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Effect of sediment slurry application on selected aspects of sulfur, iron, and manganese biogeochemistry in a coastal Louisiana marsh

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EFFECT OF SEDIMENT SLURRY APPLICATION ON SELECTED ASPECTS OF SULFUR, IRON, AND MANGANESE BIOGEOCHEMISTRY IN A COASTAL LOUISIANA MARSH

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DEDICATION

This Master of Science thesis is dedicated in loving memory of my father, Larry Maxwell.

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ABSTRACT

Coastal wetlands, long recognized to be among the most productive ecosystems on the planet, are being lost at a disturbingly high rate in coastal Louisiana due to both eustatic sea-level rise and land subsidence. A number of approaches have been proposed for reducing wetland loss and restoring deteriorated wetlands, among which the addition of sediment to increase marsh surface elevation is promising. However, little is known about how the added sediment affects the biogeochemistry of marsh sediment. The objective of this study was to determine the effects of sediment slurry addition on sulfur, iron, and manganese biogeochemistry in a subsiding *Spartina patens* dominated marsh in coastal Louisiana. The study site was located inside the Paul J. Rainey Wildlife Sanctuary in Vermillion Parish, Louisiana where low, medium, or high levels of sediment slurry were added to each study plot in July of 2008. Sediment and porewater samples were collected from the control (i.e. no sediment addition), low, medium, and high sediment treatment plots approximately on a seasonal basis from February 2009 to June 2011. Laboratory incubation of sediment using the radioisotope ^{35}S technique showed that there was no significant difference ($p=0.2201$) among the treatments in the rate of sulfate reduction 3 years after sediment slurry addition. However, significant increases ($p=0.0007$) in average concentrations of sulfate in sediment and decreases ($p<0.0001$) in sulfide in porewater with sediment addition over the 3 years' measurements indicate that there likely was a decrease in sulfate reduction rate with increasing sediment addition during the preceding 3 years. Concentrations of sediment and porewater iron and manganese significantly increased when sediment addition increased, which was primarily attributed to the high levels of these two elements in the added sediment. The increased iron and manganese concentrations could, in part, explain the lower level of sulfide observed in the sediment-treated plots over the 3 year study.

Additionally, average pH and redox increased significantly with sediment addition ($p<0.0001$ and $p=0.0084$, respectively). More samplings are needed to better understand the long-term impacts of sediment slurry addition on the rate of sulfate reduction in marsh sediment.

1. INTRODUCTION

Coastal wetlands, long recognized to be among the most productive ecosystems on the planet, are being lost at a disturbingly high rate in coastal Louisiana (Mitsch and Gosselink, 2007; Barras, J.A., 2003). Anthropogenic disruption of natural flooding regimes necessary for healthy marsh sustainability and growth are the major force behind this destruction (Day et al., 1995). Sediment addition in order to increase elevation is a useful technique to combat the subsidence of marshes and resulting formation of open water (DNR, 2000; Stagg and Mendelssohn, 2010). Sulfate reducing bacteria are considered a principle component of the carbon cycle in salt marshes because of their ability to utilize many different low molecular weight carbon substrates. A number of studies have found sulfate reduction to be the dominant source of organic matter mineralization in salt marshes (Howarth and Teal, 1979; Howes et al., 1984; Howarth and Merkel, 1984; King, G.M., 1988; Hines et al., 1989). The main product of sulfate reduction is hydrogen sulfide, a phytotoxin that has been linked to diminished vigor in wetland macrophytes (Mendelssohn and McKee, 1988; Koch et al., 1989). This toxicity can be mediated by reactive minerals such as iron in the sediment that bind sulfide to form insoluble compounds (King et al., 1982). Additionally, microbial reduction of manganese(IV) and iron(III) can play a role in the carbon cycle of moderately reduced sediments.

The goal of this research project is to determine how the rate of sulfate reduction and resulting sulfide concentrations in a brackish marsh would be affected by the addition of sediment slurry to the marsh surface as part of a larger effort to determine the viability of this method in restoring a rapidly subsiding coastal marsh to higher elevation relative to sea level. Previous research has shown that sediment addition can play a positive role in the health of

degraded marshes (Stagg, C.L., 2009; Mendelssohn and Kuhn, 2003). Our hypothesis was that the rate of sulfate reduction would decrease as relative elevation increased owing to increased aeration of the sediment due to decreased time under flooded conditions. This decrease in sulfate reduction would correspond to a decrease in toxic hydrogen sulfide. In order to achieve this increase in elevation, sediment from a nearby oil-well access canal was pumped atop a number of sections of marsh to various depths. A comprehensive seasonal analysis of the recovery of the marsh was begun after a period of acclimation. This study focused on microbial sulfate reduction, hydrogen sulfide, and sediment physical-chemical characteristics.

2. LITERATURE REVIEW

2.1 LOUISIANA COASTAL WETLANDS

Coastal marshes have long been recognized as some of the most productive ecosystems in the world (Dawes, C.J., 1998; Mitsch and Gosselink, 2007). In Louisiana, home to 41% of the wetlands in the continental United States, these wetlands play important roles as habitat for juvenile fish and crustaceans, nesting grounds for migratory bird species, and cultural treasures (Turner and Gosselink, 1975). Built over many millennia, the health of Louisiana wetlands has deteriorated rapidly over the past century because of anthropogenic interference of the natural hydrology and eustatic sea-level rise (Day et al., 1995). The result of this interference has been the loss of wetlands at rates of greater than 125 km^2 yr^{-1} from 1955-1978 and 77 km^2 yr^{-1} from 1978-2000 (Baumann and Turner, 1990; Barras, J.A., 2003). An additional 1745 km^2 of the coastal zone is projected to be lost to erosion, subsidence, and eustatic sea level rise by 2050 (Barras, J.A., 2003).

2.2 LOUISIANA WETLAND LOSS

Much of the loss and degradation of Louisiana's wetlands can be attributed to the disruption of the annual overland flooding of the Mississippi River and the loss of sediment and nutrients that this flooding provided (Mendelssohn et al., 1983, Turner et al., 1988). Impoundment of wetlands has been shown to decrease the rate of sediment accretion when compared to natural systems (Bryant and Chabreck, 1998; Cahoon, D.R., 1994). Dams and levees built along the Mississippi River and its tributaries are the main cause of this disruption. Most of these impoundment structures were built in the early 20^{th} century to enhance the livability and prosperity of the regions adjacent to the river in the aftermath of the Great

Mississippi Flood of 1927. To the detriment of wetland systems, dams also collect and store sediments as they fall out of suspension when the river current slows in reservoirs. Further exacerbating this problem at a local level are containment structures such as levees and spoil banks that limit overland flooding by rivers and canals (Turner, R.E., 1997; Swenson and Turner, 1987).

Levees and spoil banks are present and affect lands adjacent to the Mississippi River as well as coastal wetlands. In many parts of south Louisiana oil-well access canals have been dug through coastal wetlands so that oil drilling and extraction equipment can be positioned to reach subsurface crude oil pockets. The spoil left from the dredging of these canals is typically piled alongside the newly created canal. These spoil banks block the natural hydrologic cycle that regularly inundates marshes, providing sediment and nutrients (Baumann and Turner, 1990; Swenson and Turner, 1987). A consequence of the removal of the natural flooding, and its associated sediment deposition, is that these wetlands can no longer maintain the proper elevation relative to sea-level. Also of consequence to the hydrologic cycle are levees built along waterways specifically for the purpose of limiting overland flooding during natural high water events.

Another detrimental cause of marsh instability is subsidence. Sediments will naturally subside over time through organic matter decomposition and compaction if not replenished. Wetlands naturally compensate for this through organic matter buildup owing to the extremely high primary productivity along with deposition of sediment during flooding events. Subsidence can also be enhanced by human-influenced activities such as withdrawal from subsurface aquifers as well as oil and gas extraction. These activities, in addition to eustatic sea-level rise, can greatly affect the relative sea-level rise rate affecting a particular coastal marsh. For these

reasons, relative sea-level rise on the Louisiana coast can be much greater than the current 1.7 ± 0.3 mm yr^{-1} rate of worldwide eustatic sea-level rise (Church and White, 2006).

The minerals associated with natural sediment deposition also play a role in stimulating organic matter production. Both organic matter and mineral matter are necessary for the development of a healthy wetland soil (Nyman et al., 1990). Extended periods of inundation caused by decreasing elevation relative to sea-level decrease wetland plant productivity by decreasing oxygen availability, thus causing stress (Reed and Cahoon, 1992). Prolonged submergence and the resulting anoxic conditions can also lead to a buildup of toxic compounds such as hydrogen sulfide, the major product of sulfate reducing microorganisms, in the sediment that can affect plant vigor by limiting ATP production through suppression of both anaerobic and aerobic metabolic pathways (Mendelssohn and McKee, 1988; Koch et al., 1990).

2.3 WETLAND RESTORATION

Many methods to help coastal wetlands maintain elevation in the face of sea-level rise and subsidence have been suggested in the decades since the problem was first recognized. Removal of spoil banks into the canals from which they were taken is a way to restore a more natural grade to a disturbed wetland so that overbank flooding can effectively deposit sediment. This is accomplished by using heavy equipment stationed on either water or land to pull vegetation and sediment into the existing canal from which it was originally taken. Great care must be taken to ensure damage to the wetland or workers because of the nature of the large equipment used (Neill and Turner, 1987; Baustian et al., 2009).

In locations where spoil bank removal is not feasible, natural flooding has been restored using crevasses cut into levees and pipeline diversions built through levees. A crevasse is simply

a break or channel in a levee through which natural waters can flow. The rate of flow diminishes as the water moves into the large, flat receiving body and sediment drops out. This accretion aids in maintaining sediment elevation in the face of subsidence (Boyer et al., 1997). Long term deposition of sediment at the mouth of the crevasse decreases the effectiveness of this technique over time. Also, sedimentation on the river side of these structures necessitates regular dredging to safely handle ship traffic. This method of restoration has been used regularly by the U.S. Fish and Wildlife Service at the Delta National Wildlife Refuge (Bohannon, J., 2008).

Diversion structures provide a controllable method of introducing river waters into confined or flow-restricted wetlands. These structures enable the operator to control the rate of flow from the source body to the receiving body through the use of gates and pumps. An example of this is the Davis Pond Freshwater Diversion Structure. This structure is operated by the U.S. Army Corp of Engineers for the stated purpose of imitating annual flooding in order to restore the historic freshwater flow that provided nutrients and sediment to the Barataria Bay basin (DNR, 2002).

Another substitute for the natural supply of sediment is to mechanically pump dredged material onto the marsh surface. There are a number of techniques that can be used to apply this sediment. One technique involves the deposition of a thin layer of sediment dredged from the bottom of a water body and sprayed onto the adjacent wetland from what may be a considerable distance (Cahoon and Cowan, 1988; Ford et al., 1999). Another method, the one used in this study, involves the use of sediment slurry. This slurry is a solution with a water-to-sediment ratio high enough to allow the slurry to spread easily and without additional assistance to increase elevation and bulk density. Sediment slurry has the advantages of being able to spread sediment over a large area while obtaining sediments from nearby bodies of water (DNR, 2000). This

method has been shown to decrease sulfide concentrations and the duration of inundation while increasing bulk density, sediment nutrient concentrations, aboveground biomass, plant density and cover, and redox potential (Stagg and Mendelssohn, 2010; Schrift et al., 2008; Mendelssohn and Kuhn, 2003; Slocum et al., 2005).

2.4 SULFUR CYCLE

Sulfur is a ubiquitous element required for all known life on Earth. Accounting for roughly 1% percent of the dry mass of all organisms, it is a major constituent in a number of amino acids (Howarth, R.W., 1984). Estimations of the global pool of sulfur in the oceans generally agree at 1.3 x 10^{21} g dissolved as sulfate (Li, Y., 1972; Schidlowski et al., 1977; Bottrell and Newton, 2006). Other major sulfur reservoirs include ancient evaporite deposits in the form of sulfate and marine clastic deposits of sulfide (Bottrell and Newton, 2006).

The range of oxidation states available to sulfur, from +6 to -2, mean that it is available in many different forms that can be useful to biota of all kinds (Figure 1). Most important among these oxidation states as far as biogeochemical cycling is concerned are the most reduced form (S^{2-}), elemental form (S^0), and fully oxidized form (S^{6+}). The change in oxidation states is useful to microorganisms that use theses atoms as electron donors or acceptors. There are four stable isotopes of sulfur (^{32}S, ^{33}S, ^{34}S, and ^{36}S) and one man-made radioactive isotope (^{35}S). This radioactive form is useful in determining rates of reduction by bacteria in the laboratory and in situ (Reddy and Delaune, 2008).

