

DETERMINING THE IMPACT OF OIL CONTAMINATION ON COUPLED  
NITRIFICATION-DENITRIFICATION PROCESSES IN JUNCUS ROEMERIANUS AND  
SPARTINA ALTERNIFLORA MARSHES: A GREENHOUSE STUDY

by

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## ABSTRACT

Salt marshes along the Gulf of Mexico coast can potentially experience frequent exposure to contamination associated with petroleum hydrocarbons. Oiling may affect rates of coupled nitrification-denitrification (CND) in coastal wetlands, thereby affecting nitrogen (N) cycling and nutrient retention. To examine impacts of oil contamination on CND within different vegetation types, I conducted a greenhouse study using two common salt marsh species, *Juncus roemerianus* and *Spartina alterniflora*. Vegetated mesocosms containing one of the two plant species and exposed to one of three oil addition treatments (none, low, and high) were injected with  $^{15}\text{N}$  label to quantify the rate of CND over a 24 hour period. Rates of N retention and loss were determined from aboveground biomass and sediment. Total loss of N was partitioned into losses due to translocation, diffusion, and CND. Results indicated varying responses depending on oil exposure and plant species. CND rates were higher in mesocosms containing *S. alterniflora* than *J. roemerianus*, and when exposed to oil treatments, CND rates in *S. alterniflora* mesocosms decreased with increasing oil exposure, while they increased with increasing oil exposure for *J. roemerianus*. These species-specific differences were also observed for total loss and diffusion of the tracer from surface to deeper sediments. However, plant uptake of N did not differ between species, perhaps due to the short timeframe of the experiment limiting translocation. These results inform our understanding of oil impacts and species-specific differences in N cycling and nutrient retention, and provide insight for determining the fate of excess nutrients in coastal wetlands experiencing eutrophication.

Wetlands can mitigate nutrient pollution; however, the extent to which this occurs may depend on the species present and may vary in response to disturbances such as oil contamination.

## DEDICATION

This thesis is dedicated to my father, Joseph Schneider, whose passion and admiration for the outdoors is responsible for my growing drive to study the environment.

## LIST OF ABBREVIATIONS AND SYMBOLS

$\alpha$	Alpha significance value
ANOVA	Analysis of variance
C <sub>3</sub>	3 carbon-atoms
C <sub>4</sub>	4 carbon-atoms
cm	Centimeter
CND	Coupled Nitrification-Denitrification
CO <sub>2</sub>	Carbon dioxide
°C	Celsius
d	Day
DN	Denitrification
DN <sub>PG</sub>	Denitrification per gram of aboveground biomass per day
g	Gram
K <sub>T</sub>	Total Loss of nitrogen
K <sub>DN</sub>	Loss due to denitrification
K <sub>DF</sub>	Loss due to diffusion
K <sub>TR</sub>	Loss due to vegetative uptake
K <sub>TPG</sub>	Total Loss of nitrogen per gram of aboveground biomass per day
K <sub>DNPG</sub>	Loss due to denitrification per gram of aboveground biomass per day
K <sub>DFPG</sub>	Loss due to diffusion per gram of aboveground biomass per day

$K_{TRPG}$	Loss due to vegetative uptake per gram of aboveground biomass per day
mg	Milligram
mL	Milliliter
mM	Millimoles
mmhos	Millimhos
$\mu\text{g}$	Micrograms
$\mu\text{mol}$	Micromoles
MC252	Mississippi Canyon Block 252
N	Nitrogen
$\text{NH}_4^+$	Ammonium
$^{15}\text{NH}_4^+$	Ammonium- $^{15}\text{N}$
$^{15}\text{NH}_4\text{Cl}$	Ammonium- $^{15}\text{N}$ chloride
$\text{NO}_3^-$	Nitrate
$\text{O}_2$	Oxygen
pH	Power of hydrogen
SLCO	Sweet Louisiana Crude Oil
SE	Standard Error

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## CHAPTER 1

### INTRODUCTION

Coastal wetlands worldwide are subject to oil contamination because of their geographical proximity to oil rigs and transportation pathways (Garrity et al. 1994, Levings et al. 1994). Oil and gas production represents an important sector of many economies, including those of the U.S. Gulf of Mexico states, and as such, ecological impacts of oil and gas development can be high (Ko and Day 2004). In fact, Gulf of Mexico coastal areas experience oil spills at a rate of about 150 incidents per year (NOAA 2014). Such oil contamination can act as a common stressor to many coastal ecosystems, changing the fate, pathways, and rates of production in salt marshes (Hershner and Lake 1980, Pezeshki and DeLaune 1993, Lin and Mendelssohn 1996, Ko and Day 2004, Lin and Mendelssohn 2012).

Plant species in oil-contaminated coastal wetlands are at risk from the direct and indirect effects of oil exposure. Effects of oil exposure in coastal wetlands have been studied for changes in above and belowground biomass production, reduced gas exchange through plants, and increased plant mortality (Hershner and Lake 1980, Li et al. 1990, Pezeshki and DeLaune 1993, Lin and Mendelssohn 1996, Pezeshki et al. 2000, DeLaune et al. 2003, Ko and Day 2004, DeLaune and Wright 2011, Lin and Mendelssohn 2012). However, possible alterations to the nitrogen (N) cycle from oil exposure, including impacts to N uptake and coupled nitrification-denitrification (CND) rates, have yet to be studied in salt marshes along the Gulf Coast.

Because of their role as hotspots of biogeochemical transformations, it is important to understand the impact that oil contamination may have on N uptake and retention in coastal salt

marshes. Increased primary productivity in many coastal wetlands often results from increased N availability (Vitousek et al. 1997), which is occurring directly and indirectly as a result of human activities. Elevated rates of N input can enhance production of some species, which in turn, can alter ecosystem structure and function (Morris 1991, Galloway 1998), including N fluxes. The threats of this excess N loading can be mitigated by coastal wetlands that act as filters of nutrient pollution in the landscape.

The presence of both anaerobic and aerobic conditions in wetland soils makes them effective sites for CND, and thus, exchange between the ecosystem and the atmosphere. Generally, there are three fates for N reaching coastal wetlands: it can be 1) stored within the ecosystem in sediment, vegetation, roots and microbial pools; 2) lost from the ecosystem via denitrification and subsequent emission to the atmosphere as N<sub>2</sub> gas; or 3) hydrologically exported from the ecosystem with daily tidal fluctuations (Valiela et al. 1978, Deegan et al. 2012). During CND, products of nitrification produced in the oxidized rhizosphere diffuse into the surrounding anaerobic sediments where they can be denitrified (Kremen and Bear 2005). In unvegetated sediments, rates of CND are diminished or eliminated because of the absence of oxygen transport through plant tissues to wetland sediments (Reddy et al. 1989, Hamersley and Howes 2005). Thus, healthy vegetation capable of creating an oxidized rhizosphere is important to sustain high rates of CND in wetland soils, and therefore, to promote the loss of excess N from the ecosystem. If plants are negatively affected by environmental degradation or stress, the presence of adjacent anaerobic and aerobic conditions necessary for CND in the rhizosphere may be diminished. Environmental conditions that alter plant productivity in coastal marshes may affect how much N can be incorporated into plant biomass. Degradation of plant function may affect the ability of plants to compete with nitrifying bacteria for available NH<sub>4</sub><sup>+</sup>, or the extent to

which the rhizosphere is oxidized, and consequently, the ability of these marshes to act as effective removers of nutrient pollution.

Physiological adaptations facilitating the formation of oxidized rhizospheres, and therefore promoting CND, may not function as effectively when plants are stressed or killed by oil contamination. Increased stress on the vegetation could reduce the ability of oxygen to be transported through the plants, which in turn reduces aerobic soil conditions and may limit CND. In fact, rates of nitrification and denitrification are often significantly lower in unvegetated sediments compared to vegetated sediments (Reddy et al. 1989, Hamersley and Howes 2005), highlighting the importance of plant-soil feedbacks for the biogeochemical cycling of N. If plants are negatively affected by environmental degradation, conditions necessary for CND may be reduced.

Given the frequency with which coastal ecosystems worldwide, and Gulf Coast marshes in particular, are exposed to oil contamination, it is also important to understand the impact that oil exposure can have on plant performance and ecosystem functions, including N cycling, and to what extent these responses may vary with species. Coastal wetlands of the northern Gulf of Mexico often are characterized by mixed communities of C<sub>3</sub> rushes or sedges (e.g. *Juncus roemerianus*; Battaglia et al. 2012) and C<sub>4</sub> grasses (e.g., *Spartina alterniflora*) that differ physiologically and functionally (e.g., Morison and Gifford 1983, Schulze et al. 2005). These differences may result in predictable and differing responses to oil exposure. Under oiled conditions, the amount of gas exchange between the sediment-air interface or via plants may be limited, as plants may respond to reductions in gas exchange from oil exposure as they respond to limited gas exchange under other scenarios (e.g., high light, temperature or salinity). When C<sub>3</sub> plants are exposed to conditions of high light or temperature, they close their stomata to limit

water loss, which results in reduced carbon dioxide (CO<sub>2</sub>) intake and an increase in internal oxygen (O<sub>2</sub>) concentrations. Rubisco, an enzyme involved in carbon fixation, has an affinity for both O<sub>2</sub> and CO<sub>2</sub>, and when CO<sub>2</sub> is limiting, binds to O<sub>2</sub> resulting in photorespiration. To eliminate elevated O<sub>2</sub> content, more Rubisco is needed, the production of which occurs at a high N cost (Schulze et al. 2005). C<sub>4</sub> species, on the other hand, have adaptations to tolerate hotter or more saline conditions. Using the enzyme PEP carboxylase during carbon fixation, which does not have an affinity for O<sub>2</sub>, C<sub>4</sub> plants eliminate photorespiration (Schulze et al. 2005). By coating aboveground plant material or raising surface temperatures, oil exposure may indirectly or directly restrict gas exchange in marsh plants, thereby mimicking the effects of high light, high temperature or high salinity environments. Thus, C<sub>4</sub> plants may be better able to deal with the stress of oil contamination than C<sub>3</sub> plants, which may result in species-specific differences in CND rates under oiled conditions.

