IMPACTS AND RECOVERY OF THE DEEPWATER HORIZON OIL SPILL ON VEGETATION STRUCTURE AND FUNCTION OF PHRAGMITES AUSTRALIS

A Thesis

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ABSTRACT

The aim of this study was to determine the impacts of the *Deepwater Horizon* (*DWH*) oil spill on the common reed *Phragmites australis*, and the processes controlling species effects and recovery, via a greenhouse mesocosm study. In the greenhouse *DWH* source oil, weathered approximately 40% by weight and emulsified, was applied to the aboveground shoots of *P. australis* growing in marsh sods to produce the following treatment-levels: (1) oil coverage of the lower 30% of shoot-height, (2) the lower 70% of shoot-height, (3) repeated oil coverage of the lower 70% of shoot-height, (4) 100% oil coverage of shoots, (5) oil applied to the soil at a rate of 8 L m^{-2}, and (6) unoiled controls. I quantified a strong resilience of *P. australis* when oil was applied only to aboveground biomass, with negative impacts becoming apparent when oil was added to the soil profile. The Total biomass and stem cumulative length were both impacted by the addition of 8 L m^{-2} of weathered *DWH* source oil to the soil profile. Due to the apparent negative results of adding oil directly to the soil, a second experiment was designed to better understand impacts from soil oiling. Aboveground biomass was harvested from the sods that had received only shoot oiling and allowed to regrow for two months, at which point weathered *DWH* source oil was applied to the marsh sods at rates of (1) 0 L m^{-2} (control), (2) 4 L m^{-2}, (3) 8 L m^{-2}, (4) 12 L m^{-2}, and (5) 16 L m^{-2}. This experiment verified that increased oiling to the soil profile increased negative impacts to *P. australis*, reducing stem cumulative length, aboveground biomass, and belowground biomass at the highest oiling rates. Higher oiling doses resulted in higher rates of soil respiration and reduced soil Eh. Based on my research, complete mortality of *P. australis* is unlikely from exposure to weathered and emulsified *DWH* source oil. However,

vertical growth, above and belowground biomass, and other plant processes will be impacted, with oiling to the soil having much greater impact than oiling to the aboveground shoots.

INTRODUCTION

On April 20, 2010 the *Deepwater Horizon (DWH)* oil drilling platform exploded and released over the next 3 months a government-estimated 4.9 million barrels of oil (Macondo MC252), becoming the largest marine oil spill in United States history (Oil Spill Commission, 2011; Camilli, 2011). The sheer volume of oil caused widespread impacts including the oiling of approximately 1040 km of Gulf Coast marshes; this included approximately 208 km of Louisiana salt marsh being classified as moderately to heavily oiled (Oil Spill Commission, 2011). Although a majority of the impacted shoreline was salt marsh dominated by *Spartina alterniflora, Juncus roemarianus,* and *Avicennia germinans*, oiling at the mouth of the Mississippi River impacted a sizable area of fresh and oligohaline marsh dominated by the common reed, *Phragmites australis*. *Phragmites australis* is a valuable species in south Louisiana because of its exceptional ability to accumulate and stabilize sediments as well as being adaptable to a wide variety of environmental conditions (Alizai & McManus, 1980; Hartog *et al*., 1989; Gorai *et al.,* 2006).Also, the *Phragmites australis* marshes at the mouth of the Mississippi River are unique in the United States in forming expansive deltaic wetlands similar to the expansive *Phragmites*-dominated deltas of Europe, such as the Volga and Danube.

Although a relatively large body of literature has described the impact and recovery of a number of wetland species to oiling (e.g., Pezeshki *et al.*, 2000; DeLaune *et al.*, 2003; Mendelssohn *et al.*, 2012), *Phragmites australis* has received much less attention. Species such as *Spartina alterniflora, Spartina patens,* and *Juncus roemarianus* have been negatively impacted by oil exposure to soil and to aboveground tissues; these impacts range from reduced photosynthesis, stem density, and biomass to complete mortality (Lin & Mendelssohn, 1996;

Pezeshki *et al.*, 2000; Dowty *et al.*, 2001; Anderson & Hess, 2012). However, not all wetland species show negative impacts to oiling. For example, when *Sagittaria lancifolia*, a dominant freshwater species in the southeastern United States, was exposed to soil oiling, regrowth was stimulated up to an oil dosage of 24 L m^{-2} (Lin & Mendelssohn, 1996). The limited research on *P. australis* and oiling has shown negative impacts occurring from oiling to the soil. These impacts include a reduction in photosynthetic rates and above/belowground biomass, and reduced oxygenation of phyllospheres and rhizospheres causing failure of new buds to emerge (Dowty *et. al*, 2001; Armstrong, 2009; Nie *et al.*, 2010; Zhu *et al.*, 2013). However the response of *Phragmites* to oiling requires further examination given its demonstrated resilience to various pollutants as evidenced by its use in wastewater treatment facilities, for sludge stabilization (Cole, 1998; Bianchi, 2011; Borin, 2011), and for the phytoremediation of a number of pollutants (Toyama *et al*., 2011; Toyama, 2011).

Impacts from the *DWH* oil spill have been documented for marsh species such as *Spartina alterniflora* and *Juncus roemarianus*, showing negative impacts at varying degrees of oiling applied to both the aboveground biomass and the soil profile in field and greenhouse studies (Lin & Mendelssohn, 2012; Wu *et al.*, 2012; Biber, In Press). Impacts on these species range from minor, when oil is applied to the aboveground biomass at low doses, to severe with the addition of oil to the soil. In contrast to the documentation of impacts on salt marsh species, no research has been published concerning the response of *Phragmites* to the *DWH* oil spill. Due to the relatively extensive weathering and emulsification of the Macondo oil before it made landfall, toxic components of the oil were reduced, thereby making for a unique situation compared to most spills of un-weathered oil (Mendelssohn, *et al.*, 2012). The effects of such

weathered oil on *P. australis* requires investigation so that information on the sensitivity of this species to oiling can be applied to cleanup and restoration efforts during future oil spills in the Gulf of Mexico and elsewhere. In addition, much of the oil contact occurred aboveground, but with varying degree of plant coverage. The effects of differential aboveground oiling have received little attention, especially for this species.

The objectives of this study were to (1) quantify the impacts of weathered and emulsified Macondo oil on *P. australis* across a wide range of possible modes of contact with the plant material, both above and belowground, (2) determine the effects of different volumes of oil on plant response to soil oiling, and (3) use this information to clarify how oil exposure (aboveground versus belowground) controls plant response. I predicted that *Phragmites* would eventually recover from all levels of oiling to aboveground plant oil-exposure, but recovery time would slow with increasing oil doses and physiological function would be impaired at the highest oil doses.