Sulfate (SO_4^{2-}) is a commonly available form of inorganic oxidized sulfur that is supplied to salt and brackish marshes through the ebb and flow of tidal seawater. Marine systems can contain sulfate at concentrations up to 28mM, while freshwater systems may have concentrations

below 2mM (Reddy and Delaune, 2008). Microbial sulfate reduction may occur through two different pathways, either assimilatory or dissimilatory. Simply, assimilative reduction involves the reduction of sulfate for incorporation into biosynthetic processes within the cell (proteins, amino acids, etc.), while dissimilative reduction involves the use of sulfate as an electron acceptor with the product, sulfide, being released as waste. The dissimilatory pathway is discussed in more detail in the next section on sulfate reducing bacteria.

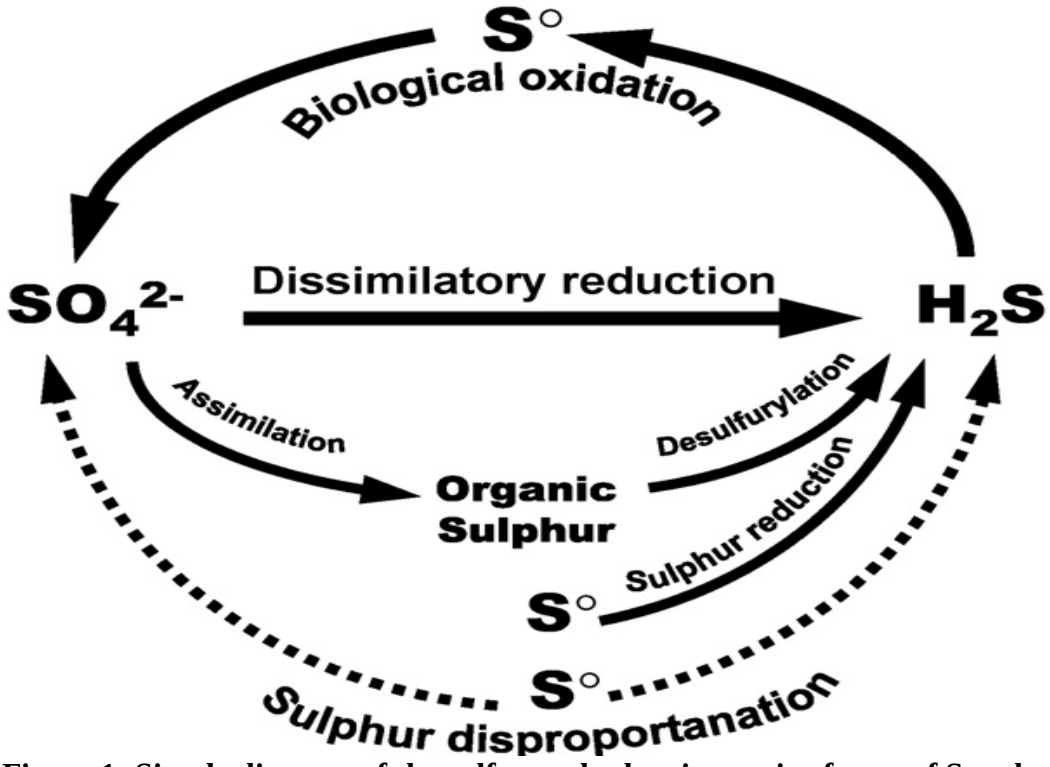


Figure 1: Simple diagram of the sulfur cycle showing major forms of S and methods of transformation (Tang, 2009)

Reduced sulfur is involved in many important sulfur compounds. Two commonly found biological forms are the amino acids methionine and cysteine. In cysteine, the S^{2-} is part of a thiol group, whereas in the methionine it is part of a thioether (Carey, F.A., 2008). As a constituent of humic acids, reduced sulfur compounds have been shown to represent up to 51% of the total sulfur content of wetland sediment (Ferdelman et al., 1991). In soil and sediment

systems where sulfide is produced, the stable product will depend on the pH of the media. Below pH 6 sulfides will mainly be available as H_2S, between ca. pH 6 and pH 10 HS^- is the predominant form, and above pH 10 S^{2-} predominates (Reddy and Delaune, 2008). Sulfide is also utilized by colorless sulfur bacteria, autotrophic sulfur bacteria, and heterotrophic bacteria as an electron donor (Madigan et al., 2008; Reddy and Delaune, 2008). Elemental sulfur (S^0) is also utilized by a number of microorganisms (Madigan et al., 2009). Sulfur disproportionation is a process by which a microorganism utilizes sulfur molecules as both electron acceptors and donors, oxidizing and reducing the same molecules.

2.5 SULFATE REDUCING BACTERIA

Sulfate reducing bacteria (SRB) are those prokaryotes that are capable of utilizing sulfate (SO_4^{2-}) as their terminal electron acceptor in energy metabolism. Consequently, these bacteria are considered obligate anaerobes. Ideally SRB prefer reducing conditions below -100mV for optimal growth (Connell and Patrick, 1968; Reddy and Delaune, 2008). The different species of SRB have been classified into distinct groups based on analysis of rRNA sequences. These groups are the Gram-negative mesophilic SRB, the Gram-positive spore forming SRB, the thermophilic bacterial SRB, and the thermophilic archaeal SRB (Castro et al., 2000). Using both geological and biological data, SRB are theorized to have evolved ca. 3.4 billion years ago. This figure is based on a comparison of the time frame of the accumulation of certain biologically produced minerals in the geologic record and branching patterns of the evolution of *16S* small sub-unit rRNA of a large number of bacteria, archaea, and eukaryotes (Canfield and Raiswell, 1999). The early evolution of the anoxic respiration is in line with current theories of the evolution of life from anaerobic respiration to aerobic respiration.

Sulfate reduction occurs in the cytoplasm and periplasm of the SRB. Transportation of sulfate (SO_4^{2-}) across the cytoplasmic membrane occurs via an ion gradient with different species using different ions (Cypionka, H., 1987; Warthmann and Cypionka, 1990). Once in the cytoplasm, sulfate is activated by ATP sulfurylase to make the molecule more easily reducible (Peck, H.D., 1959). The products of the activation of sulfate are adenosine-5'-phosphosulfate (APS) and orthophosphate (P_i). The transformation of pyrophosphate, an intermediary in the hydrolytic formation of orthophosphate (P_i), by the action of the enzyme pyrophosphatase makes the product side of the reaction more energetically favorable (Wilson and Bandurski, 1958; Fauque et al., 1991). The reaction proceeds as follows:

$$SO_4^{2-} + ATP + 2H^+ + H_2O \rightarrow APS + 2P_i$$

APS is then utilized as an electron acceptor in its conversion to sulfite (SO_3^{2-}) and adenosine monophosphate (AMP).

$$APS + 2e^- \rightarrow SO_3^{2-} + AMP$$

APS reduction is catalyzed by the enzyme APS reductase, a nonheme iron-sulfur flavoprotein (Bramlett and Peck, 1975; Stille and Trüper, 1984; Fritz, G., 1999). Finally, sulfite (or bisulfite) is catalyzed to sulfide (S^{2-}) by dissimilatory sulfite reductase according to the following equation where the sulfide product actually produced is pH dependent as mentioned earlier:

$$SO_3^{2-} + 6e^- + 8H^+ \rightarrow H_2S + 6H_2O$$

This process involves a number of metallic cofactors, a reduced porphyrin, a siroheme, and an iron-sulfur compound to transfer electrons from the donor to the substrate (Murphy and Siegel, 1973; Murphy et al., 1974).

The gene responsible for encoding dissimilatory sulfite reductase is known as *dsrAB*. Its amplification is the most common method for detection of SRB using polymerase chain reaction (PCR)-based methods (Karkhoffschweizer et al., 1995; Stahl et al., 1998). This gene has been sequenced in a number of quite different SRB species including *Desulfovibrio vulgaris*, *Archaeglobus fulgidus*, and *Chromatium vinosum* and found to be homologous among them all (Dahl et al., 1993; Hipp et al., 1997; Karkhoffschweizer et al., 1995). The relative and absolute abundance of this gene is quantifiable using real-time PCR.

A number of SRB are able to utilize compounds other than sulfate as their terminal electron acceptor. Two common electron acceptors utilized are sulfite (SO_3^{2-}) and thiosulfate ($S_20_3^{2-}$). These are intermediate species in the reduction of sulfate so their utilization is easily understood (Postgate, J.R., 1984; Widdel and Pfennig, 1982). Dimethylsulfoxide (C_2H_6OS) utilization has been demonstrated in a number of species in the genera *Desulfovibrio* and *Desulfuromusa* resulting in the dimethylsulfide (C_2H_6S) as the product (Liesack and Finster, 1994; Jonkers et al., 1996). Nitrate (NO_3^-) is also available to SRB as an electron acceptor under certain conditions (Liesack and Finster, 1994; Widdel and Pfennig, 1982). The presence of at least 0.75 mM sulfide had a complete inhibitory effect on the reduction of nitrate in the laboratory. Interestingly, the product of nitrate (via nitrite) reduction is ammonia (NH^{3-}) as opposed to bimolecular nitrogen (N_2; Moura et al., 1997). Iron(III) is another possible electron acceptor that can be utilized by certain *Desulfovibrio* species (Lovley et al., 1993; Bale et al., 1997). Uranium(VI) can be reduced by *Desulfovibrio vulgaris* (Lovley et al., 1993). Most surprisingly, bimolecular oxygen (O_2) has been shown to be an electron acceptor in certain *Desulfovibrio sp.* when hydrogen was the donor, though no growth was observed (Dilling and Cypionka, 1990; Dannenberg et al., 1992). Other researchers have shown that O_2 concentrations

above 1 µM and 15 µM stopped growth in a number of different SRB strains (Johnson et al., 1997; Marschall et al., 1993).

Electron donors utilized by SRB are restricted to low molecular weight compounds. An important donor is bimolecular hydrogen (H_2). A number of genera including *Desulfovibrio*, *Desulfobulbus*, *Desulfobacter*, and *Thermodesulfobacterium* have demonstrated growth using H_2 as the sole electron donor via a hydrogenase pathway (Fauque et al., 1991; Kremer and Hansen, 1988; Schauder et al., 1986, and Fauque et al., 1992). When H_2 is utilized as an electron donor, acetate and carbon dioxide are used as carbon sources for growth (Rafus et al., 2006). Other electron donors that are either completely or incompletely oxidized to CO_2 include formate (Fauque et al., 1991), propionate (Kremer and Hansen, 1988), butyrate (Widdel and Pfenning, 1981), lactate (Ogata et al., 1981), ethanol and acetaldehyde (Postgate, J.R., 1984), fructose (Klemps et al., 1985; Ollivier et al., 1988), glycolate (Friedrich et al., 1996; Friedrich and Schink, 1995), certain dicarboxylic acids (Postgate, J.R., 1984), amino acids (Coleman, G.S., 1960; Zellner et al., 1989), certain aromatic hydrocarbons (Edwards et al., 1992; Lovely and Lonergan, 1990), and certain polar aromatic compounds (Bak and Widdel, 1986)

The first SRB to have its genome completely sequenced was *Desulfovibrio vulgaris* Hildenborough (Heidelberg et al., 2004). Though 22 other SRB have since been sequenced, this organism remains a model organism in the study of adaptations necessary for growth in the many environments where SRB are found (Zhou et al., 2011). Recent studies have given important insights into *D. vulgaris* Hildenborough's use of different electron donors and the metabolic pathways necessary for such use at the genomic level. One such study elucidated the transcriptional changes in the microorganism while H_2 was the sole electron donor and sulfate the receptor compared to carbon molecules as the donor utilizing sulfate (Louro et al., 2008). A

change in the transcription of >500 genes was observed using whole-genome microarray technology. This illustrates that plasticity that could be available to many of the SRB due to the wide variety of environments they occupy. Another study identified osmotic and nitrate stress response mechanisms as important factors in the growth inhibition of *D. vulgaris* under high nitrate levels (Zhou et al., 2010).

2.6 Iron and Manganese Biogeochemistry

Iron and manganese play important roles in microbial metabolism and in mitigating sulfide toxicity in wetlands. The major oxidation states of iron in wetlands are in the oxidized Fe(III) form and the reduced Fe(II) form. Similarly, manganese is abundant in 2 major oxidation states in wetlands, oxidized Mn(IV) and reduced Mn(II). A major biotic adaptation that utilizes the availability of these different oxidation states is the microbial reduction of these metals. Dissimilatory reduction of Fe(III) and Mn(IV) have been recognized as major anaerobic pathways, in some places they have been shown to dominate over sulfate reduction (Canfield et al., 1993; Myers and Nelson, 1988; Thamdrup et al., 2000). The reduced products of these reactions are water soluble compounds that are measureable by ion chromatography of filtered water. The ideal redox potential range for the microbial reduction of Fe(III) is between 0 and +100 mV while the ideal range for microbial Mn(IV) reduction is between +200 and +300 (Reddy and Delaune, 2008). Abiotic reduction is possible for these atoms in the presence of hydrogen sulfide where Mn(IV) and Fe(III) are reduced to form insoluble sulfide complexes. This pathway is important in mitigating potential sulfide toxicity in reduced environments where sulfate is also present.