These physiological and functional differences between marsh plants, as well as the differences in the hydro-edaphic conditions in which they are found, may support distinct microbial communities that differ in CND capacity. Vegetation can influence microbial communities directly and indirectly due to differences in root density and type, above and below ground biomass, nutrient type and availability, inorganic and organic matter content, and abiotic conditions such as salinity, sulfides, and oxygen availability, all of which can differ with plant zonation (Ribeiro et al. 2013). Studies have shown that the composition of microbial communities can be determined by vegetation, sediment characteristics, and the type of oil contaminating wetland soils (Franklin et al. 2002, Blum et al. 2004, Cordova-Kreylos et al. 2006). In fact, a recent study (Ribeiro et al. 2013) found differences in microbial communities of denitrifiers and hydrocarbon degraders associated with different plant species. In this case,

vegetation type, and not oil addition alone, was more important for understanding how the microbial community responded to oil and N additions. Given the potential differences in microbial communities related to plant species presence, differences in denitrification potentials may be expected for *J. roemerianus* and *S. alterniflora*, and may differ depending on the extent of oil exposure.

Species-specific responses to oil contamination, including differences in plant stem density, canopy height, aboveground biomass, and photosynthetic rates, have been observed for *J. roemerianus* (C<sub>3</sub>) and *S. alterniflora* (C<sub>4</sub>) along the Gulf of Mexico (Lin and Mendelsohn 2012). Given that these plants differed in response to oil contamination in the Lin and Mendelsohn (2012) study, it is likely that N retention and loss rates will also differ between *J. roemerianus* and *S. alterniflora* dominated marshes. Both soil microbial communities and gas exchange between plant roots and leaves may be impeded by the added stress of oil contamination, which would have consequences for CND rates. However, alterations of plant N uptake and rates of nitrification-denitrification in salt marshes along the Gulf Coast have yet to be studied as a side effect of oil contamination.

To test for possible species-specific differences in CND under varying oil contamination scenarios, I conducted a controlled greenhouse experiment utilizing a <sup>15</sup>NH<sub>4</sub><sup>+</sup> tracer approach. This approach has been used successfully in prior experiments to determine CND rates in *S. alterniflora* marshes in New England (White and Howes 1994, Hamersley and Howes 2005). Prior studies of this sort revealed rapid uptake of labeled N within hours to days, indicating that microbial communities respond quickly to the introduction of N to sediments (White and Howes 1994, Hamersley and Howes 2005). Thus, I designed a short-term experiment to assess immediate species-specific CND responses to oil contamination. In this experiment, I addressed

the following questions: (1) Are there species-specific differences in CND rates between mesocosms vegetated with *J. roemerianus*, a C<sub>3</sub> rush, and *S. alterniflora*, a C<sub>4</sub> grass; (2) How does oil exposure affect short-term CND rates in vegetated mesocosms; and (3) Do CND rates in *J. roemerianus* and *S. alterniflora* mesocosms respond similarly to oil exposure? I hypothesized that the stress of oiling to vegetation negatively affects CND rates relative to un-oiled controls, and due to species-specific differences in physiology and function, that *S. alterniflora* (C<sub>4</sub>) CND rates are higher than those of *J. roemerianus* (C<sub>3</sub>).

## CHAPTER 2

### METHODOLOGY

#### 2a. SITE DESCRIPTION AND MESOCOSM ESTABLISHMENT

Twenty-one (21) sods of intact soil and monocultures of either *J. roemerianus* or *S. alterniflora* plants were collected from Point Aux Pines marsh (30° 22' 42.863" N, 88° 18' 14.85" W) near Bayou La Batre, AL for use in a controlled greenhouse experiment. Point Aux Pines is a low wave energy, microtidal marsh with a mixed plant community characterized by monotypic stands of *S. alterniflora* along the shoreline and transitioning to monotypic stands of *J. roemerianus* farther inland. Canal channels separate the marsh from upland pine savanna. In addition, field studies were conducted for seasonal comparison of vegetation N uptake rates between summer and winter months in a *S. alterniflora* marsh on Dauphin Island, a small barrier island south of Mobile, Alabama (30° 15' 26.688" N, 88° 7' 26.371" W).

Each study site is located in the Gulf Barrier Islands and Coastal Marshes district of the Southern Coastal Plain Physiographic Section. Near-surface geology is comprised of beach sand and alluvium facies of the Holocene age alluvial, coastal and low terrace deposits, with residual soils derived from this parent material (Szabo et al. 1988). A site-specific soil report was generated using the Web Soil Survey from the National Resources Conservation Service. In summary, the soil present within the area from which sods were collected belongs to the Axis mucky sandy clay loam, 0 to 1 percent slopes. Axis is generally found along tidal flats, toeslope and bank, and is concave in shape going down and concave across slopes. Its parent material is loamy marine deposits derived from sedimentary rock. Overall, it is very poorly drained soil

type. Salinity ranges from 4.0 to 8.0 mmhos/cm. The typical soil profile demonstrates mucky sandy clay loam from 0 to 17.78 cm and sandy loam depths greater than 17.78 cm. Sods (15cm in depth) were collected from this upper mucky sandy clay loam layer.

Vegetated sods collected from Point Aux Pines were placed in mesocosms and transported to the University of Alabama greenhouse in Tuscaloosa, AL. Mesocosms (15.2 cm diameter x 20 cm deep PVC containers) contained a 5 cm layer of pea gravel at the bottom and were equipped with a tubing system to facilitate water drainage. Thus, mesocosms were hydrologically independent, which permitted manual adjustments of water levels to mimic diurnal tides while also isolating isotope-labeled water for later analysis. Mesocosms were randomly positioned within the greenhouse, and plants were allowed to acclimate to greenhouse conditions for at least 3 months prior to the initiation of the experiment in May 2012. All mesocosms received the same, unmanipulated diurnal light exposure throughout the study. Average temperature was 36.3 °C and the average relative humidity was 31.1% during the experiment. In addition, pH, sulfides, and salinity were collected for each mesocosm before the experiment to understand initial differences among mesocosms and to interpret responses to treatments; additionally stem density and aboveground biomass were determined for each mesocosm at the time of collection (Table 2.1).

	JURO			SPAL		
	None	Low	High	None	Low	High
<b>Sulfides (mM)</b>	3.72 ± 0.01 <sup>d</sup>	5.25 ± 0.01 <sup>cd</sup>	4.95 ± 0.01 <sup>cd</sup>	13.58 ± 0.06 <sup>a</sup>	6.66 ± 0.02 <sup>bc</sup>	10.15 ± 0.01 <sup>ab</sup>
<b>Salinity (ppt)</b>	0.73 ± 0.10 <sup>b</sup>	0.13 ± 0.01 <sup>b</sup>	1.18 ± 0.21 <sup>b</sup>	8.29 ± 0.10 <sup>a</sup>	5.70 ± 0.03 <sup>a</sup>	6.23 ± 0.02 <sup>a</sup>
<b>pH</b>	6.23 ± 0.01 <sup>a,B</sup>	7.43 ± 0.01 <sup>a,A</sup>	6.49 ± 0.01 <sup>a,AB</sup>	5.03 ± 0.01 <sup>b,A</sup>	5.33 ± 0.01 <sup>b,A</sup>	5.33 ± 0.01 <sup>b,AB</sup>
<b>Density (stems/mesocosm)</b>	24.42 ± 0.29 <sup>a</sup>	38.31 ± 0.01 <sup>a</sup>	33.10 ± 0.12 <sup>a</sup>	5.26 ± 0.04 <sup>b</sup>	5.97 ± 0.01 <sup>b</sup>	5.59 ± 0.04 <sup>b</sup>
<b>AGBM (g/mesocosm)</b>	38.76 ± 1.20 <sup>a</sup>	51.85 ± 0.58 <sup>a</sup>	46.48 ± 0.78 <sup>a</sup>	26.41 ± 0.70 <sup>b</sup>	33.61 ± 0.36 <sup>b</sup>	29.14 ± 0.15 <sup>b</sup>

Table 2.1 Mean summary table of environmental and plant variables for each species and oil treatment combination. Stem density and aboveground biomass were collected at the time of harvesting. JURO = *J. roemerianus*, and SPAL = *S. alterniflora*. None = un-oiled controls, Low = 20 mL oil/ 20 mL water, and High = 40 mL oil/ 40 mL water. AGBM = aboveground biomass. Values represent reverse-transformed means ± 1 SE (n=3). Letters denote significant differences at  $\alpha=0.1$  for all pairwise comparisons for sulfides (*Species x Oil interaction*), and between species for salinity, density and AGBM (*Species main effects*). For pH, lower case and upper case letters distinguish species and oil treatment main effects, respectively.

## 2b. EXPERIMENTAL DESIGN

The experiment represented a 3 x 2 full-factorial design, with three oil treatments (none, low, high) and two species treatments (*J. roemerianus*, *S. alterniflora*), and three replicates per treatment combination for a total of 18 experimental mesocosms. Given the time and expense required to perform this type of labeling experiment, replication was less than ideal, yet sufficient to explore patterns of oil and species effects on CND. In addition to the 18 experimental mesocosms, we included three time-0, un-oiled controls (2 *J. roemerianus* and 1 *S. alterniflora*), which were sacrificed immediately to determine a baseline CND level for comparison to experimental un-oiled controls sacrificed after 24 hours. All 21 mesocosms were randomly assigned to one of the 18 treatment groups or to one of three time-0 controls. Thus, the experiment was designed to test for effects of species and oil exposure on CND rates after 24 hours by comparing CND rates after 24 hours to baseline readings. Furthermore, the mesocosm

approach permitted isolation of CND occurring in the plant rhizosphere. Therefore, it allowed for the examination of plant-soil interactions and determination of whether or not CND responses across oil treatments depended on plant species.

To achieve oiling treatments, I used Sweet Louisiana crude oil (SLCO), a Deepwater Horizon MC252 surrogate. Prior to application, oil underwent weathering by placing it in a fume hood allowing volatilization of light hydrocarbons until a constant weight was achieved. The oil was then emulsified by combining 50% water and 50% oil. Emulsified oil was then applied to assigned mesocosms in quantities representing high (40 mL oil/ 40 mL water) or low (20 mL oil/ 20 mL water) levels of contamination (*sensu* Lin and Mendelsohn 2012). Before the initial application of emulsified oil, water was added to the soil surface to simulate high tide. After the initial application of emulsified oil, water levels were lowered to simulate low tides and the drained water was collected in mesocosm-specific containers. This collected water was reapplied to the mesocosms over the course of 5 days to partially expose stems to oiling and to ensure oil had percolated into the rhizosphere of the marsh sods. Control mesocosms were treated similarly using water without any emulsified oil.

