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Impacts of the Deepwater Horizon Oil Spill on Microbial-Mediated Cellulose Decomposition in Mississippi Gulf Coast Salt Marshes

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IMPACTS OF THE DEEPWATER HORIZON OIL SPILL ON MICROBIAL-MEDIATED CELLULOSE DECOMPOSITION IN MISSISSIPPI GULF COAST SALT MARSHES

by

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ABSTRACT

IMPACTS OF THE DEEPWATER HORIZON OIL SPILL ON MICROBIAL-MEDIATED CELLULOSE DECOMPOSITION IN MISSISSIPPI GULF COAST SALT MARSHES

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Field studies were conducted to examine the effects of the Deepwater Horizon oil spill on rates of marsh organic matter decomposition. Decomposition in surface and subsurface marsh sediments was assessed in stands of *Spartina alterniflora* and *Juncus roemerianus* in 9 Mississippi Gulf Coast marshes exposed to differing oiling intensities. The cotton strip bioassay technique was used as a proxy for cellulose decomposition. In addition, rates of microbial respiration, fungal biomass (ergosterol), and nutrients (C:N, C:P) of surface sediment cotton strips were also quantified. Subsurface cotton strip decay, as determined by losses in tensile strength, were significantly different among marsh sites, with higher overall rates being observed in oiled versus unoiled *S. alterniflora* plant zones ($p<0.05$). No differences were observed in subsurface sediments between oiled and unoiled *J. roemerianus* plant zones ($p>0.05$). In contrast to subsurface sediments, cotton strip decay in surface sediments displayed an opposite pattern, with significantly ($p>0.05$) higher rates of decay in unoiled versus oiled *S. alterniflora* and *J. roemerianus* plant zones. Cotton strip C:N and C:P ratios were negatively correlated with losses in cotton strip tensile strength. In addition, both fungal ergosterol concentrations and microbial respiration rates were positively correlated with cotton strip decay and negatively correlated with C:N and C:P ratios, providing evidence that N and P availabilities in

marsh sediments may have limited the activity of microbial communities. Although conducted ~1.5 years after the Deep Water Horizon oil spill, this study suggests that both subsurface and surface microbial processes may still be affected by oil.

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TABLE OF CONTENTS

LIST OF TABLES

Table

LIST OF ILLUSTRATIONS

Figure

CHAPTER I

INTRODUCTION

In the United States alone, approximately 62,000 km^2 of wetland area occur along the Gulf Coast (Stedman and Dahl 2008) and assessing the health and stability of these ecosystems is of vital importance. These coastal wetlands provide a variety of ecological and economic services to the region, which include water quality enhancement, flood protection, defense of coastal shorelines from erosion, and the provision of habitat and food resources for wildlife. Furthermore, these wetlands provide an important source and sink for carbon (C) and nutrients (nitrogen and phosphorus), and their processing and storage is widely recognized as a critical ecosystem service (McLeod et al. 2011).

Globally, salt marshes are recognized as among the most productive ecosystems on earth, with rates of annual net primary productivity ranging from 800-4,000 $g/m^2/y$ (Mitsch and Gosselink 2007). In the Gulf Coast region, the emergent vascular plants *Spartina alterniflora* and *Juncus roemerianus* are the two most common marsh species and frequently form the largest fraction of the annual marsh plant biomass produced (White et al. 1978; Christian et al. 1990; Dai and Wiegert 1996; Ewe et al. 2006). Most of this plant biomass enters the detrital pool following senescence and death of the plant shoot, where the accumulation and subsequent decomposition of plant litter is important for marsh soil development and accretion, storage of C and nutrients, and supply of detrital-based food resources that fuels both estuarine and near-shore food webs (Cebrian and Lartigue 2004; Moore et al. 2004; Craft 2007; Mitsch and Gosselink 2007; Neubauer 2008).

There are justifiable concerns over the effects of the Deepwater Horizon Oil Spill and its unintended consequences on salt marsh ecological processes (DeLaune and Wright 2011, Mendelssohn et al. 2012). Prior studies have examined the effects of oil-spills on marsh vegetation (DeLaune et al. 1984; Lin and Mendelssohn 1996; Hester and Mendelssohn 2000; Pezeshki et al. 2000; DeLaune et al. 2003) and marsh sediment microbial community structure (Wright et al. 1997; Paisse et al. 2008; Bordenave et al. 2009). However, very few have addressed sub-lethal effects of oil exposure on the physiology of marsh vegetation (Smith et al. 1984; Pezeshki and DeLaune 1993; DeLaune et al. 2003), or its potential impacts on salt marsh ecosystem functional processes. Recently, efforts have been made to assess the specific impacts of the Deepwater Horizon oil spill on various components of salt marsh plant productivity (e.g. Lin and Mendelssohn 2012; Mishra et al. 2012; Khanna et al. 2013; Judy et al. 2014). Mishra et al. (2012) observed reduced marsh canopy chlorophyll contents in response to oiling in Louisiana marshes, but did not assess the exact mechanisms behind this response. Additional studies have also reported alterations in marsh sediment microbial communities in response to oil intrusion (Bik et al. 2012; Cravo-Laureau and Duran 2014; Mason et al. 2014; Newell et al. 2014), but very little is known about how these shifts in microbial communities may have impacted marsh biogeochemical processes and the detrital-based ecosystem services they provide (Delaune and Wright 2011; Mendelssohn et al. 2012).

Currently, no published study has examined effects of the Deepwater Horizon oil spill on decomposition and nutrient cycling processes within Mississippi Gulf Coast salt marshes. The present study was conducted to examine organic matter decomposition

patterns in Mississippi Gulf Coast marshes exposed to different oiling intensities as a result of the Deepwater Horizon oil spill. In this study, we assessed both surface and subsurface marsh sediment cellulose decomposition in nine coastal salt marshes (6 oiled, 3 unoiled) using a standardized cotton strip bioassay technique as a proxy for organic matter decomposition (French 1988b; Slocum et al. 2009, Tiegs et al. 2013). In addition, rates of microbial respiration, fungal biomass (ergosterol) and nutrient ratios (CNP) associated with surface sediment cotton strips were also quantified in order to examine their relationship with cotton strip decomposition (i.e., tensile strength loss) and their potential importance to nutrient cycling functions. Overall, this research hypothesized that oiling would have a significant impact on organic matter decomposition and predicted that sites impacted by oiling would exhibit altered cotton strip decomposition rates in comparison to unoiled sites.

CHAPTER II

MATERIALS AND METHODS

Study Sites

This study was conducted at 9 saltmarsh sites along the Mississippi Gulf Coast, which differed in their degree of oiling exposure (Figure 1). Study sites were initially screened based on the degree of oiling exposure, as determined by cumulative maximum observed oiling data gathered from Shoreline Cleanup Assessment Team (SCAT) maps (Environmental Response Management Application [ERMA], 2014). Sites were also initially ground-truthed based on observations of oiling and from on-site interviews with personnel from the MS Department of Marine Resources and Emergency Management Agencies of Jackson and Hancock County. A total of six oiled and three unoiled sites were examined (Table 1), with the final site selections chosen for similarity in plant physiognomy and tidal influences.

Field Procedures

The effects of oiling on cellulose decomposition in 2 differing salt marsh vegetative zones, *J. roemerianus* and *S. alterniflora*, was evaluated by the standardized cotton strip bioassay using Fredrix-brand #548 style art canvas fabric (see Slocum et al. 2009, Tiegs et al. 2013). Cotton strips (9 x 2.5 cm) were enclosed in fiberglass mesh (200 µm) litter bags, transported to the field sites (September 2011), and placed in three randomly selected plots within monotypic stands of *S. alterniflora* or *J. roemerianus*. At each plot and within each vegetation zone, five replicate litter bags were placed at and within the marsh sediments to assess the influence of oiling on both surface (i.e. aerobic)

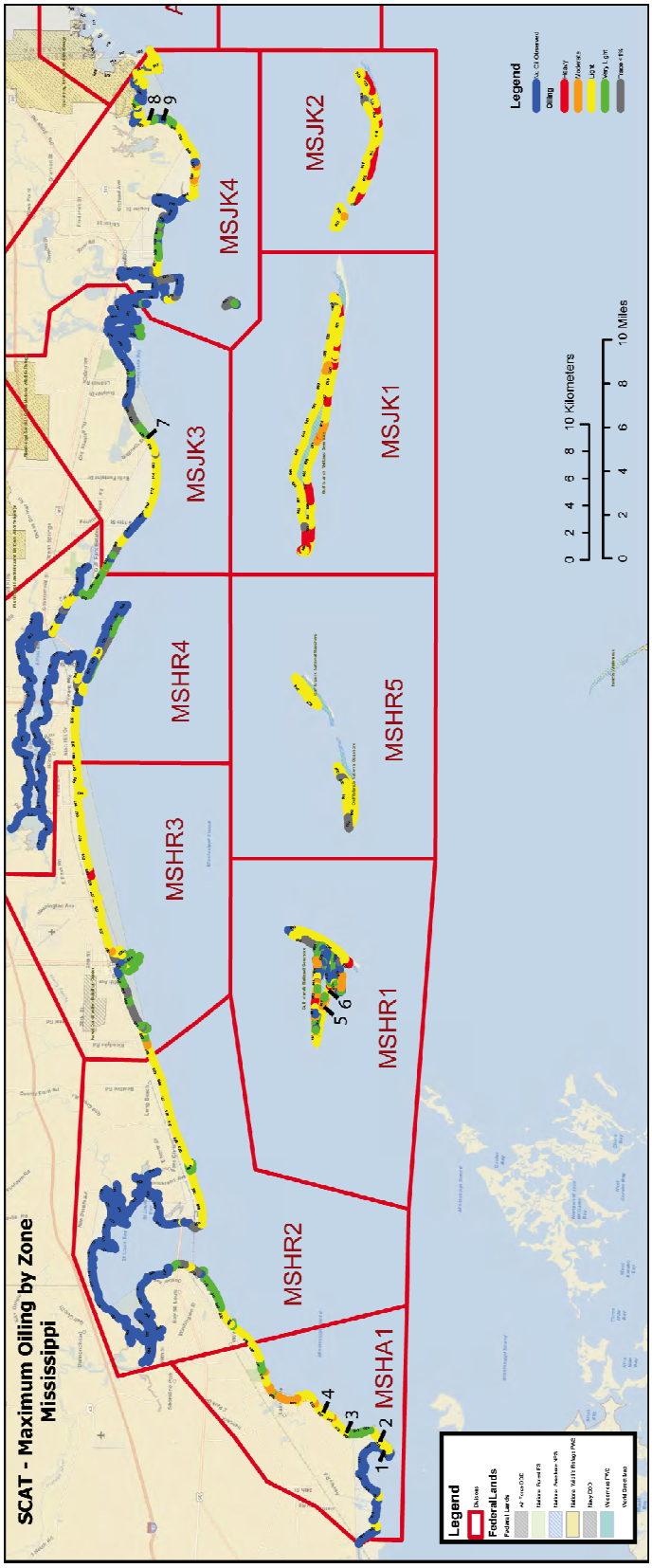

Figure 1. Map of the Mississippi Gulf Coast illustrating the sample study sites and the known oiling impacts based on the Shoreline Cleanup Assessment Team (SCAT) surveys conducted immediately after the oil spill.

