem functions and services include denitrification and nitrogen mineralization. Denitrifying bacteria are a specialized microbial group which reduces bioavailable nitrate through their respiratory pathways by converting nitrate (NO₃⁻) to

nitrous oxide (N₂O) and nitrogen gas (N₂). Denitrification is an important ecosystem function because it reduces the nitrate content in coastal waters which have the capability to promote large phytoplankton blooms resulting in hypoxia and anoxia. Nitrogen mineralization is a process by which heterotrophic microbes play a key role in the oxidation of complex organic compounds and regeneration of available nitrogen. This process regulates primary productivity in wetlands and is therefore linked to the ecosystem services of providing habitats and carbon sequestration.

Research interests include determining the impacts the BP DWH oil spill has had on LA coastal marsh soil denitrification, nitrogen mineralization, and microbial biomass. The main goal of this study was to compare dispersant and crude oil impacts to microbial processes. The objectives were to 1) compare the effect of fresh (non-weathered), weathered crude oil, and Corexit EC9500A on LA coastal marsh soil denitrification rates following immediate and 2 week exposure, and 2) identify the impact of Corexit additions on microbial biomass and potentially mineralizable N.

Soil cores (0-10 cm of soil) were collected from an unimpacted salt marsh located in the upper Barataria basin along the LA coast and brought back to the lab. The soil cores were homogenized and samples were subjected to anaerobic conditions with varying additions of dispersant and BP DWH crude oil. The 1:10 ratio of crude oil:wet soil fully coated the soil surface, mimicking a heavy oiling scenario. Potential denitrification rates at the 1:10 ratio for weathered south LA light sweet crude oil were $46 \pm 18.4\%$ of the control immediately after exposure and $62 \pm 8.0\%$ of the control following a two week incubation period. Denitrification rates of soil exposed to fresh (non-weathered) crude oil were $51.5 \pm 5.3\%$ of the control after immediate exposure

and significantly lower, at $10.9 \pm 1.1\%$, after a 2 week exposure period. Potential denitrification rates after immediate exposure to Corexit:wet soil ratios of 0:10 (control), 1:10, 1:100, 1:1,000, and 1:10,000 were below detection for the 1:10 treatment, while the 1:100 was $7.6 \pm 2.7\%$ of the control, and the 1:1,000 was $33 \pm 4.3\%$ of the control. The 1:10,000 treatment was not significantly different from the control. Denitrification rates measured after 2 weeks exposure showed the 1:10 treatment still below detection limit and the 1:100 treatment was $12 \pm 2.6\%$ of the control.

Dispersants were used in response to the BP DWH oil spill to mitigate environmental impacts from the spill. Comparing the results of the immediate exposure denitrification rates for the 1:10 additions of wet soil weight to weathered crude oil and fresh crude oil were $46 \pm 18.4\%$ and $51.5 \pm 5.0\%$ of the control, while Corexit was non-detectable (Figure 4.1). The 2 week exposure denitrification rates for the same dispersant to soil ratio for weathered and fresh crude oil were $62 \pm 8.0\%$ and $10.9 \pm 1.1\%$ of the control, while Corexit was still non-detectable. Corexit EC9500A had a greater impact on denitrification rates than either fresh or weathered crude oil at equal ratios.