METHODS

Experiment #1 - Effects of Most Common Oiling Scenarios on *Phragmites australis* Growth Response

A greenhouse mesocosm study was conducted to determine the impacts of weathered and emulsified Macondo oil from the *DWH* spill on common reed, *Phragmites australis*. *Phragmites australis* sods (28 cm in diameter and approx. 30 cm deep), were collected from an unoiled marsh site at 30° 23.205' North latitude by 90° 09.551' West longitude in Madisonville, Louisiana in October 2011. The sods contained intact vegetation and soil with a pH of 6.37 ± 1.09, a salinity of 1.64 ± 0.45 psu, and 22.4 ± 2.8% organic matter (means and standard errors); the texture class of the soil was loam with 27.9 ± 3.6% sand, 45.3 ± 3.3% silt, and 26.8 ± 2.9% clay. After collection the sods were immediately transported to a greenhouse (21-40° C) at Louisiana State University (LSU) where they acclimated for two months before the oil treatment began. Soil samples for analyses of the above soil physiochemical properties were collected using a 7 x 41cm coring piston; they were dried whole at 60° C for a week and sent to the Coastal Wetland Soils Characterization Lab, School of Plant, Environmental and Soil Sciences, LSU Agricultural Center for the analyses.

Deepwater Horizon Oil and Weathering Process

The oil used for the study was Macondo source oil (MC252), the same oil that was spilled during the *DWH* event. The Macondo MC252 oil has an API gravity of approximately 37, containing a relatively large proportion of lighter hydrocarbon compounds, as typical of south Louisiana crude (SLC) (Platts Oilgram, 2010). To simulate the weathering process the oil would have undergone between its entry into Gulf of Mexico waters and contact with the plant material, the oil was artificially weathered 40% by weight using wind (greenhouse fan) and sunlight. After

weathering, the oil was then emulsified to 50% water content by pouring the oil over 15 cm of 3% salt water (using Instant Ocean™) and mixing with submersible pumps (Lin & Mendelssohn, 2012).

Experimental Design and Procedures

The most common field oiling scenarios, as used in a previous study (Lin & Mendelssohn, 2012), were simulated in the greenhouse on and the effects on *P. australis* were examined. The experiment design was a randomized block with five replicate blocks. A one way analysis of variance (ANOVA) with six treatment-levels (oiling categories) was used to analyze the measured response variables. Each of the 30 marsh sods (six oil treatment-levels times five replicate blocks) was an experimental unit. The six oil treatment-levels were (1) oil coverage of the lower 30% of the shoot-height of *P. australis* (30-oil), (2) oil coverage of the lower 70% of the shoot-height (70-oil), (3) repeated oil coverage of the lower 70% of the shoot-height (70-rep-oil), (4) 100% oil coverage of shoots (100-oil), (5) soil oiling with 8 L m^{-2} of oil added to the soil surface and allowed to penetrate the sediment (Oil-Soil), and (6) no oil treatment as the control. For the soil oiling treatment, 8 L m^{-2} of weathered and emulsified oil was applied over standing water in each sod for even dispersal, and the water was then drained through the soil column allowing the oil to contact the soil and encouraging penetration into the sediment. The drained water from each experimental unit was collected in a receptacle and added back to the same experimental unit at the end of each day, resulting in approximately eight hours of drainage each day. The process of draining the sods and adding water back was repeated each day until the oil was no longer clearly visible on water surface, implying it had successfully adsorbed into the soil profile. For the 70% repeated oil coverage treatment, oiling was repeated every four days

for two and a half months. Oil was applied to aboveground biomass by turning the sod on its side and applying oil with a paint brush, making sure no oil contacted the soil. All oil used in the mesocosm study was weathered and emulsified as previously described.

Analysis of Plant Responses and Oil Chemistry

Data collection occurred four times over the course of the experiment with each collection-event taking place over a five-day period.

Stem Density, Cumulative Stem Length, and Relative Growth Rate. Plant stem density was determined by directly counting the number of individual stems in each sod. Cumulative stem length (green and brown) in each sod was determined by measuring the length of each stem from the sod surface to the tip of the stem's newest unfolded leaf. These lengths were then summed for the entire sod and expressed in millimeters (mm). Cumulative stem length was determined separately for green (indicating live) and brown (indicating oiled or dead) plant material. Relative growth rate was determined by selecting and tagging two stems from each sod and measuring their heights. Five days later a second measurement was taken on the same tagged stems. Relative growth rate (RGR) was determined from the two measurements with the following equation:

$$RGR = (\ln W2 - \ln W1)/(t2 - t1)$$

where W2 is the second height measurement, W1 is the first (both in mm), t2 minus t1 is the duration of growth (in days), and ln is the natural logarithm. Relative growth rate was expressed as mm of growth per mm of stem length per day (mm mm^{-1} d^{-1})

Soil Redox and Chlorophyll. Redox potential was measured with an Accument AP71 pH/mV/°C meter (Fisher Scientific, Waltham, MA). The meter was connected to a corning hi-stab calomel

reference electrode and bright platinum electrodes at two soil depths, 1 cm and 15 cm into the soil column. Measurements were taken from each sod with six electrodes, three at each depth. The electrodes were left in place for 24 hours before data were collected to assure a stable reading. Due to shortage of electrodes only one of the five blocks could be done each day, and because of this, redox measurements were taken over a five-day period. The Ec values were converted to Eh values by adding +244 to each reading, and the redox was reported in mV. Plant chlorophyll rate was measured on five stems in each sod using a MINOLTA chlorophyll meter SPAD-502. For each stem the newest, completely unfolded leaf was chosen. The leaf was inserted into the measuring cell, and the measuring cell was pressed closed, automatically taking a SPAD reading (SPAD units) (Spectrum Technologies, Aurora, IL).

Final Aboveground Biomass and Final Stem Density. Final aboveground biomass (Live, Dead, and total) was collected at the conclusion of the experiment, 24 and nine weeks after oiling for Experiments 1 and 2, respectively. All aboveground plant material was cut from the sods one node above the soil surface. Collected plant material was separated into living and dead tissue. If there were dead leaves on living stems, they were stripped and added to the dead tissue portion. Plant material was then dried in an oven at 60° C for three days until all moisture had been removed. Dried plant material was weighed in grams (g) as live, dead, and total (the sum live and dead) biomass. Final plant stem density data (Live and Dead) were collected at the same time as the final aboveground biomass, which simply required counting the total number of both living and dead stems.

Residual Total Petroleum Hydrocarbon (TPH). Residual TPH was measured by collecting approximately 2cm of surface soil from each sod, extracting the oil with dichloromethane

(DCM), and analyzing gravimetrically (Lin and Mendelssohn, 1996). Briefly, approximately 5g of soil was placed into a glass vial, the oil extracted from the soil using DCM, and the DCM extract transferred to a pre-weighed foil dish. Once in the dish the DCM evaporated leaving only the oil in the dish which was weighed to the nearest 0.0001 g. The TPH concentration was calculated and expressed as mg g^{-1} dry soil.

Experiment #2 - Effects of Oil Applied to the Soil on *Phragmites australis* Growth Response

At the conclusion of experiment #1, a second experiment was initiated to further the effects of adding oil directly to the soil. The aboveground biomass was harvested from the sods that had received shoot oiling and the control, and then the sods were allowed to regrow for two months, at which point *DWH* source oil was applied to the marsh sods at rates of (1) none/control, (2) 4 L m^{-2}, (3) 8 L m^{-2}, (4) 12 L m^{-2}, and (5) 16 L m^{-2}. This time the randomized block design used a one way ANOVA and five levels of one treatment (oiling), five replicate blocks were used (20 sods in total). The same process was used to prepare the oil as it was for the first experiment, along with the process of draining and adding water to the oiled sods to encourage oil penetration into the soil profile.