Table 1

Marsh study sites examine and their varying degrees of oil and contaminant exposure as determined from Shoreline Cleanup Assessment Team (SCAT) surveys.

Site	Maximum oil observed*	Latitude	Longitude
Site 1, Herron Bay	No oil observed	30.193646°	-89.481996°
Site 2, Lower Point Clear	Light	30.176753°	-89.462508°
Site 3, St. Joseph Point	Very light	30.189712°	-89.446815°
Site 4, Grand Bayou	No oil observed	30.263125°	-89.355899°
Site 5, Cat Island, Little Bay	Moderate	30.223953°	-88.756339°
Site 6, Cat Island	Moderate	30.224328°	-84.113933°
Site 7, Graveline Bay	No oil observed	30.364200°	-88.668912°
Site 8, Chevron Refinery	Moderate	30.320676°	-88.492312°
Site 9, Point Aux Chenes Bay	Very light	30.207264°	-88.418591°

and subsurface (anaerobic) cellulose decay, respectively (9 marsh sites x 2 vegetation zones x 3 plots x 2 sediment zones x 5 replicates = 540 total litter bags).

Subsurface litter bags containing 1 cotton strip were buried at ~5 cm beneath the marsh sediment surface by using a standard dibble bar to ensure their placement at a consistent uniform depth. Corresponding surface litter bags contained two cotton strips were placed in direct contact with surface sediment. These litter bags included one cotton strip for determination of cellulose decomposition rates and a second strip to examine

cotton strip associated fungal biomass (ergosterol), rates of microbial respiration (CO_2 evolution) and associated nutrients (carbon, nitrogen and phosphorus). Five litter bags from each vegetative zone were immediately collected returned to the laboratory for determination of initial cotton strip tensile strength. Thereafter, litter bags were retrieved at 2, 3 and 4 weeks, placed in clean zip lock bags on ice in a cooler, and immediately returned to laboratory for processing.

On each sampling date, interstitial pore water within each vegetative zone was monitored in established peziometers (n=3, ~60 cm depth) for pH, oxidation-reduction potential (mV), salinity (ppt) using a YSI professional plus multiparameter meter. In addition, surface and subsurface temperatures at each site were continuously monitored at 30 min intervals throughout the entire study period using two Onset HOBO water temp Pro V2 series data loggers.

Cellulose Decomposition Rates

Rates of cotton strip cellulose decomposition were inferred from losses in tensile strength of cotton strip cellulose fibers. Upon return to the laboratory, collected strips were gently cleaned with deionized water to remove adhering sediment, dried at 60˚C, and stored in clean ziplock bags within a desiccator until analyzed. Cotton strip tensile strengths were subsequently determined using a Mark-10 Tensiometer following protocols described by Tiegs et al. (2013). Losses in tensile strength are expressed as the percent of initial tensile strength lost per day during the incubation period using the equation:

$$\%\text{CTSL} = 1 - [(TS_{treatment\ strips} / TS_{reference\ strips}) \times 100\ ] / \text{incubation time}$$

where $TS_{treatment\ strips}$ is the maximum tensile strength recorded for each cotton strip incubated in the field, and $TS_{reference\ strips}$ is the mean tensile strength recorded for initial cotton strips that were transported to the field during litterbag deployment and immediately returned to the laboratory for processing.

Rates of Microbial Respiration

Rates of microbial respiration (CO_2 evolution) from collected surface cotton strips were determined using a LiCor LI-6400 Portable Infrared Gas Analyzer. Upon return to the laboratory, collected surface cotton strips were removed from litter bags and placed into sterile Petri dishes lined with sterile (autoclaved) filter paper. Cotton strips and filter paper were wetted with ~10 mL of 0.22 µm membrane filtered marsh surface water collected from each respective site and incubated in darkness for 2 h at 15˚C. After 2 hours of incubation, rates of CO_2 evolution were measured by enclosing cotton strips into a LI 6400-89 insect respiration chamber connected to the LiCor LI-6400 Infrared Gas Analyzer. Following respiration measurements, cotton strips were stored frozen (-20˚C) and later lyophilized and weighed to determine hourly rates of microbial respiration. Subsamples of lyophilized cotton strips were also used to determine cotton strip associated fungal biomass and nutrient concentrations (see below).

Fungal Ergosterol and Nutrient Concentrations

The extent of fungal colonization of collected cotton strips was estimated from ergosterol concentrations (Gessner 2005). Following lyophilization, subsamples were immediately cut from cotton strips, weighed, placed into clean 20 ml glass scintillation vial containing 5 ml of HPLC grade methanol, and stored at -20˚C until analyzed. Later,

ergosterol in stored samples were extracted in alcoholic KOH (0.8% KOH in HPLC grade methanol, total extraction volume 10 ml) for 30 min at 80˚C in tightly capped thick-walled Pyrex digestion tubes with constant stirring. Sample extracts were partially cleaned by solid-phase extraction (Gessner and Schmitt 1996), and ergosterol quantified by a Shimadzu high-pressure liquid chromatography (HPLC) system using a LichroSpher 100 RP-18 column (0.46 × 25 cm, mobile phase HPLC-grade methanol, flow rate of 1.5 mL min^{-1}). Ergosterol was detected at 282 nm and identified and quantified on the basis of comparison with known ergosterol standards (Fluka Chemicals). Ergosterol concentrations were determined as the average of two HPLC injections per sample extract.

Subsamples of cotton strips were also analyzed for carbon, nitrogen and phosphorus concentrations. The carbon and nitrogen concentrations of cotton strips were determined using a Costech 4010 elemental combustion analyzer. Phosphorus concentrations were determined using a SEAL AA3 Flow Injection Nutrient Analyzer (molybdate-ascorbic acid method) following combustion (500˚C) of cotton strip subsamples and hot 1N HCl solubilization of the resulting ash.

Data Analyses

Statistical analyses of the data was conducted using JMP software (SAS Institute Inc., Cary, NC, USA, version 11), with differences at the $p<0.05$ level being considered significant. If necessary, data were transformed prior to analysis to ensure normality and, heteroscedasticity. All data were analyzed using a nested ANOVA, with sites nested within oiling as the random factor and species zones and oiling as fixed factors. A

Tukey's HSD multiple comparison test was used to test for differences among treatments when significant main or interactive effects of ANOVAs were found.

CHAPTER III

RESULTS

Cementation Effects

Unfortunately, all sites examined in this research project experienced some degree of cotton strip cementation during the study period (French 1988a). Cementation is a phenomenon whereby the tensile strength of cotton fibers tends to increase during incubation in the field. This phenomenon is caused by a variety of biotic and abiotic factors, such as the production of polysaccharides and resins by inhabitant microorganisms or the physical binding of fine sediments to the cotton strip. Correction for cementation effects is often difficult, and any cementation correction when comparing multiple experimental sites must ensure that both physical and chemical conditions at the sites are similar (French 1988c). However, even if correction is possible, the resulting data should be interpreted with caution.

In the present study, both surface and subsurface cotton strips were deployed at the marsh study sites and retrieved at 2, 3 and 4 week intervals. The initial objective of this study was to calculate a rate of cotton strip tensile strength loss through time. However, due to cementation issues it was not possible to use all of the cotton strip tensile strength data collected. To deal with the effects of cementation the single sampling date that displayed the highest mean tensile strength loss (i.e. less cementation) at each site was chosen for analysis and inclusion in this thesis.

Environmental Parameters

Both surface and subsurface temperatures remained relatively consistent during the study period (Figure 2), and did not significantly vary among sites (surface sediments:

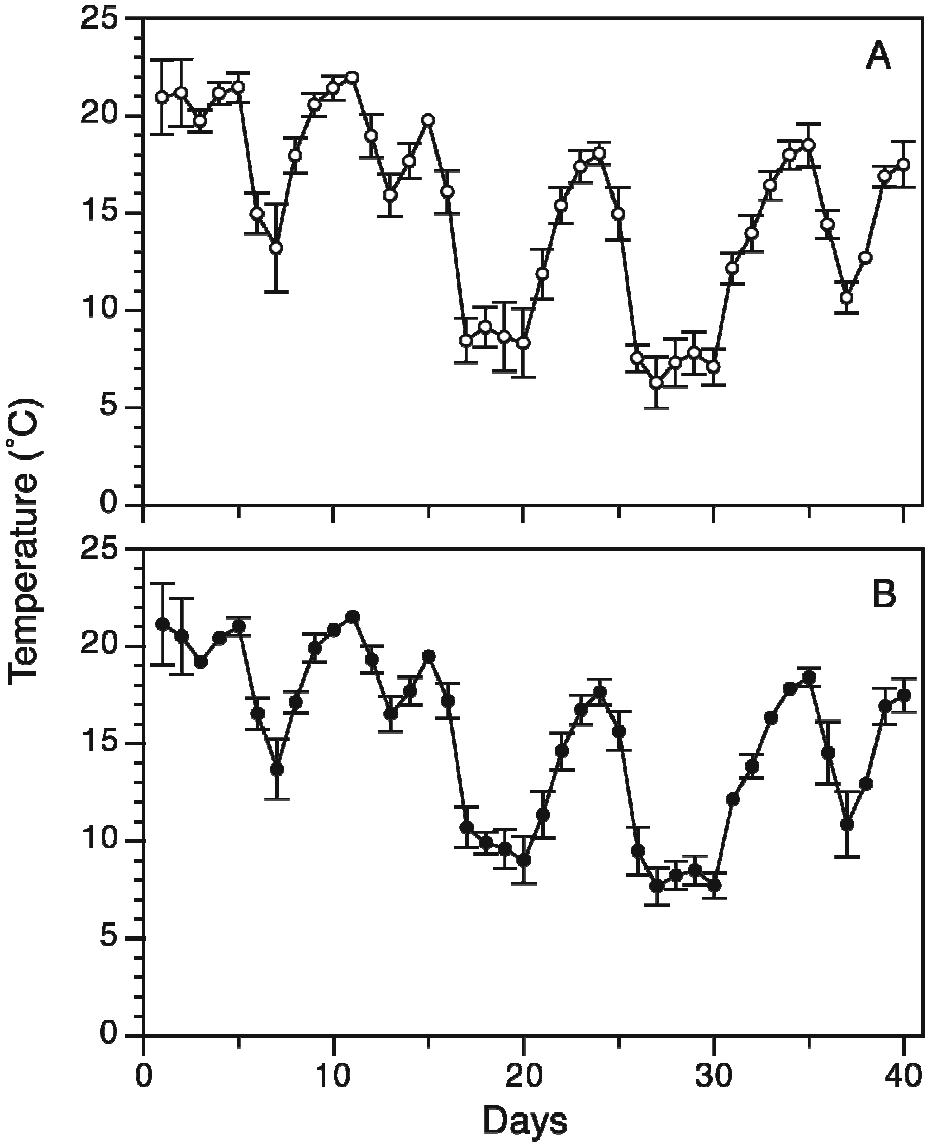

Figure 2. Changes in surface (A) and subsurface (B) sediment temperatures during the study period. Values are the overall mean±SD for all study sites combined

$F_{(7,230)}$ =0.276, p=0.96, subsurface sediments: $F_{(7,230)}$ =0.341, p=0.934). In contrast, marsh pore water salinity, pH and reduction potential (ORP) varied significantly among sites and plant vegetative zones (Table 2). However, oiling showed no significant effects on any of the measured parameters (Table 3). In addition, no significant interactions between

Table 2

Salinity, pH and reduction potential (ORP) of sediment pore water within S. alterniflora and J. roemerianus plant zones. Values are the mean±1SD (n=12,except site 4 for J. roemerianus where n=1).