Five days after initial oil exposure, all mesocosms were evenly injected with 0.82 mL  $\text{cm}^{-1}$  of  $^{15}\text{NH}_4\text{Cl}$  solution throughout the upper 10 cm of soil, beginning at 10 cm and moving up to the soil surface. Label applications and procedures mirrored those presented in Hamersley and Howes (2005). The addition of ammonium ( $\text{NH}_4^+$ ) permits examination of CND, as opposed to direct denitrification of nitrate ( $\text{NO}_3^-$ ), because the labeled ammonium must first be oxidized before it can be denitrified. Time 0 controls (n=3) were harvested immediately; the remaining cores were harvested after 24 hours (n=18). During the final harvest, aboveground plant material was collected for determination of stem density (live and dead), stem height, standing biomass,

and for isotope analysis to determine rates of N uptake by plants. Sediment cores, comprised of both marsh sediment and belowground plant material, were collected and sectioned by depth (0-10 cm, 10-15 cm), and then immediately frozen until processing. To minimize  $^{15}\text{NH}_4^+$  volatilization during the drying process, frozen cores were submerged in 0.2 N sulfuric acid to achieve a pH of 2. Sediment cores and aboveground biomass were then dried at 60°C, ground through a 40 micron mesh using a Wiley Mill, homogenized, and sub-sampled for isotopic analysis to determine the amount of N remaining in the sediments and belowground biomass.

A similar protocol was followed to quantify seasonal vegetation uptake of labeled N at the Dauphin Island marsh. Transects established in *S. alterniflora* stands consisted of 3 replicates per 5 time points (1 meter spacing in between time point locations), for a total of 15 plots. The upper 10 cm of sediment in each plot was line-injected with 0.1602 and 0.1558 grams of  $^{15}\text{NH}_4\text{Cl}$  in 250 mL of water in July 2011 and March 2012, respectively, and N uptake by vegetation was measured using the same approach as in the greenhouse. All plots were sampled within a 55 hour time frame to capture plant N uptake, as in White and Howes (1994) and Hamersley and Howes (2005).

To determine N isotope ratios, sub-samples of 2.5 mg of ground and homogenized greenhouse and field vegetation, and 0.30 mg of ground greenhouse sediment were packed into tin and silver cups, respectively, and analyzed using isotope mass spectrometry at the Washington State Stable Isotope Lab or the Utah State Isotope Lab. Results of these aboveground biomass and sediment samples were used to calculate N losses from the upper 0-10 cm of sediment over time and rates of denitrification as described below.

Atom % (how much N is present relative to the control) was examined relative to the background N level (0.3669%) measured from cores not injected with label. Grams of N in

biomass were determined based on the % N in vegetation and the total weight of aboveground biomass in the mesocosm. The amount of N in biomass that was in excess of the background was compared to the amount of N label added (0.000754795 grams of  $^{15}\text{N}$ , 0.82 ml  $\text{cm}^{-1}$  of  $^{15}\text{NH}_4\text{Cl}$ ). The percent loss relative to the proportion of grams of  $^{15}\text{N}$  recovered in controls was used to determine the various pathways of N loss during the experiment. The calculated recovered grams of  $^{15}\text{N}$  in controls were used as a baseline for available  $\text{NH}_4^+$  to be denitrified post application until the point of harvest (time 1). Baseline available  $\text{NH}_4^+$  was determined to account for variability in recovery. Losses were determined by comparing available N at time 0 (0 hours after injection) and time 1 (24 hours after injection) using the following equation:

$$K_T = K_{\text{TR}} + K_{\text{DF}} + K_{\text{DN}}$$

where  $K_T$  represents total loss of  $^{15}\text{N}$  over time from the 0-10 cm sediment layer;  $K_{\text{TR}}$ , or vegetative uptake/translocation, represents loss of  $^{15}\text{N}$  from the 0-10 cm layer to aboveground biomass;  $K_{\text{DF}}$ , or advection/diffusion, represents loss of  $^{15}\text{N}$  over time from the upper 0-10 cm to the bottom 10-15 cm of sediment; and  $K_{\text{DN}}$ , which was calculated by subtracting all other losses from  $K_T$ , represents loss due to denitrification. When baseline values were lower than those at time 1, negative values were reported and represent 0 or no loss for the given pathway. The rate of denitrification (DN) through the pathway of CND was then calculated using the following equation:

$$\text{DN } (\mu\text{mol N g}^{-1}\text{d}^{-1}) = K_{\text{DN}} [\text{NH}_4^+]$$

where  $[\text{NH}_4^+]$  is the natural abundance of  $\text{NH}_4^+$  in sediments. The natural abundance of  $\text{NH}_4^+$  was determined using a KCL extraction methods on non-injected sediment cores (n=3) collected from Point Aux Pines, as described in Hamersley and Howes (2005). The average concentration of  $\text{NH}_4^+$  in the sediment cores ( $306.60 \pm 34.10 \mu\text{mol}$ ; n=3) was used to determine denitrification.

## Statistical Analysis

Two-way analysis of variance (ANOVA) was used to test for differences in environmental and plant variables among mesocosms (Table 2.1), as well as treatment effects on N losses and DN. Because of low replication and high variability, main effects of oil (none, low, high), species (*J. roemerianus* and *S. alterniflora*), and their interactions were all tested at  $\alpha = 0.1$  level. When significant differences were detected, Students t or Tukey's HSD tests were performed to compare means among species and oil treatments, respectively, and their interactions. Because plant biomass differed between mesocosms, and was significantly greater in JURO vs. SPAL mesocosms (Table 2.1), losses ( $K_T$ ,  $K_{TR}$ ,  $K_{DF}$ , and  $K_{DN}$ ) were standardized by grams of aboveground biomass per day (% loss  $g^{-1} d^{-1}$ ) prior to analysis, and abbreviated as  $K_{TPG}$ ,  $K_{TRPG}$ ,  $K_{DFPG}$ , and  $K_{DNPG}$ , respectively. Environmental and plant data were square-root transformed to meet assumptions of normality and equal variances; reverse-transformed means and standard errors are reported as indicated in Table 2.1, referenced above. All loss and DN data met assumptions of normality and equal variances; no data were transformed. Relationships between environmental or plant variables and  $K_{DNPG}$  were examined using linear regression. Sulfide concentrations in JURO mesocosms were square-root transformed to meet normality assumptions as indicated in Figure 3.5; all other datasets met assumptions for linear regression. All analyses were performed in JMP 10 (SAS Institute, Cary, NC, USA).

## CHAPTER 3

### RESULTS

#### Pathways of N Loss: Total Loss ( $K_T$ )

Denitrification represents one pathway of overall N loss of from marsh sediments during the 24 hour experiment. To examine the fate of N, I examined the various components contributing to total N loss ( $K_T$ ) from the upper 10 cm of sediment. Although not significant,  $K_T$  per gram of aboveground biomass per day ( $K_{TPG}$ ) increased in *J. roemerianus* with increasing oil exposure, whereas it decreased in *S. alterniflora* with increasing oil (Table 3.1, Figure 3.1 *Species x Oil*), a pattern similar to that observed for DN (see below). Total losses per gram of aboveground biomass per day ( $\% \text{ g}^{-1} \text{ d}^{-1}$ ) for *J. roemerianus* and *S. alterniflora* mesocosms averaged  $-0.04 \pm 0.35$  and  $1.32 \pm 0.21$  for no oil,  $0.35 \pm 0.22$  and  $1.12 \pm 0.24$  for low oil, and  $0.58 \pm 0.15$  and  $0.83 \pm 0.19$  for high oil, respectively (Table 3.1). In the absence of oil, *S. alterniflora*  $K_{TPG}$  was significantly greater than *J. roemerianus*  $K_{TPG}$  ( $F_{1,12}=17.39$ ,  $p=0.0013$ ; Table 3.2, Figure 3.1 *Species*). On average, rates of  $K_{TPG}$  for *J. roemerianus* and *S. alterniflora*, regardless of the amount of added oil, were  $0.29 \pm 0.16$  and  $1.09 \pm 0.13$ , respectively (Table 3.2).

	JURO			SPAL		
	None	Low	High	None	Low	High
<b>K<sub>TPG</sub></b>	-0.04 ± 0.35 <sup>C</sup>	0.35 ± 0.22 <sup>BC</sup>	0.58 ± 0.15 <sup>ABC</sup>	1.32 ± 0.21 <sup>A</sup>	1.12 ± 0.24 <sup>AB</sup>	0.83 ± 0.19 <sup>ABC</sup>
<b>K<sub>TRPG</sub></b>	0.18 ± 0.10	0.25 ± 0.06	0.25 ± 0.02	0.26 ± 0.05	0.26 ± 0.15	0.45 ± 0.26
<b>K<sub>DFPG</sub></b>	-0.27 ± 0.10	-0.16 ± 0.03	-0.09 ± 0.12	0.33 ± 0.13	0.36 ± 0.13	0.14 ± 0.28
<b>K<sub>DNPG</sub></b>	0.05 ± 0.20 <sup>B</sup>	0.26 ± 0.19 <sup>AB</sup>	0.41 ± 0.06 <sup>AB</sup>	0.73 ± 0.03 <sup>A</sup>	0.50 ± 0.21 <sup>AB</sup>	0.25 ± 0.14 <sup>AB</sup>
<b>DN<sub>PG</sub></b>	0.15 ± 0.61 <sup>B</sup>	0.80 ± 0.58 <sup>AB</sup>	1.25 ± 0.18 <sup>AB</sup>	2.24 ± 0.10 <sup>A</sup>	1.55 ± 0.64 <sup>AB</sup>	0.75 ± 0.43 <sup>AB</sup>

Table 3.1 Nitrogen losses ( $K_{TPG}$ ,  $K_{TRPG}$ ,  $K_{DFPG}$ , and  $K_{DNPG}$ ) expressed as %  $g^{-1} d^{-1}$ . Values were standardized by grams of aboveground biomass for each species and oil treatment combination. JURO = *J. roemerianus*, and SPAL = *S. alterniflora*. None = un-oiled controls, Low = 20 mL oil/ 20 mL water, and High = 40 mL oil/ 40 mL water. Values represent means  $\pm$  1 SE (n=3). Letters denote significant differences at  $\alpha = 0.1$  for all pairwise comparisons for each variable.

