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Impacts of the *Deepwater Horizon* oil spill on the salt marsh vegetation of Louisiana☆

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ABSTRACT

The coastal wetland vegetation component of the *Deepwater Horizon* oil spill Natural Resource Damage Assessment documented significant injury to the plant production and health of Louisiana salt marshes exposed to oiling. Specifically, marsh sites experiencing trace or greater vertical oiling of plant tissues displayed reductions in cover and peak standing crop relative to reference (no oiling), particularly in the marsh edge zone, for the majority of this four year study. Similarly, elevated chlorosis of plant tissue, as estimated by a vegetation health index, was detected for marsh sites with trace or greater vertical oiling in the first two years of the study. Key environmental factors, such as hydrologic regime, elevation, and soil characteristics, were generally similar across plant oiling classes (including reference), indicating that the observed injury to plant production and health was the result of plant oiling and not potential differences in environmental setting. Although fewer significant impacts to plant production and health were detected in the latter years of the study, this is due in part to decreased sample size occurring as a result of erosion (shoreline retreat) and resultant loss of plots, and should not be misconstrued as indicating full recovery of the ecosystem.

1. Introduction

The *Deepwater Horizon* (*DWH*) oil spill, which began on April 20, 2010, is the largest marine oil spill in U.S. waters recorded to date (McNutt et al., 2012), with millions of gallons of oil estimated to have been spilled in the Gulf of Mexico. Subsequent to oil spills, a Natural Resource Damage Assessment (NRDA) is initiated to provide information on the degree of resultant injury to natural resources, which informs actions related to Trustee compensation and ecological restoration (Ofiara, 2002). As part of the NRDA process, a damage assessment plan for the *DWH* oil spill was developed and implemented to evaluate the impact of oiling associated with the *DWH* spill on coastal wetland vegetation (CWV) in different habitat types in the northern Gulf of Mexico (GOM) (Hester and Willis, 2011). We report herein the impacts specific to Louisiana mainland salt marshes dominated by *Spartina alterniflora* documented through the CWV component of the *DWH* NRDA. Note that we are not including salt marshes on the bayward edge of barrier islands, which are often referred to as back-barrier marshes, as these salt marshes can differ substantially in soil characteristics and plant community composition.

Salt marshes are a primary component of Louisiana's coastal zone and are well recognized for the multitude of important ecosystem services they provide, including nursery habitat, primary production, coastal protection, and carbon sequestration, among others (Costanza et al., 1997; Mitsch and Gosselink, 2000; Mitsch et al., 2015). The provision of many of these ecosystem services is greatly influenced by the health and abundance of the dominant plant species, which in Louisiana salt marshes is *Spartina alterniflora* (Ocean Studies Board, 2013; Zedler and Kercher, 2005). The impacts of oiling on salt marsh plant species, including *S. alterniflora*, have been extensively studied (Baker, 1970; Pezeshki et al., 2000; DeLaune and Wright, 2011; Mendelssohn et al., 2012), allowing the general mechanisms of oiling injury to be understood. However, thorough and specific assessments of individual oiling

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events are required to directly determine impacts because, as described below, the degree of injury can be modulated by the unique attributes of the oil spill, such as oil type, amount or degree of oiling, degree of weathering, and season of occurrence, among others.

Oiling can affect vegetation through a number of different physical and chemical mechanisms, which are dependent on whether the oil exposure is through direct contact with aboveground tissues, soil oiling, or both (Baker, 1970; Pezeshki et al., 2000; Hester and Mendelssohn, 2000; DeLaune and Wright, 2011; Mendelssohn et al., 2012; Michel and Rutherford, 2014). The specific mechanisms and severity of vegetation injury vary in regard to many factors, including degree of oil weathering (Biber et al., 2015), seasonality of exposure (Pezeshki et al., 2000), soil type and exposure (Lin and Mendelssohn, 1996), and degree of oil coverage of aboveground tissues (Pezeshki et al., 2000; Lin and Mendelssohn, 2012; Michel and Rutherford, 2014). The impacts of vegetation oiling are generally thought to increase as the relative extent of the aboveground vegetation covered by oil increases (Pezeshki et al., 2000). An inflection point of dramatically increased injury to vegetation once a certain extent of oiling has been exceeded has been reported in some instances (Silliman et al., 2012). Contamination of wetland soils by oil also impacts vegetation through exposure of belowground tissues and oiling of new shoots as they emerge through the soil oiling layer (Ferrell et al., 1984; Pezeshki et al., 2000; DeLaune and Wright, 2011). In addition to direct toxic effects, soil oiling may alter the local edaphic environment by limiting gas exchange (Pezeshki et al., 2000), which can impede plant growth by lowering soil redox potential and increasing the concentration of sulfides in soil pore water.