Site	Species	Salinity (ppm)	pH	ORP (mV)
1	Spartina	14.81±2.32	6.79±0.15	-254±13
	Juncus	14.82±1.33	6.59±0.07	-233±39
2	Spartina	13.75±2.19	6.87±0.17	-290±23
	Juncus	17.83±1.30	6.63±0.10	-242±47
3	Spartina	18.47±1.15	7.08±0.15	-178±57
	Juncus	19.68±0.55	7.16±0.15	-180±41
4	Spartina	15.93±5.65	6.91±0.18	-154±37
	Juncus	22.04	6.67±0.03	-69±31
5	Spartina	27.26±2.29	6.66±0.18	-180±97
	Juncus	28.58±1.81	6.89±0.22	-90±34
6	Spartina	25.51±2.92	6.94±0.44	-147±73
	Juncus	24.17±1.43	6.68±0.10	-174±12
7	Spartina	18.98±4.27	6.90±0.15	-298±33
	Juncus	26.31±1.07	6.61±0.06	-262±24
8	Spartina	24.96±0.83	6.95±0.09	-268±51
	Juncus	27.47±0.05	6.87±0.07	-263±11

Table 2 (continued).

Site	Species	Salinity (ppm)	pH	ORP (mV)
9	Spartina	28.28±1.97	6.85±0.28	-7±102
	Juncus	30.10±2.64	6.84±0.27	-139±95

Table 3

Nested ANOVA summary table indicating the effect of site, plant species zone, and oiling on marsh sediment pore water salinity, pH and reduction potentials (ORP) at each marsh site.

Effect	Salinity			pH			Reduction Potentials (ORP)		
	df	F-Ratio	p-value	df	F-Ratio	p-value	df	F-Ratio	p-value
Site [Oiling]	7,84	50.13	<0.001	7,94	3.85	<0.001	7,90	35.42	<0.001
Plant species zone	1,84	28.26	<0.001	1,94	11.12	0.001	1,90	8.32	0.005
Oiling	1,84	2.01	0.199	1,94	2.21	0.178	1,90	0.47	0.514
Plant species zone *Oiling	1,84	3.08	0.083	1,94	5.42	0.022	1,90	0.15	0.703

oiling and plant species zones were observed, except for pH where *J. roemerianus* zones had significantly lower pore water pH at oiled sites (Tukey's HSD, $p>0.05$).

Cotton Strip Decay - Subsurface Sediments

In the present study, tensile strength loss of cotton strips (CTSL %/d) in subsurface marsh sediment varied significantly among study sites (ANOVA, $F_{(7,76)} =10.46$, $p<0.001$) and between plant species zones (ANOVA, $F_{(1,76)} =7.62$, $p<0.01$), with a slightly higher rate of tensile strength loss being observed within sediments at the *J. roemerianus* versus *S. alterniflora* plant zone (Figure 3, Tables 4 and 5). Although oiling showed no significant effect (ANOVA, $F_{(1,76)} =1.37$, $p=0.280$) on tensile strength loss, there was a significant interaction between oiling and plant species zone (ANOVA, $F_{(1,76)} =5.33$, $p=0.024$), suggesting that tensile strength losses varied between vegetation zones (*S. alterniflora* vs. *J. roemerianus*) in response to oiling (Table 4). When analyzed for oiling effects across study sites and by overall plant species zone, tensile strength losses within oiled *S. alterniflora* plant zones were significantly higher (~26%, Tukey's HSD, $p<0.05$) than corresponding losses observed in unoiled *S. alterniflora* zones (Figure 4). In contrast, only minor differences in tensile strength losses were observed between oiled and unoiled *J. roemerianus* plant zones, which were not significantly different (Tukey's HSD, $p>0.05$) (Figure 4, Table 5).

Cotton Strip Decay - Surface Sediments

Tensile strength losses of cotton strips at the marsh surface sediments (CTSL %/d) were markedly lower than observed cotton decay rates in subsurface sediments, and did not vary significantly (ANOVA, $F_{(7,79)} =7.80$, $p=0.271$) among the study sites examined (Figure 5, Table 4). However, similar to marsh subsurface sediments,

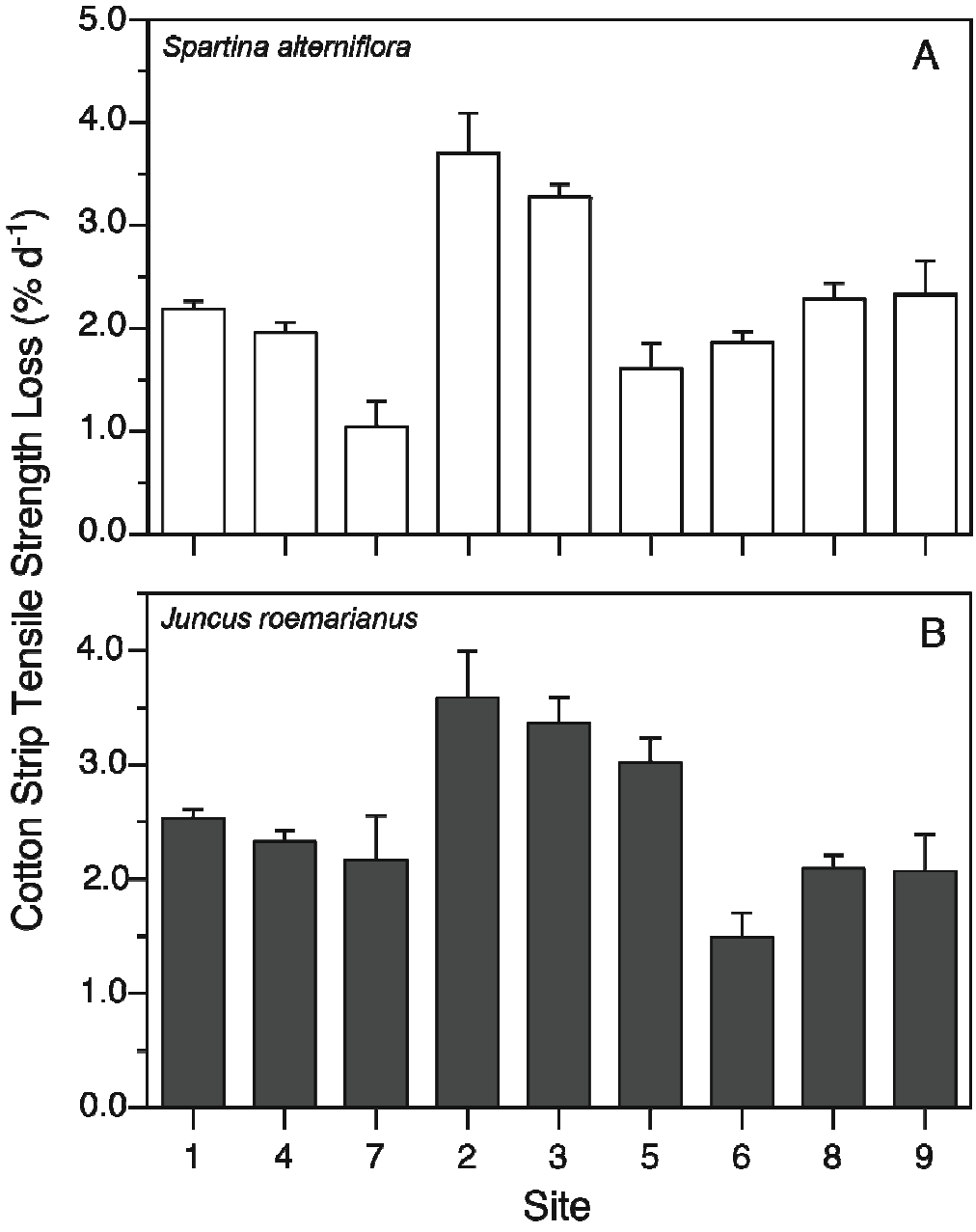

Figure 3. Losses in cotton strip tensile strength observed in subsurface sediments in the *S. alterniflora* (A) and *J. roemerianus* (B) plant zones at each marsh site. Values are the mean±SE (n=5).

Table 4

Nested *ANOVA summary indicating the effect of site, plant species zone, and oiling on subsurface and surface percent cotton strip tensile strength loss.*

Effect	Subsurface (%CTSL/d)			Surface (%CTSL/d)		
	df	F-Ratio	p-value	df	F-Ratio	p-value
Site [Oiling]	7,76	10.46	<0.001	7,79	7.80	0.271
Plant species zone	1,76	7.62	0.007	1,79	9.43	0.003
Oiling	1,76	1.37	0.280	1,79	3.99	0.086
Plant species zone *Oiling	1,76	5.33	0.024	1,79	5.98	0.071

Table 5

Overall tensile strength loss (%CTSL/d) of surface and subsurface cotton strips in oiled and unoiled S. alterniflora and J. roemerianus plant zones. Values are the mean±1SD (n=15-30).