	JURO	SPAL
<b>K<sub>TPG</sub></b>	0.29 ± 0.16 <sup>B</sup>	1.09 ± 0.13 <sup>A</sup>
<b>K<sub>DFPG</sub></b>	-0.17 ± 0.05 <sup>B</sup>	0.28 ± 0.10 <sup>A</sup>
<b>K<sub>DNPG</sub></b>	0.24 ± 0.10 <sup>B</sup>	0.49 ± 0.10 <sup>A</sup>
<b>DN<sub>PG</sub></b>	0.73 ± 0.30 <sup>B</sup>	1.54 ± 0.31 <sup>A</sup>

Table 3.2 Nitrogen losses for *J. roemerianus* (JURO) and *S. alterniflora* (SPAL) mesocosms, regardless of oil treatment, standardized by grams of aboveground biomass. Values are the mean  $\pm$  1 SE (n=9). Letters denote significant differences at  $\alpha = 0.1$  between species for each variable.

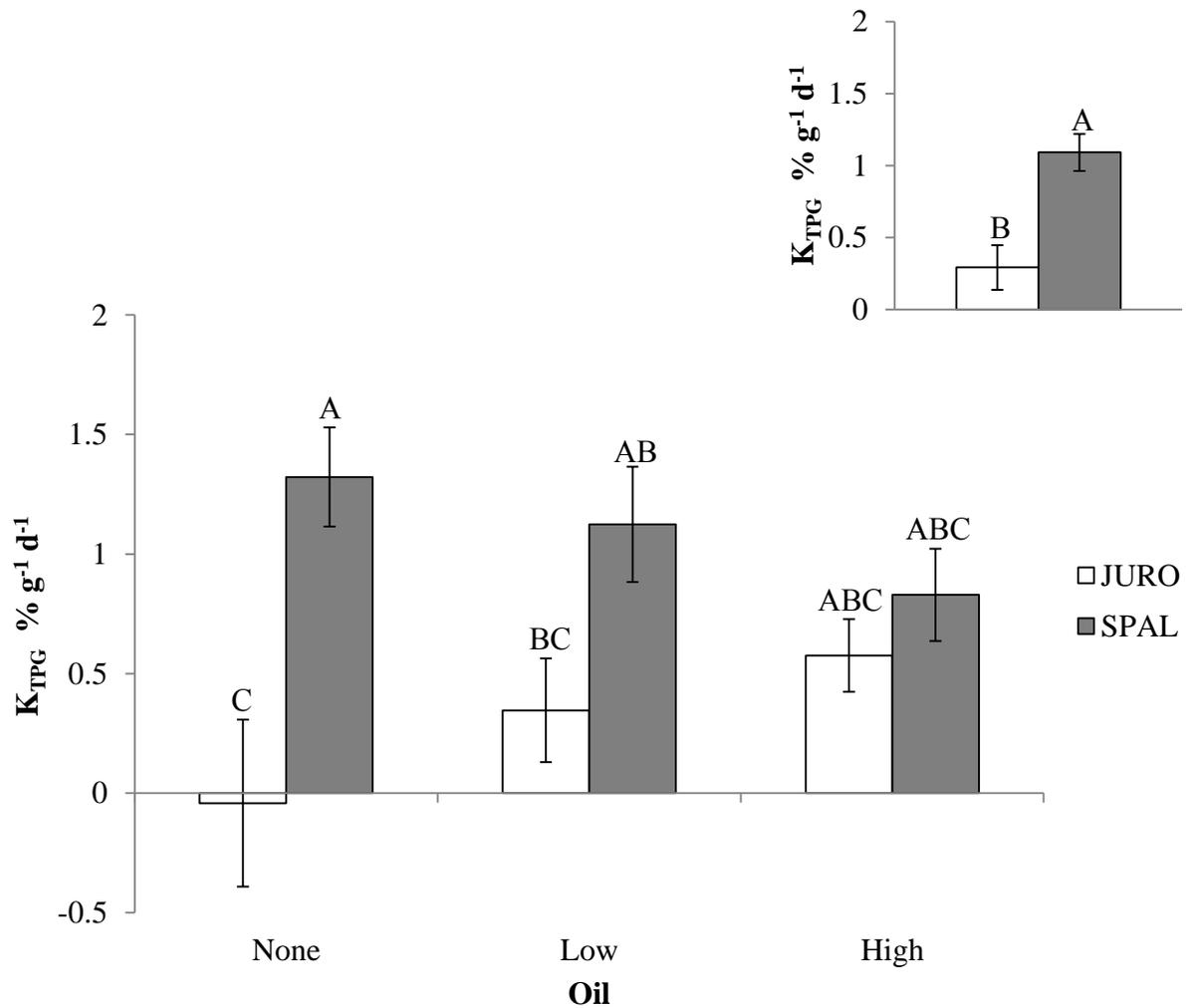


Figure 3.1 *Species x Oil*: Total Loss of nitrogen per gram of aboveground biomass per day ( $K_{TPG}$ ) across species and oil treatments ( $F_{2,12}=2.80$ ,  $p=0.10$ ;  $n=3$ ). *Species*:  $K_{TPG}$  between species ( $F_{1,12}=17.39$ ,  $p=0.0013$ ;  $n=9$ ). JURO = *J. roemerianus*, and SPAL = *S. alterniflora*. None = un-oiled controls, Low = oil treatments of 20 mL oil/ 20 mL water, and High = oil treatments of 40 mL oil/ 40 mL water. Values represent means  $\pm 1$  SE. Letters denote significant differences at  $\alpha=0.1$  for all pairwise comparisons (*Species x Oil*) or between species (*Species*).

### Pathways of N loss: Translocation ( $K_{TR}$ )

$K_{TR}$  per gram of aboveground biomass per day ( $K_{TRPG}$ ) did not differ among oiling treatments or between species following label injections ( $F_{2,12}=0.24$ ,  $p=0.79$ ; Figure 3.2). On average, uptake of labeled N ( $\% g^{-1} d^{-1}$ ) for *J. roemerianus* and *S. alterniflora* was  $0.18 \pm 0.10$  and  $0.21 \pm 0.05$  in unoiled-controls,  $0.25 \pm 0.06$  and  $0.26 \pm 0.15$  for low oiling, and  $0.25 \pm 0.02$  and  $0.45 \pm 0.26$  for high oiling, respectively (Table 3.1).

Uptake by vegetation in the greenhouse fell within the ranges documented in July 2011 and March 2012 for the *S. alterniflora* marsh on Dauphin Island (Table 3.3). In this case of field trials, values represent the fraction of injected  $^{15}N$  label taken up by plants, and are not standardized by aboveground biomass or daily rate of change. The average percent uptake of  $^{15}N$  by vegetation in the greenhouse study ranged from  $4.86 \pm 0.74$  to  $9.12 \pm 1.15$ , which was within the range observed for *S. alterniflora* in the field ( $3.84 \pm 0.70$  to  $12.19 \pm 4.63$ ) (Table 3.3).

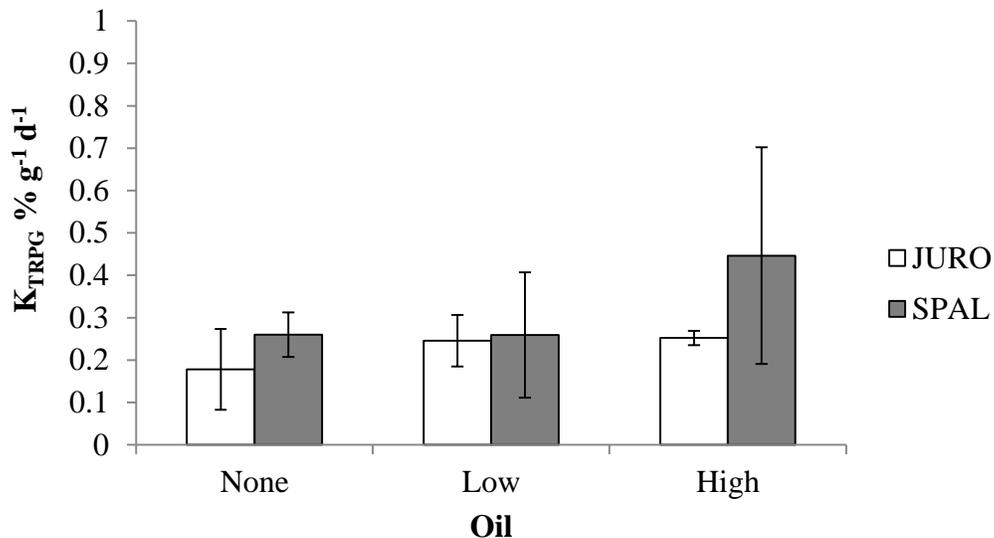


Figure 3.2 Loss due to vegetative uptake per gram of aboveground biomass per day ( $K_{TRPG}$ ) across species and oil treatments ( $F_{2,12}=0.24$ ,  $p=0.79$ ;  $n=3$ ). JURO = *J. roemerianus*, and SPAL = *S. alterniflora*. None = un-oiled controls, Low = 20 mL oil/ 20 mL water, and High = 40 mL oil/ 40 mL water. Values represent means  $\pm 1$  SE.

<b>Field</b>	<b>Time (24 hours)</b>	<b>Greenhouse</b>	<b>Time (24 hours)</b>
July 2011	12.19 ± 4.63	SPAL High	8.49 ± 3.80
March 2012	3.84 ± 0.70	SPAL Low	5.96 ± 2.60
		SPAL None	4.86 ± 0.74
		JURO High	9.00 ± 1.90
		JURO Low	9.12 ± 1.15
		JURO None	6.38 ± 3.13

Table 3.3 Average % <sup>15</sup>N taken up by *S. alterniflora* in July 2011 and March 2012 at the Dauphin Island field site and by *J. roemerianus* and *S. alterniflora* in the greenhouse after 24 hours. JURO = *J. roemerianus*, and SPAL = *S. alterniflora*. None = un-oiled controls, Low = 20 mL oil/ 20 mL water, and High = 40 mL oil/ 40 mL water. Values represent mean ± 1 SE (n=15 in field; n=3 in greenhouse).

### Pathways of Loss: Diffusion Downward ( $K_{DF}$ )

$K_{DF}$  per gram of aboveground biomass per day ( $K_{DFPG}$ ) differed between species. Diffusive losses from the 0-10 cm layer in *S. alterniflora* sediments ( $0.28 \pm 0.10 \% \text{ g}^{-1} \text{ d}^{-1}$ ) were higher than in *J. roemerianus* sediments ( $-0.17 \pm 0.05 \% \text{ g}^{-1} \text{ d}^{-1}$ ), regardless of oil exposure (Table 3.1 and 3.2, Figure 3.3 *Species*). The mesocosm design allowed for only one possible diffusion pathway of the labeled N from the upper 0-10 cm to the bottom 10-15 cm of sediment. However, because label was injected at 10 cm, some label immediately crossed this boundary and entered the lower 10-15 cm layer at time 0. Consequently, when diffusive losses were calculated as the change in label amount relative to the 0-10 cm sediment layer, some negative  $K_{DFPG}$  values were obtained, particularly for *J. roemerianus*, because larger diffusion values had been deducted from smaller baseline averages. In such cases, these values should be interpreted as no loss from the 0-10 cm layer over time.