The *DWH* spill differs from many of the previous spills in the northern GOM for a variety of reasons beyond its scale (Mendelssohn et al., 2012; Peterson et al., 2012; Ocean Studies Board, 2013). The *DWH* wellhead was located both at a considerable oceanic depth and at a substantial distance offshore (McNutt et al., 2012; Mendelssohn et al., 2012; Peterson et al., 2012). Therefore, *DWH* oil experienced a high degree of weathering prior to reaching the shore (Reddy et al., 2011; Mendelssohn et al., 2012) as opposed to oil originating from many other recorded spills in the northern GOM where the point of release was often shallow and more proximate to coastal habitats. Although weathered oil typically contains a lower fraction of light weight hydrocarbons than fresh oil of the same source, it has still been shown to substantially impact vegetation health and production through both chemical and physical mechanisms (Ferrell et al., 1984; Anderson and Hess, 2012; Lin and Mendelssohn, 2012). Stranding of oil in marshes during the *DWH* incident was typically patchy in nature, with some areas experiencing persistent oiling (Michel et al., 2013).

As detailed by Hester and Willis (2011) the NRDA of *DWH* oiling impacts on coastal wetland vegetation employed a large number of both plant and soil metrics in the following habitat types: Louisiana mainland salt marshes, Alabama-Mississippi coastal salt marshes, Louisiana back-barrier marshes, Louisiana mangrove marshes, and the *Phragmites australis*-dominated marshes of the Mississippi River Delta. This manuscript describes the impacts of oiling to the Louisiana mainland salt marsh habitat by utilizing a subset of the data most relevant to wetland plant production and oil exposure (e.g., vegetation cover, peak standing crop, vegetation health index, and soil total polycyclic aromatic hydrocarbons (tPAH)). We sought to determine the relationship between the degree of oiling of aboveground plant tissue by *DWH* oil (i.e., the vertical extent of oiling on aboveground tissues expressed as a percentage of mean canopy height) and vegetation cover, biomass, and health. We further evaluated whether key environmental factors that can influence vegetation production and health (e.g., marsh surface elevation, percentage of time flooded, soil bulk density, etc.) differed among plant oiling classes. Although a number of studies have documented a variety of impacts of the *DWH* incident on Louisiana salt marshes exposed to oiling (see Lin and Mendelssohn, 2012; Silliman et al., 2012; Fleeger et al., 2015; Zengel et al., 2015, 2016a,b), this study is unique because it spanned a much larger geographic area and the sampling effort encompassed five categories of oil exposure over a four-year period.