Analysis of Plant Responses and Oil Chemistry

Data collection was carried out three times over the course of the experiment with each round of collection taking place over a five day period. In addition to all the variables measured during the first experiment, the second experiment also measured additional variables described below.

Final Belowground Biomass and Soil Respiration. At the conclusion of the second experiment, a coring piston (7 x 41cm) was used to collect belowground biomass samples. The samples were

filtered through a 2 mm wire sieve and then separated into categories of live root, live rhizome, dead root, and dead rhizome. The samples were then placed in an oven at 60° C for three days to remove moisture and weighed in grams (g). Soil respiration rate (carbon dioxide exchange rate, expressed in g CO_2 m^{-2} hr^{-1}, was measured using a EGM-4 environmental gas monitor after aboveground biomass was collected but before the soil was disturbed during the coring process. The respiration data were collected while the soil was drained of water by placing the sensor firmly against the soil surface and waiting for a steady reading.

Statistical Analyses

All statistical analyses were conducted using SAS (version 9.2, SAS Institute, Cary, NC). I used univariate mixed-model ANOVAs (PROC MIXED) to determine the effects of oil treatment, time, and their interaction on the following dependent variables for Experiments 1 and 2 individually: stem density, total stem length, relative growth rate, soil redox, chlorophyll, aboveground biomass, belowground biomass, soil respiration, and TPH. When ANOVAs were significant at $p < 0.05$, treatment means in PROC MIXED were tested using the least- square (LS) means procedure with a Tukey-Kramer post-host adjustment to maintain an experiment-wise error rate of 5%. When necessary, these data were logarithmically or square transformed prior to analysis to improve ANOVA assumptions of normality and homogeneity of variance. All measures of significance were identified at $p<0.05$ unless otherwise stated.

RESULTS

Experiment #1 - Effects of most common oiling scenarios on *Phragmites australis* growth response

In this greenhouse mesocosm study, I investigated what had previously been identified as the most common oiling scenarios to coastal wetlands during the *Deepwater Horizon* oil release (Lin & Mendelssohn, 2012). As expected, soil TPH was significantly higher ($p<0.0001$) when oil was added directly to the soil (27 ± 2.7 mg g^{-1}) compared to the control and those treatment-levels that received oil coating of aboveground shoots/leaves alone (control=1.1 ± 0.1 mg g^{-1}; 30-oil=1.1 ± 0.1 mg g^{-1}; 70-oil=1.3 ± 0.2 mg g^{-1}; 70-rep-oil=1.3 ± 0.2 mg g^{-1}; 100-oil=1.7 ± 0.4 mg g^{-1}). Soil redox, which could be affected by oiling, was significantly lower ($p<0.0104$), i.e., more reduced, for the oil-soil treatment-level than the control (-63.9 ± 13.1 mV and -28.1 ± 10.6 mV, respectively). Also, the redox of the 30-oil treatment-level (-61.3 ± 10.9 mV) was significantly lower than the control, but the other treatment-levels with even more shoot oiling (70-oil, 70-rep-oil, and 100-oil) had redox levels (-42.7 ± 11.7 mV, -41.8 ± 10.8 mV, and -49.5 ± 13 mV, respectively) that were not statistically different from the control.

Oil exposure affected total (green plus brown tissue) cumulative stem length, but this effect significantly varied over time (significant Treatment*Week interaction, $p<0.0001$; Figure 1a). During the first six weeks following exposure, cumulative stem length was similar for all treatment-levels, but thereafter diverged. The 100-oil treatment-level, where 100% of the shoot tissue was oiled, had the greatest increase in cumulative stem length over time (11961 ± 1585 mm), while those sods receiving soil-oiling (with no shoot exposure), had the smallest increase (7737 ± 551 mm) (Figure 1a). Side-branching, the production of new shoots from the nodes of

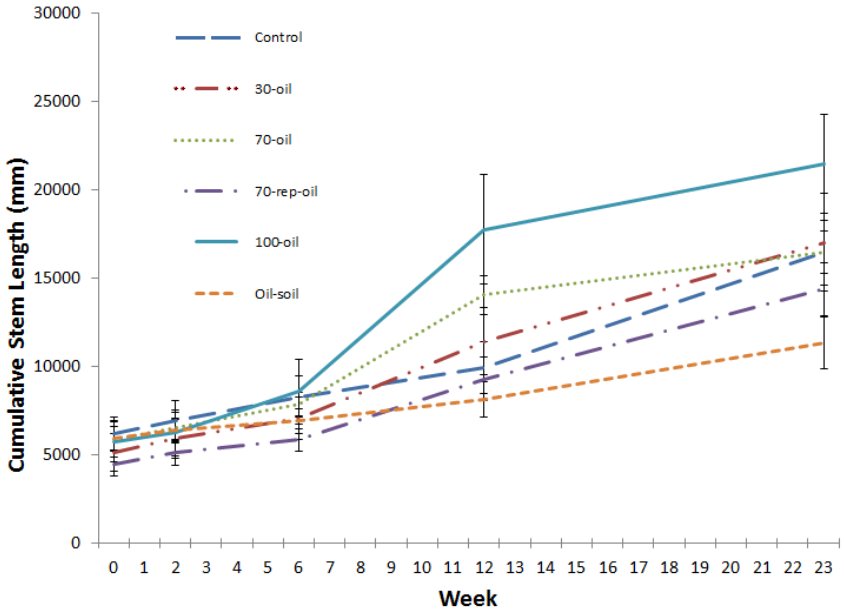

Figure 1a. Effects of oil treatment-levels on the cumulative stem length from 0-23 weeks after oiling. The values are means ± standard errors (n=5).

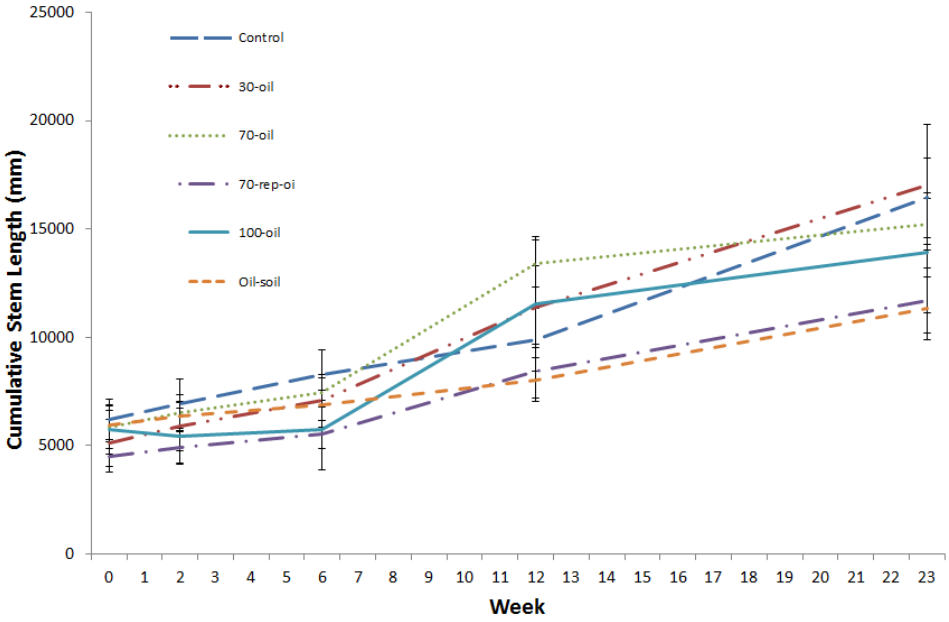

Figure 1b. Effects of oil treatment-levels on the cumulative stem length, excluding side-branches, from 0-23 weeks after oiling. The values are means ± standard errors (n=5).