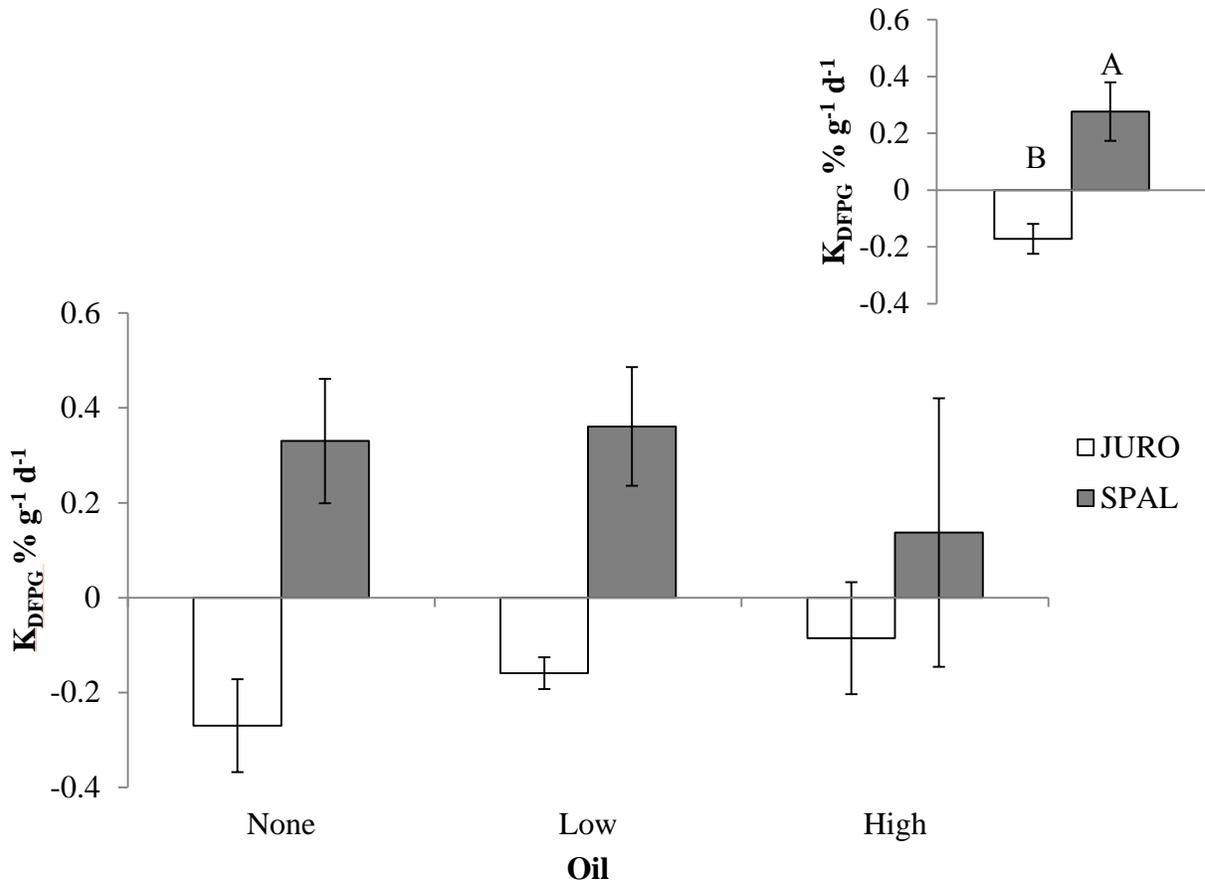


Figure 3.3 *Species x Oil*: Loss due to downward diffusion per gram of aboveground biomass per day ( $K_{DFFPG}$ ) across species and oil treatments ( $F_{2,12}=0.86$ ,  $p=0.45$ ;  $n=3$ ). *Species*:  $K_{DFFPG}$  between species ( $F_{1,12}= 13.10$ ,  $p=0.0035$ ;  $n=9$ ). JURO = *J. roemerianus*, and SPAL = *S. alterniflora*. None = un-oiled controls, Low = 20 mL oil/ 20 mL water, and High = 40 mL oil/ 40 mL water. Values represent means  $\pm$  1 SE. Letters denote significant differences at  $\alpha=0.1$  between species (*Species*).

### Pathways of Loss: Denitrification ( $K_{DN}$ )

$K_{DN}$  was the dominant pathway for loss of labeled N (Table 3.1), with responses between species and among oil treatments similar to those observed for  $K_T$ . Averages for  $K_{DN}$  per gram of aboveground biomass per day ( $K_{DNPG}$ ) ( $\% \text{ g}^{-1} \text{ d}^{-1}$ ) for *J. roemerianus* no oil, low oil, and high oil were  $0.05 \pm 0.20$ ,  $0.26 \pm 0.19$ ,  $0.41 \pm 0.06$ , respectively. Averages observed for *S. alterniflora* no oil, low oil, and high oil were  $0.73 \pm 0.03$ ,  $0.50 \pm 0.21$ ,  $0.25 \pm 0.14$ , respectively (Table 3.1). *S. alterniflora*  $K_{DNPG}$  values were significantly greater than those for *J. roemerianus*, regardless of oil exposure ( $F_{1,12}=4.05$ ,  $p=0.07$ ; Table 3.1, Figure 3.4 *Species*). Negative  $K_{DNPG}$  values occurred when larger loss values at time 1 were subtracted from smaller baseline averages, and ultimately signify no loss via  $CND$ . Species-specific  $K_{DNPG}$  responses were not constant across oil treatments ( $F_{2,12}=3.72$ ,  $p=0.06$ ; Figure 3.4 *Species x Oil*).  $K_{DNPG}$  was greater for *S. alterniflora* than *J. roemerianus* in un-oiled controls, and as  $K_{DNPG}$  values decreased with increasing oil exposure for *S. alterniflora*, values increased with increasing oil exposure for *J. roemerianus* (Table 3.2, Figure 3.4 *Species x Oil*). Species and oil treatment effects on  $K_{DNPG}$  mirrored trends observed for  $K_{TPG}$  (Table 3.1, Figure 3.1), which suggests that  $CND$  drove patterns of total loss.

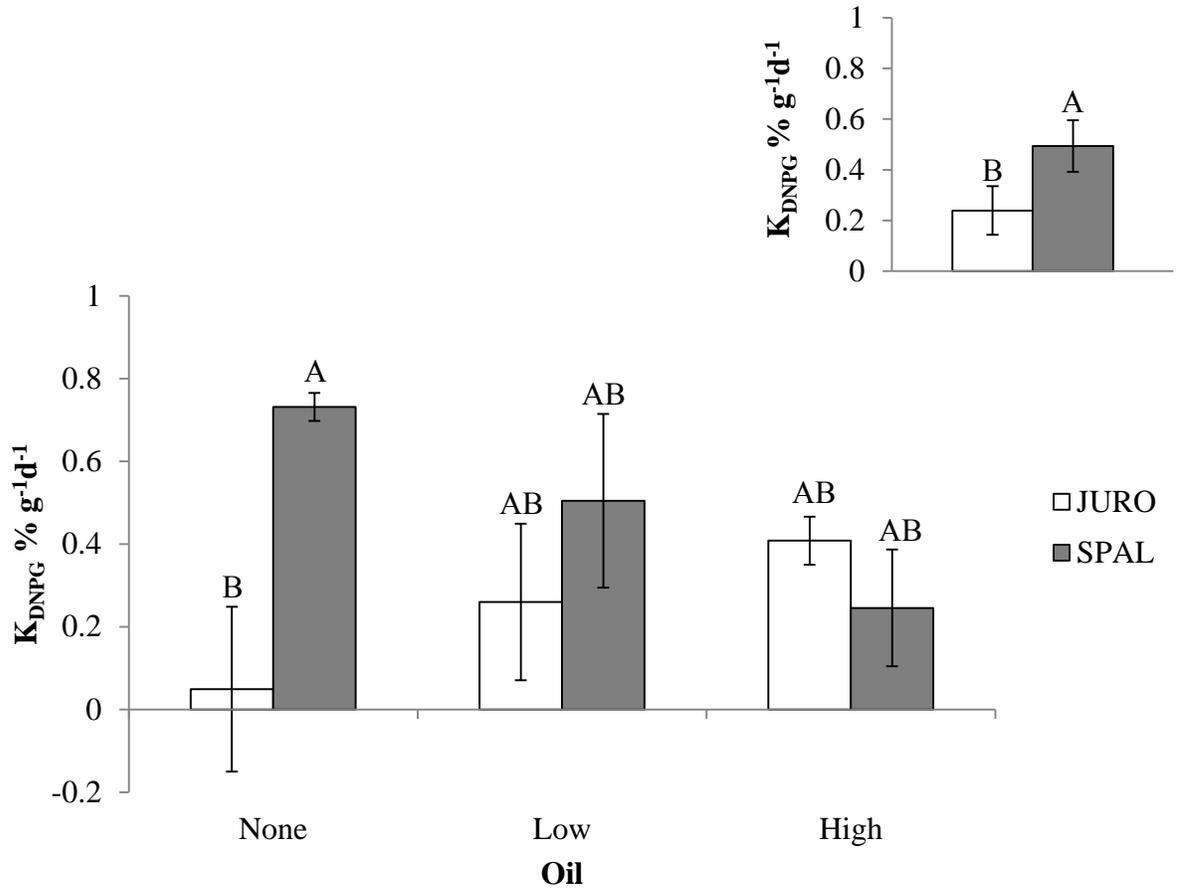


Figure 3.4 *Species x Oil*: Loss due to denitrification per gram of aboveground biomass per day ( $K_{DNPG}$ ) across species and oil treatments ( $F_{2,12}=3.72$ ,  $p=0.06$ ;  $n=3$ ). *Species*:  $K_{DNPG}$  between species ( $F_{1,12}=4.05$ ,  $p=0.07$ ;  $n=9$ ). JURO = *J. roemerianus*, and SPAL = *S. alterniflora*. None = un-oiled controls, Low = 20 mL oil/ 20 mL water, and High = 40 mL oil/ 40 mL water. Values represent means  $\pm 1$  SE. Letters denote significant differences at  $\alpha=0.1$  for all pairwise comparisons (*Species x Oil*) or between species (*Species*).