2. Materials and methods

2.1. Study approach

For the Coastal Wetland Assessment component of the NRDA that was employed in Louisiana mainland salt marshes, a stratified random sampling approach was implemented based on point surveys conducted at 709 marsh sites during the early portion of the *DWH* spill by the natural resource Trustees and the Responsible Party, hereafter referred to as the pre-assessment data set. This pre-assessment data set described the shoreline type and vertical extent of oiling on vegetation, along with other pertinent characteristics at spatially explicit points (NOAA, 2010). To best capture the effects of plant oiling extent, the following plant oiling classes were designated for the CWV assessment: no visible oiling (0–0% oiling, also referred to as "reference"), trace (<1%)–10%, 11%–50%, 51%–90%, and 91%–100% vertical oil coverage on the vegetation. These plant oiling classes were selected as it was felt that they would effectively represent both plant oiling conditions and the resulting degree of plant injury. Potential CWV assessment sites were selected at random from a pool of pre-assessment sampling locations for each of the oiling classes. No sites employed for this study were affected by *DWH* intensive shoreline cleanup operations (Zengel and Michel, 2013). Upon arrival, the shoreline was examined for representativeness of herbaceous marsh habitat, sufficient marsh area for transect installation, and the extent of vegetation oiling prior to the establishment of transects. Once a site was selected, transects in oiled areas were installed perpendicular to the shoreline to a length the longer of either the pre-assessment shore perpendicular distance of oiling into the marsh or the shore perpendicular distance of observed oiling at the time of transect installation. The final numbers of transects in each plant oiling class were as follows: reference (0–0%) = 16; trace–10% = 13; 11%–50% = 18; 51%–90% = 16; and 91%–100% = 15; yielding a total of 78 transects (Fig. S1). Transects at reference (0–0%) sites were installed to a length of 20 meters (m), which was the 95th percentile of reported pre-assessment oiling penetration distances. All transects were divided into three shore parallel zones. Each transect zone contained a cover plot for repeated, nondestructive sampling, and a paired production plot area for destructive sampling of above- and belowground biomass over time. The center of the most shoreward plot pair was located 1.5 m inland from the shoreline (zone 1), the center of the second plot pair was located inland of the shoreline at 50% of the transect length (zone 2; average length of 8.2 m), and the third plot pair was located inland of the shoreline at 80% of the transect length (zone 3; average length of 13.9 m), with a minimum of a 1 m buffer maintained between plots. Cover plots, 1 m × 1 m in area, were always established to the left of the transect looking inland. Production plots, 1 m × 2 m in area, were always established to the right of the transect (looking inland), and were subdivided into eight 0.5 m × 0.5 m subplots for harvesting of peak standing crop over time. Once established, all plot locations were geospatially fixed relative to the fall 2010 shoreline position and were not relocated inland as shoreline erosion occurred.

2.2. Variables measured

This NRDA evaluated an extensive number of vegetation and soil metrics; however, this paper focuses on key metrics that represent overall trends in marsh vegetation cover, biomass, and health responses to this oiling event. For a full review of both the sampling approach and the complete list of metrics assessed, readers are referred to the CWV sampling plan for the *DWH* spill (Hester and Willis, 2011). Vegetation cover, biomass partitioning, and vegetation health index were selected for this paper due to their frequent usage in wetland ecology literature and direct linkage to ecological processes. During each sampling event, live and dead vegetation cover by species were visually estimated (1% increments up to 10%, 5% increments thereafter) in the 1 m^2 cover plots by NRDA teams consisting of natural resource Trustee and Responsible Party representatives. To minimize potential bias, sampling teams received training on estimating cover by utilizing templates of known cover within 1 m^2 training quadrats. In the field, Trustee and Responsible Party representatives were required to reach consensus on cover values (Hester and Willis, 2011). Aboveground biomass was collected by NRDA teams in randomly assigned 0.25 m^2 subplots in the 1 m $\times$ 2 m production plot area by clipping all vegetation rooted within the subplot immediately above the soil surface, placing the vegetation into labeled bags, storing on ice, and transporting them to the laboratory. Upon arriving at the laboratory, vegetation was rinsed of mud/soil, sorted into live and dead partitions by species and wet weights determined. Vegetation samples with remnant oil were first sorted into live and dead partitions by species and then cleaned with a commercial soap to remove oily residue. Subsamples of each partition were taken, dried to a constant weight, and used to calculate the dry weight of the entire partition based on the ratio of dry subsample to wet subsample mass.

To evaluate the degree of whole plant chlorosis, which is not captured by plant cover or aboveground biomass estimates, the vegetation condition index (VCI) from Mendelssohn et al. (1993) was employed. In this approach, a consensual categorical index value of plant visual condition was agreed upon by representatives of the natural resource Trustees and Responsible Party for all plant leaf area in the 1 m^2 cover plot for each station according to the following rules. Plots having live vegetation with a natural appearance were designated a value of 0, those with intense speckled chlorosis were designated as 0.5, those with considerable but less than 50% chlorosis were designated as 1.0, and those with live vegetation with greater than 50% chlorosis were designated as 2.0, and where all the vegetation was dead or where the plot was denuded, a VCI value of 3.0 was assigned. These reported index values were then expressed as percentages of 100% vegetation health to generate a vegetation health index (VHI) as described in the supporting information section.