Species Zone	Site	Subsurface Sediments	Surface Sediments
S. alterniflora	Oiled	2.51±0.82	0.35±0.34
	Unoiled	1.73±0.60	0.93±0.36
J. roemerianus	Oiled	2.60±0.84	0.21±0.12
	Unoiled	2.34±0.18	0.27±0.20

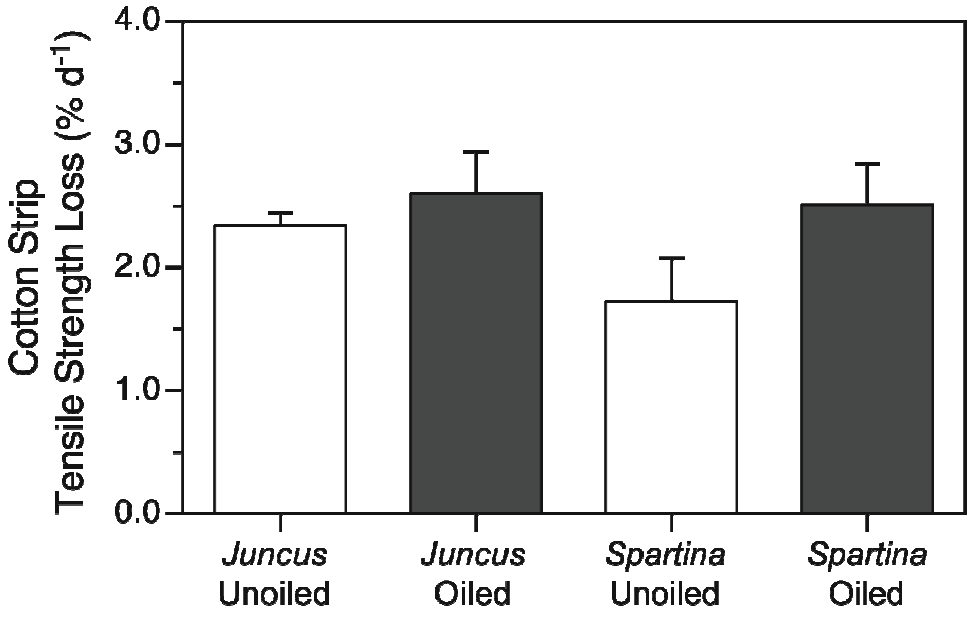

Figure 4. Overall patterns of cotton strip tensile strength loss observed in subsurface sediments in the *S. alterniflora* and *J. roemerianus* plant zones within oiled and unoiled marsh sites. Values are the mean±SE (n=15-30).

significant differences (ANOVA, $F_{(1,79)}$ =9.43, p=0.003) in tensile strength losses were noted between vegetative zones (*S. alterniflora* vs. *J. roemerianus*) (Table 4). These differences displayed an opposite pattern to those observed in marsh subsurface sediments, with on average higher tensile strength losses observed in the *S. alterniflora* versus the *J. roemerianus* plant zone. Similar to subsurface sediment cotton strips, oiling displayed no significant effect on tensile strength loss between sites; however, note that the p-value was low (ANOVA, $F_{(1,79)}$ =3.99, p=0.086) (Table 4). Furthermore, the p-value for the interaction between oiling and plant species zone was also low (ANOVA, $F_{(1,79)}$ =5.98, p=0.071), suggesting that tensile strength loss rates in surface sediments may have also varied in vegetative zones according to oiling exposure (Table 4). When analyzed for

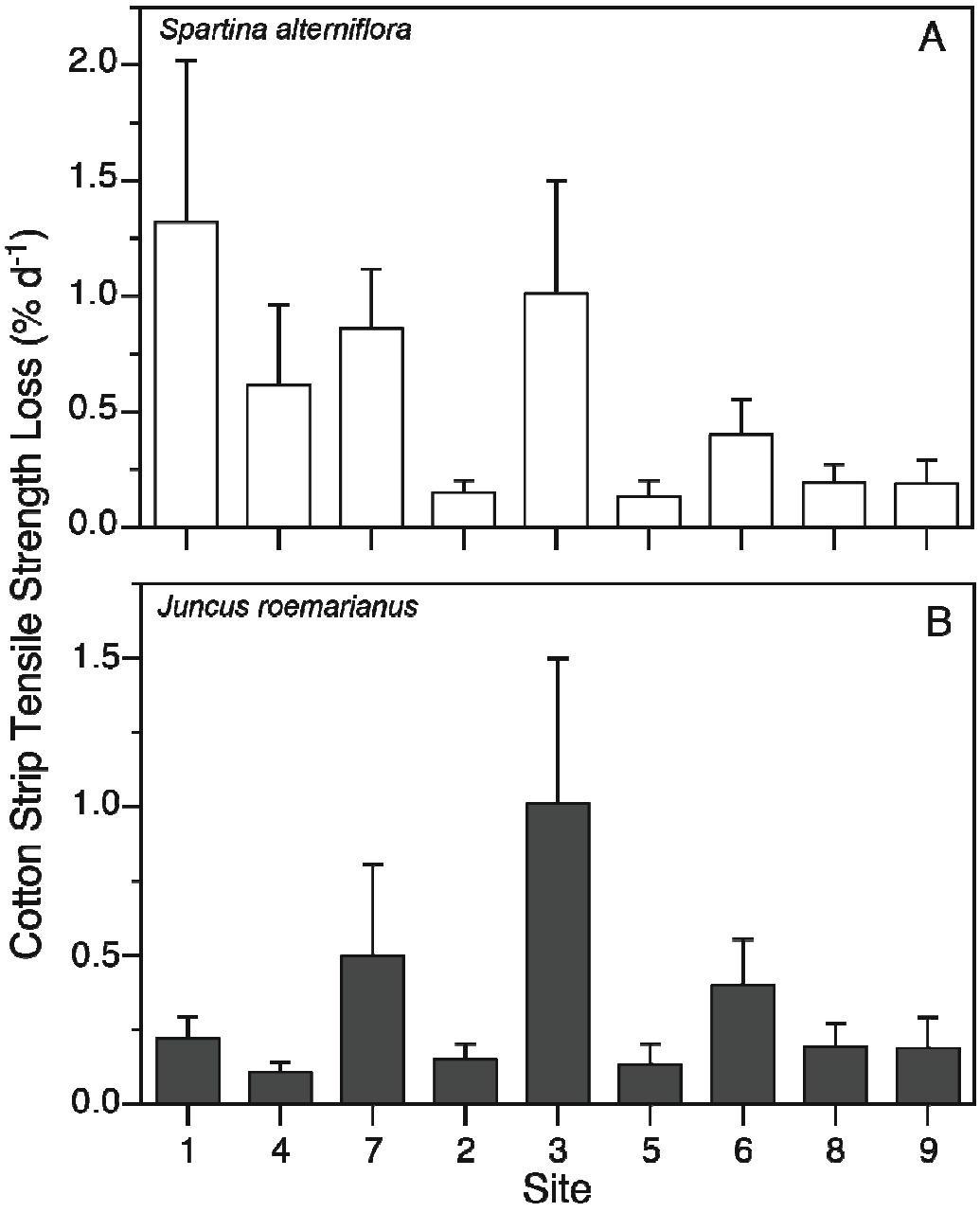

Figure 5. Losses in cotton strip tensile strength observed in surface sediments in the *S. alterniflora* (A) and *J. roemerianus* (B) plant zones at each marsh site. Values are the mean±SE (n=5).

oiling effects across study sites and by overall plant zone, tensile strength losses within surface marsh sediments displayed an opposite pattern when compared to corresponding cotton strip data from marsh subsurface sediments, with cotton strips in unoiled *S. alterniflora* plant zone having the significantly higher rates of decay (Tukey's HSD, $p>0.05$). Surface sediment tensile strength losses of cotton strips were on average ~63% and ~24% lower in the oiled versus unoiled *S. alterniflora* and *J. roemerianus* plant zones, respectively (Figure 6, Table 5), which implies that oiling might have differential effects on microbial decay dynamics between subsurface (anaerobic) and surface (aerobic) sediment environments.

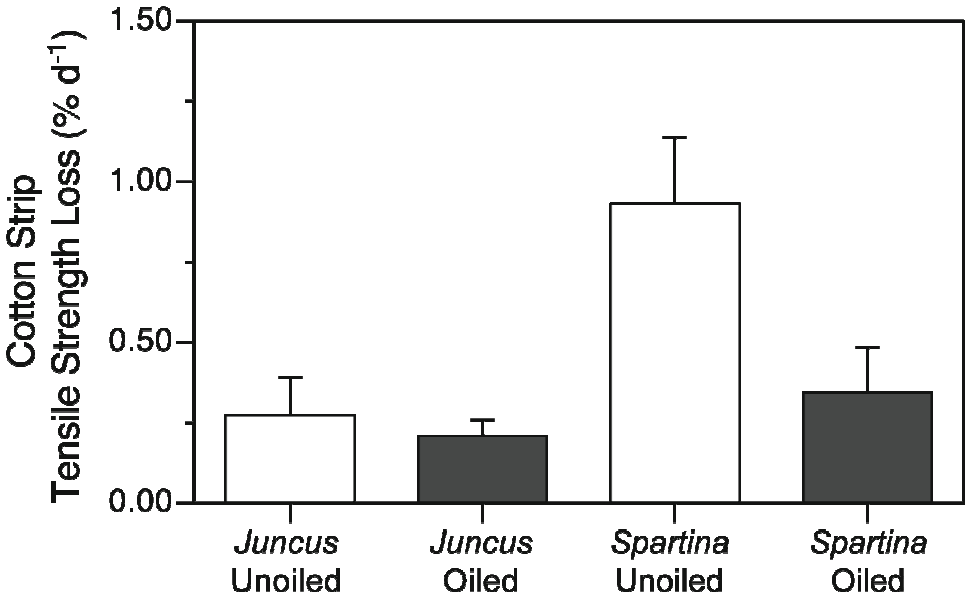

Figure 6. Overall patterns of cotton strip tensile strength loss observed in surface sediments in the *S. alterniflora* and *J. roemerianus* plant zones within oiled and unoiled marsh sites. Values are the mean±SE (n=15-30).

Cotton Strip Nutrient Dynamics

Significant differences in the carbon:nitrogen (C:N) ratios of surface sediment cotton strips were observed among study sites (ANOVA, $F_{(7,79)}$ =7.80, p=0.041) and between plant vegetative zones (ANOVA, $F_{(1,79)}$ =4.85, p=0.031) (Figure 7, Table 6). Site oiling also had a significant effect on cotton strip C:N ratios (ANOVA, $F_{(1,79)}$ =10.80, p=0.013), and there was a significant interaction between oiling and plant species zone (ANOVA, $F_{(1,79)}$ = 7.11, p=0.009) (Table 6). When analyzed across study sites and by plant species zone, C:N ratio's of cotton strips were on average significantly higher (~38%, Tukey's HSD, p<0.05) in oiled versus unoiled *S. alterniflora* plant zones (Figure 8, Table 7). Similarly, the C:N ratio's of cotton strips in *J. roemerianus* plant zones were also higher in oiled versus unoiled sites, with cotton strips in unoiled *J. roemerianus* zones having on average lower C:N ratios (Figure 8, Table 7), however, this difference was not significant (Tukey's HSD, p<0.05). Across study sites, cotton strip C:N ratios were negatively correlated with losses in cotton strip tensile strength (Table 8).