Environmental variables and live stem density measured prior to oiling and labelling, as well as standing aboveground biomass measured immediately following the experiment, were analyzed to identify potential differences among mesocosms that could influence patterns of CND during the 24 hour experiment. Sulfide, salinity, and pH were not consistent across mesocosms (Table 2.1). Generally, sulfides were higher in *S. alterniflora* mesocosms than in *J. roemerianus* ones, with *S. alterniflora* mesocosms assigned to un-oiled controls and high oiling having significantly higher sulfides than all *J. roemerianus* mesocosms. Salinity was significantly higher, and pH significantly lower, in *S. alterniflora* mesocosms than in *J. roemerianus* ones. In addition, mesocosms assigned to low oiling had significantly higher pH than those assigned to un-oiled controls. These differences in sulfides and pH among oil treatments were not due to oil applications, but instead demonstrated that initial environmental conditions were not equivalent across oil treatments. In addition, live stem density and aboveground biomass were greater in *J. roemerianus* mesocosms than in *S. alterniflora* ones. Given the significant differences in the live stem density and AGBM between species, losses and DN rates were standardized by gram of biomass to ensure comparability of means across treatments. Differences in environmental variables between species, however, may have contributed to observed loss patterns and driven the species-specific differences in denitrification.

To assess the potential effects of salinity, sulfides, and pH on denitrification, relationships between environmental variables and  $K_{DN}$  were examined. Overall,  $K_{DNPG}$  rates increased with increasing sulfides ( $R_2=0.31$ ,  $p=0.02$ ,  $y=0.05x +0.02$ ) and salinity ( $R_2=0.23$ ,  $p=0.04$ ,  $y=0.04x+0.20$ ), regardless of oil or species. These patterns were not apparent when examined separately by species, with one exception. In *J. roemerianus* mesocosms,  $K_{DNPG}$

significantly increased with increasing sulfides, regardless of oil (Figure 3.5). No relationship was evident for pH and  $K_{\text{DNPG}}$ .

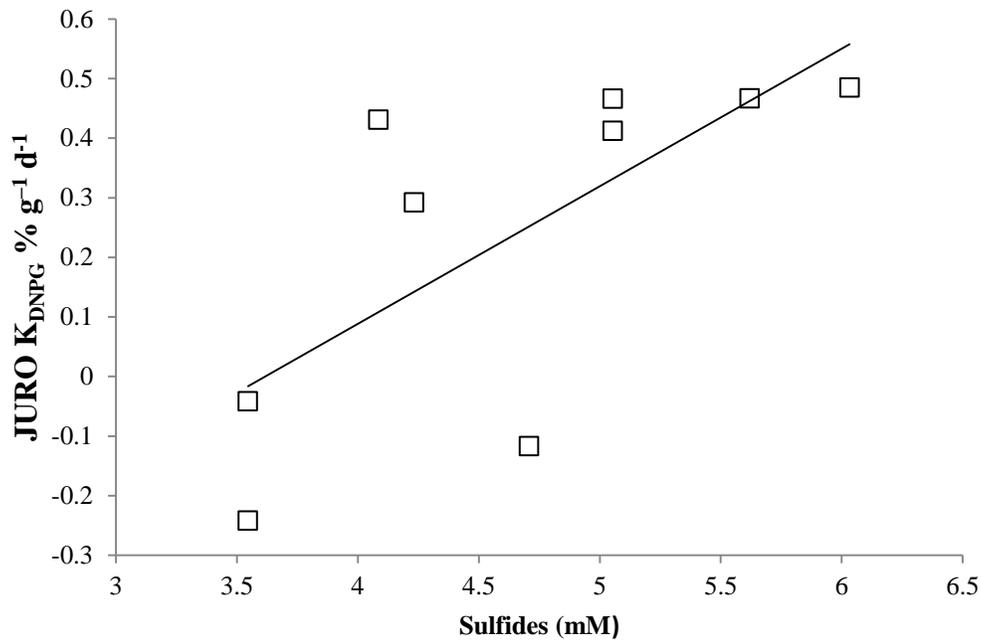


Figure 3.5 Relationship between sulfide concentration (mM) and  $K_{\text{DNPG}}$  for *J. roemerianus* mesocosms, regardless of oil exposure ( $R^2 = 0.50$ ,  $p = 0.03$ ,  $y = 1.00x - 1.92$ ,  $n = 9$ ). Data were square-root transformed for analysis to meet assumptions of normality.

### Rates of Denitrification (DN)

Patterns of denitrification rates per gram of aboveground biomass per day ( $DN_{PG}$ ) mimicked those observed for  $K_{DN_{PG}}$ , identifying CND as an important pathway of N loss. Denitrification rates in *S. alterniflora* mesocosms were significantly greater than in *J. roemerianus* mesocosms, regardless of oil exposure ( $F_{1,12}=4.05$ ,  $p=0.07$ ; Figure 3.6 *Species*). However, species-specific  $DN_{PG}$  responses were not consistent across oil treatments ( $F_{2,12}=3.72$ ,  $p=0.06$ ; Table 3.1, Figure 3.6 *Species x Oil*). In the absence of oil, *S. alterniflora*  $DN_{PG}$  rates were greater than those for *J. roemerianus*, and while *S. alterniflora*  $DN_{PG}$  rates decreased with increasing oil exposure, they increased with increasing oil exposure for *J. roemerianus* (Table 3.2, Figure 3.6 *Species*). After the 24 hour study, average  $DN_{PG}$  rates ( $\mu\text{mol N g}^{-1} \text{d}^{-1}$ ) for *J. roemerianus* and *S. alterniflora* were  $0.15 \pm 0.61$  and  $2.24 \pm 0.10$ ,  $0.80 \pm 0.58$  and  $1.55 \pm 0.64$ , and  $1.25 \pm 0.18$  and  $0.75 \pm 0.43$  in none, low, and high oil treatments, respectively (Table 3.1).

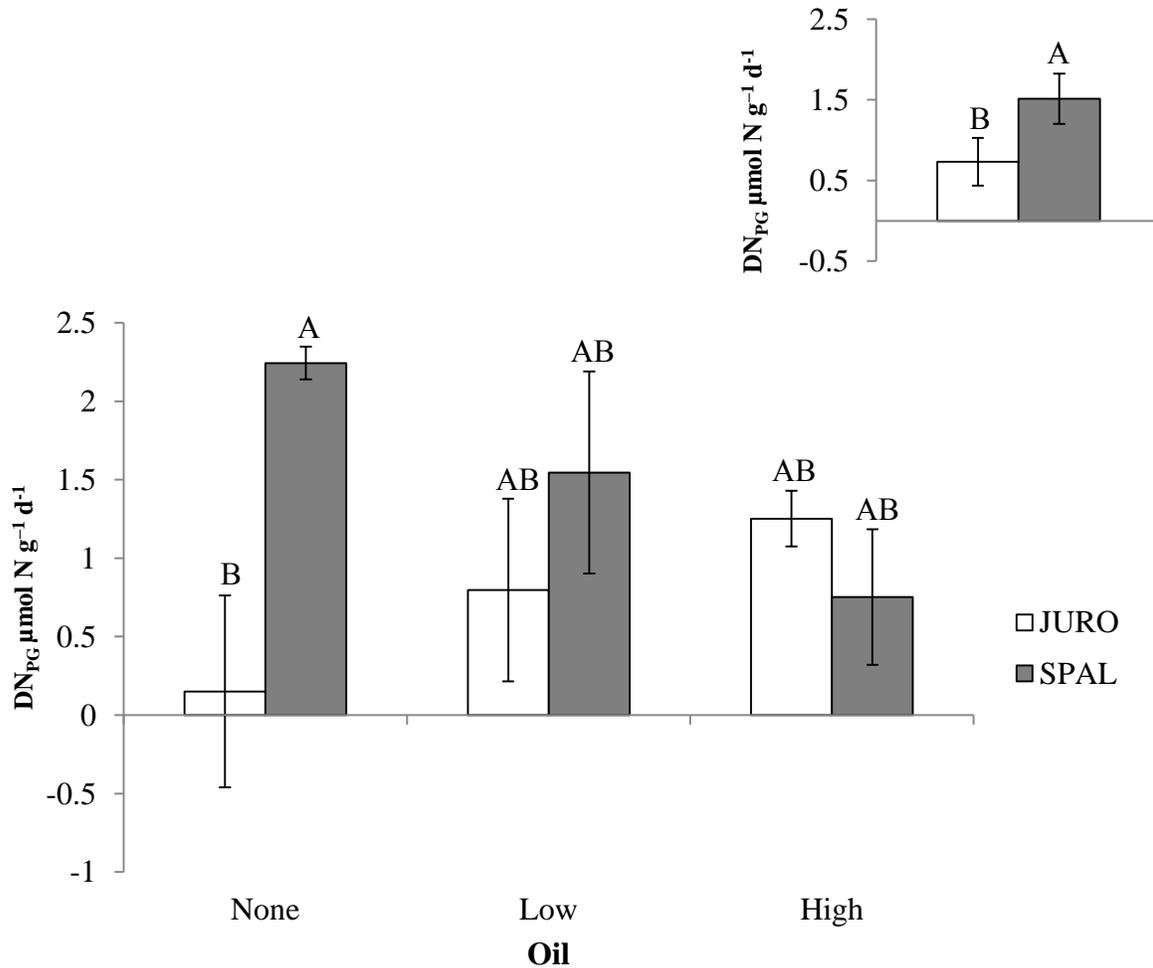


Figure 3.6 *Species x Oil*: Rates of denitrification per gram of aboveground biomass per day ( $DN_{PG}$ ) across species and oil treatments ( $F_{2,12}=3.72$ ,  $p=0.06$ ;  $n=3$ ). *Species*:  $DN_{PG}$  between species ( $F_{1,12}=4.05$ ,  $p=0.07$ ;  $n=9$ ). JURO = *J. roemerianus*, and SPAL = *S. alterniflora*. None = un-oiled controls, Low = 20 mL oil/ 20 mL water, and High = 40 mL oil/ 40 mL water. Values represent means  $\pm$  1 SE. Letters denote significant differences at  $\alpha=0.1$  for all pairwise comparisons (*Species x Oil*) or between species (*Species*).