3. MATERIALS AND METHODS

3.1 STUDY SITE

The study site for this particular project is located inside Paul J. Rainey Wildlife Sanctuary in Vermillion Parish, Louisiana. This 105 km^2 wildlife refuge is owned by the National Audubon Society and has been under its management since 1924 (Kemp, P., 2010). It is bordered by the Vermillion Bay to the east, Freshwater Bayou Canal to the west and north, and the Gulf of Mexico the south. The area was utilized for many decades to facilitate oil and gas extraction; it was still in use for this purpose as late as 1999 (Snyder and Shaw, 1995; DeGregorio, J., 2010). Still present and a significant part of the modern landscape of the sanctuary are the access canals and spoil banks associated with these decades of use.

Boardwalks were constructed around sections of *Spartina patens* dominated marsh within the National Audubon Society's Paul J. Rainey Sanctuary (Figure 2; 29°41'34.45"N, 92°13'42.95"W) on the south central coast of Louisiana, USA in July of 2008. These sections of marsh were subdivided by water permeable membrane into 3m by 4m plots in order to ensure confinement of sediment. The boardwalks provided easy access with minimal disturbance to the vegetation and underlying sediment. A Piranha PS-135-E mini-dredge positioned on a flat boat was used to extract and pump sediment slurry from the bottom of a nearby oil-well access canal level (Piranha Pumps & Dredges, Albuquerque, NM). A pipeline was used to transfer the sediment slurry from the boat onto each plot to achieve different sediment depths and, consequently, relative height above sea. A valve-controlled distribution manifold was used to control the distribution of the sediment slurry into the plots.

Figure 2: Study site at Rainey Wildlife Sanctuary. Labels in white indicate plot locations and types. The sediment source canal is at the top of the image. (Image: Google Earth, Mountain View, CA).

Hurricane Gustav passed very close to the study site during its progression north just weeks after the completion of sediment slurry application. For results dealing with the comparison of treatments, certain plots were grouped into high, medium, low, and control categories that reflect their post-hurricane relative elevations. These plots were at a NAVD88 determined elevation of 36±3 cm prior to sediment addition. The low elevation group (n=2) contains plots that received 0-10 cm while the medium elevation group (n=3) contains plots receiving 10-15 cm of sediment. The high elevation group (n=4) contains plots to which 15-20 cm of sediment were added. The control plots (n=3) did not have any sediment addition (Figure 3).

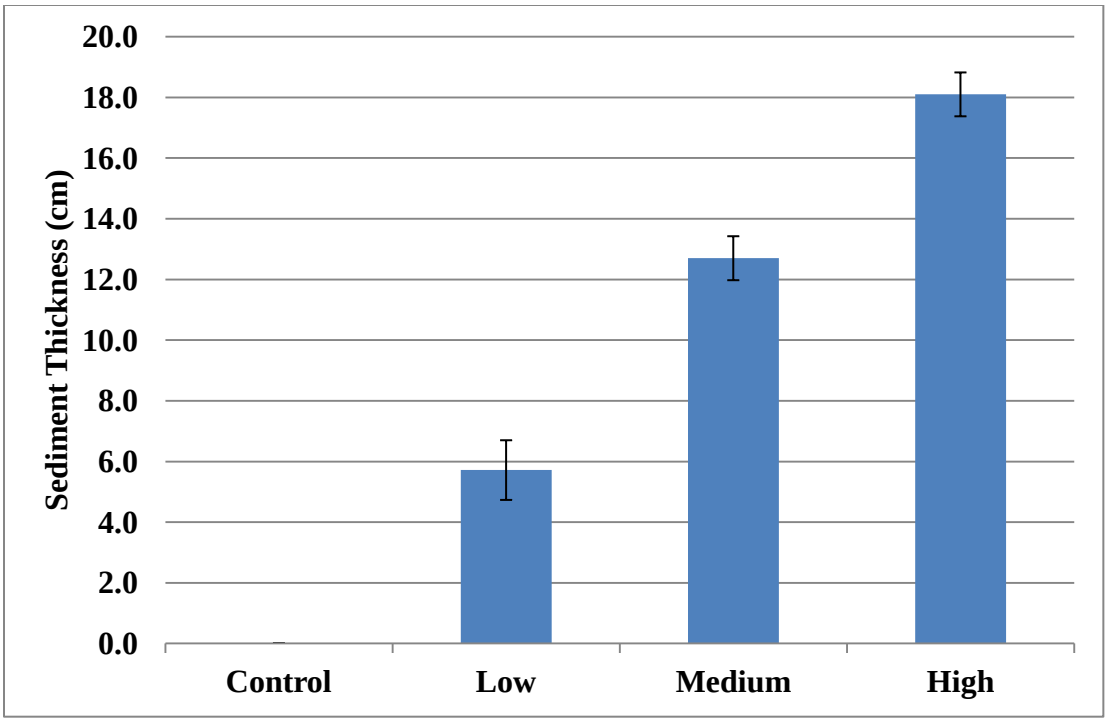


Figure 3: Thickness of sediment addition (with standard error) in each treatment as recorded on Feb. 5, 2009.

3.2 SAMPLE COLLECTION

Sediment samples were collected in February 2009, May 2009, August 2009, December 2009, March 2010, July 2010, October 2010, and June 2011 at the Rainey Sanctuary. Duplicate sediment samples were extracted from the top 15cm of sediment in each plot using a russian peat corer and transferred to sterile plastic with all efforts made to exclude any headspace. Sediment porewater for analysis was collected from 10-20 cm deep using the syringe sipper method during May 2009, December 2009, and July 2010 samplings (McKee et al., 1988). Porewater was extracted by centrifugation of a 15 cm deep whole sediment core stored under a nitrogen headspace for the June 2011 sampling. In the field, samples were stored in a cooled ice chest until return to the LSU Dept. of Environmental Science where they were transferred to storage at 4°C.

3.3 MICROBIAL COUNTING BY FLUORESCENT MICROSCOPY

Microbial counting was carried on sediments collected in February 2009 out using a method developed by Hobbie et al. (1977) and modified by Kepner and Pratt (1994). One gram of sediment was combined with 10% formalin solution to fix bacteria. Just prior to counting, this sediment solution was further diluted in filtered deionized water, vortexed for 30 seconds, and sonicated for 10 minutes to disperse the sediment and break bonds between mineral or organic matter and the microbes. A 1:2000 dilution of sediment stained with Acridine Orange was filtered onto a non-fluorescing 0.22 µm pore size Nucleopore filter (Whatman PLC, Kent, UK). The filters were rinsed with filtered deionized water to remove excess dye and mounted on glass microscope slides. Forty images per plot were taken at the LSU Department of Biological Science's Socolofsky Microscopy Center using a Leica DM RXA2 upright microscope that is equipped with a SensiCam QE 12-bit, cooled CCD camera (Leica Microsystems, Inc, Buffalo Grove, NY). A no-neighbors deconvolution algorithm was run on each image using Slidebook 4.0 software in order to remove out of focus objects from the image (Intelligent Imaging Inc., Denver, CO). Red and green points were counted on each image with red representing RNA-bound fluorophore and green representing DNA-bound fluorophore.

3.4 SULFATE REDUCTION RATE

The potential rate of bacterial sulfate reduction (SRR) was determined in sediment collected in June 2011 using the method developed by Ulrich et al. (1997) as modified by Babenzein et al. (2000) for quantifying reduced inorganic sulfur compounds. In a properly functioning Coy anaerobic chamber (Coy Laboratory Products, Inc, Grass Lake, MI), duplicate cores were combined and homogenized. One gram of homogenized sediment, 4 ml of 0.45 µm

filtered, autoclaved water collected from the study marsh, and a small test tube containing 2.5 ml of 10% zinc acetate (ZnAc) was placed in autoclaved, brown boston round bottles in duplicate for each plot. Each bottle was then capped with an air-tight septum and cap. The bottles were removed from the anaerobic chamber and 1 µCurie of $Na^{35}SO_4$ dissolved in 1 ml of filtered deionized water was added through the septum to the sediment slurry.

The reaction bottles were then incubated for 4 hours in a darkened rotary shaker (ca. 100 rpm) at room temperature. After incubation, 8 ml of anoxic 6 M HCl and 8 ml of 1 M Cr(II)Cl in 0.5 M HCl was then added to the bottle. The bottles were incubated in a darkened rotary cabinet operating at ca. 100 rpm for 24 hours at room temperature in order to extract reduced inorganic sulfur products. The ZnAc acted as a trap for the acid-volatile sulfide (AVS) and chromium-reducible sulfide (CRS) fractions, which precipitated as ZnS. Immediately afterwards the test tubes were removed from the bottles. A 1 ml aliquot from each test tube and bottle was then mixed with 9 ml of UltimaGold AB in scintillation vials (PerkinElmer, Waltham, MA). The CPM data was collected using a PerkinElmer Tri-Carb 3110TR Liquid Scintillation Counter (PerkinElmer, Waltham, MA). Results were calculated such that they are based on the dry weight of the sediment analyzed.

Seasonal measurements were made of the rate of sulfate reduction from February 2009 to March 2010 but these data are not included because of a flaw in the procedure used that invalidated the data. The sulfide volatilization agents, 6 M HCl and 1 M Cr(II)Cl, were added at the same time as the radioisotope $^{35}SO_4$. This error did not allow the sulfate to be properly cycled through the microbial sulfate reduction process necessary to measure the product, $H_2{}^{35}S$, produced. This explains why the measured rates were so low. It was obvious in the ZnAc traps that sulfides were being volatilized and precipitating as they were supposed to but the liquid

scintillation values did not reflect any significant amount of volatilization of the radioisotope. This experience taught me a valuable lesson in properly understanding and undertaking procedures and protocols in experimentation.

3.5 PHYSICAL-CHEMICAL DATA

For sulfate determination, 1 g of wet sediment was added to a 15 ml centrifuge tube along with 10 ml of deionized water. This solution was centrifuged at ca. 3000 g for 15 minutes. The supernatant was extracted, filtered through 0.45 µm syringe filters, and stored at 4°C prior to analysis. Sulfate levels were determined at the LSU Ag Center's Central Analytical Instruments Research Laboratory using a Dionex ICS 2000 Ion Chromatograph as per EPA method 300.0 (Dionex, Sunnyvale, CA; Pfaff, 1993). Sulfate concentrations were determined in the months of February, May, August, and December of 2009 as well as March 2010 and June 2011.

Redox potential measurements were made *in situ*. Three bright platinum electrodes and a calomel reference electrode were inserted into the sediment of each plot to a depth of 15 cm and allowed to equilibrate for at least 30 minutes prior to reading the potential using a digital pH meter. The readings for each plot were averaged and corrected for the reference electrode value.

Bulk density, sediment iron, and sediment manganese were determined from sediment samples taken from the top 15 cm of each plot. Bulk density was determined according to Method 3B6 of the USDA Soil Survey Laboratory Methods Manual (National Soil Survey Center, 1996). DTPA-extractable iron and manganese were measured by the LSU AgCenter's Soil Testing and Plant Analysis Lab using an inductively-coupled plasma spectrophotometer (Leggett and Argyle, 1983; Spectro Analytical Instruments, Germany).

Porewater for sulfide analysis was mixed 1:1 by volume with an antioxidant buffer and stored on ice for transport back to LSU. In the lab, an Orion sulfide selective probe was used to determine total soluble sulfides (Thermo-Fisher Scientific, Waltham, MA; American Public Health Association et al., 2005). Porewater iron and manganese concentrations were determined using an inductively-coupled plasma spectrometer after 0.45µm filtration and acidification to <2 pH (Spectro Analytical Instruments, Germany; Lindsay and Norvell, 1978). Dissolved organic carbon was determined using a Shimadzu TOC-V CSH/CSN after filtration using 0.45 µm nylon filters and acidification (Shimadzu Corp., Kyoto, Japan). Porewater measurements are only included for May 2009, December 2009, July 2010, and June 2011 as these were the only months that porewater from the 0-15 cm depth was accessible.

3.6 STATISTICAL ANALYSIS

The data analysis for this paper was generated using SAS software, Version 9.2 of the SAS System for Windows (SAS Institute Inc., Cary, NC). Proc Mixed was used to test ANOVA relationships and Proc Reg was used to test regression analyses. PDMIX800 was used to convert pdiffs data from Proc Mixed into letter groups for differences with a p-value of <0.05 (Saxton, A.M., 1998). The Tukey-Kramer adjustment was used in ANOVA tests as well. Normality of residuals was tested using Stem-Leaf plots, Normal Probabilities plots, and the Shapiro-Wilk test in Proc Univariate. Normality was achieved in through the use of log transformation when necessary. To test for outliers, DFFIT, DFBETA, rStudent, and Hat Diagonal values were analyzed within Proc Reg.