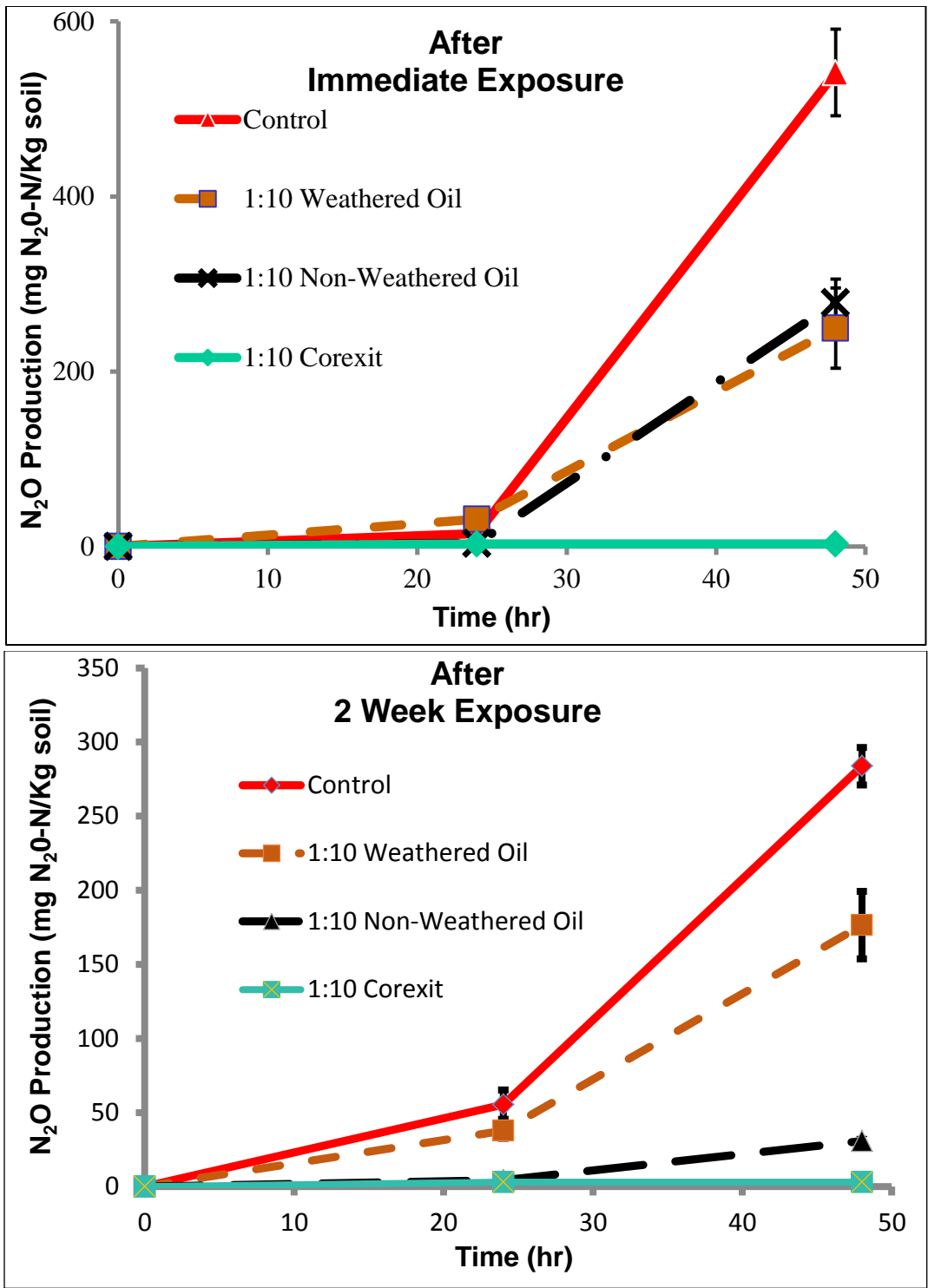


Figure 4.1 Effects of the 1:10 additions of crude oil and Corexit:wet soil weight ratio on N₂O production potential following immediate (Top) and 2 weeks exposure (Bottom).

Microbial biomass nitrogen (N) values were below detection for the 1:10, 1:100 and 1:1,000 Corexit:wet soil treatments. Potentially mineralizable N correlated with microbial biomass with decreased activity for 1:10 and 1:100 Corexit:wet soil additions. These results suggest that not only does Corexit inhibit microbial processes, but also diminishes microbial populations.

This is the first study the author is aware of which demonstrates that crude oil and Corexit EC9500A can significantly affect the important marsh function of denitrification in LA's coastal salt marshes, a function linked to the ecosystem service of water quality improvement. Results from this experiment suggest that an crude oil spill at close proximity to the coastal wetlands can have a significantly greater impact on the wetland soil microbial processes due to less weathering, following longer term exposure, than an crude oil spill more distally located which would incur greater weathering time. Regardless of the weathering status of the crude oil, there appeared to be a similar impact immediately suppressing denitrification rates with negative impacts of fresh crude oil increasing over time. The results conclude that microbial processes which regulate water quality and primary productivity in southern LA salt marsh soil were impacted by the BP DWH oil spill. Additional studies should be focused on investigating longer term exposure effects and changes in the microbial consortia as a result of oiling and dispersant use.

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VITA

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