## CHAPTER 4

### DISCUSSION

The capacity of coastal marshes to remove excess N may depend on the plant species present, as *J. roemerianus* and *S. alterniflora* mesocosms differed in total N loss, losses via diffusion and CND. These species-specific differences were most apparent in the absence of oil, where losses for *S. alterniflora* were higher than for *J. roemerianus*. With the addition of oil, however, the observed differences between species diminished such that N losses and CND rates for *J. roemerianus* increased with increasing oil exposure while they decreased with increasing oil exposure for *S. alterniflora*. For *J. roemerianus*, N removal may be unaffected or may even be enhanced in the presence of oil. While in the absence of oil, N removal in *S. alterniflora* may be better than *J. roemerianus*, but with oil additions, the capacity for N removal in *S. alterniflora* is diminished.

These short-term, species-specific responses to oiling were not consistent with the expected longer-term responses to oiling. In a study examining coastal salt marsh responses to oil spills, *J. roemerianus* experienced greater negative effects following oiling in terms of productivity, while *S. alterniflora* had a greater potential to resist mortality and showed signs of faster recovery (Lin and Mendelssohn 2012). Assuming that *J. roemerianus* responds more negatively to long-term oil exposure, the opposite pattern of declining CND by *J. roemerianus* with increasing oil would have been expected. Had there been more time in this study for oil to directly affect plant growth and performance, as has been observed in previous studies (Lin and Mendelssohn 2012), there may have been similar results of oil on plant health, which would

likely have influenced patterns of N loss and CND between species. However, instead of documenting these long-term effects of oil on plant health, this study isolated microbially-mediated processes that can differ with plant species and the sediment conditions plants help create.

Species-specific differences in denitrification rates may reflect differing responses to oil exposure by the different microbial communities associated with each plant species (Ribeiro et al. 2013). Because different plant species can support different microbial communities, the potential for N transformations in marshes may depend on the dominant plant species present. Microbial communities have been shown to vary with changes in soil characteristics, redox potentials, vegetation type, root system type, abiotic environmental parameters, and nutrient type and concentrations (Cao et al. 2008, Ribeiro et al. 2013). Although not investigated here, differences in microbial community between *J. roemerianus* and *S. alterniflora* dominated marshes may have driven the observed differences in CND responses to oil in this study.

The process of CND also depends on the presence of adjacent oxic and anoxic areas in sediments, the creation of which is facilitated by oxygen exchange through plants that alters soil redox potentials (Durand et al. 2011). Nitrification requires the presence of O<sub>2</sub>, while denitrification relies on O<sub>2</sub> depletion, as O<sub>2</sub> provides more energy to microbes compared to NO<sub>3</sub><sup>-</sup> when used as an electron acceptor (Dong et al. 2009). This preference in energy source causes differences in microbial communities within different plants zones based on the variability in O<sub>2</sub> levels (Peralta et al. 2010). With oiling, marshes would likely experience reduced oxygen exchange, reduced soil redox potential, and therefore, reduced CND rates, the extent to which would likely increase with increasing oil exposure and could vary with plant species. Thus, CND rates were expected to decrease in oiled marshes, as was observed for *S. alterniflora* mesocosms.

*J. roemerianus* mesocosms, however, did not demonstrate this expected decrease in CND rates with short-term exposure to oil.

When compared to CND rates across ecosystem types, rates in this study were generally lower than those reported in other studies (Reddy et al. 1989, Hamersley and Howes 2005, UMCES 2005, Koop-Jakobsen and Giblin 2010, Sousa et al. 2012). Because different factors control denitrification of N from different sources, it is important to note the source of N being denitrified (water column N or N produced by nitrification) when comparing rates of denitrification among studies (Rivera-Monroy et al. 2010). Many studies either do not distinguish the N source or combine the two sources contributing to denitrification, and as a result, total denitrification is often reported. Because the current study excluded water column supported denitrification, it may have underestimated total denitrification rates; therefore, only CND rates among studies were compared here. For rates compiled in UMCES (2005), mean CND rates ( $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ) were  $8,040.00 \pm 720.00$  (7,320.00-8,760.00; n=2 studies) for freshwater marshes;  $3,142.38 \pm 918.79$  (2.40-197,016.00; n=248) for estuaries;  $7,996.35 \pm 4,829.60$  (16.80-77,000.00; n=16) for saltmarshes;  $1,261.71 \pm 139.93$  (912.00-1,872.00; n=7) for mudflats;  $1,032.00 \pm 200.67$  (96.00-1,920.00; n=11) for lakes;  $488.23 \pm 129.20$  (72.00-1,104.00; n=7) for creeks;  $221.21 \pm 48.76$  (7.92-840.00; n=21) for coastal open waters; and  $75.98 \pm 18.04$  (40.08-139.92; n=5) for seagrass beds. CND rates ( $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ) in this study were  $34.60 \pm 14.34$  (-27.05-94.82; n=9) for *J. roemerianus*, and  $46.15 \pm 10.27$  (4.57-80.18; n=9) for *S. alterniflora*, falling in the lower end of levels reported in the literature. Negative rates observed in this study were considered to represent denitrification values of  $0 \mu\text{mol N m}^{-2} \text{d}^{-1}$ .

Although several factors have been shown to cause high variability in denitrification rates including N availability, seasonality, presence or absence of vegetation, temperature, microbial

community (presence and competition of nutrients), aerobic/anaerobic soil layers, and ecosystem type N availability is one of the most important (Seitzinger 1988, Reddy et al. 1989, Seitzinger 1994, Joye and Hollibaugh 1995, Rysgaard et al. 1999, Risgaard-Petersen 2003, Hamersley and Howes 2005, Hayatsu et al. 2008, Dong et al. 2009, Koop-Jakobsen and Giblin 2010, Sousa et al. 2012, Ribeiro et al. 2013). For example, in their  $^{15}\text{NH}_4^+$  tracer study in salt marshes in the Great Sippewissett March Cape Cod, MA, Hamersley and Howes (2005) reported that CND rates in unfertilized stands were considerably lower than those in fertilized stands. CND in unfertilized and fertilized marsh stands ranged from 400 to 11,900  $\mu\text{mol N m}^{-2} \text{ d}^{-1}$  and 22,000 to 77,000  $\mu\text{mol N m}^{-2} \text{ d}^{-1}$ , respectively. Similar findings were reported in Koop-Jakobsen and Giblin's study (2010) comparing fertilized and unfertilized creek and marsh CND rates. Rates observed in fertilized creek were 681.60 to 1,104.00  $\mu\text{mol N m}^{-2} \text{ d}^{-1}$  compared to 206.40 to 321.60  $\mu\text{mol N m}^{-2} \text{ d}^{-1}$  for unfertilized creek, while rates in the fertilized marsh were 40.80 to 122.40  $\mu\text{mol N m}^{-2} \text{ d}^{-1}$  compared to 16.80 to 93.60  $\mu\text{mol N m}^{-2} \text{ d}^{-1}$  in the unfertilized marsh (Koop-Jakobsen and Giblin 2010). Lower CND rates were found in this study compared to some salt marshes studies, including the companion Hamersley and Howes (2005) study in which similar methods and label quantities were used. Rates from this study were most similar to rates reported for salt marsh by Jakobsen and Giblin (2010), as well as the lower range of values observed in estuaries, coastal open waters, and seagrass beds. Given the exclusion of water column N in this study, it is likely that these rates represent underestimates of total denitrification in these marshes.

Regardless of oil exposure, total loss of N was significantly greater for *S. alterniflora* than *J. roemerianus*, and was driven by losses via diffusion and CND, demonstrating strong species-specific effects for all N loss routes except translocation. The differences in CND between species may have been driven in part by differences in abiotic conditions between plant

zones. In marshes, sediment type, salinity and sulfides change along an elevation gradient, contributing to a predictable zonation of vegetation. In this case, *S. alterniflora* dominates lower elevation environments along the shore, while *J. roemerianus* dominates higher elevation, marsh interior. As a result, hydro-edaphic conditions related to diffusion and CND may have differed between the two species in this study.

Greater diffusion in *S. alterniflora* sediments compared to *J. roemerianus* sediments may reflect differences in sediment characteristics linked to the elevation from which samples were collected. Based on the national elevation dataset (NED) 1/9th arc-second: Topobathy for Alabama-Mississippi, there were slight elevation differences between areas from which the vegetated sods were harvested. Elevation increased slightly from the shoreline towards upland areas, and with that, the marsh would experience differences in microtidal action. Located nearer the shore, *S. alterniflora* more frequently experienced the fluctuating deposits of the sandy marine sediments from tides than *J. roemerianus*. As such, sediments along the shoreline would consist of coarser textures resulting in more permeable soils allowing greater diffusion than interior *J. roemerianus* dominated marshes.

CND rates have been shown to vary with hydro-edaphic conditions, including salinity and sulfides (Seitzinger 1988, Joye and Hollibaugh 1995), which may have contributed to the observed species-specific differences in denitrification. Lower elevation *S. alterniflora* stands typically endure higher salinity and sulfide concentrations compared to *J. roemerianus* stands located further inland (Touchette et al. 2009). While studies of species-specific differences in CND rates are lacking, Seitzinger (1988) suggested the range of denitrification rates is greater in marine ecosystems with N loading activities; such as agricultural runoff, compared to freshwater ecosystems such as lakes or rivers with no N loading activities. Higher N availability has been

shown to increase denitrification (Hamersley and Howes 2005, Koop-Jakobsen and Giblin 2010). The abiotic difference, particularly salinity, for these two target systems has been reported to have an effect on CND ability for N removal. Rysgaard et al. (1999) reported lower denitrification rates in Randers Fjord estuary sediments in Denmark at higher salinity concentrations. As salinity increased in their study, nitrification and denitrification rates decreased due to the reduced  $\text{NH}_4^+$  concentrations from increased ion exchange and competition of cations and  $\text{NH}_4^+$  to negative binding sites (Rysgaard et al. 1999). Because of the geographical distribution of species in the present study along a salinity gradient, *S. alterniflora* was at higher salinity concentrations compared to *J. roemerianus*; however, DN was significantly higher in *S. alterniflora* than *J. roemerianus*, which suggests that salinity did not inhibit CND in this study.