Soil cores, 7.2 cm in diameter, were collected to a depth of 10 cm immediately outside vegetation cover plots and from appropriate subplots within production plots for determination of soil physicochemical properties following standard analytical methods (Hester and Willis, 2011) in each sampling season. Soil samples (approximately 5.3 ounces) for petroleum hydrocarbon characterization were collected to a depth of 2 cm from each plot by hand (wearing nitrile gloves), preserved, then homogenized prior to analysis at a certified laboratory (Hester and Willis, 2011). The determination of tPAH in soils employed GC/MS-SIM based on EPA Method 8270 and included the sum of 50 PAHs, including alkylated homologues and were expressed on a per weight basis. The elevation of the marsh surface at each CWV plot was determined via a Real-Time Kinematic survey (NOAA, 2012). These plot elevations were used in conjunction with existing water level gauge infrastructure to provide a hydrologic analysis at each mainland herbaceous CWV site by the National Oceanic and Atmospheric Administration (NOAA) Center for Operational Oceanographic Products and Services (CO-OPS), which was expanded to each CWV plot in a subsequent analysis.

2.3. Statistical analysis

Analysis of targeted variables was performed within each sampling season (i.e., sampling season and year) using an analysis of variance (ANOVA) approach with plant oiling class and zone as factorial treatments. A repeated measures component was not included because shoreline retreat would have greatly affected the inclusion of plots in the analysis from year to year, and because the primary comparisons of interest were between oiling classes within a sampling period. For vegetation cover and biomass, we analyzed total values for all species combined, as well as for *Spartina alterniflora* and *Juncus roemerianus* separately. For ease of visual representation in figures, all minor species were combined into an “other species” category that was not statistically analyzed. Data were only collected when the water depth in a plot was 15 cm or less; if this depth was exceeded, sampling was postponed and sites were revisited. In instances where no data could be collected, whether because of excessive water depth or plot erosion, these data were treated as missing values. Additionally, two sites originally established as reference (0–0% oiling) experienced oiling after site set-up. Because these sites no longer represented reference conditions, all data from these sites were removed from analyses from the time of oiling forward. Also, one site was noted as being buried by 20 cm–23 cm of shell and sediment after Hurricane Isaac in August of 2012; therefore, data associated with this site were removed from analyses from fall 2012 and fall 2013. Finally, one biologically implausible set of biomass values for a plot in fall 2010 was excluded from statistical analyses. For significance evaluations, alpha was set at 0.10, which is considered appropriate for this type of environmental assessment in terms of balancing the rate of type I and II errors (Mapstone, 1995). One tailed tests were employed for all vegetation metrics, where an *a priori* expectation of a negative effect of plant oiling existed (i.e., values for all vegetation metrics were predicted to decrease with plant oiling). Two-tailed tests were employed for environmental metrics, where the directionality of the plant oiling effect was not predicted. *A priori* contrasts (0% vs all oiling levels combined, 0% vs >10% oiling levels combined, 0% vs >50% oiling levels combined, and 0% vs >90%) were implemented within a sampling period by each zone (i.e., contrasts were performed on the plant oiling class by zone interaction). Assumptions of normality and homogeneity of variance were not specifically tested; however, ANOVA is generally considered robust regarding departures from these assumptions (Neter et al., 1990). Results in this manuscript that are presented as percentage reductions are made relative to mean reference values for a given variable. To investigate whether the environmental setting of field sites differed by factors other than plant oiling class, ANOVAs were performed for the initial sampling period (fall 2010) for the following potentially influential variables: marsh surface elevation, percentage of time the marsh surface was flooded, extractable-soil salinity, and soil bulk density.

3. Results

3.1. Vegetation cover

Total (live and dead combined) vegetation cover in zone 1 of oiled mainland salt marshes (i.e., contrast of reference vs. all oiling classes combined) was significantly decreased by 18% in fall 2010 (Fig. 1; $F_{1,184} = 1.956$, $p = 0.08$), 20% in fall 2011 (Fig. 1; $F_{1,172} = 1.945$,