For ANOVA tests, treatment groups were created based on the pretreatment elevation and thickness of sediment added to each plot. These plots were at a NAVD88 determined

elevation of 36±3 cm prior to sediment addition. The low elevation group (n=2) contains plots that received 0-10 cm while the medium elevation group (n=3) contains plots receiving 10-15 cm of sediment. The high elevation group (n=4) contains plots to which 15-20 cm of sediment were added. The control plots (n=3) did not have any sediment addition (Figure 3). These 12 plots do not encompass the full extent of the study, only those that fit in the defined criteria (See appendix for full list of plots). For the regression analysis of SRR and DOC, all plots were used except for one that violated tests for significant outliers (n=22).

4. RESULTS

Sulfate reduction rates (SRR) ranged from 44.3 mmol m^{-2} day^{-1} in the High treatment to 82.0 mmol m^{-2} day^{-1} in the Medium treatment (see Appendix 1 for all SRR data). Statistical analysis of SRR by one-way ANOVA did not reveal a significant treatment effect three years after sediment addition (p=0.2201; n=12). The mean rate by treatment was higher on average in Medium plots compared to other treatments but the standard error is too high to declare it significant (Figure 4). A one-way ANOVA did not reveal a significant treatment effect in SRR when all plots, not only plots that fit into the defined treatment criteria, were included (p=0.7910; n=23).

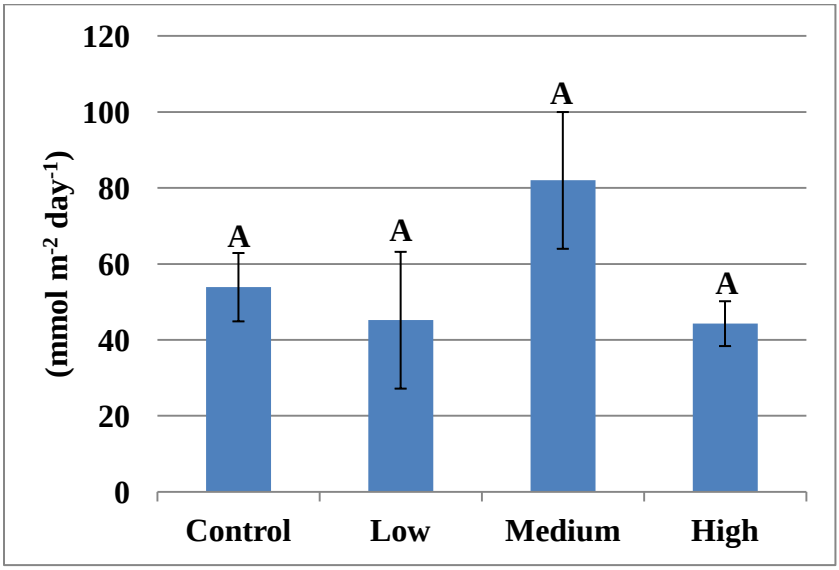


Figure 4: Mean rate of sulfate reduction (mmol m^{-2} day^{-1}) by treatment in June 2011. Vertical bars indicate standard error. Letter groupings as determined by the PDMIX800 macro in the Mixed Procedure with Tukey-Kramer adjustment. Groups with the different letters are significant with an alpha of 0.05.

A significant treatment effect was found between the time-averaged, mean sulfate concentrations measured during February, May, August, and December of 2009 as well as March 2010 and June 2011 sampling trips (p=0.0007; Figure 5 Left). The High and Medium treatments

were not significantly different from one another and were both significantly higher than the Control treatment. The Low treatment mean was intermediate and was not significantly different compared to any other treatments.

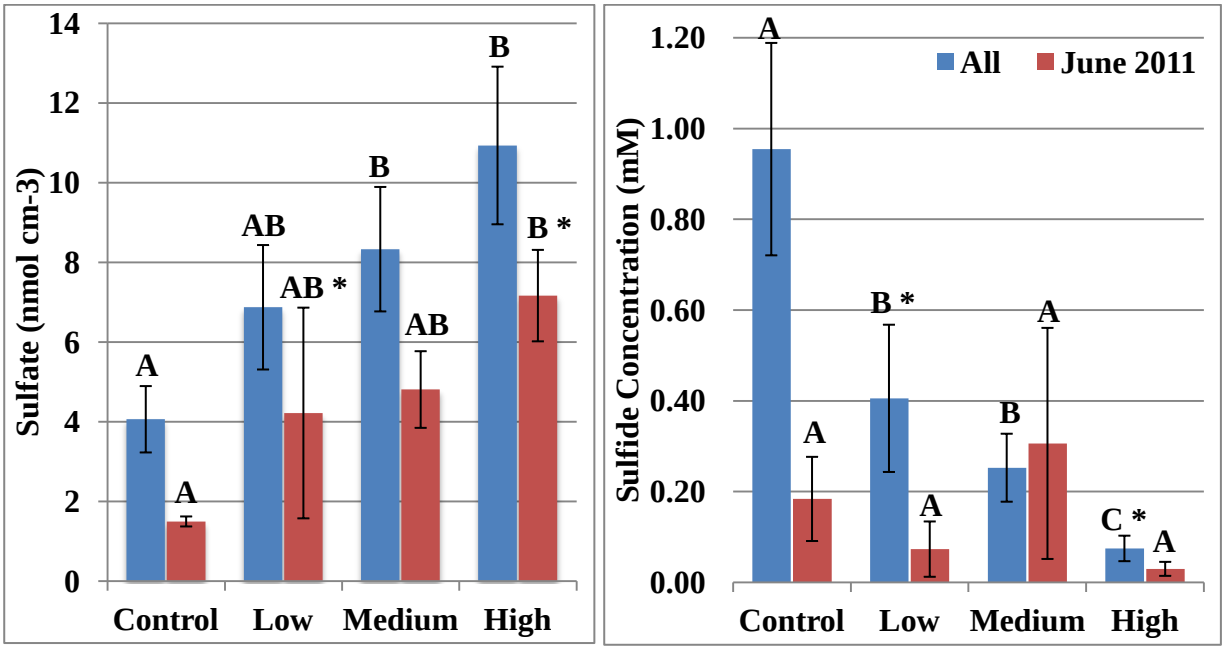


Figure 5: (Left) Mean, time-averaged sulfate (SO_4^{2-}) concentration (nmol cm^{-3} dry sediment), standard error (S.E.), and letter group. Sampling occurred in February, May, August, and December of 2009 as well as March 2010 and June 2011. (Right) Mean, time-averaged porewater sulfide concentration (mM), standard error (S.E.), and letter group. Red column sampling occurred in May 2009, December 2009, July 2010, and June 2011. Letter groupings as determined by the PDMIX800 macro in the Mixed Procedure with Tukey-Kramer adjustment. Groups with the different letters are significant with an alpha of 0.05. (*) denotes non-normal distribution.

A significant treatment effect was found between time-averaged, mean sulfide concentrations ($p<0.0001$; Figure 5 Right). Normality could not be achieved for High and Low treatments in a One-Way ANOVA examining the sulfide porewater concentrations measured during May 2009, December 2009, July 2010, and June 2011 sampling trips, though a number of transformations were attempted (W=0.836 and W=0.898, respectively). The highest levels were found in the lowest elevation control plots and concentrations decreased as the amount of sediment increased. For the June 2011 sampling, which correlates in time with the measured

SRR, there was no significant difference in sulfide concentrations by treatment in agreement with the lack of difference found in SRR (Figure 5 Right; p=0.4484).

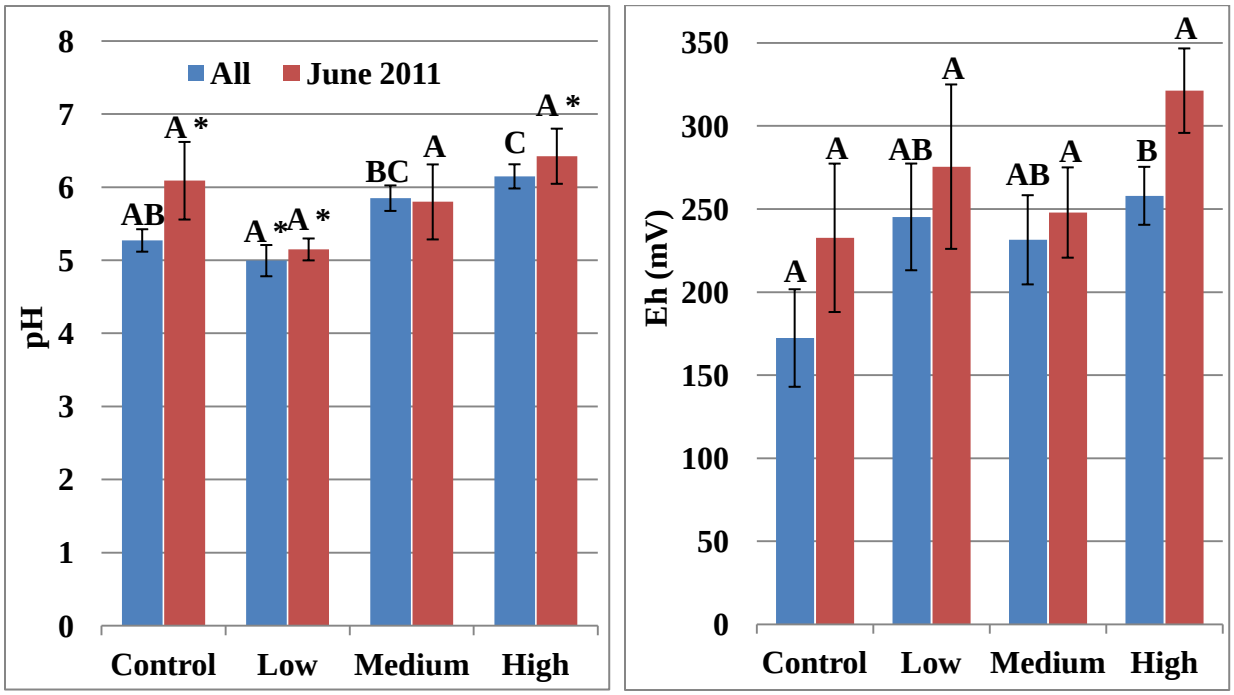


Figure 6: (Left) Porewater pH in June 2011 (blue) and all months sampled (red; quarterly 2009-2011). (Right) Sediment redox potential (mV) in June 2011 (blue) and all months sampled (red; quarterly 2009-2011). Vertical bars represent standard error. Letter groupings as determined by the PDMIX800 macro in the Mixed Procedure with Tukey-Kramer adjustment. Groups with the different letters are significant with an alpha of 0.05. (*) denotes non-normal distribution.

These trends in mean, time-averaged sulfate and sulfide concentrations could indicate that, contrary to the single estimate of sulfate reduction measured in June 2011, sulfate reduction was higher in the Control treatment relative to the Medium and High treatments in the preceding years after sediment addition. Sulfate input to all plots is identical as its source is the overland flow of water. A difference in porewater concentration indicates that it is being utilized by sulfate reducing bacteria in anaerobic respiration. The product of this respiratory pathway, hydrogen sulfide, shows a corresponding increase in concentration in lower elevation plots where sulfate is being utilized at a higher rate.

Reduction-oxidation potential averaged over all sampling periods was significantly higher in the High treatment plots (258 mV) compared to the Control plots (172 mV; Figure 6 Right; p=0.0084). This is much higher than the range needed by sulfate reducing bacteria but is in the range of manganese reduction. Porewater pH followed the same trend with higher pH found in Medium (5.9) and High (6.2) plots compared to the Control plots (5.3; Figure 6 Left; p<0.0001). Eh and pH measured in June 2011 did not differ significantly though in three treatments normality could not be achieved.

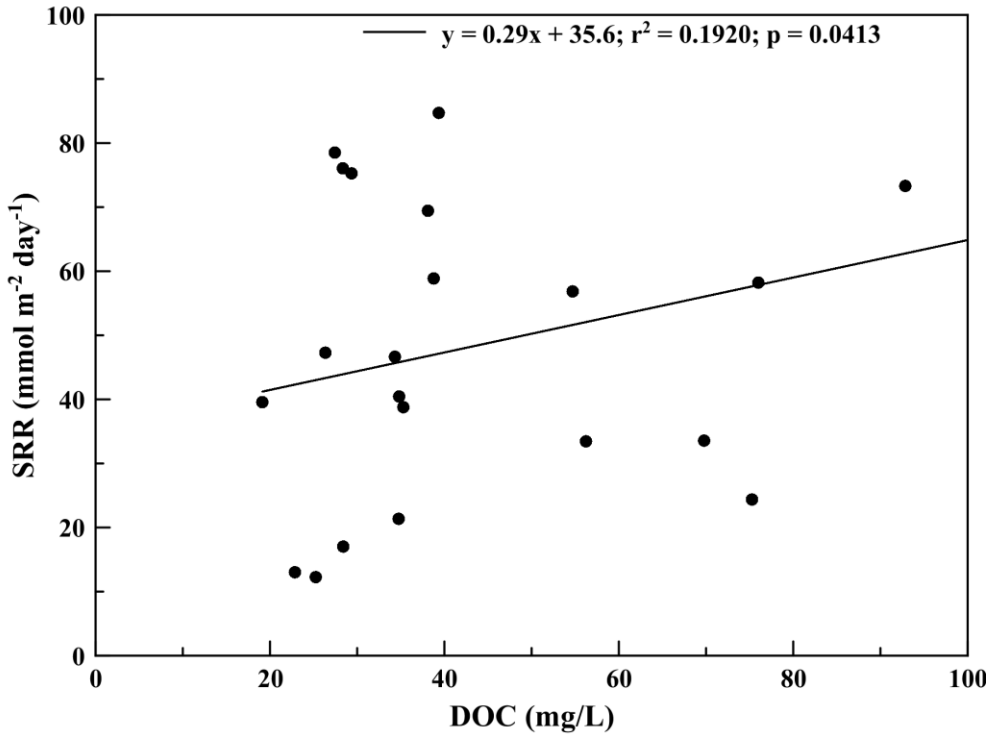


Figure 7: Simple linear regression of sulfate reduction rate (SRR) and dissolved organic carbon (DOC).