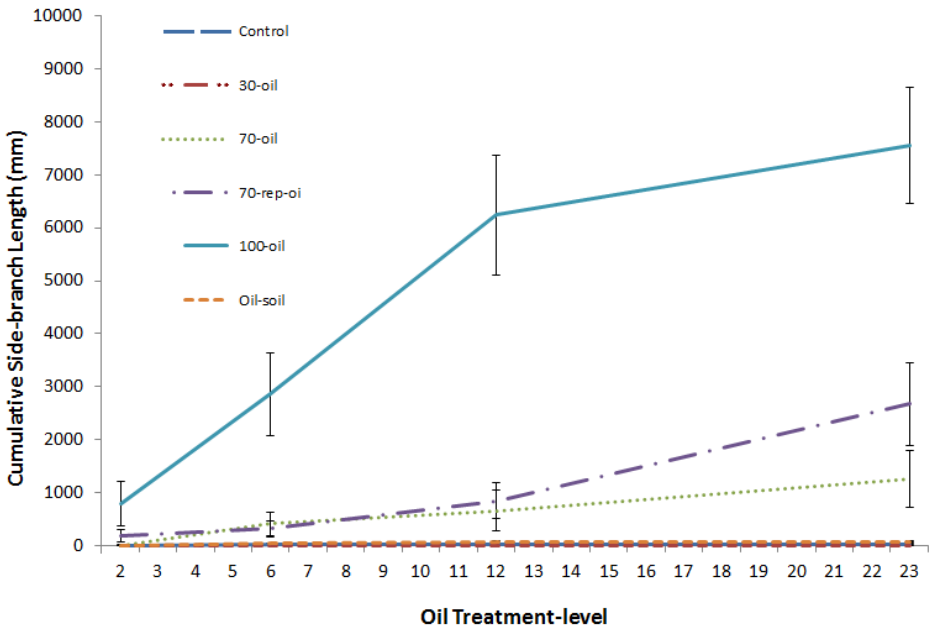

Figure 2a. Effects of oil treatment-levels on cumulative side-branch length, from 2-23 weeks after oiling. The values are means ± standard errors (n=5).

Figure 2b. The 100-oil sod treatment-level from Experiment 1 showing stem-branching from nodes at week 23 (whole mesocosm on left and close up of the side-shoots on right).

oiled stems, was dramatic in the 100-oil treatment-level (Figures 2a and 2b). By week 23, at the end of the experiment, cumulative stem length was significantly greater in the 100-oil treatment-level than in the oil-soil condition ($p<0.0021$), which, however, had the lowest cumulative stem length of all treatment-levels. Trends were similar for both live ($p<0.0001$) and brown ($p<0.0001$) tissue cumulative stem length (data not shown). When side-branch lengths were subtracted from total cumulative stem length, the impact of oiling over time was most apparent in the oil-soil and 70-rep-oil treatment-levels (significant Treatment*Week interaction, $p<0.0001$; Figure 1b).

Oil treatment also had significant effects on end-of-the-experiment live ($p<0.0377$) and dead ($p<0.0584$) biomasses, respectively (Figure 3). The live biomass for the oil-soil treatment-level was significantly lower than that for the 70-oil treatment-level ($p<0.0217$), which did not significantly differ from the control ($p=0.1215$) or the other shoot exposure conditions (Figure 3). The oil-soil treatment-level significantly affected dead aboveground biomass at a probability of 0.0584; dead biomass tended to be higher when the shoots and leaves were exposed to oil compared to the control and oil applied to the soil (Figure 3). Total aboveground biomass showed similar trends ($p<0.0391$) as the live (data not shown).

The other growth variables that were measured during the experiment were not affected by oiling. Relative growth rate was unaffected by oiling treatment ($p=0.8503$) and by the interaction of oiling treatment with time ($p=0.4733$) with an overall mean and standard error of 0.009 ± 0.001 mm^{-1} day^{-1}. Stem density was similarly unaffected (live treatment effect, $p=0.3717$ (17 ± 1 stems sod^{-1}); dead treatment effect, $p=0.5583$ (1 ± 0.25 stems sod^{-1}); total treatment effect, $p=0.5002$ (18 ± 1 stems sod^{-1}).

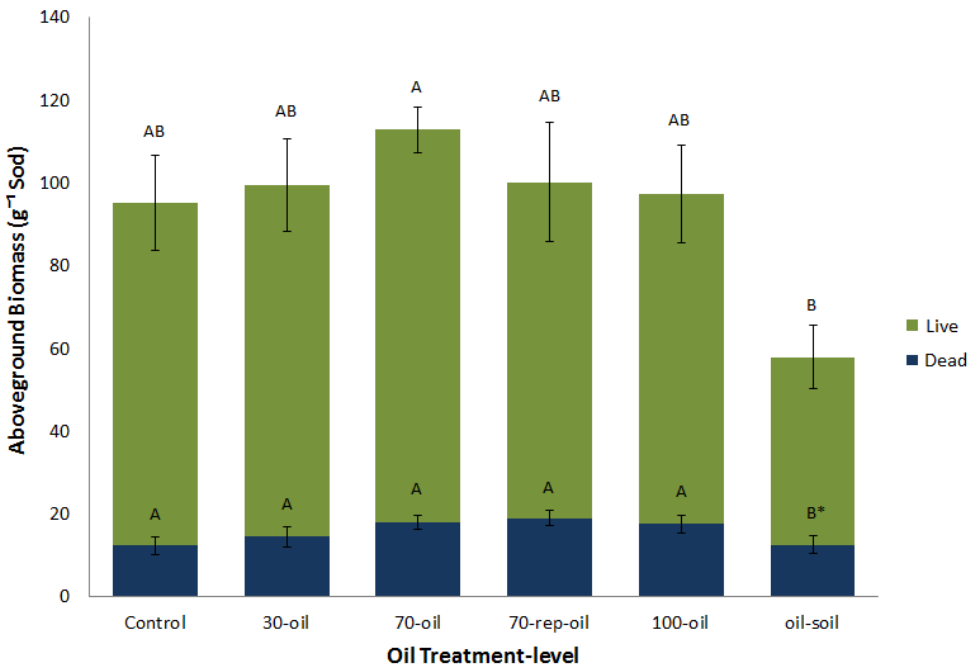

Figure 3. Effects of oil treatment-levels on aboveground biomass (live and dead) 23 weeks after oiling. The values are means ± standard errors (n=5). Different letters indicate significant differences between treatment-level means ($p<0.05$). *Significantly different at $p=0.0584$.

Oil treatment significantly affected leaf chlorophyll ($p<0.0139$), although there was no interaction with time. The 70-rep-oil treatment-level (30.3 ± 1.1 SPAD units) had significantly higher chlorophyll levels than the 30-oil treatment-level (23.6 ± 1.4 SPAD units). The other four treatment-levels yielded intermediate chlorophyll levels with values of 26.4 ± 0.7 SPAD units (control), 26.5 ± 1.3 SPAD units (70-oil), 25.3 ± 1.5 SPAD units (100-oil), and 26.1 ± 0.8 SPAD units (oil-soil).