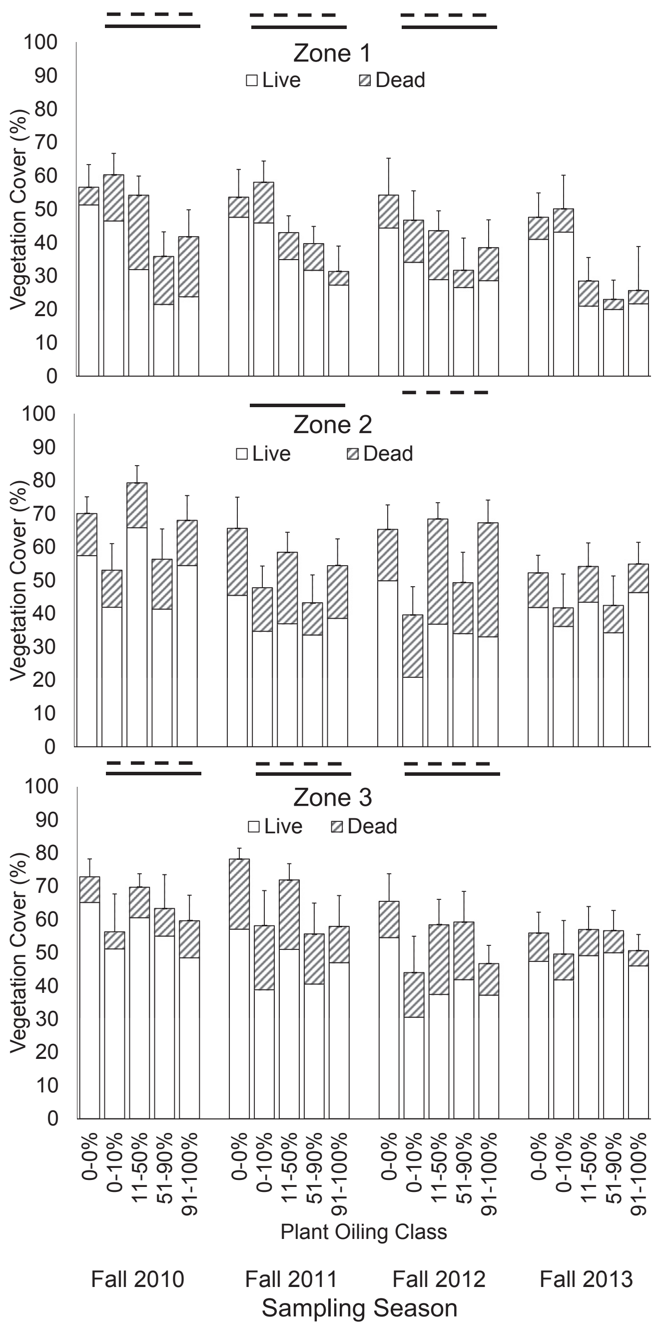

Fig. 1. The effect of plant oiling class and zone within a sampling season on live, dead, and total vegetation cover for all species combined (mean ± 1 standard error for total cover). Horizontal bars represent combined groupings that are significantly ($p \leq 0.1$) different from reference by contrast. Significant differences in total vegetation cover are represented by solid horizontal bars, and live vegetation cover by dashed horizontal bars.

$p = 0.08$), and 25% in fall 2012 (Fig. 1; $F_{1,136} = 1.859$, $p = 0.09$). This corresponds with a zone 1 average total cover of 59% for reference and an average across oiling classes of 49% in fall 2010. It should be noted that the fall 2010 total cover estimate included standing dead vegetation that died in 2010. Oiling significantly reduced zone 2 total vegetation cover in fall 2011 by 19% (Fig. 1; $F_{1,172} = 2.298$, $p = 0.07$), but no effects of plant oiling on total vegetation cover were detected in zone 2 for any other sampling season. However, significant reductions in total vegetation cover with oiling were detected in zone 3, with reductions of 13% in fall 2010 (Fig. 1; $F_{1,184} = 1.647$, $p = 0.10$), 21% in fall 2011 (Fig. 1; $F_{1,172} = 4.656$, $p = 0.02$), and 20% in fall 2012 (Fig. 1; $F_{1,136} = 2.682$ $p = 0.05$).