Significant differences (ANOVA, $F_{(7,79)}$ =6.57, p<0.001) in the carbon:phosphorus (C:P) ratios of cotton strips were also observed among study sites (Figure 9, Table 6). In contrast to C:N ratios, no significant differences were observed between plant species zones (ANOVA, $F_{(1,79)}$ = 0.56, p=0.457), nor was there a significant oiling effect (ANOVA, $F_{(1,79)}$ =4.105, p=0.082). However, a significant interaction (ANOVA, $F_{(1,79)}$ =4.93, p=0.029) was observed between plant species zone and oiling, suggesting that cotton strip C:P ratios may have also varied with vegetative zones according to oiling exposure (Table 6). Overall, the C:P ratios of cotton strips followed a similar pattern as C:N ratios, with cotton strips in oiled *S. alterniflora* plant zones having much higher

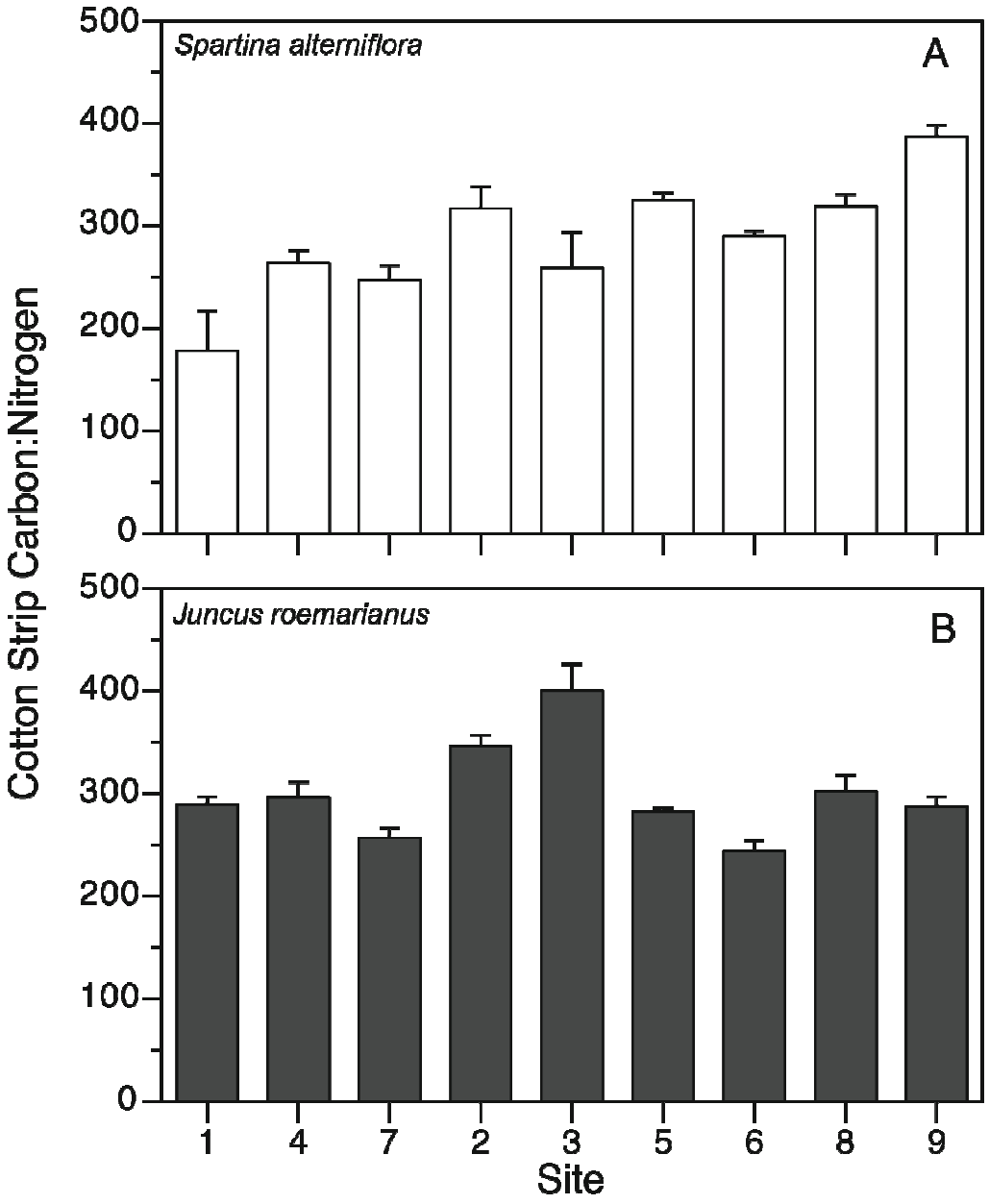

Figure 7. Carbon:nitrogen ratio patterns for cotton strips incubated at the marsh surface sediments in the *S. alterniflora* (A) and *J. roemerianus* (B) plant zones at each marsh site. Values are the mean±SE (n=5).

Table 6

Nested ANOVA summary table indicating the effect of site, plant species zone and oiling on the carbon:nitrogen and carbon:phorsphorus ratios of cotton strips incubated at the marsh sediment surface.

Effect		Carbon:Nitrogen		Carbon:Phosphorus	
	df	F-Ratio	p-value	F-Ratio	p-value
Site [Oiling]	7,79	7.80	0.041	6.57	<0.001
Plant species zone	1,79	4.85	0.031	0.56	0.457
Oiling	1,79	10.80	0.013	4.11	0.082
Plant species zone *Oiling	1,79	7.11	0.009	4.93	0.029

Table 7

Overall patterns of carbon:nitrogen and carbon:phosphorus ratios for cotton strips incubated at the marsh surface sediments in S. alterniflora and J. roemerianus plant zones within oiled and unoiled marsh sites. Values are the mean±1SD (n=15-30).

Species Zone	Site	Carbon:Nitrogen	Carbon:Phosphorus
S. alterniflora	Oiled	316±42	22,991±9,653
	Unoiled	230±46	12,450±5,428
J. roemerianus	Oiled	310±55	20,377±6,577
	Unoiled	281±21	16,143±4,703

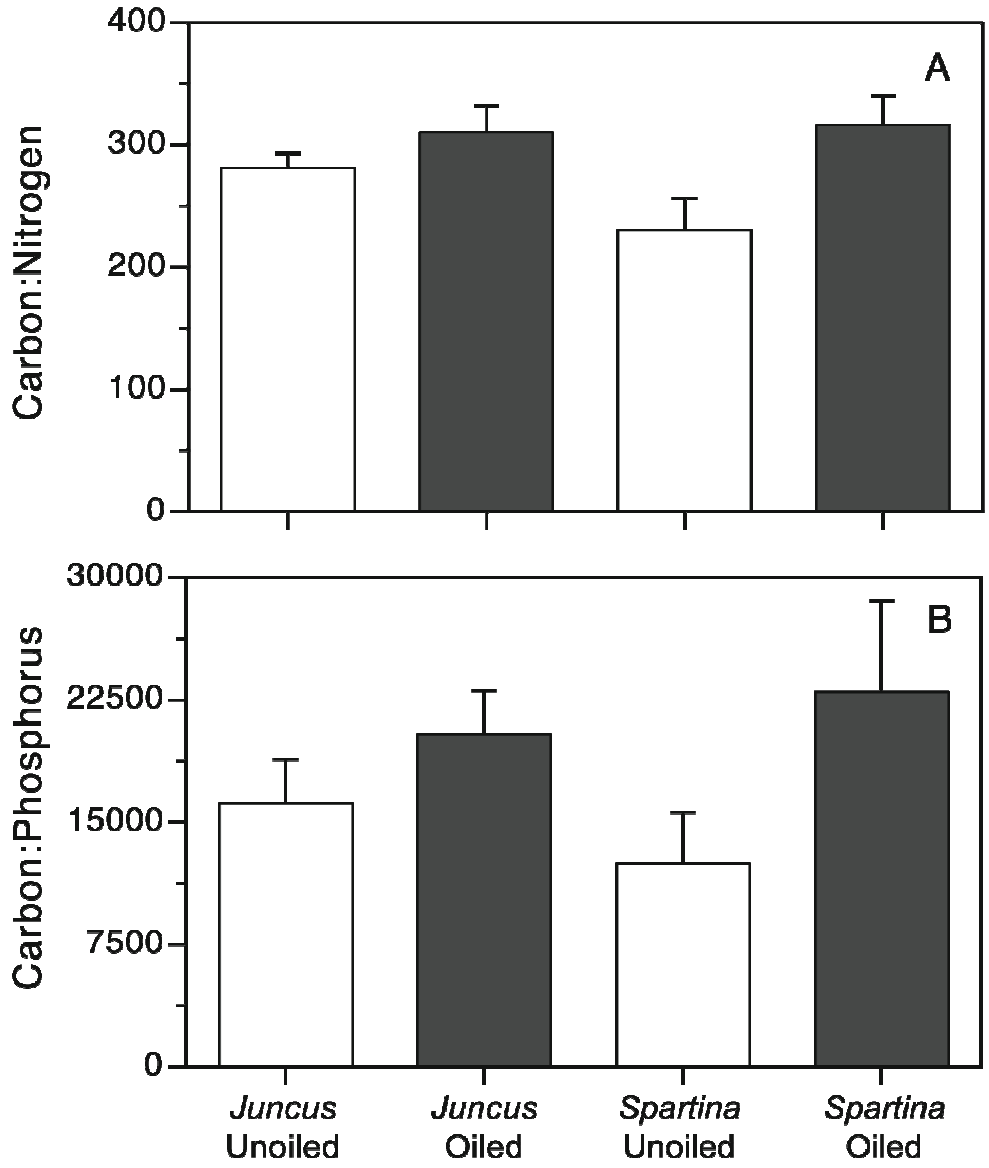

Figure 8. Overall patterns of carbon:nitrogen (A) and carbon:phosphorus (B) ratios for cotton strip incubated at the marsh surface sediments in the *S. alterniflora* and *J. roemerianus* plant zones within oiled and unoiled marsh sites. Values are the mean±SE (n=15-30).