Experiment #2 - Effects of oil applied to the soil on *Phragmites australis* growth response

During the first mesocosm study it became clear that there was more of a negative impact caused by oil being added directly to the soil profile than when it was applied to the aboveground tissue. Based on these findings, I designed a second greenhouse mesocosm study to investigate

the effects of oil added to the soil profile, with five increasing dosages, including a control. Soil TPH levels significantly increased with each increasing treatment-level ($p<0.0001$) (Figure 4).

Just as in the first greenhouse mesocosm study, soil redox was affected by oiling. Soil redox was significantly lower($p<0.0011$) for oil added at 4 L m^{-2}, 8 L m^{-2}, and 16 L m^{-2} (-111.9 ± 12.2 mV, -125.1 ± 10.5 mV, and -145.7 ± 13.2 mV, respectively) than the control (-68.5 ± 15.3 mV). The redox at 12 L m^{-2}, as opposed to all other oiling treatment-levels, was not statistically different from the control (-110.2 ± 14.5 mV). Soil respiration, which was not measured during the first mesocosm study was significantly affected by oiling ($p<0.0189$). The highest oil dosage

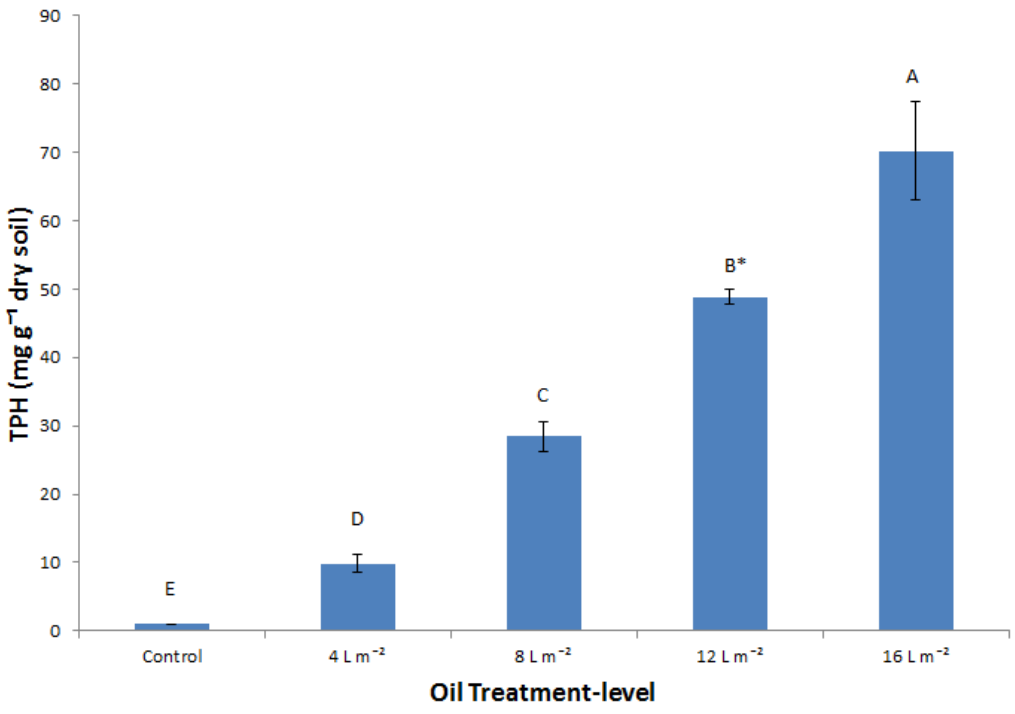

Figure 4. Effects of oil treatment-levels on soil total petroleum hydrocarbons (TPH) eight weeks after oiling. The values are means and standard errors (n=5). Different letters indicate significant differences between treatment-level means ($p<0.05$). *Significantly different at $p=0.0526$.

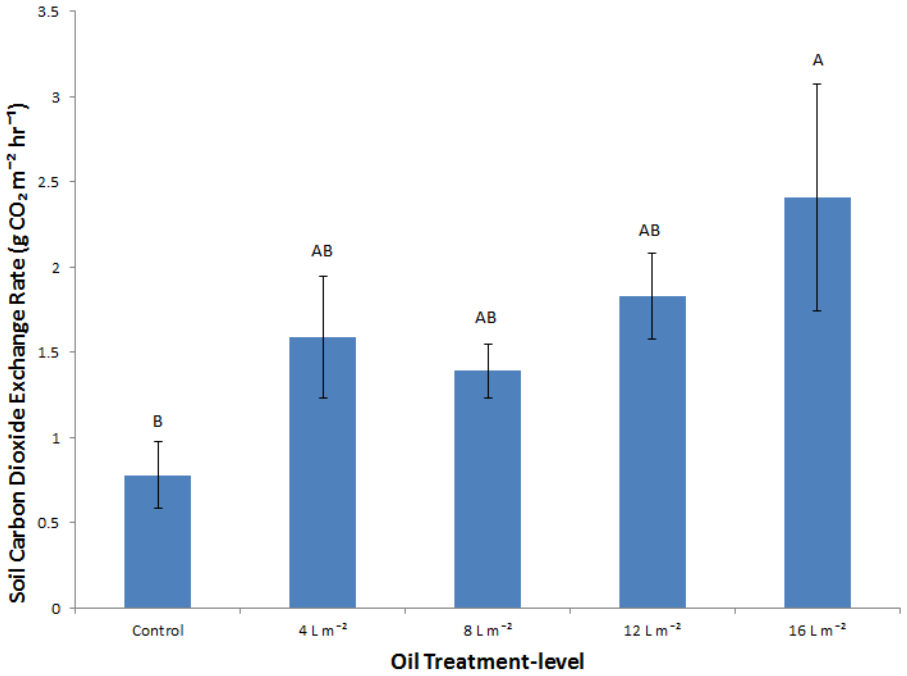

Figure 5. Effects of oil treatment-levels on the soil respiration eight weeks after oiling. The values are means and standard errors (n=5). Different letters indicate significant differences between treatment-level means ($p<0.05$).

(16 L m^{-2}) had significantly higher soil respiration rates than the control ($p<0.0111$) while the other oil treatment-levels were intermediate (Figure 5).

Oil application to the soil significantly affected the change in total (green plus brown tissue) relative cumulative stem length over an eight week period (significant treatment effect, $p<0.0002$; Figure 6). The control treatment-level had a significantly greater change in total cumulative stem length compared to all oiling treatment-levels ($p<0.0002$), and the 4 L m^{-2} treatment-level had a greater change in cumulative stem length than the 16 L m^{-2} treatment-level ($p<0.0332$). The control treatment-level showed the greatest increase in cumulative stem length over time (15718 ± 846 mm) with the 16 L m^{-2} and 12 L m^{-2} treatment-levels having the two lowest (6235 ± 612 mm and 7520 ± 879 mm, respectively; Figure 6).