Dissolved organic carbon measured in porewater collected in June 2011 ranged from 19-192 mg-C L^{-1}. A positive correlation was found between the rates of sulfate reduction and dissolved organic carbon (p=0.0413, r^2=0.1920; Figure 7). Dissolved organic carbon in the form

of low molecular weight compounds such as acetate, ethanol, formate, and fructose are the main sources of carbon for anaerobic microbes so its correlation with increase activity is expected.

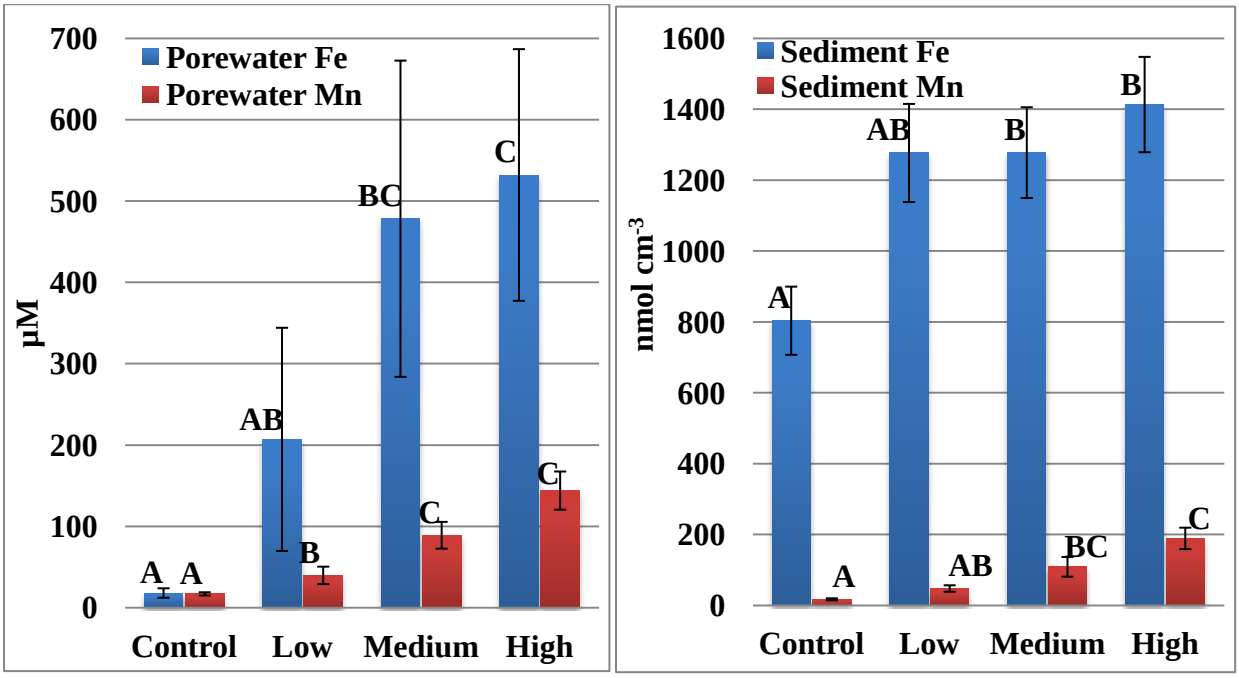


Figure 8: (Left) Mean, time-averaged porewater concentrations (µM) with standard error of iron and manganese by treatment. (Right) Mean, time-averaged sediment concentrations (nmol cm^{-3}) with standard error of iron and manganese by treatment. Letter groupings as determined by the PDMIX800 macro in the Mixed Procedure with Tukey-Kramer adjustment. Groups with the different letters are significant with an alpha of 0.05.

A significant treatment effect was observed in sediment and porewater fractions of manganese ($p<0.0001$) Significant variation was found in porewater and sediment fractions of iron as well ($p<0.0001$ and $p=0.0091$, respectively). For both elements in both phases, the concentrations in the Control plots was always significantly lower than in the Medium and High plots (Figure 8). Analysis of the sediment Fe and Mn levels in canal sediments prior to application to the marsh showed that Fe was present at 3.2±0.2 nmol g^{-1} while Mn was present at 0.6±0.08 nmol g^{-1}. Bulk density increased significantly with increasing sediment slurry addition ($p<0.0001$; Figure 9). All treatments were significantly different from each other with value ranging from 0.10 for the control to 0.46 for the high treatment.

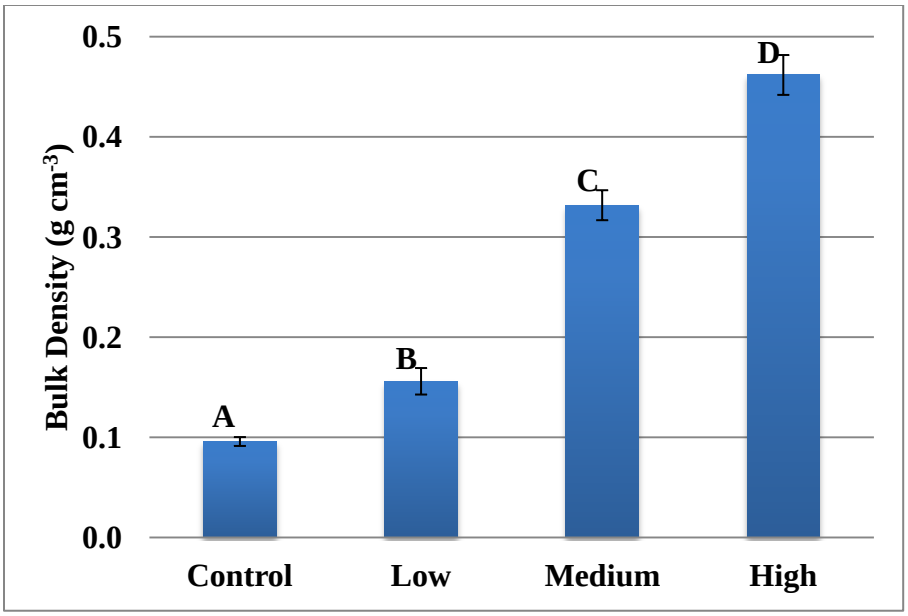


Figure 9: Mean, time-averaged bulk density (g cm^{-3}) by treatment and standard error (S.E.). Letter groupings as determined by the PDMIX800 macro in the Mixed Procedure with Tukey-Kramer adjustment. Groups with the different letters are significant with an alpha of 0.05.

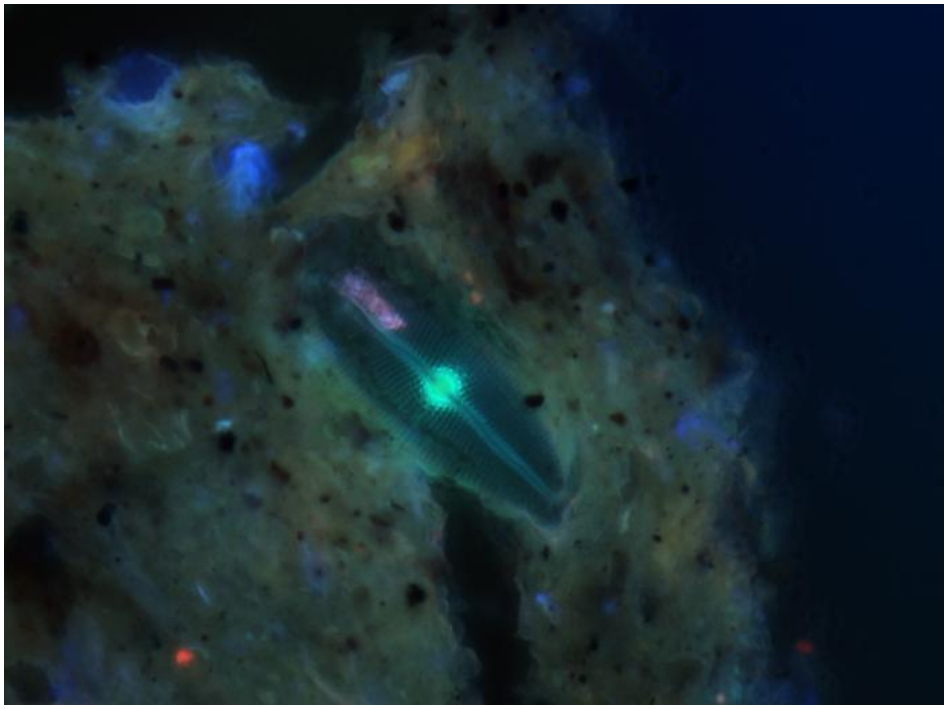


Figure 10: Fluorescent microscope image of Rainey wetland sediment without fluorescent dye added.

Fluorescent microscopy results were deemed invalid and are not included in this work. Analysis of a sediment sample that had not been treated with acridine orange dye revealed that the sediment contained silica content sufficient to cause auto-fluorescence (Fig. 10; Brown, M., Personal Communication). High dilutions and physical disruption were utilized to attempt to dislodge microbes from particulate matter but in the end, that was not the problem to overcome. Silicates, presumably washed in with tidal flux, fluoresce in the same wavelengths as intercalated Acridine Orange. This background fluorescence makes microbial counting through the use of acridine orange invalid because the fluorescence of particulate matter cannot be distinguished from that of the DNA- and RNA-bound intercalating dye.

5. DISCUSSION

The rates of sulfate reduction measured in the present study are within the range found in other manuscripts. However, overall the rates measured in this study were lower than the rates measured in *Spartina patens* dominated marshes by Delaune et al. (2002) and Hines et al. (1989) in Louisiana and New Hampshire coastal wetlands, respectively (Table 1). To our knowledge, this was the first study to determine the rate of sulfate reduction in a coastal wetland after addition of sediment slurry. An article by Kostka et al. (2002) indirectly studied elevation dependant SRR with their analysis of a mudflat, levee, and middle marsh. It lacked specific mention of the range of elevation in relation to the rates of reduction measured and instead focused on spatial variability and vegetation density. A number of research articles have demonstrated differences in SRR among stands of different wetland plant species or subspecies (King, G.M., 1982; Gribsholt, B., 2002; Hines et al., 1999).

Being that these rate measurements were made 34 months after sediment slurry application, we assume that the plots had normalized from a disturbed state affected by the sediment addition. Therefore, sulfate reduction rates measured in these plots can be viewed as a proxy for potential near-term effects of sediment slurry addition on other wetlands. The results indicate that there is no significant change in the rate of sulfate reduction after 34 months across different sediment treatments that increased the elevation of a subsiding coastal wetland. This lack of a significant change in SRR with sediment addition does not tell us the effect on the rate of bacterial carbon metabolism as a whole though because we do not yet know enough about the change in bacterial community structure across the sediment treatments.

Table 1: Sulfate reduction rates from selected articles focusing on coastal wetlands

Dominant Plant	Sulfate Reduction Rate (mmol m^{-2} d^{-1})	Depth (cm)	Source
S. alterniflora (tall)	Avg: 40	0-10	King, G.M., 1982
S. alterniflora (short)	Avg: 25.7	0-10	
S. patens	High: 380; Low: 10	0-20	Hines et al., 1989
S. alterniflora	High: 1000; Low: 15	0-20	
S. alterniflora (Tall)	High: 50; Low: 2	0-10	King, G.M., 1988
S. alterniflora (Short)	High: 94; Low: 4	0-10	
S. patens	High: 280; Low: 10		Delaune et al., 2002
S. alterniflora	High: 200; Low: 55	0-50	Kostka et al., 2002
S. anglica (mesocosm)	High: 35; Low: 31	0-50	Gribsholt and Kristensen, 2002
N. diversicolor (mesocosm)	High: 24; Low: 22		
S. anglica	High: 160; Low: 10	0-18	Gribsholt and Kristensen, 2003
S. alterniflora (non-flooded)	Avg: 85.5	0-15	Shin et al., 2000
S. alterniflora (flooded)	Avg: 93.1		
J. roemerianus	High: 117; Low: 15.2	0-20	Miley and Kiene, 2004
S. patens	High: 112.4; Low: 12.3	0-15	Present study

Factors affecting the rate of sulfate reduction in a wetland include availability of electron acceptors and donors, proper pH, and reducing conditions sufficient to allow the reduction of sulfate to proceed favorably. Electron acceptors could be limiting at this site. The sulfate concentration in the above ground water flowing through the marsh was measured at 5.3 mM while porewater concentrations were found to be from 0.2 – 1.2 mM. Levels of sulfate in freshwater systems tend to be below 2 mM and up to 28 mM in seawater. The low sulfate concentrations found are likely due to the limited hydrologic exchange capability of the marsh due to impoundment as well as exceptional drought conditions (Appendix 2). Additionally,

oxidized iron, an alternate electron acceptor for dissimilatory sulfate reducers, is available in the sediment at levels ranging from 196-2596 nmol cm^{-3}. This could be a method of respiration utilized by SRB that would not produce toxic sulfides as a byproduct.