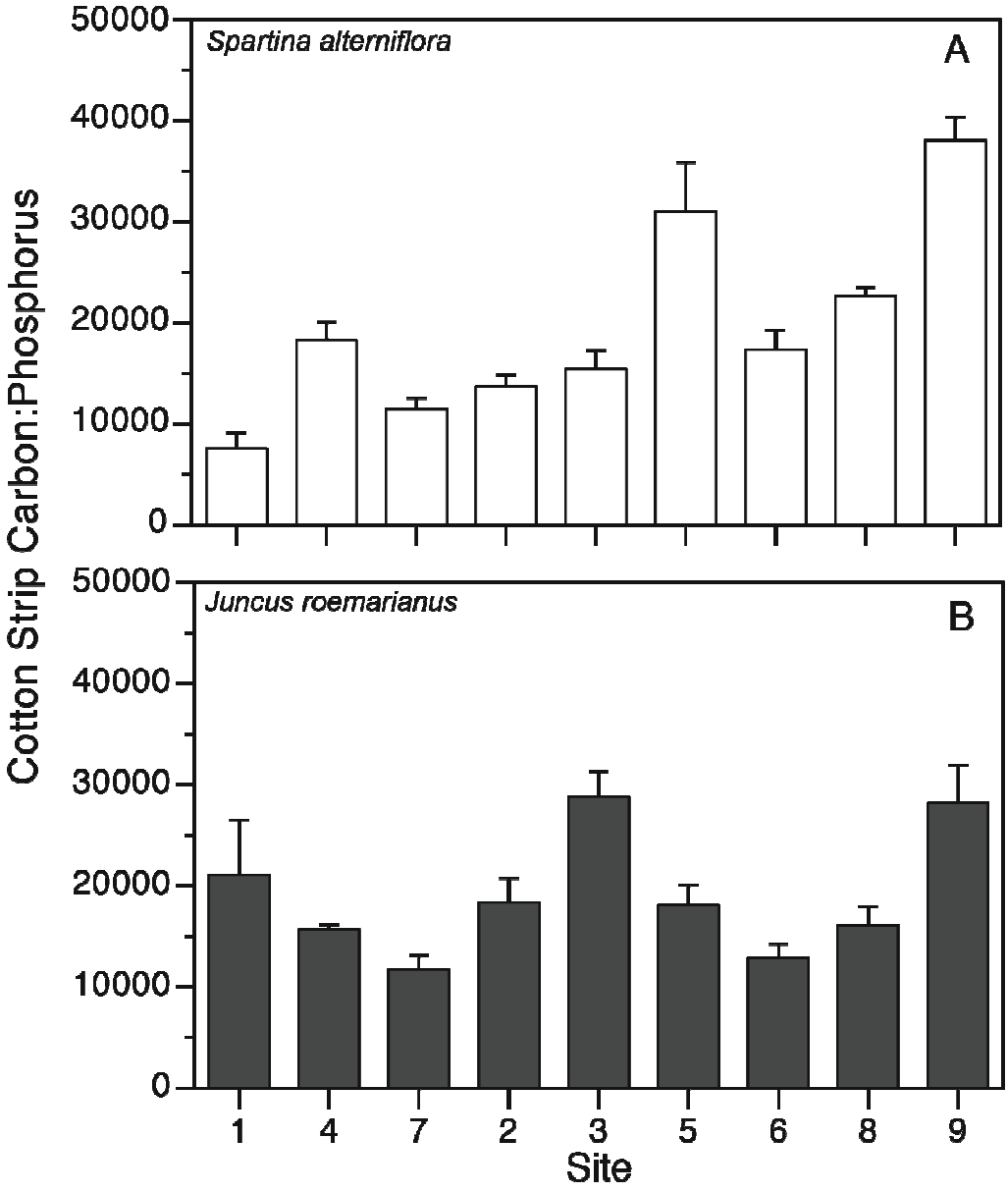

Figure 9. Carbon:phosphorus ratio patterns for cotton strips incubated at the marsh surface sediments in the *S. alterniflora* (A) and *J. roemerianus* (B) plant zones at each marsh site. Values are the mean±SE (n=5).

Table 8

Pearson product-moment intercorrelation matrix showing the relationships between rates of surface sediment cotton strip decay (%CTSL/d) and cotton strip-associated fungal ergosterol concentrations, rates of microbial respiration and carbon:nitrogen and carbon:phosphorus ratios.

	Cotton strip (%CTSL/d)	Ergosterol	Microbial Respiration	Carbon:Nitrogen	Carbon:Phosphorus
Cotton strip *(%CTSL/d)*	1.000				
Ergosterol	0.514***	1.000			
Microbial Respiration	0.672***	0.604***	1.000		
Carbon:Nitrogen	-0.628***	-0.629***	-0.616***	1.000	
Carbon:Phosphorus	-0.380**	-0.332*	-0.349*	0.637***	1.000

Note: ***p< 0.001, **p<0.01, and *p<0.05

ratios than corresponding unoiled sites (Figure 8, Table 6). When analyzed for oiling across study sites and by vegetative zone, average C:P ratios of cotton strips were significantly higher in oiled *S. alterniflora* (~85%) and *J. roemerianus* (~26%) plant zones versus cotton strips in unoiled zones, respectively (Tukey's HSD $p<0.05$) (Figure 8, Table 7). In addition, similar to cotton strip C:N ratios, C:P ratios were also negatively correlated with observed losses in cotton strip tensile strength, however, the resulting correlation coefficient was low (Table 8).

Cotton Strip Fungal Colonization and Microbial Respiration Rates

Fungal colonization of cotton strips, as determined by ergosterol concentrations, were not significantly different among study sites (ANOVA, $F_{(7,79)}=7.80$, $p=0.171$) or between plant zones (ANOVA, $F_{(1,79)}=0.085$, $p=0.77$) (Figure 10, Table 9). However, marsh oiling did have a significant effect on ergosterol concentrations (ANOVA, $F_{(1,79)}=8.12$, $p=0.025$), and there was a significant interaction between plant species zone and oiling (ANOVA, $F_{(1,79)}=5.98$, $p=0.015$). When analyzed across study sites and by vegetative zone, cotton strip ergosterol concentrations were on average significantly lower (~76%, Tukey's HSD, $p<0.05$) in oiled *S. alterniflora* zones versus unoiled zones (Figure 11, Table 10). In contrast, cotton strip ergosterol concentrations in the *J. roemerianus* plant zones were only 7% lower in oiled versus unoiled sites and were not significantly different (Tukey's HSD, $p>0.05$).

Rates of microbial respiration associated with cotton strips varied significantly among marsh sites (ANOVA, $F_{(7,79)}=8.41$, $p<0.0001$) (Figure 12, Table 9). Consistent with patterns of fungal colonization (ergosterol), no significant difference was observed between plant zones (ANOVA, $F_{(1,79)}=1.85$, $p=0.177$). Unlike ergosterol concentrations,

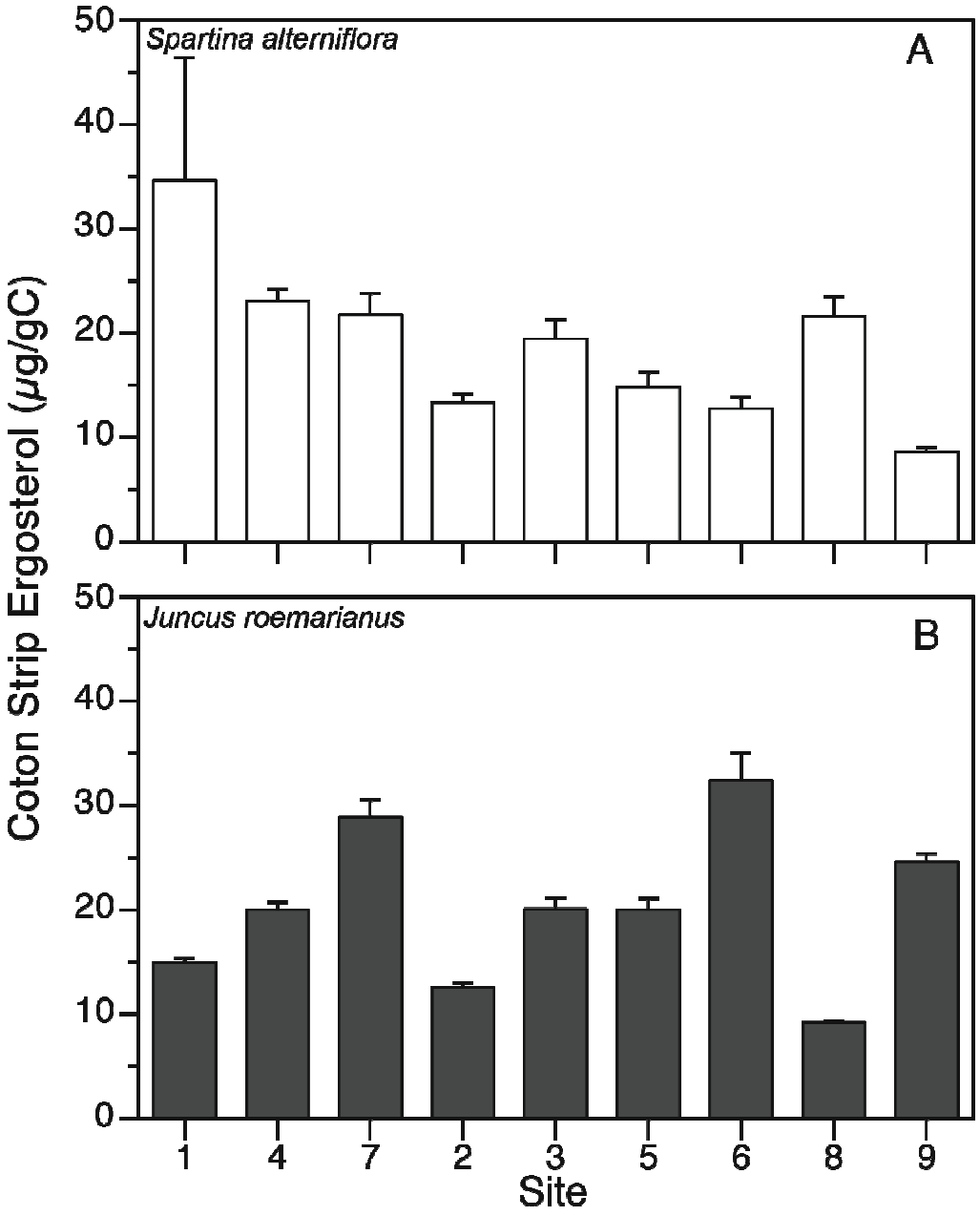

Figure 10. Fungal ergosterol concentrations associated with cotton strips incubated at the marsh surface sediments in the *S. alterniflora* (A) and *J. roemerianus* (B) plant zones at each marsh site. Values are the mean±SE (n=5)

Table 9

Nested ANOVA summary table indicating the effect of site, plant species zone and oiling on cotton strip-associated fungal ergosterol concentrations and microbial respiration rates in cotton strips incubated at the marsh sediment surface.

Effect		Ergosterol		Microbial Respiration	
	df	F-Ratio	p-value	F-Ratio	p-value
Site [Oiling]	7,79	7.80	0.171	8.41	<0.001
Plant species zone	1,79	0.09	0.772	1.85	0.177
Oiling	1,79	8.12	0.025	0.17	0.692
Plant species zone *Oiling	1,79	5.98	0.015	5.32	0.024

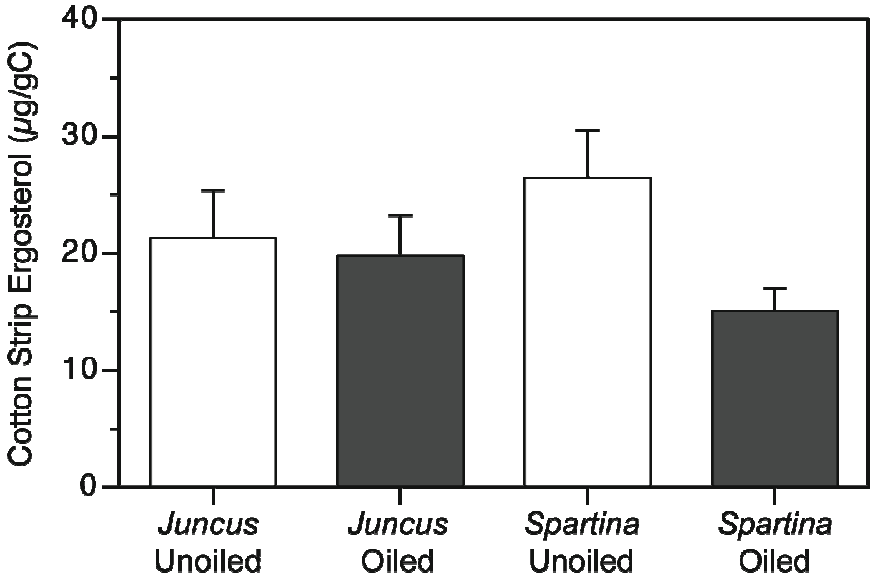

Figure 11. Overall patterns of fungal ergosterol concentrations associated with cotton strips incubated at the marsh surface sediments in the *S. alterniflora* and *J. roemerianus* plant zones within oiled and unoiled marsh sites. Values are the mean±SE (n=15-30).