Sulfide concentrations can also affect denitrification processes in marshes. Salt marsh ecosystems are characterized by higher sulfide concentrations than freshwater systems because sulfate availability is higher and it is reduced in highly anaerobic marsh sediments. Denitrification and reduction of sulfate to sulfide both occur in the absence of oxygen. Sulfides have been found to directly inhibit nitrification processes with varying effects on denitrification (Seitzinger 1988, Joye and Hollibaugh 1995). A study of crude oil effects on denitrification rates found that long term oiling in marsh sediments decreased denitrification rates in Alaskan subarctic marine sediments due to changes in redox potentials, which was attributed to higher sulfate reduction rates in oiled sediments (Griffiths et al. 1982). Sulfides also adversely affect vegetation, which may reduce oxygen transport to sediments and the subsequent oxidation of sulfides. An accumulation of sulfides would likely inhibit nitrification and denitrification by microbes, and reduce denitrification by lowering the supply of nitrate for CND.

In this study, sulfides were significantly higher in *S. alterniflora* than in *J. roemerianus* mesocosms. Overall, loss via *CND* increased with increasing sulfides for both species, but when this relationship was examined separately for each species, the increase in *CND* with increasing sulfides was only observed for *J. roemerianus*. Sulfide concentrations observed in this experiment were higher than concentrations seen in other salt marshes (McKee et al. 1988, Caffrey 2007). N transformations, particularly N uptake by vegetation, can be limited by high sulfide concentrations (Bradley and Morris 1990). However, N uptake by vegetation in this study (FIELD Winter, *S. alterniflora*:  $3.84 \pm 0.69$  %  $d^{-1}$  to Summer, *S. alterniflora*:  $12.19 \pm 4.63$  %  $d^{-1}$  and GREENHOUSE *J. roemerianus*:  $6.38 \pm 3.13$  to  $9.12 \pm 1.15$  %  $d^{-1}$ ; *S. alterniflora*:  $4.86 \pm 0.74$  to  $8.49 \pm 3.80$  %  $d^{-1}$ ) was higher than vegetation uptake rates reported in the Hamersley and Howes (2005) study ( $0.9 \pm 1.4$  %  $d^{-1}$  unfertilized,  $2.0 \pm 0.8$  %  $d^{-1}$  fertilized). Thus, it does not appear that sulfides inhibited *CND* in *J. roemerianus* and *S. alterniflora* mesocosms in this study.

Unlike diffusion and *CND*, plant uptake of N did not differ between species and was not responsible for the observed patterns of N loss and *CND* in this study. Plant uptake of N did not differ between the two species likely because the short 24 hour time frame was more conducive to capturing microbially driven losses, such as *CND*, over plant-mediated responses such as translocation. Even so, plant uptake can represent an important pathway of temporary N removal from marsh sediments and can vary with plant species (Mozdzer et al. 2011). Translocation rates in this study were higher than those measured by Hamersley and Howes (2005), suggesting that plant uptake may be an important pathway of N removal in these sites. Furthermore, plant N uptake has been shown to be directly related to geographic position in the marsh, which influences inundation, plant height, N availability, and salinity (Mozdzer et al. 2011). Position

within the marsh can also cause differences in redox potentials, which play a major role in the reduction of nitrate (Jenkins and Kemp 1984). Thus, greater N uptake by vegetation may occur at lower marsh elevations that are exposed to more frequent inundation periods (Mozdzer et al. 2011). Plant uptake of N also has been proposed as a limiting factor for denitrification in marshes (Hamersley and Howes 2005) because vegetation are important for providing oxygen via diffusion through the roots for nitrate production via nitrification (Reddy et al. 1989, Hamersley and Howes 2005). Longer-term studies of species-specific differences in N uptake would likely reflect these differences between *J. roemerianus* and *S. alterniflora* that did not emerge in the short timeframe of this study. In addition, greater stress on vegetation with longer-term exposure to oil would likely diminish not only N removal via CND, but potentially N uptake by the vegetation.

### *Conclusions*

N loading contributes to eutrophication and the formation of dead zones, declines in biodiversity, and shifts in primary producer communities (Vitousek and Howarth 1991, Vitousek et al. 1997, Schlesinger 2009). In many cases, the threats of N loading to estuaries and coasts can be mitigated by coastal wetlands, which can act as buffers eliminating excess N (VanZomerem et al. 2013). By eliminating excess N before it reaches bays and oceans, wetlands act as buffers from N loading. In wetlands, CND is an efficient way to remove anthropogenic derived N from the ecosystem, and thus, enhance N exchange between the ecosystem and the atmosphere. Wetlands commonly experience oil spills along the Gulf coast that can influence the capacity of wetlands to perform this important ecological function. Understanding how certain marshes react to oil contamination in regards to the efficiency of N removal is applicable to coastal ecosystem management. A review of studies examining denitrification determined there is a research need

for the understanding of CND and its ecological role in coastal marshes (Rivera-Monroy et al. 2010).

This study was conducted to determine immediate species-specific CND responses under varying oil scenarios and to answer the following questions: (1) Are there species-specific differences in CND rates between mesocosms vegetated with *J. roemerianus*, a C<sub>3</sub> rush, and *S. alterniflora*, a C<sub>4</sub> grass; (2) How does oil exposure affect short-term CND rates in vegetated mesocosms; and (3) Do CND rates in *J. roemerianus* and *S. alterniflora* mesocosms respond similarly to oil exposure? Excess N can be removed via CND, the extent of which differs between species with higher rates of removal possible by the C<sub>4</sub> species. Rates of CND were affected more by species than oil effects, but *J. roemerianus* and *S. alterniflora* responses differed depending on oil exposure. With increasing oil exposure, CND rates decreased for the C<sub>4</sub>-higher stress tolerant species, *S. alterniflora*, while increasing for the C<sub>3</sub> species, *J. roemerianus*.

The species-specific CND responses to oiling differed in this short-term study from what was expected based on results of longer-term studies of oil effects on plant health (Lin and Mendelssohn 2012). Negative effects of longer-term oil exposure on plant health would likely affect N removal processes, as the health of the plant community is linked to biogeochemical processes, particularly N cycling (Reddy et al. 1989). Wetlands can differ in their capacity to remove N and respond to oil stresses depending on vegetation, and denitrification and vegetation uptake are important sinks for excess N loading in coastal marshes. Loss via CND dominated total N losses in this study and represents an important pathway by which these marshes remove excess N.

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APPENDIX

Source	DF	F ratio	P value
K <sub>T</sub> Oil	2, 12	0.5577	0.5867
K <sub>TPG</sub> Oil	2, 12	0.0857	0.9184
K <sub>DF</sub> Oil	2, 12	0.2649	0.7716
K <sub>DFPG</sub> Oil	2, 12	0.1536	0.8593
K <sub>TR</sub> Oil	2, 12	0.8185	0.4643
K <sub>TRPG</sub> Oil	2, 12	0.5348	0.5991
K <sub>DN</sub> Oil	2, 12	0.0797	0.9239
K <sub>DNPG</sub> Oil	2, 12	0.099	0.9065
DN Oil	2, 12	0.0797	0.9239
DN <sub>PG</sub> Oil	2, 12	0.099	0.9065

Table A1 Two-way ANOVA results of the oil main effects on all nitrogen losses (K<sub>T</sub>, K<sub>TR</sub>, K<sub>DF</sub>, K<sub>DN</sub>, DN) and all nitrogen losses standardized by grams of aboveground biomass (K<sub>TPG</sub>, K<sub>TRPG</sub>, K<sub>DFPG</sub>, K<sub>DNPG</sub>, DN<sub>PG</sub>). Separate two-way ANOVAs were performed for each response variable and the results for the main effects tests for oil are shown here.

Source	DF	F ratio	P value
K <sub>T</sub> Spp vs Oil	2, 12	2.0179	0.1756
<b>K<sub>TPG</sub> Spp vs Oil</b>	2, 12	2.8016	<b>0.1004</b>
K <sub>DF</sub> Spp vs Oil	2, 12	0.9109	0.4282
K <sub>DFPG</sub> Spp vs Oil	2, 12	0.8615	0.4471
K <sub>TR</sub> Spp vs Oil	2, 12	0.1479	0.8641
K <sub>TRPG</sub> Spp vs Oil	2, 12	0.241	0.7895
K <sub>DN</sub> Spp vs Oil	2, 12	2.4746	0.126
<b>K<sub>DNPG</sub> Spp vs Oil</b>	2, 12	3.7158	<b>0.0555</b>
DN Spp vs Oil	2, 12	2.4746	0.126
<b>DN<sub>PG</sub> Spp vs Oil</b>	2, 12	3.7158	<b>0.0555</b>

Table A2 Two-way ANOVA results of the species x oil interaction effects on all nitrogen losses (K<sub>T</sub>, K<sub>TR</sub>, K<sub>DF</sub>, K<sub>DN</sub>, DN) and all nitrogen losses standardized by grams of aboveground biomass (K<sub>TPG</sub>, K<sub>TRPG</sub>, K<sub>DFPG</sub>, K<sub>DNPG</sub>, DN<sub>PG</sub>). A bold number represents a significant effect at  $\alpha = 0.1$ . Separate two-way ANOVAs were performed for each response variable and the results for the interaction tests for oil and species are shown here.

Source	DF	F-ratio	Prob>F
<b>KT Spp</b>	<b>1, 12</b>	<b>4.1988</b>	<b>0.063</b>
<b>KTPG Spp</b>	<b>1, 12</b>	<b>17.3875</b>	<b>0.0013*</b>
<b>KDF Spp</b>	<b>1, 12</b>	<b>16.8502</b>	<b>0.0015*</b>
<b>KDFPG Spp</b>	<b>1, 12</b>	<b>13.0975</b>	<b>0.0035*</b>
KTR Spp	1, 12	0.7361	0.4077
KTRPG Spp	1, 12	0.8159	0.3842
KDN Spp	1, 12	0.4583	0.5113
<b>KDNPG Spp</b>	<b>1, 12</b>	<b>4.049</b>	<b>0.0672</b>
DN Spp	1, 12	0.4583	0.5113
<b>DNPG Spp</b>	<b>1, 12</b>	<b>4.049</b>	<b>0.0672</b>

Table A3 Two-way ANOVA results of species main effects on all nitrogen losses ( $K_T$ ,  $K_{TR}$ ,  $K_{DF}$ ,  $K_{DN}$ , DN) and all nitrogen losses standardized by grams of aboveground biomass with species effects ( $K_{TPG}$ ,  $K_{TRPG}$ ,  $K_{DFPG}$ ,  $K_{DNPG}$ ,  $DN_{PG}$ ). A bold number represents a significant effect at  $\alpha = 0.1$ . A bold number and asterisk represents a significant effect at  $\alpha = 0.05$ . Separate two-way ANOVAs were performed for each response variable and the results for the main effects tests for species are shown here.