Though organic carbon is abundant in wetlands, the forms necessary for utilization by sulfate reducers may not necessarily be abundant. A weak correlation was found between rates of sulfate reduction and dissolved organic carbon in porewater samples (Figure 7). The measured DOC concentrations are within the range commonly found in wetlands but still might be a limiting factor because no information is known of the structural characteristics of the available carbon. The use of root exudates such as acetate, ethanol, and malate as major carbon sources for SRB growth has been suggested by a number of authors but only tested in association with *Spartina alterniflora* (Hines et al., 1989; Whiting et al., 1986). One source of these organic exudates could be from root leakage while growing (Rovira, A.D., 1969; Weston et al., 2003). Another source of these exudates has been demonstrated in studies by Mendelssohn et al. (1981) and Mendelssohn and McKee (1987) showing that *S. alterniflora* can produce low molecular weight compounds such as ethanol and malate that may diffuse into the surrounding porewater when under anaerobic stress.

The pH was below the optimal circumneutral range for SRB in all treatments. This factor could have an effect on the rate of sulfate reduction (Connell and Patrick, 1968). On the other hand, the measured redox potential of the plots was higher than optimal for sulfate reduction in all treatments (Figure 6). Additionally, during the June sampling there was no significant difference in redox potential between treatments ($p=0.2907$). A likely cause of this occurrence could be the exceptional drought conditions this part of Louisiana experienced for a month prior to sampling (Appendix 2). The ideal range for sulfate reducing bacteria is at Eh values of -100 or

less (Connell and Patrick, 1968). Generally, this may indicate that there were fewer micro-sites with highly reducing conditions in which the SRB could colonize and have access to electron donors and acceptors. A lack of precision is inherent in the measurement of Eh in comparison to microbial communities because there is such a difference in scale caused by the microscopic nature of microbial communities but it can still be useful as a broad spectrum tool. The redox potential tells us that in general the sediment is slightly reducing instead of highly reducing, the optimal condition for sulfate reduction. Redox probes were allowed to equilibrate in sediment for at least 30 minutes prior to measurement so it is possible that there was not enough time for equilibration but care was taken to check for drift when readings were made.

Mean, time-averaged hydrogen sulfide levels in the control plots were very close to the >1.0 mM concentrations that have been shown to inhibit plant growth (Figure 5; Koch et al., 1989). Single measurements of sulfide levels in control plots were very high, 2.0 and 2.6 mM, during one sampling. However, the decrease in sulfide levels as sediment addition increases is a good indicator that plant growth will be less restricted by the stress caused by sulfides on the physiological processes necessary for nutrient uptake. The increased concentrations of iron and manganese in treated plots could have provided a method of remediation through the formation of insoluble Fe-S and Mn-S compounds should higher sulfide levels occur.

As a proxy for direct measurement of the rate of sulfate reduction, the significant differences in sulfide levels across the treatments during the 34 months it was measured indicate that there could have been a difference in the rate of sulfate reduction. Further evidence for this theory comes from the finding that sulfate concentrations followed a corresponding pattern. Sulfate concentrations declined significantly in the control treatment compared to the medium and high treatments indicating that it was being reduced more rapidly as all plots have identical

mechanisms of replenishment. Continued measurement of the SRR and sulfide will help elucidate the longer-term affects that sediment slurry addition had on the marsh.

Biogeochemical cycling of manganese and iron may be occurring as well given the significant differences in the concentrations of these metals found between treatments. A confounding factor in this assessment is that DTPA-extractable levels of both Fe(III) and Mn(IV) in oxidized form follow the same trend as mobile fractions. One would expect that extractable levels would have the opposite trend as the dissolved fraction because the oxidized fraction should be depleted as it is reduced while the reduced fraction increased in concentration. One explanation for the significant increases in iron and manganese concentrations of both sediment and porewater with increasing sediment addition may be the higher levels of these two elements present in the added sediment (3.2±0.2 nmol g^{-1} for Fe and 0.6±0.08 nmol g^{-1} for Mn). Sulfide concentrations are highest in the control plots and decrease with increased elevation which corresponds with the opposite trend in sediment Fe and Mn. This could indicate that sulfide is forming insoluble, reduced molecules such as FeS and MnS. A number of studies have found that sulfide – Fe(III) interaction is the major source of Fe(III) reduction (Jacobson, M.E., 1994; Kostka and Luther, 1995).

6. CONCLUSIONS

The sulfate reduction experiment conducted 34 months after sediment slurry addition does not support my hypothesis that sediment addition decreases the rate of sulfate reduction. On the other hand, it does appear from the sulfate and sulfide data collected that in the time period leading up to the near-term SRR measurement that sulfate reduction rates may have been lower in the medium and high sediment treatments. Although the decrease in sulfide concentrations could be due to the increased concentration of iron that binds with sulfides, decreased sulfate concentrations support the idea that a change in the rate of sulfate reduction occurred. Additionally, the drought conditions that occurred in the spring and summer of 2011 may have masked the treatment effect on the rate of sulfate reduction measured in June 2011. Regardless of the mechanism, the decrease in sulfide concentration is a good indication that plants will be less stressed in the sediment amended marsh. More continuous measurements of the rate of sulfate reduction along with the sulfide concentration would help elucidate the long-term effects of the sediment addition.

Future work on microbial counting in wetland sediment may benefit from the use of the disintegration method studied by J. Boenigk (2004) that relies on hydrofluoric acid to dissolve certain mineral compounds in the sediment while leaving bacteria intact for counting. A method of visually performing counts of microbes is still an important technique in modern microbiology. As we expand our repertoire of modern microbial techniques from PCR-based methods to genomics, we still rely on tried and true methods such as culturing and microscopy to fill in the gaps and provide quality assurance that the more detached computer controlled methods are focusing on the organisms of interest.

Analysis of anaerobic respiration pathways that occur at higher redox potentials (iron and manganese reduction) may be more useful at this and similar sites considering the redox potentials recorded as well as a result of the additional iron and manganese introduced to the site by the sediment itself. The methods for determining these rates are simple and effective. The standard tests performed on the sediment and soluble manganese and iron fractions are one such method of easily determining whether or not this is occurring. Reduction rates can be determined with simple incubation experiments to determine the change in these fractions over time in a controlled environment.

A better understanding of the dissolved organic carbon forms and concentrations available in the sediment may help in understanding the spatial variability found in this study. The growing library of information relating stress responses of sulfate reducing bacteria provides a good background of the particular organic compounds to be looked for in the field. A greenhouse study focusing on the utilization of various dissolved organic carbon compounds by microbes as well as those secreted by macrophytes would also be valuable. This work, with the aid of functional genomics tools, could help provide more insight into the microbial responses related to global climate change. This could be performed with water-level manipulated greenhouse experiments.

REFERENCES

American Public Health Association, American Water Works Association., et al. (2005). Standard methods for the examination of water and wastewater: including bottom sediments and sludges. New York, American Public Health Association.

Babenzien, H. D., J. Meier, et al. (2000). "A comparison of S-35-SO42- radiotracer techniques to determine sulphate reduction rates in laminated sediments." Journal of Microbiological Methods **41**(1): 9-18.

Bak, F. and F. Widdel (1986). "Anaerobic Degradation of Indolic Compounds by Sulfate-Reducing Enrichment Cultures, and Description of Desulfobacterium-Indolicum Gen-Nov, Sp-Nov." Archives of Microbiology **146**(2): 170-176.

Bale, S. J., K. Goodman, et al. (1997). "Desulfovibrio profundus sp nov, a novel barophilic sulfate-reducing bacterium from deep sediment layers in the Japan Sea." International Journal of Systematic Bacteriology **47**(2): 515-521.

Barras, J. A. (2003). Historical and projected coastal Louisiana land changes : 1978-2050. Reston, Va., United States Geological Survey.

Barton, L. (1995). Sulfate-reducing bacteria. New York, Plenum Press.

Barton, L. and W. A. Hamilton (2007). Sulphate-reducing bacteria: environmental and engineered systems. Cambridge, Cambridge University Press.

Baumann, R. H. and R. E. Turner (1990). "Direct Impacts of Outer Continental-Shelf Activities on Wetland Loss in the Central Gulf of Mexico." Environmental Geology and Water Sciences **15**(3): 189-198.

Baustian, J. J., R. E. Turner, et al. (2009). "Restoration of dredged canals in wetlands: a comparison of methods." Wetlands Ecology and Management **17**(5): 445-453.

Boenigk, J. (2004). "A disintegration method for direct counting of bacteria in clay-dominated sediments: dissolving silicates and subsequent fluorescent staining of bacteria." Journal of Microbiological Methods **56**(2): 151-159.

Bohannan, J. (2008). Delta and Breton National Wildlife Refuges: Comprehensive Conservation Plan. U. S. D. o. t. Interior. Atlanta.

Bottrell, S. H. and R. J. Newton (2006). "Reconstruction of changes in global sulfur cycling from marine sulfate isotopes." Earth-Science Reviews **75**(1-4): 59-83.

Boyer, M. E., J. O. Harris, et al. (1997). "Constructed crevasses and land gain in the Mississippi River delta." Restoration Ecology **5**(1): 85-92.

Bramlett, R. N. and H. D. Peck (1975). "Some Physical and Kinetic-Properties of Adenylyl Sulfate Reductase from Desulfovibrio-Vulgaris." Journal of Biological Chemistry **250**(8): 2979-2986.

Brown, M. (2010). Background fluorescence in wetland sediment samples. M. Maxwell. Baton Rouge.

Bryant, J. C. and R. H. Chabreck (1998). "Effects of impoundment on vertical accretion of coastal marsh." Estuaries **21**(3): 416-422.

Cahoon, D. R. (1994). "Recent Accretion in 2 Managed Marsh Impoundments in Coastal Louisiana." Ecological Applications **4**(1): 166-176.

Cahoon, D. R. and J. H. Cowan (1988). "Environmental Impacts and Regulatory Policy Implications of Spray Disposal of Dredged Material in Louisiana Wetlands." Coastal Management **16**(4): 341-362.

Canfield, D. E., B. B. Jorgensen, et al. (1993). "Pathways of Organic-Carbon Oxidation in 3 Continental-Margin Sediments." Marine Geology **113**(1-2): 27-40.

Canfield, D. E. and R. Raiswell (1999). "The evolution of the sulfur cycle." American Journal of Science **299**(7-9): 697-723.

Carey, F. A. (2008). Organic chemistry. Boston, McGraw-Hill Higher Education.

Castro, H. F., N. H. Williams, et al. (2000). "Phylogeny of sulfate-reducing bacteria." Fems Microbiology Ecology **31**(1): 1-9.

Church, J. A. and N. J. White (2006). "A 20th century acceleration in global sea-level rise." Geophysical Research Letters **33**(1).

Coleman, G. S. (1960). "A Sulphate-Reducing Bacterium from the Sheep Rumen." Journal of General Microbiology **22**(2): 423-436.

Connell, W. E. and W. H. Patrick (1968). "Sulfate Reduction in Soil - Effects of Redox Potential and Ph." Science **159**(3810): 86-87.

Cypionka, H. (1987). "Uptake of Sulfate, Sulfite and Thiosulfate by Proton-Anion Symport in Desulfovibrio-Desulfuricans." Archives of Microbiology **148**(2): 144-149.

Dahl, C., N. M. Kredich, et al. (1993). "Dissimilatory Sulfite Reductase from Archaeoglobus-Fulgidus - Physicochemical Properties of the Enzyme and Cloning, Sequencing and Analysis of the Reductase Genes." Journal of General Microbiology **139**: 1817-1828.

Dannenberg, S., M. Kroder, et al. (1992). "Oxidation of H-2, Organic-Compounds and Inorganic Sulfur-Compounds Coupled to Reduction of O-2 or Nitrate by Sulfate-Reducing Bacteria." Archives of Microbiology **158**(2): 93-99.

Dawes, C. J. (1998). Marine botany. New York, John Wiley.