Oil treatment significantly reduced live aboveground biomass ($p<0.0002$; Figure 7). The control treatment-level had significantly more live mass than 4 L m^{-2} ($p<0.0420$), 12 L m^{-2} ($p<0.0018$), and 16 L m^{-2} ($p<0.0002$), while 8 L m^{-2} had significantly more mass than 16 L m^{-2} ($p<0.0382$; Figure 7). Total aboveground biomass showed similar trends ($p<0.0003$) as the live, while the dead aboveground biomass did not significantly vary ($p=0.203$) among treatment-levels (Figure 7).

At the conclusion of the experiment, I measured belowground biomass and found significant differences in live root ($p<0.0066$; Figure 8) and total biomasses ($p<0.0399$; Figure 9). The control and the 4 L m^{-2} treatment-levels both had significantly higher live root mass than the 16 L m^{-2} treatment-level ($p<0.0599$ and $p<0.0065$, respectively). Total belowground biomass of the control was significantly greater ($p<0.0853$) than that for the 16 L m^{-2} ($p<0.0852$) and

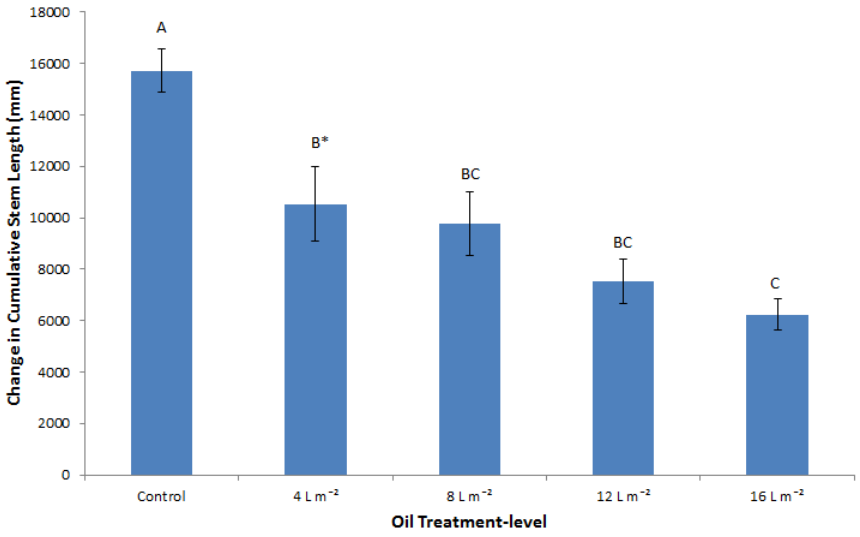

Figure 6. Effects of oil treatment-levels on change in cumulative stem length, from 0 to 8 weeks after oiling. The values are means and standard errors (n=5). Different letters indicate significant differences between treatment-level means ($p<0.05$). *Significantly different at $p=0.0552$).

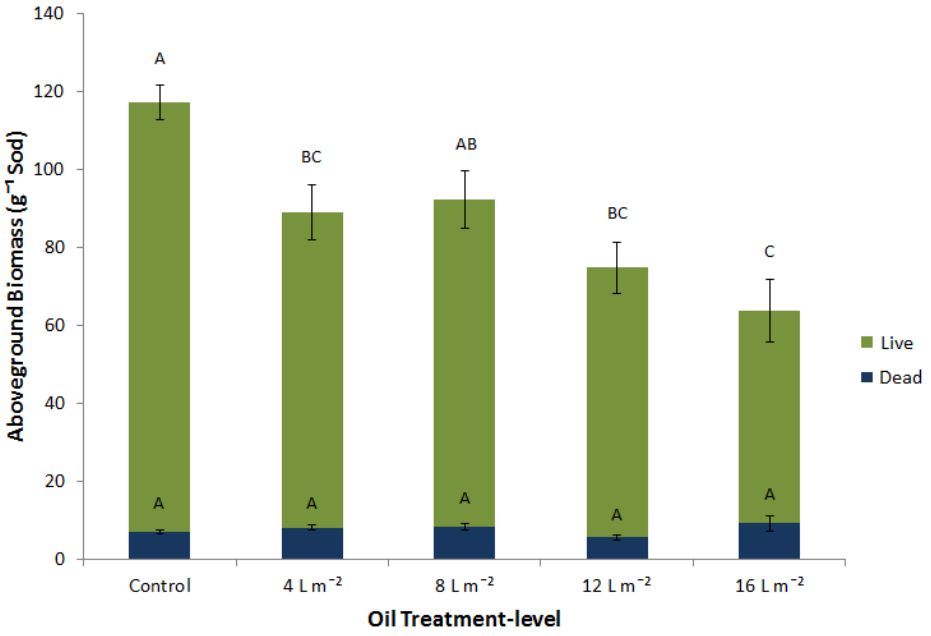

Figure 7. Effects of oil treatment-levels on aboveground biomass (live and dead) eight weeks after oiling. The values are means and standard errors (n=5). Different letters indicate significant differences between treatment-level means ($p<0.05$).

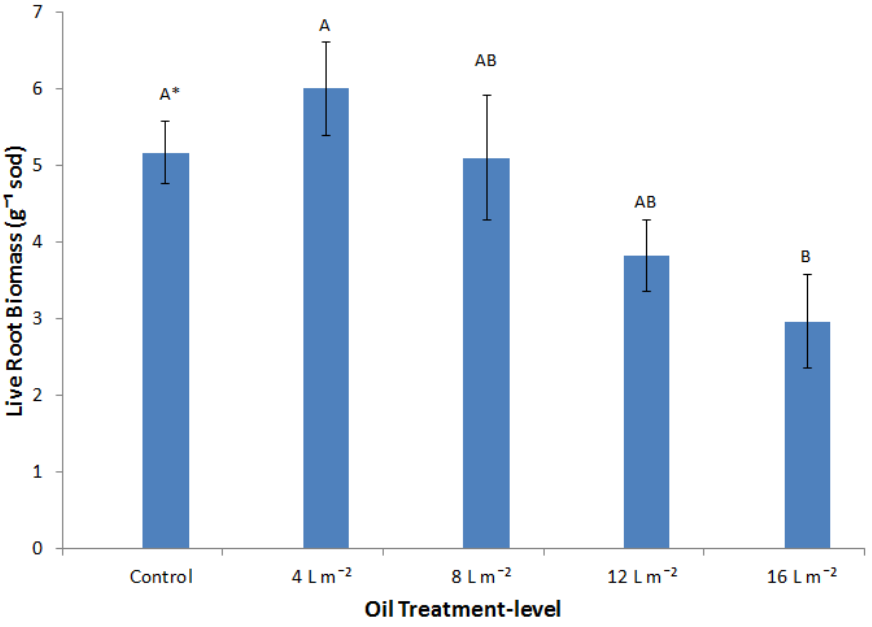

Figure 8. Effects of oil treatment-levels on live root biomass eight weeks after oiling. The values are means and standard errors (n=5). Different letters indicate significant differences between treatment-level means ($p<0.05$). *Significantly different at $p=0.0599$.