Plant oiling (i.e., contrast of reference vs. all oiling categories combined) significantly decreased zone 1 total live vegetation cover (i.e., total live cover across all species) by 44% in fall 2010 (Fig. 1; $F_{1,184} = 11.932$, $p < 0.01$), 27% in fall 2011 (Fig. 1; $F_{1,172} = 3.210$, $p = 0.04$), and 34% fall 2012 (Fig. 1; $F_{1,136} = 3.330$, $p = 0.04$). This reflects an average zone 1 live vegetation cover of 54% for reference marshes compared to 30% average live vegetation cover across oiling classes in fall 2010. A reduction in zone 2 total live vegetation cover was only observed in fall 2012, with a decrease of 36% in oiled marshes (Fig. 1; $F_{1,136} = 7.373$ $p < 0.01$). In zone 3, however, oiled marshes had significantly reduced total live vegetation cover in all sampling periods through fall 2012, with reductions of 16% in fall 2010 (Fig. 1; $F_{1,184} = 2.226$, $p = 0.07$), 20% in fall 2011 (Fig. 1; $F_{1,172} = 2.417$ $p = 0.06$), and 33% in fall 2012 (Fig. 1; $F_{1,136} = 7.079$, $p < 0.01$).

Plant oiling reduced *S. alterniflora* live cover in zone 1 by 45% in fall 2010 (Fig. 2; $F_{1,183} = 8.953$ $p < 0.01$) and 27% in fall 2011 (Fig. 2; $F_{1,172} = 2.737$, $p = 0.10$). Oiling significantly reduced *S. alterniflora* live cover in zone 2 in fall 2012 by 47% (Fig. 2; $F_{1,136} = 8.678$, $p < 0.01$), and in zone 3 in fall 2011 by 24% (Fig. 2; $F_{1,172} = 2.868$, $p = 0.09$) and in fall 2012 by 31% (Fig. 2; $F_{1,136} = 4.238$, $p = 0.04$). No other significant impacts on live *S. alterniflora* cover were detected. No significant impacts of plant oiling on *J. roemerianus* live cover in any zone or season were detected (Fig. 2).

3.2. Vegetation biomass

Total (live and dead combined) aboveground biomass of oiled marshes (i.e., contrast of reference vs. all oiling categories combined) in zone 1 was reduced by 27% relative to the reference in fall 2010 (Fig. 3; $F_{1,181} = 3.175$, $p = 0.04$) and by 43% in fall 2012 (Fig. 3; $F_{1,128} = 4.579$, $p = 0.02$). Fall 2010 zone 1 average total aboveground biomass in reference marsh sites was 1120 g m^{-2}, whereas the average across oiling classes was 813 g m^{-2}. Greater than 10% plant oiling significantly diminished total aboveground biomass in zone 1 by 29% in fall 2011 (Fig. 3; $F_{1,142} = 3.419$, $p = 0.03$). No effect of plant oiling on total aboveground biomass was detected in fall 2013 for any zone. Similarly, significant reductions in zone 2 total aboveground biomass of oiled marshes were 28% in fall 2010 (Fig. 3; $F_{1,181} = 5.807$, $p = 0.01$), 40% in fall 2011 (Fig. 3; $F_{1,169} = 12.929$, $p < 0.01$), and 36% in fall 2012 (Fig. 3; $F_{1,128} = 6.971$, $p < 0.01$). No significant effect of plant oiling on total aboveground biomass was detected in zone 3 in fall 2010; however, significant reductions in total aboveground biomass of 32% in fall 2011 (Fig. 3; $F_{1,169} = 7.756$, $p < 0.01$) and 22% in fall 2012 (Fig. 3; $F_{1,128} = 1.797$, $p = 0.09$) in oiled marshes were detected.