Day, J. W., D. Pont, et al. (1995). "Impacts of Sea-Level Rise on Deltas in the Gulf-of-Mexico and the Mediterranean - the Importance of Pulsing Events to Sustainability." Estuaries **18**(4): 636-647.

DeGregorio, J. (2010). Audubon Society considers allowing oil and gas drilling at Sanctuary in Vermillion Parish. Times-Picayune. New Orleans.

DeLaune, R. D., I. Devai, et al. (2002). "Sulfate reduction in Louisiana marsh soils of varying salinities." Communications in Soil Science and Plant Analysis **33**(1-2): 79-94.

Dilling, W. and H. Cypionka (1990). "Aerobic Respiration in Sulfate-Reducing Bacteria." Fems Microbiology Letters **71**(1-2): 123-127.

DNR, L. (2000). Closure Report: Initial Funding Allocation DNR Dedicated Dredging Program (LA-1). L. D. o. N. R. C. R. Division. Baton Rouge.

DNR, L. (2002). Davis Pond Freshwater Diversion Project: Annual Report 1998-2001. L. D. o. N. Resouces.

Edwards, E. A., L. E. Wills, et al. (1992). "Anaerobic Degradation of Toluene and Xylene by Aquifer Microorganisms under Sulfate-Reducing Conditions." Applied and Environmental Microbiology **58**(3): 794-800.

Fauque, G., M. Czechowski, et al. (1992). "Partial-Purification and Characterization of the 1st Hydrogenase Isolated from a Thermophilic Sulfate-Reducing Bacterium." Biochemical and Biophysical Research Communications **184**(3): 1256-1260.

Fauque, G., J. LeGall, et al. (1991). Sulfate-reducing and sulfur-reducing bacteria. Variations in autotrophic life. J. M. Shively and L. L. Barton. London, Academic**:** 271-337.

Ferdelman, T. G., T. M. Church, et al. (1991). "Sulfur Enrichment of Humic Substances in a Delaware Salt-Marsh Sediment Core." Geochimica Et Cosmochimica Acta **55**(4): 979-988.

Ford, M. A., D. R. Cahoon, et al. (1999). "Restoring marsh elevation in a rapidly subsiding salt marsh by thin-layer deposition of dredged material." Ecological Engineering **12**(3-4): 189-205.

Friedrich, M. and B. Schink (1995). "Isolation and Characterization of a Desulforubidin-Containing Sulfate-Reducing Bacterium Growing with Glycolate." Archives of Microbiology **164**(4): 271-279.

Friedrich, M., N. Springer, et al. (1996). "Phylogenetic positions of Desulfofustis glycolicus gen nov, sp nov, and Syntrophobotulus glycolicus gen nov, sp nov, two new strict anaerobes growing with glycolic acid." International Journal of Systematic Bacteriology **46**(4): 1065-1069.

Fritz, G. (1999). Structure and function of redox proteins involved in dissimilatory sulfate reduction: adenosine 5'-phosphosulfate reductase and multiheme cytochromes, University of Konstanz.

Gribsholt, B. and E. Kristensen (2002). "Effects of bioturbation and plant roots on salt marsh biogeochemistry: a mesocosm study." Marine Ecology-Progress Series **241**: 71-87.

Gribsholt, B. and E. Kristensen (2003). "Benthic metabolism and sulfur cycling along an inundation gradient in a tidal Spartina anglica salt marsh." Limnology and Oceanography **48**(6): 2151-2162.

Heidelberg, J. F., R. Seshadri, et al. (2004). "The genome sequence of the anaerobic, sulfate-reducing bacterium Desulfovibrio vulgaris Hildenborough." Nature Biotechnology **22**(5): 554-559.

Hines, M. E., R. S. Evans, et al. (1999). "Molecular phylogenetic and biogeochemical studies of sulfate-reducing bacteria in the rhizosphere of Spartina alterniflora." Applied and Environmental Microbiology **65**(5): 2209-2216.

Hines, M. E., S. L. Knollmeyer, et al. (1989). "Sulfate Reduction and Other Sedimentary Biogeochemistry in a Northern New-England Salt-Marsh." Limnology and Oceanography **34**(3): 578-590.

Hipp, W. M., A. S. Pott, et al. (1997). "Towards the phylogeny of APS reductases and sirohaem sulfite reductases in sulfate-reducing and sulfur-oxidizing prokaryotes." Microbiology-UK **143**: 2891-2902.

Hobbie, J. E., R. J. Daley, et al. (1977). "Use of Nuclepore Filters for Counting Bacteria by Fluorescence Microscopy." Applied and Environmental Microbiology **33**(5): 1225-1228.

Howarth, R. W. (1984). "The Ecological Significance of Sulfur in the Energy Dynamics of Salt-Marsh and Coastal Marine-Sediments." Biogeochemistry **1**(1): 5-27.

Howarth, R. W. and S. Merkel (1984). "Pyrite Formation and the Measurement of Sulfate Reduction in Salt-Marsh Sediments." Limnology and Oceanography **29**(3): 598-608.

Howarth, R. W. and J. M. Teal (1979). "Sulfate Reduction in a New-England Salt-Marsh." Limnology and Oceanography **24**(6): 999-1013.

Howes, B. L., J. W. H. Dacey, et al. (1984). "Carbon Flow through Oxygen and Sulfate Reduction Pathways in Salt-Marsh Sediments." Limnology and Oceanography **29**(5): 1037-1051.

Jacobson, M. E. (1994). "Chemical and Biological Mobilization of Fe(Iii) in Marsh Sediments." Biogeochemistry **25**(1): 41-60.

Johnson, M. S., I. G. Zhulin, et al. (1997). "Oxygen-dependent growth of the obligate anaerobe Desulfovibrio vulgaris Hildenborough." Journal of Bacteriology **179**(17): 5598-5601.

Jonkers, H. M., M. J. E. C. vanderMaarel, et al. (1996). "Dimethylsulfoxide reduction by marine sulfate-reducing bacteria." Fems Microbiology Letters **136**(3): 283-287.

Karkhoffschweizer, R. R., D. P. W. Huber, et al. (1995). "Conservation of the Genes for Dissimilatory Sulfite Reductase from Desulfovibrio-Vulgaris and Archaeoglobus-Fulgidus Allows Their Detection by Pcr." Applied and Environmental Microbiology **61**(1): 290-296.

Kemp, P. (2010). "Audubon's Dr. Paul Kemp testifies at Gulf recovery gathering." 2011, from http://gulfoilspill.audubon.org/newsroom/news-stories/2010/audubons-dr-paul-kemp-testifies-gulf-recovery-gathering.

Kepner, R. L. and J. R. Pratt (1994). "Use of Fluorochromes for Direct Enumeration of Total Bacteria in Environmental-Samples - Past and Present." Microbiological Reviews **58**(4): 603-615.

King, G. M. (1988). "Patterns of Sulfate Reduction and the Sulfur Cycle in a South Carolina Salt-Marsh." Limnology and Oceanography **33**(3): 376-390.

King, G. M., M. J. Klug, et al. (1982). "Relation of Soil-Water Movement and Sulfide Concentration to Spartina-Alterniflora Production in a Georgia Salt-Marsh." Science **218**(4567): 61-63.

Klemps, R., H. Cypionka, et al. (1985). "Growth with Hydrogen, and Further Physiological-Characteristics of Desulfotomaculum Species." Archives of Microbiology **143**(2): 203-208.

Koch, M. S., I. A. Mendelssohn, et al. (1990). "Mechanism for the Hydrogen Sulfide-Induced Growth Limitation in Wetland Macrophytes." Limnology and Oceanography **35**(2): 399-408.

Kostka, J. E. and G. W. Luther (1995). "Seasonal Cycling of Fe in Salt-Marsh Sediments." Biogeochemistry **29**(2): 159-181.

Kostka, J. E., A. Roychoudhury, et al. (2002). "Rates and controls of anaerobic microbial respiration across spatial and temporal gradients in saltmarsh sediments." Biogeochemistry **60**(1): 49-76.

Kremer, D. R. and T. A. Hansen (1988). "Pathway of Propionate Degradation in Desulfobulbus-Propionicus." Fems Microbiology Letters **49**(2): 273-277.

Leggett, G. E. and D. P. Argyle (1983). "The DTPA-Extractable Iron, Manganese, Copper, and Zinc from Neutral and Calcareous Soils Dried under Different Conditions." Soil Science Society of America Journal **47**(3): 518-522.

Li, Y. (1972). "Geochemical Mass Balance among Lithosphere, Hydrosphere, and Atmosphere." American Journal of Science **272**(2): 119-&.

Liesack, W. and K. Finster (1994). "Phylogenetic Analysis of 5 Strains of Gram-Negative, Obligately Anaerobic, Sulfur-Reducing Bacteria and Description of Desulfuromusa Gen-Nov, Including Desulfuromusa-Kysingii Sp-Nov, Desulfuromusa-Bakii Sp-Nov, and Desulfuromusa-Succinoxidans Sp-Nov." International Journal of Systematic Bacteriology **44**(4): 753-758.

Louro, R. O., P. M. Pereira, et al. (2008). "Energy metabolism in Desulfovibrio vulgaris Hildenborough: insights from transcriptome analysis." Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology **93**(4): 347-362.

Lovley, D. R. and D. J. Lonergan (1990). "Anaerobic Oxidation of Toluene, Phenol, and Para-Cresol by the Dissimilatory Iron-Reducing Organism, Gs-15." Applied and Environmental Microbiology **56**(6): 1858-1864.

Lovley, D. R., E. E. Roden, et al. (1993). "Enzymatic Iron and Uranium Reduction by Sulfate-Reducing Bacteria." Marine Geology **113**(1-2): 41-53.

Madigan, M. T. and T. D. Brock (2009). Brock biology of microorganisms. San Francisco, CA, Pearson/Benjamin Cummings.

Marschall, C., P. Frenzel, et al. (1993). "Influence of Oxygen on Sulfate Reduction and Growth of Sulfate-Reducing Bacteria." Archives of Microbiology **159**(2): 168-173.

McKee, K. L., I. A. Mendelssohn, et al. (1988). "Reexamination of Pore Water Sulfide Concentrations and Redox Potentials near the Aerial Roots of Rhizophora-Mangle and Avicennia-Germinans." American Journal of Botany **75**(9): 1352-1359.

Mendelssohn, I. A. and N. L. Kuhn (2003). "Sediment subsidy: effects on soil-plant responses in a rapidly submerging coastal salt marsh." Ecological Engineering **21**(2-3): 115-128.

Mendelssohn, I. A. and K. L. McKee (1988). "Spartina-Alterniflora Dieback in Louisiana - Time-Course Investigation of Soil Waterlogging Effects." Journal of Ecology **76**(2): 509-521.

Mendelssohn, I. A., R. E. Turner, et al. (1983). "Louisiana Eroding Coastal Zone - Management Alternatives." Journal of the Limnological Society of Southern Africa **9**(2): 63-75.

Miley, G. A. and R. P. Kiene (2004). "Sulfate reduction and porewater chemistry in a gulf coast Juncus roemerianus (Needlerush) marsh." Estuaries **27**(3): 472-481.

Mitsch, W. J. and J. G. Gosselink (2007). Wetlands. Hoboken, N.J., Wiley.

Moura, I., S. Bursakov, et al. (1997). "Nitrate and nitrite utilization in sulfate-reducing bacteria." Anaerobe **3**(5): 279-290.

Murphy, M. J. and L. M. Siegel (1973). "Siroheme and Sirohydrochlorin - Basis for a New Type of Porphyrin-Related Prosthetic Group Common to Both Assimilatory and Dissimilatory Sulfite Reductases." Journal of Biological Chemistry **248**(19): 6911-6919.

Murphy, M. J., L. M. Siegel, et al. (1974). "Siroheme - New Prosthetic Group Participating in 6-Electron Reduction Reactions Catalyzed by Both Sulfite and Nitrite Reductases." Proceedings of the National Academy of Sciences of the United States of America **71**(3): 612-616.

Myers, C. R. and K. H. Nealson (1988). "Microbial Reduction of Manganese Oxides - Interactions with Iron and Sulfur." Geochimica Et Cosmochimica Acta **52**(11): 2727-2732.

National Soil Survey Center (U.S.) (1996). Soil survey laboratory methods manual. Washington, D.C.

Neill, C. and R. E. Turner (1987). "Backfilling Canals to Mitigate Wetland Dredging in Louisiana Coastal Marshes." Environmental Management **11**(6): 823-836.

Nyman, J. A., R. D. Delaune, et al. (1990). "Wetland Soil Formation in the Rapidly Subsiding Mississippi River Deltaic Plain - Mineral and Organic-Matter Relationships." Estuarine Coastal and Shelf Science **31**(1): 57-69.

Ogata, M., K. Arihara, et al. (1981). "D-Lactate Dehydrogenase of Desulfovibrio-Vulgaris." Journal of Biochemistry **89**(5): 1423-1431.

Ollivier, B., R. Cordruwisch, et al. (1988). "Characterization of Desulfovibrio-Fructosovorans Sp-Nov." Archives of Microbiology **149**(5): 447-450.