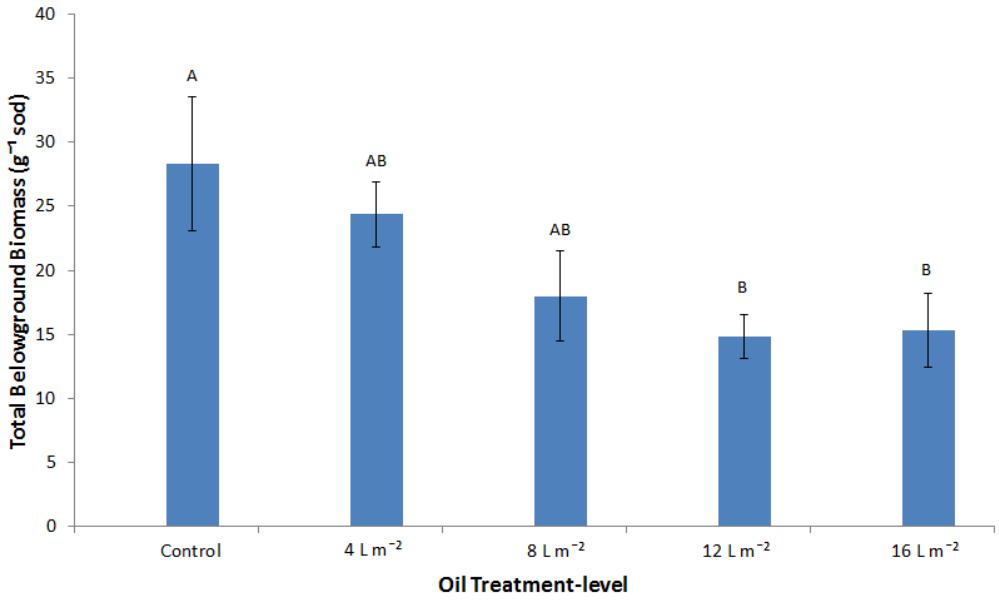

Figure 9. Effects of oil treatment-levels on total (live and dead, roots and rhizomes) biomass eight weeks after oiling. The values are means and standard errors (n=5). Different letters indicate significant differences between treatment-level means ($p<0.05$).

12 L m^{-2} ($p<0.0686$) treatment-levels. No significance differences were found in dead root biomass ($p=0.4085$), live rhizome ($p=0.401$), or live total biomass ($p=0.2183$).

Just as in Experiment 1, there were variables that were not affected by oiling. Relative growth rate once again was unaffected by the oiling treatment ($p=0.2376$) or by an interaction of oil treatment with time ($p=0.3396$) with an overall mean and standard error of 0.004 ± 0.001 mm^{-1} day^{-1}. The same was true for stem density (live treatment effect, $p=0.1809$ (25 ± 2 stems sod^{-1}), and total treatment effect, $p=0.1215$ (26 ± 1 stems sod^{-1})). Unlike Experiment 1, chlorophyll was unaffected by oiling treatment ($p=0.2828$) having an overall mean and standard error of 23.7 ± 1 SPAD units. Soil shear strength was also unaffected by soil oiling ($p=0.3423$) or by an interaction of oiling and depth ($p=0.7878$) with an overall mean and standard error of 31 ± 3 kPa.

DISCUSSION

This research demonstrated the resilience of *P. australis* to weathered and emulsified Macondo oil when applied to aboveground biomass and to low levels of oiling when applied to the soil. It became clear during the 23-week study period of Experiment 1 that most of the growth in response to the 100-oil treatment-level, and to a lesser degree the 70-rep-oil and 70-oil treatment-levels, occurred from the production of new shoots from the nodes of oiled stems. This production of side-shoots has not been noted in previous studies investigating the effects of oiling on *P. australis* but has been known to occur as an effect of insect damage.

The production of side-shoots can result from *Phragmites* in response to a number of stressors. It was reported by van der Toorn and Mook (1982) that stemborers of the genus *Archanara* can cause heavy damage to *P. australis*, but most of the shoots in their study area survived because of side-shoot production. The side-shoot production was reported as occurring early in the growing season and to be a response to apical meristem damage caused by the stem-borer. Tscharntke (1990) also noted this, stating that the stem boring caterpillar *Archanera geminipuncta* reduced shoot length greatly and induced the production of narrow side-shoots. Tscharntke concluded that the abundance of insect-damaged shoots was correlated with the number of side-shoots. The present study and the aforementioned entomological work suggest that the production of side-shoots by *P. australis* is an effective response to environmental stressors. There was no visually recognizable stress to the shoots of *P. australis* when oil was added only to the soil. The absence of an immediate shoot impact with this treatment-level was likely the reason why there was little side-shoot branching. One might hypothesize that if there

was an aboveground oil exposure in conjunction with the belowground oiling, the production of new side-shoots might have reduced the negative impacts of the belowground oiling on total plant biomass.

Spartina alterniflora has also shown rapid recovery of aboveground biomass when weathered and emulsified oil is applied to its shoots. Lin and Mendelssohn (2012) reported that after seven months under the same oil treatment-levels and using Macondo oil prepared in a similar fashion way as the present study, *S. alterniflora* showed no difference in canopy height compared to the control and only showed a negative impact to live stem density in the 70-rep-oil treatment-level. In the same study *J. roemarianus* only showed recovery of canopy height at the 30-oil and 70-oil treatment levels while live stem density only recovered from the 30-oil treatment level. Another report showed that South Louisiana "sweet" crude oil (SLC) weathered to 87% and 67% of its original weight and applied to the bottom 30cm of *S. alterniflora* stems caused rapid death but new stems began growing within seven days of oiling (Pezeshki *et al.*, 1995). These results suggest that oiling to the aboveground plant tissues of multiple Louisiana marsh species does not cause lasting damage to plant vigor. Just as *Phragmites* produces side-branches and quickly recovers from aboveground tissue oiling, *S. alterniflora* produces new growth from its underground rhizomes.

In addition to cumulative stem length, total and living aboveground biomasses were also most impacted when oil was applied to the soil compared to the other treatment-levels; this result was re-affirmed in Experiment 2, which demonstrated that increases in oil dosages caused decreases in cumulative stem length, aboveground biomass, and total and live-root belowground biomass. These findings are supported by other papers investigating the effects of oil on *P.*

australis. Zhu *et al*. (2013) showed that increasing TPH levels, associated with seepage around oil wells at the Chengdong oilfield in Eastern China, were directly correlated with reduced aboveground biomass of *P. australis.* Dowty *et al.* (2001) found a decrease in both above and belowground biomass of *Phragmites* collected from the Manchac Wildlife Management Area in south Louisiana when soil was exposed to 5 L m^{-2} and 10 L m^{-2} of un-weathered SLC oil in a greenhouse mesocosm study; Nie *et al*. (2010) also found a decrease in above and belowground biomass of *Phragmites* grown from seed in the greenhouse when un-weathered crude oil from the Shengli oilfield in China was mixed with *Phragmites* soil at oil dosages of 6000 mg kg^{-1} of soil and 12000 mg kg^{-1} of soil. However, both of these studies used un-weathered oil, which would likely be much more toxic than the weathered Macondo oil used in my research. Similar reductions with respect to shoot length were observed when shoots alone were exposed to liquid paraffin and un-weathered diesel oils (Armstrong *et al*., 2009). These results indicate that exposure of *Phragmites* to oiling is directly related to negative impacts on plant growth, but that the intensity of impact and the ability to rapidly recover is dependent on the extent of oil weathering, the volume/concentration of the oil, and the mode of exposure (aboveground versus belowground).