Live aboveground biomass (i.e., total live biomass across all species) in zone 1 was significantly impacted by oiling. The reference marsh zone 1 fall 2010 average live aboveground biomass value was 943 g m^{-2}, whereas the average live aboveground biomass for all oiling classes was 514 g m^{-2}. Specifically, live aboveground biomass in zone 1 was reduced by 45% in fall 2010 (Fig. 3; $F_{1,181} = 13.187$, $p < 0.01$) and 31% in fall 2012 (Fig. 3; $F_{1,131} = 2.482$, $p = 0.06$), and 25% with >10% plant oiling in fall 2011 (Fig. 3; $F_{1,142} = 2.533$, $p = 0.06$). No effect of plant oiling in zone 1 was detected in fall 2013. Similarly, in zone 2 plant oiling significantly diminished live aboveground biomass by 32% in fall 2010 (Fig. 3; $F_{1,181} = 9.833$, $p < 0.01$), 31% in fall 2011 (Fig. 3; $F_{1,169} = 5.447$, $p = 0.01$), and 34% in fall 2012 (Fig. 3; $F_{1,131} = 5.368$, $p = 0.01$), but not in fall 2013. Live aboveground biomass in zone 3 of oiled marshes was significantly reduced by 16% in fall 2010 (Fig. 3; $F_{1,181} = 2.254$, $p = 0.07$), 28% in fall 2011 (Fig. 3; $F_{1,169} = 4.256$, $p = 0.02$), and 21% in fall 2012 (Fig. 3; $F_{1,131} = 1.861$, $p = 0.09$), but not in fall 2013.

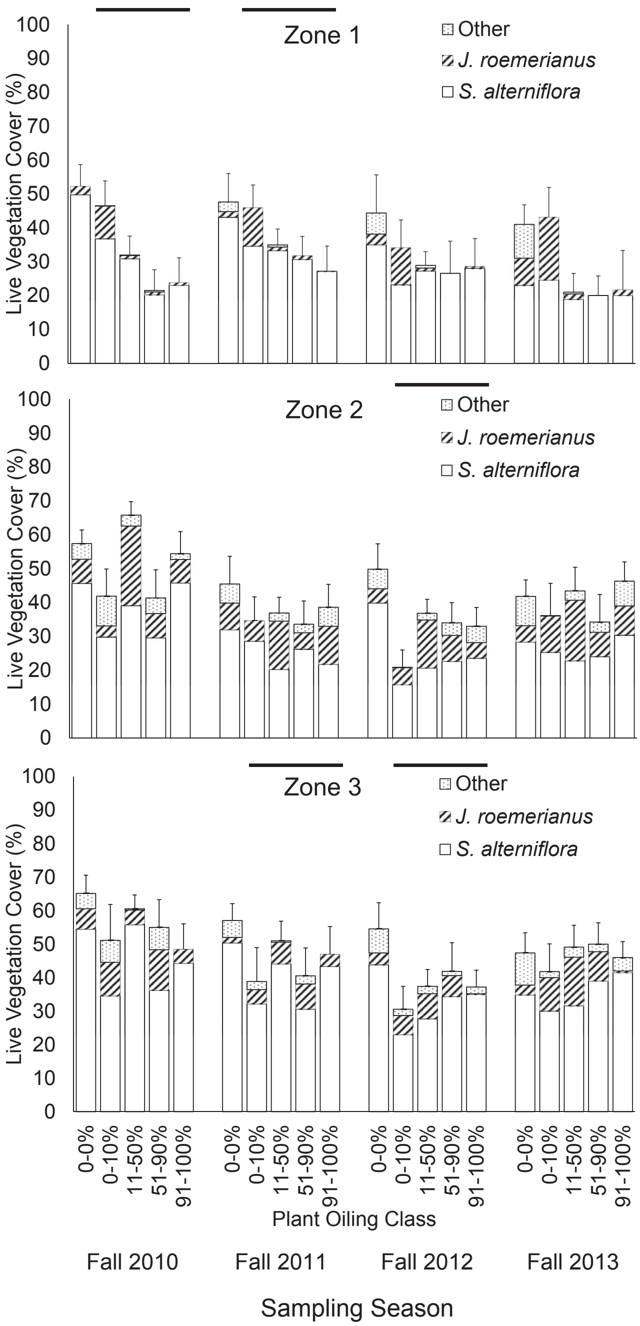

Fig. 2. The effect of plant oiling class and zone within a sampling season on live vegetation cover of *S. alterniflora*, *J. roemerianus*, and all other species (mean ± 1 standard error for total live cover). Solid horizontal bars represent combined groupings significantly ($p \leq 0.1$) different from reference in live *S. alterniflora* cover by contrast. No significant effect was detected for *J. roemerianus* cover.