Table 10

Overall patterns of fungal ergosterol concentrations and rates of microbial respiration associated with cotton strips incubated at the marsh surface sediments in S. alterniflora and J. roemerianus plant zones within oiled and unoiled marsh sites. Values are the mean±1SD (n=15-30).

Species Zone	Site	Ergosterol (µg/gC)	Microbial Respiration (µg CO2-C/gC/h)
S. alterniflora	Oiled	15.06±4.73	53.26±27.69
	Unoiled	26.45±7.08	124.26±157.71
J. roemerianus	Oiled	19.80±8.33	60.38±26.93
	Unoiled	21.26±7.03	51.72±32.37

marsh oiling had no significant effect on respiration rates (ANOVA, $F_{(1,79)}$ =0.1705, p=0.692); however, there was a significant interaction between oiling and plant species zone (ANOVA, $F_{(1,79)}$ =5.32, p=0.024) (Table 9). When analyzed across study sites by oiling and vegetative zone, rates of microbial respiration associated with cotton strips in oiled *S. alterniflora* plant zones were significantly lower (~133%, Tukey's HSD, $p<0.05$) versus corresponding cotton strips in unoiled zones (Figure 13, Table 9). Rates of microbial respiration associated with cotton strips in *J. roemerianus* plant zones were on average slightly higher in oiled versus unoiled plant zones (Figure 13, Table 10), however, this difference was not significant (Tukey's HSD, $p>0.05$). Cotton strip ergosterol concentrations and rates of microbial respiration were positively correlated with one another and positively correlated with observed losses in cotton strip tensile

strength (Table 8). Furthermore, both ergosterol concentrations and respiration rates were

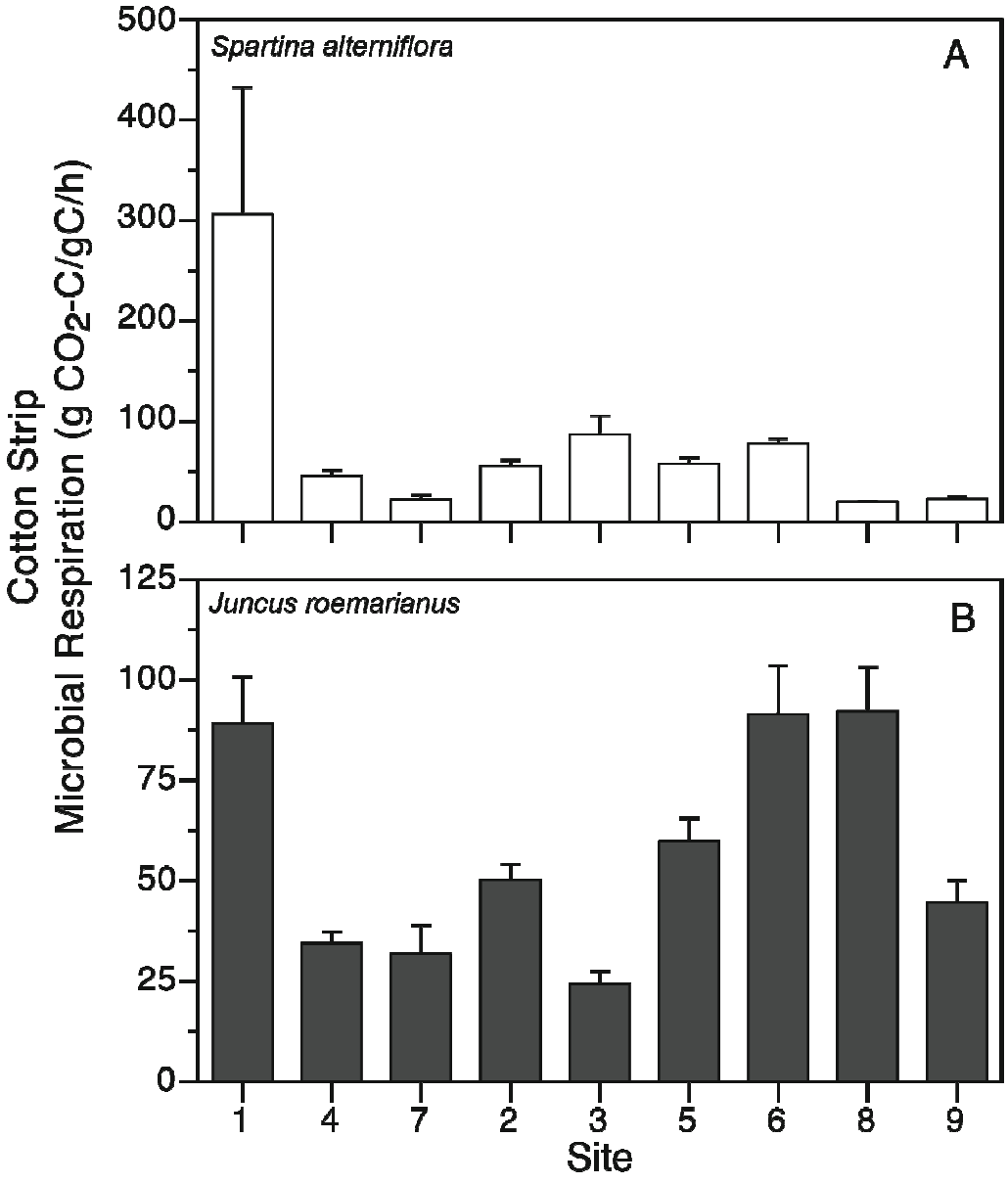

Figure 12. Rates of microbial respiration (CO_2 evolution) associated with cotton strips incubated at the marsh surface sediments in the *S. alterniflora* (A) and *J. roemerianus* (B) plant zones at each marsh site. Values are the mean±SE (n=5).

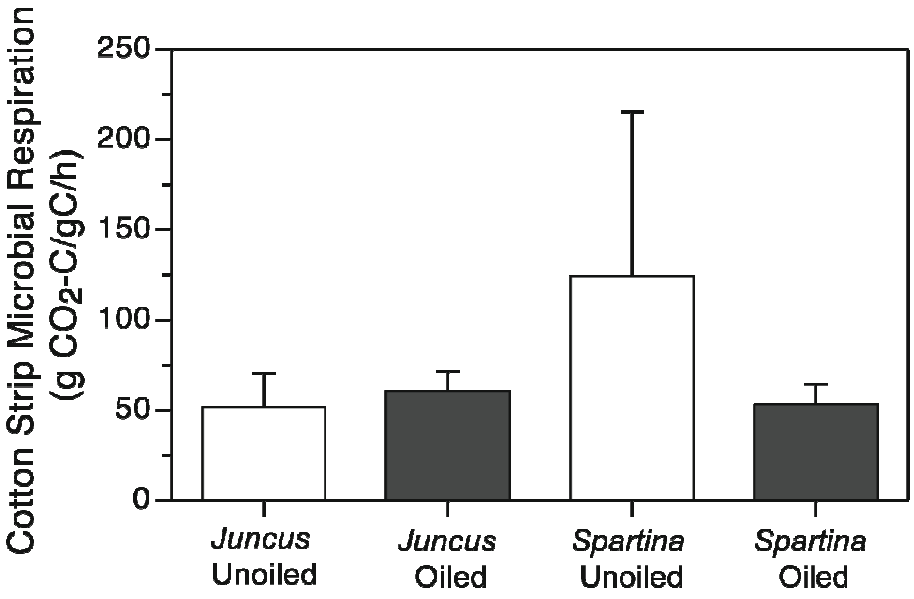

Figure 13. Overall patterns of microbial respiration rates (CO_2 evolution) associated with cotton strips incubated at the marsh surface sediments in the *S. alterniflora* and *J. roemerianus* plant zones within oiled and unoiled marsh sites. Values are the mean±SE (n=15-30).

negatively correlated with cotton strip C:N and C:P ratios, providing evidence that prevailing N and P availabilities within the marsh surface sediments may have limited the decay activity of fungal communities associated with cotton strips and hence the corresponding observed losses in cotton strip tensile strengths.

CHAPTER VI

DISCUSSION

Coastal salt marshes along the Northern Gulf of Mexico are widely regarded as among the most productive wetland-estuarine ecosystems within the United States (Mitsch and Gosselink 2007), and their conservation and management is critical due to the multitude of important economic and ecosystem services that they provide to the region. The emergent vascular plants, *Spartina alterniflora* and *Juncus roemerianus*, are the dominant vegetation within these costal marshes and provide the base for many important ecosystem services (Maltby 2009; DeLaune and Wright 2011; Mendelssohn et al. 2012). Currently, published estimates indicate that ~4.9 million barrels of oil were released into the Gulf of Mexico from the Deepwater Horizon Mississippi Canyon Block 252 oil spill (Crone and Tolstoy 2010). As a result of the oil spill, approximately 430 miles of marsh shoreline was exposed to extensively weathered MC252 crude oil (Reddy et al. 2011), of which ~41% or 176 miles were either moderately or heavily oiled (Mendelssohn et al. 2012). Although recent studies from the Deepwater Horizon spill have attempted to assess oiling impacts on salt marsh vegetation (Anderson and Hess 2012; Lin and Mendelssohn 2012; Mishra et al. 2012; Silliman et. al. 2012; Khanna et al. 2013; Judy et al. 2014), sediment microbial communities (Joye et al. 2014; Horel et al. 2012; Horel et al. 2014b), marsh fish communities (Fodrie et al. 2014; Echols et al. 2015), and benthic insect and invertebrate populations (McCall and Pennings 2012), very few studies have examine the effect of the oil spill on critical ecological processes, such as organic matter decomposition.