Research done shortly after the *DWH* oil spill, using similar weathered and emulsified Macondo oil as in the present experiment, showed that aboveground biomass and live stem density of both *S. alterniflora* and *Juncus roemarianus*, two dominant salt marsh plants, were significantly impacted by moderate to heavy soil oiling (Lin & Mendelssohn, 2012). This research was the first to show that the weathered and emulsified *DWH* oil was still toxic enough to impact plant growth, and it agrees with previous work done on *S. alterniflora* after an oil spill

in a Galveston Bay, Texas bayou that occurred January 1984 (Alexander & Webb, 1987). Although this oil had much less time to weather and emulsify before contacting the salt marsh than the Macondo oil, it still shows that 5-51 mg/g of oil in the sediment of a natural salt marsh caused significantly reduced growth of *S. alterniflora* after 18 months. Un-weathered Empire mix and Saudi Arabian crude oils, used in a study of oil effects on *Juncus roemarianus*, showed that a single dose of 1.5 L m^{-2} or 6-10 successive monthly doses of 0.6 L m^{-2} completely killed the plants and continued to suppress growth for up to two years; only an initial impact was seen at single low doses ranging from 0.25-0.6 L m^{-2} (De La Cruz, 1981).

Field plots examined by Lin and Mendelssohn (2012), post *DWH*, contained TPH concentrations of approximately 500 mg g^{-1} in heavily oiled Louisiana salt marshes and approximately 80 mg g^{-1} dry soil for moderately oiled marshes. They also estimated that if the TPH concentrations in the heavily oiled marsh TPH were converted to L m^{-2}, in order to compare to the dosages used in their mesocosm study, the TPH level would be approximately 20 L m^{-2}. This dosage is much higher than that for the oil-soil treatment in Experiment 1 and even for the highest oil treatment-level (16 L m^{-2}) in Experiment 2. If these oil dosages held true at coastal salt marsh sites populated by *P. australis* in the Mississippi River Birdfoot delta and if these volumes penetrated the soil, negative impacts on plant response could have occurred at some of the sites classified as heavily oiled. Field data on *Phragmites* response to the *DWH* event are presently lacking

When oil penetrates soil, the increase in labile carbon can promote microbial activity and affect soil biogeochemistry (Ellis & Adams, 1961). I found that with greater oil dosages to the

soil and resultant higher soil TPH concentrations, soils respiration rates were higher and soils became more reducing.

The presence of oil in the soil has previously been shown to decrease soil Eh (Ellis & Adams, 1961). However, more reducing soil conditions associated with oil additions to the soil can be an initial impact that does not persist (Nyman *et al.*, 1999; Nyman, 1999). Other studies found no effect on soil Eh by additions of oil to the soil (Lin & Mendelssohn, 1999; DeLaune *et al.*, 1979) possibly due to transient nature of oil impacts on soil Eh as previously mentioned. An increase in soil respiration in response to the addition of oil to the soil has also been previously observed, which was correlated with the decrease in soil Eh. Oil additions to the soil can stimulate microbial activity and soil respiration, which in turn reduces oxygen and other electron acceptors and thus lowers soil Eh (Nie, 2010; Llangovanand & Vivekanandan, 1992; Ellis and Adams, 1961). However, when oil levels in the soil are too high, the microbes die due to oil toxicity, resulting in reduced soil respiration (Li *et al*, 1990; Nyman, 1999). A consequence of oil-induced microbial toxicity is a probable reduction in bioremediation potential, given that microbes associated with the rhizosphere of the *P. australis* are known to contribute to bioremediation (Toyama *et al.*, 2011; Toyama, 2011).

Phragmites australis exhibited its resilience to oiling with chlorophyll levels that were relatively unaffected, especially when oil was added to the soil (Experiment 2). This finding suggests, at least at the treatment-levels used in this research, that the *Phragmites* stems were largely unharmed by oiling, thereby allowing for their normal physiological function. The maintenance of leaf chlorophyll concentrations of *Phragmites* with shoot-oiling can be explained by the rapid production of new side-shoots after oiling. Regeneration of new shoots was also

credited to the photosynthetic recovery of *S. alterniflora*, *Spartina patens*, and *Sagittaria lancifolia* when un-weathered SLC and Arabian medium crude oils were added to the soil at a rate of 2 L m^{-2} (DeLaune *et al.*, 2003)*;* this shoot regeneration response was also noted when Pezeshki *et al.* (1995) applied weathered SLC to the lower 30cm of *S. alterniflora* stems. The fact that soil oiling in my study did not affect *Phragmites* leaf chlorophyll was interesting given that Dowty *et al.* (2001) reported greatly reduced photosynthetic rates of *Phragmites* after 18 months of soil-exposure with 5 L m^{-2} and 10 L m^{-2} un-weathered SLC oil. This suggests that had I run the experiment longer, lower chlorophyll levels might have resulted in the oil-soil treatment-level for Experiment 1 and perhaps all oil treatment-levels in Experiment 2.

Recovery of chlorophyll content and photosynthetic rates have been observed for *S. alterniflora* at multiple locations along the Mississippi Gulf coast following the *DWH* spill; plants exposed to this oil in the field recovered in as little as two months, relative to a control (Biber *et al.*, In Press). Wu (2012) also found rapid recovery of *S. alterniflora* photosynthesis in Mississippi following the *DWH* spill, reporting photosynthetic recovery of heavily oiled marsh sites in about 140 days. Lin and Mendelssohn (2012) showed a recovery of *S. alterniflora* photosynthetic rate in seven months after weathered and emulsified Macondo oil was applied to aboveground biomass in a greenhouse study. Somewhat similar to the present work, they found an increase in photosynthesis at the 70-rep-oil treatment-level. Photosynthesis of the *S. alterniflora*, in the Lin and Mendelssohn paper, was still significantly lower than the control when oil was applied to the soil at a rate of 8 L m^{-2}. It appears that studies with longer oil-exposure times, especially when oil is applied to the soil, are more likely to yield negative

impacts to photosynthesis. The Lin and Mendelssohn research also showed little recovery in photosynthetic rates of *Juncus roemarianus*, which was partially due the dramatic decrease in live stem numbers caused by the oiling.

CONCLUSIONS

The present study demonstrated that applying weathered and emulsified Macondo oil to the aboveground tissue of *Phragmites australis* had no lasting major impact on overall plant health. In fact, shoot oiling increased plant growth via vegetative side-branches. Production of side-branches by *Phragmites* is a stress response to aboveground oiling that compensates for the loss of photosynthetic tissue due to direct mortality of oiled leaves. If this weathered and emulsified oil penetrates the soil, impacts to *P. australis* can be significant, however this requires relatively high volumes of oil. An increase in soil respiration and a decrease in soil Eh after oiling is likely an effect of increased microbial activity; microbial communities associated with the oiled soils of *P. australis* and their efficiency of TPH degradation from the soil is a possibility for further research. Oiling intensity of *Phragmites* was quite variable in the Birdfoot Delta of the Mississippi River, and consequently this research evaluated a suite of oiling scenarios and intensities. However, future research encompassing an even broader range of oiling conditions with an endpoint of complete plant mortality would quantify the maximum oiling that this species can survive. Based on comparable research (Lin & Mendelssohn, 2012), *P. australis* shows similar resilience to the Macondo oil as *S. alterniflora*, both being more resilient than *J. roemarianus*.

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VITA

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