Peck, H. D. (1959). "The Atp-Dependent Reduction of Sulfate with Hydrogen in Extracts of Desulfovibrio Desulfuricans." Proceedings of the National Academy of Sciences of the United States of America **45**(5): 701-708.

Pfaff, J. D. (1993). Method 300.0: Determination of Inorganic Anions by Ion Chromatography. E. P. Agency. Cincinnati.

Postgate, J. R. (1984). Genus Desulfovibrio. Bergey's manual of systematic bacteriology. D. H. Bergey and J. G. Holt. Baltimore, Md., Williams & Wilkins**:** 666-672.

Rafus, R., T. A. Hansen, et al. (2006). Dissimilatory Sulfate- and Sulfur-Reducing Prokaryotes. The prokaryotes : a handbook on the biology of bacteria. M. Dworkin and S. Falkow. New York ; London, Springer. **2:** 659-768.

Reddy, K. R. and R. D. DeLaune (2008). Biogeochemistry of wetlands: science and applications. Boca Raton, CRC Press.

Reed, D. J. and D. R. Cahoon (1992). "The Relationship between Marsh Surface-Topography, Hydroperiod, and Growth of Spartina-Alterniflora in a Deteriorating Louisiana Salt-Marsh." Journal of Coastal Research **8**(1): 77-87.

Rovira, A. D. (1969). "Plant Root Exudates." Botanical Review **35**(1): 35.

Saxton, A. M. (1998). A macro for converting mean separation output to letter groupings in Proc Mixed. 23rd SAS Users Group International, Gary, NC, SAS Institute.

Schauder, R., B. Eikmanns, et al. (1986). "Acetate Oxidation to Co2 in Anaerobic-Bacteria Via a Novel Pathway Not Involving Reactions of the Citric-Acid Cycle." Archives of Microbiology **145**(2): 162-172.

Schidlowski, M., C. E. Junge, et al. (1977). "Sulfur Isotope Variations in Marine Sulfate Evaporites and Phanerozoic Oxygen Budget." Journal of Geophysical Research-Oceans and Atmospheres **82**(18): 2557-2665.

Schrift, A. M., I. A. Mendelssohn, et al. (2008). "Salt Marsh Restoration with Sediment-Slurry Amendments Following a Drought-Induced Large-Scale Disturbance." Wetlands **28**(4): 1071-1085.

Shin, W. S., J. H. Pardue, et al. (2000). "Oxygen demand and sulfate reduction in petroleum hydrocarbon contaminated salt marsh soils." Water Research **34**(4): 1345-1353.

Slocum, M. G., I. A. Mendelssohn, et al. (2005). "Effects of sediment slurry enrichment on salt marsh rehabilitation: Plant and soil responses over seven years." Estuaries **28**(4): 519-528.

Snyder, P. and J. S. Shaw (1995). PC Oil Drilling in a Wildlife Refuge. The Wall Street Journal. New York.

Stagg, C. L. (2009). Remediating Impacts of Global Climate Change-Induced Submergence on Salt Marsh Ecosystem Functions. Ph.D. Dissertation, Louisiana State University.

Stagg, C. L. and I. A. Mendelssohn (2010). "Restoring Ecological Function to a Submerged Salt Marsh." Restoration Ecology **18**: 10-17.

Stahl, D. A., M. Wagner, et al. (1998). "Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration." Journal of Bacteriology **180**(11): 2975-2982.

Stille, W. and H. G. Truper (1984). "Adenylylsulfate Reductase in Some New Sulfate-Reducing Bacteria." Archives of Microbiology **137**(2): 145-150.

Swenson, E. M. and R. E. Turner (1987). "Spoil Banks - Effects on a Coastal Marsh Water-Level Regime." Estuarine Coastal and Shelf Science **24**(5): 599-609.

Tang, K., V. Baskaran, et al. (2009). "Bacteria of the sulphur cycle: An overview of microbiology, biokinetics and their role in petroleum and mining industries." Biochemical Engineering Journal **44**(1): 73-94.

Thamdrup, B., R. Rossello-Mora, et al. (2000). "Microbial manganese and sulfate reduction in Black Sea shelf sediments." Applied and Environmental Microbiology **66**(7): 2888-2897.

Turner, R. E. (1997). "Wetland loss in the northern Gulf of Mexico: Multiple working hypotheses." Estuaries **20**(1): 1-13.

Turner, R. E., D. R. Cahoon, et al. (1988). Causes of wetland loss in the coastal Central Gulf of Mexico. New Orleans, La., Minerals Management Service.

Turner, R. E. and J. G. Gosselink (1975). "Note on Standing Crops of Spartina-Alterniflora in Texas and Florida." Contributions in Marine Science **19**(Aug): 113-118.

Ulrich, G. A., L. R. Krumholz, et al. (1997). "A rapid and simple method for estimating sulfate reduction activity and quantifying inorganic sulfides (vol 63, pg 1627, 1997)." Applied and Environmental Microbiology **63**(11): 4626-4626.

Warthmann, R. and H. Cypionka (1990). "Sulfate Transport in Desulfobulbus-Propionicus and Desulfococcus-Multivorans." Archives of Microbiology **154**(2): 144-149.

Weston, L. A., C. Bertin, et al. (2003). "The role of root exudates and allelochemicals in the rhizosphere." Plant and Soil **256**(1): 67-83.

Whiting, G. J., E. L. Gandy, et al. (1986). "Tight Coupling of Root-Associated Nitrogen-Fixation and Plant Photosynthesis in the Salt-Marsh Grass Spartina-Alterniflora and Carbon-Dioxide Enhancement of Nitrogenase Activity." Applied and Environmental Microbiology **52**(1): 108-113.

Widdel, F. and N. Pfennig (1981). "Studies on Dissimilatory Sulfate-Reducing Bacteria That Decompose Fatty-Acids .1. Isolation of New Sulfate-Reducing Bacteria Enriched with Acetate from Saline Environments - Description of Desulfobacter-Postgatei Gen-Nov, Sp-Nov." Archives of Microbiology **129**(5): 395-400.

Widdel, F. and N. Pfennig (1982). "Studies on Dissimilatory Sulfate-Reducing Bacteria That Decompose Fatty-Acids .2. Incomplete Oxidation of Propionate by Desulfobulbus-Propionicus Gen-Nov, Sp-Nov." Archives of Microbiology **131**(4): 360-365.

Wilson, L. G. and R. S. Bandurski (1958). "Enzymatic Reactions Involving Sulfate, Sulfite, Selenate, and Molybdate." Journal of Biological Chemistry **233**(4): 975-981.

Zellner, G., P. Messner, et al. (1989). "Desulfovibrio-Simplex Spec-Nov, a New Sulfate-Reducing Bacterium from a Sour Whey Digester." Archives of Microbiology **152**(4): 329-334.

Zhou, J. Z., Q. He, et al. (2010). "Impact of elevated nitrate on sulfate-reducing bacteria: a comparative Study of Desulfovibrio vulgaris." Isme Journal **4**(11): 1386-1397.

Zhou, J. Z., Q. He, et al. (2011). "How sulphate-reducing microorganisms cope with stress: lessons from systems biology." Nature Reviews Microbiology **9**(6): 452-466.

APPENDIX 1: Sulfate Reduction Rates by Plot

Plot	Replicate	Sulfate Reduction Rate (mmol m^{-2} day^{-1})	Mean Sulfate Reduction Rate by Plot (mmol m^{-2} day^{-1})	Treatment Group
1C	1	110.1	84.7	
1C	2	59.3		
1H	1	34.6	33.6	High
1H	2	32.5		High
1L	1	80.0	76.1	
1L	2	72.1		
1M	1	77.7	56.8	High
1M	2	36.0		High
2H	1	23.0	46.7	
2H	2	70.3		
2L	1	126.0	112.4	Medium
2L	2	98.8		Medium
2M	1	106.7	100.2	
2M	2	93.6		
3C	1	1.5	17.0	Low
3C	2	32.6		Low
3H	1	29.5	21.4	
3H	2	13.2		
3L	1	15.2	24.4	
3L	2	33.6		
3M	1	67.5	75.3	Medium
3M	2	83.0		Medium
4C	1	10.9	13.0	
4C	2	15.2		
4H	1	28.2	39.6	High
4H	2	50.9		High
4L	1	4.2	12.3	
4L	2	20.3		
4M	1	36.5	47.3	High
4M	2	58.1		High
5C	1	84.2	73.3	Low
5C	2	62.4		Low

5H	**1**	**79.0**	**58.2**	**Medium**
5H	**2**	**37.5**		**Medium**
5L	**1**	**37.6**	**38.8**	
5L	**2**	**39.9**		
5M	**1**	**76.3**	**78.5**	
5M	**2**	**80.8**		
R1	**1**	**75.1**	**69.4**	**Control**
R1	**2**	**63.8**		**Control**
R2	**1**	**47.3**	**58.9**	**Control**
R2	**2**	**70.5**		**Control**
R3	**1**	**14.7**	**33.5**	**Control**
R3	**2**	**52.2**		**Control**
R4	**1**	**6.6**	**40.5**	
R4	**2**	**74.3**		

APPENDIX 2: Louisiana drought conditions 2 days prior to June 2011 sampling

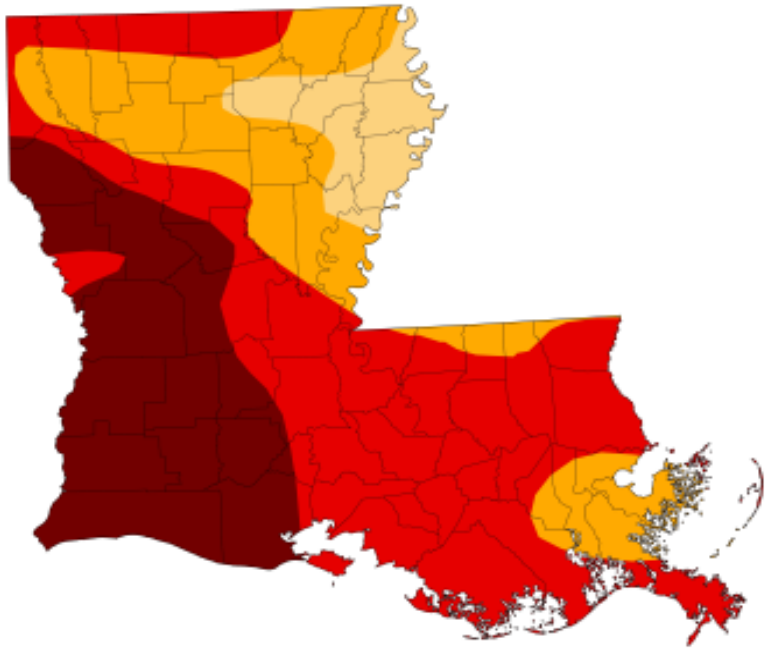
U.S. Drought Monitor

Louisiana

June 14, 2011
Valid 7 a.m. EST

Drought Conditions (Percent Area)

	None	D0-D4	D1-D4	D2-D4	D3-D4	D4
Current	0.00	100.00	100.00	92.71	70.17	27.50
Last Week (06/07/2011 map)	0.00	100.00	100.00	92.71	69.98	20.51
3 Months Ago (03/15/2011 map)	0.00	100.00	83.13	60.01	37.81	0.00
Start of Calendar Year (12/28/2010 map)	0.00	100.00	87.22	59.72	40.99	0.00
Start of Water Year (09/28/2010 map)	6.49	93.51	65.44	35.29	9.18	0.00
One Year Ago (06/08/2010 map)	34.43	65.57	44.68	26.97	0.00	0.00



Intensity:

- D0 Abnormally Dry
- D1 Drought - Moderate
- D2 Drought - Severe
- D3 Drought - Extreme
- D4 Drought - Exceptional

The Drought Monitor focuses on broad-scale conditions. Local conditions may vary. See accompanying text summary for forecast statements.

http://drought.unl.edu/dm

USDA National Drought Mitigation Center

Released Thursday, June 16, 2011
Brian Fuchs, National Drought Mitigation Center

VITA

Martin Maxwell was born in 1981 in El Dorado, Arkansas. After moving to Gulfport, Mississippi, as a young child, Martin spent many hours in the bays, estuaries, rivers and beaches that make the Mississippi Gulf Coast such a beautiful place. Along with his parents, Martin travelled around the country many times, always with an eye to the local flora and fauna encountered.

After graduating from Hancock High School in May of 2000, Martin studied at Mississippi State University and Mississippi Gulf Coast Community College but found working to be more fulfilling than school. After leaving the Mississippi Gulf Coast in the aftermath of Hurricane Katrina he began attending the University of Arkansas at Monticello. After graduating with a Bachelor of Science degree in biology Martin knew that he wanted to learn about the coastal zone of the Gulf of Mexico so that he could help protect and restore it. That led him to the Department of Environmental Science at Louisiana State University. Martin hopes to continue working in the field of wetland restoration after graduation.