In the present study, we used a standardized cotton strip bioassay to examine rates of cellulose decomposition in 9 Mississippi coastal salt marsh sites that differed in the degree of oiling intensity. All of the study sites were chosen based on their similar plant physiognomy and degree of tidal influence, in order to control for other environmental parameters (e.g., pH, salinity, moisture and temperature), which can also significantly influence rates of organic matter decomposition. Although this study was conducted ~1.5 years after the oil spill, we observed significant differences in both surface and subsurface cotton strip decay among the marsh study sites, with oiling impacts being significantly noted within *S. alterniflora* plant zones. In contrast, no significant oiling effects were observed in the *J. roemerianus* plant zone, which was consistent with initial field observations that most of the visible oil inundation in these marshes were restricted to the lower *S. alterniflora* tidal zone at the outer periphery of the marsh. Although, note that recent studies in Louisiana coastal marshes have provided evidence that oil was distributed much deeper (100 m) into oiled marshes (i.e. *J. roemerianus* plant zones), which was not noted during the rapid Shoreline Cleanup Assessment Team (SCAT) surveys immediately following the oil spill (Turner et al. 2014b; see also Ramsey et al. 2014).

A notable and unexpected finding in the present study is the observation of opposing cotton strip decay dynamics between surface (aerobic) and subsurface (anaerobic) sediment environments, particularly within oiled and unoiled *S. alterniflora* plant zones. Despite the presence of aerobic conditions, tensile strength losses of cotton strips placed at the marsh surface sediments were markedly lower than corresponding tensile strength losses for cotton strips incubated within the subsurface sediments. In

addition, the degree of oiling impact on cotton strip decay was also quite different between surface and subsurface sediments. Tensile strength losses of cotton strips in the surface sediment were markedly higher in unoiled versus oiled *S. alterniflora* plant zones, suggesting that oiling may have a negative impact on cellulose decomposition. In contrast, corresponding tensile strength losses of cotton strips in subsurface sediments were significantly higher in oiled versus unoiled *S. alterniflora* marsh sediments, suggesting that oiling inundation within sediments may have stimulated cellulose degradation. Collectively, these observations imply that oiling may have differential effects on microbial communities and hence organic matter decomposition between surface (aerobic) and subsurface (anaerobic) sediment environments.

Observations of oiling effects in the present study differ with earlier findings of Mendelssohn and Slocum (2004), which found no significance influence of oil composition and concentration on salt marsh sediment cellulose decomposition. Using the same cotton strip bioassay approach, Mendelssohn and Slocum (2004) assessed rates of subsurface cellulose decomposition at 8 brackish intertidal marshes that had been exposed to different oiling intensities as a result of the Chalk Point, Maryland fuel oil spill. Similar to the present study, Mendelssohn and Slocum (2004) conducted cotton strip decay studies ~2 years after the oil spill in order to assess potential long-term effects from the oil spill. Although no apparent oiling effect was observed, results of their study found that abiotic environmental characteristics, particularly pH and salinity, had a strong effect on cellulose decomposition rates among the study sites. In their study, strong negative effects of salinity and positive effects of pH on cellulose decomposition were observed among the sites examined. These findings led the authors to conclude that sediment decomposition in

these marshes was controlled more by natural environmental conditions than by its prior history of oiling.

As mentioned earlier, marsh sites chosen for this study all had a similar plant physiognomy and tidal influence in order to control for possible between-site differences in environmental conditions. Despite our best efforts in site selection, significant differences in some environmental parameters were observed between study sites. Although surface and subsurface temperatures did not significantly vary among sites during the study period, we did observe significant differences in both the pH and salinity of interstitial pore water. Mendelssohn and Slocum (2004) observed the slowest rates of cellulose decomposition at estuarine marsh sites with high salinities and low pH. In the present study, we observed no significant relationships between tensile strength losses of cotton strip and salinity or pH. Unlike Mendelssohn and Slocum (2004), we did not measure soil redox potentials, sediment nutrient pools, or other abiotic factors (e.g., bulk density) during the study period. As a consequence, we cannot rule out that some environmental conditions may have contributed, at least in part, to the cotton strip decay patterns observed.

Degradation of oil hydrocarbons by microorganisms is well documented, and deposition of crude oil along coastal shorelines (beaches and marshes) is often expected to alter wetland microbial processes and their important biogeochemical functions (e.g. Leahy and Colwell 1990; Atlas and Bartha 1998; Shin et al. 2000; Widdel and Rabus 2001; Horel et al. 2014a). Following contamination, crude oil typically leads to a shift in the microbial community, resulting in a decrease in microbial diversity and an increased production and abundance in microbial taxa that possess the enzymatic capacity to utilize

oil substrates as a carbon source (Shiaris 1989; Leahy and Colwell 1990; Li et al.1990; So and Young 2001; Paissé et al. 2008; Haritash and Kaushik 2009; Horel et al. 2012). However, there is no clear consensus in the literature regarding the impacts of oil contamination on microbial communities and hence the exact spatial and temporal outcomes on wetland biogeochemical cycles (DeLaune and Wright 2011; Mendelssohn et al. 2012). The overall effect of oil contamination on microbial communities appears to be variable and dependent on the composition (i.e., toxicity) and concentration of hydrocarbons in the oil and the environmental conditions present, such as temperature, oxygen, nutrient availability, pH and salinity (Mendelssohn et al. 2012; Horel et al. 2012; 2014a; 2014b). If large enough amounts of oil contaminants are introduced microbial communities may be negatively impacted due to increased toxicity (Walker et al. 1975; Siddiqui and Adams 2002; Suarez-Suarez et al. 2011), which may inhibit rates of organic matter decomposition. In contrast, if small to moderate amounts of oil contaminants are introduced it may actually stimulate or possibly prime (Bianchi 2011) microbial communities by providing a labile carbon source (Shiaris 1989; Leahy and Colwell 1990; Li et al. 1990; Nyman 1999; So and Young 2001; Haritash and Kaushik 2009), which may accelerate rates of organic matter decomposition.

Hydrocarbon degradation in salt marshes is primarily considered an aerobic bacterial process (Joye et al. 2014; McGenity 2014) where bacteria initially metabolize labile fractions of crude oil (e.g., linear, branched and cyclic alkanes) followed by limited degradation of more recalcitrant oil fractions (e.g., polycyclic aromatic hydrocarbons, PAH's), which are considered the most toxic and may potentially inhibit some microbial populations. Like bacteria, degradation of petroleum hydrocarbons by fungal organisms

(i.e. filamentous and yeast) is also well documented (e.g. Yanto and Tachibana 2014; Covino et al. 2015), however, the effects of specific crude oil fractions (e.g., PAH's) on fungal community growth and their contribution to crude oil degradation within salt marshes has not been extensively investigated. The pervasive hyphal growth of filamentous fungi suggests that they may play a role in metabolizing oil within marsh sediments (Mendelssohn et al. 2012), and recent studies have pointed to fungal-bacterial interactions as important in facilitating hydrocarbon degradation (McGenity et al. 2012; Banitz et al. 2013).

Under anaerobic conditions, microbial-mediated degradation of oil occurs much more slowly, which supports recent observations that petroleum hydrocarbons (e.g., PAH's) continue to persist in Gulf Coast salt marsh sediments after nearly 5 years since the Deep Water Horizon oil spill (Turner et al. 2014a; 2014b). Although higher under aerobic conditions, hydrocarbon degradation does occur under anaerobic conditions (Milhelcic and Luthy 1988; Widdel and Rabus 2001), where it can stimulate the activities of anaerobic bacterial populations. For example, recently Horel et al. (2014a) found that nitrogen fixation rates among sulfur reducing bacteria increased 142% in response to oiling, which demonstrated that these anaerobic microbial communities had the potential to both fix nitrogen and degrade hydrocarbons.

Prior studies have demonstrated that the addition of hydrocarbons can accelerate organic matter decomposition rates at the salt marsh sediment surface (Hershner and Lake 1980; Li et. al. 1990). For example, Hershner and Lake (1980) observed that oiling significantly increased the decomposition rate of *S. alterniflora* plant litter. This additional C source can fuel or possibly prime (Bianchi 2011) the microbial decomposer community

and accelerate rates of decomposition and the immobilization of nutrients (Jingguo and Bakken 1997). However, because oil provides little to no nitrogen or phosphorus to the system, increased decomposition and mineralization of organic matter can ultimately lead to long-term increases in C:N and C:P ratios, which can eventually limit microbial growth and production (Leah and Colwell 1990) and slow decomposition. In the present study, measured nutrient and microbial parameters associated with surface incubated cotton strips were significantly correlated with corresponding rates of cotton strip decay, with cotton strips from oiled sites collectively having much higher C:N and C:P ratios, lower ergosterol content (fungal biomass), lower respiration rates, and ultimately lower decomposition rates. This study was conducted approximately 1.5 years after the spill, and it is possible that shortly after the spill decomposition rates were possibly elevated, but as time progressed nitrogen and phosphorus may have become limited slowing microbial growth and leading to decreased rates of decomposition. Research by Hines et al. (2006) demonstrated that the addition of a labile carbon source to salt marsh ecosystems increased microbial communities' demand for nitrogen, and that this increased microbial immobilization of nitrogen negatively impacted both aboveground plant biomass (growth) and the resulting C:N ratios of plant litter. Their findings in conjunction with this study imply that oil contamination could possibly lead to progressive nitrogen limitation (and perhaps phosphorus) in aerobic marsh surface sediments, thereby impacting important biogeochemical functions.

In the present study, we did not measure nutrient and microbial parameters associated with cotton strips placed within the more anaerobic marsh sediments. Findings of faster decay rates of cotton strip decay in these zones, particularly *S. alterniflora* plant

zones exposed to oiling, suggest that anaerobic microbial communities may not be nutrient limited and that oiling enhanced their overall decay activities. If this trend is consistent and widespread, then increased rates of organic matter (i.e., cellulose) decomposition could alter the rate of organic matter accumulation and accretion within oiled Gulf Coast marsh sediments, which could further weaken the marsh soil profile and facilitate increased rates of erosion. Increased rates of marsh erosion have been documented in marsh sites exposed to a wide range of oiling intensity, despite an apparent recovery of the plant community (McClenachan et al. 2013).

In salt marshes, decomposition of organic matter is a complex process that includes an array of biotic and abiotic processes that result in the production of decomposer biomass (e.g., microbial and invertebrate), release of CO_2 and nutrients (N & P) through organic matter mineralization, as well as the release of dissolved and fine particulate organic matter (Kuehn 2008). From a microbial perspective, the rates of these decay processes are strongly influenced by the response of microbial communities to the prevailing environmental conditions, the intrinsic quality of the plant detrital resources they metabolize, and the myriad of potential interactions that may occur within detrital food web. Even though this study was conducted ~1.5 years after the Deep Water Horizon oil spill, it is evident that both the subsurface and surface microbial processes were still being affected by oil intrusion. Due to the continued persistence of oil contaminates in Gulf coast marshes, particularly PAH's in subsurface sediments (Turner et al. 2014a; 2014b), more long-term monitoring of marsh biogeochemical functions are warranted in order to understand and predict the spatial and temporal recovery of these vital ecosystems and possible restoration activities.

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