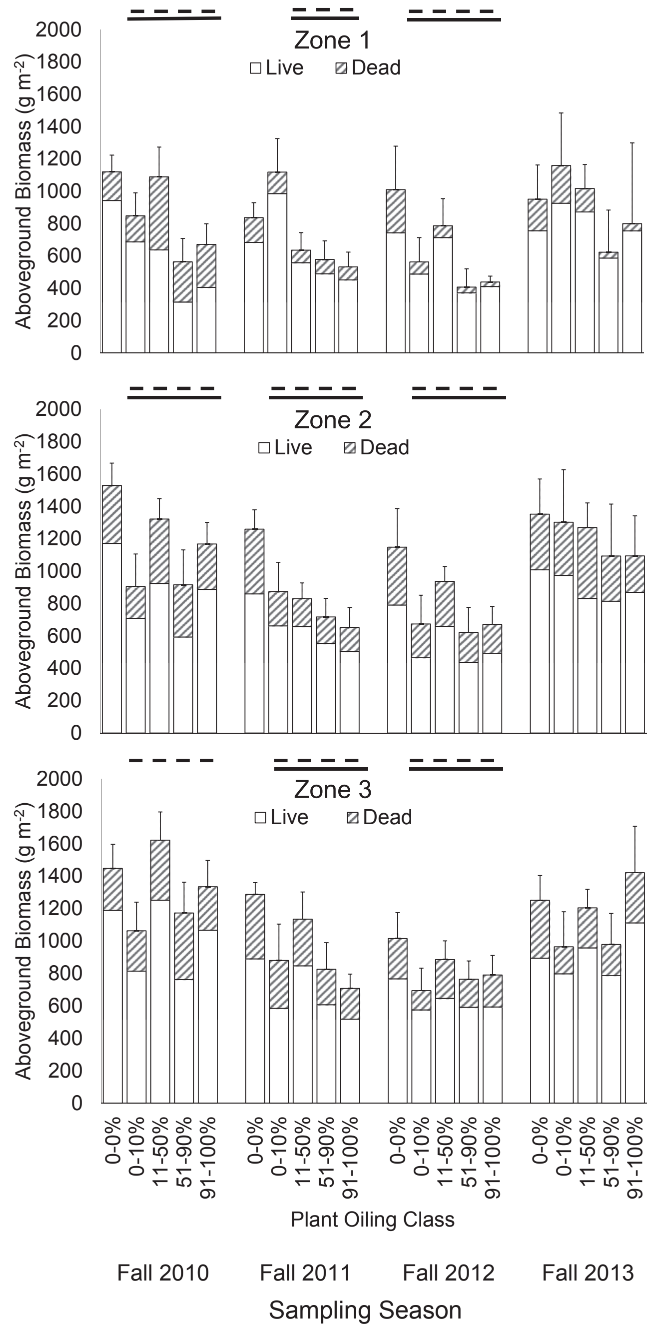

Fig. 3. The effect of plant oiling class and zone within a sampling season on live, dead, and total aboveground biomass for all species combined (mean ± 1 standard error for total aboveground biomass). Horizontal bars represent oil class combined groupings that are significantly ($p \leq 0.1$) different from reference by contrast. Significant differences in total aboveground biomass are represented by solid horizontal bars, and live aboveground biomass by dashed horizontal bars.

Plant oiling (i.e., contrast of reference vs. all oiling categories combined) significantly reduced *S. alterniflora* live aboveground biomass in zone 1 by 44% in fall 2010 (Fig. 4; $F_{1,181} = 8.372$, $p < 0.01$), but not in any other sampling season. *S. alterniflora* live aboveground biomass in zone 2 of oiled marshes was significantly reduced by 40% in fall 2010 (Fig. 4; $F_{1,181} = 11.498$, $p < 0.01$), 31% in fall 2011 (Fig. 4; $F_{1,169} = 3.339$, $p = 0.07$), 41% in fall 2012 (Fig. 4; $F_{1,131} = 4.830$, $p = 0.03$), and 32% in fall 2013 (Fig. 4; $F_{1,112} = 2.724$ $p = 0.10$). In zone 3, plant oiling significantly reduced *S. alterniflora* live aboveground biomass by 24% in fall 2010 (Fig. 4; $F_{1,181} = 4.997$, $p = 0.03$) and 30% in fall 2011 (Fig. 4; $F_{1,169} = 4.067$, $p = 0.05$), but not in fall 2012 nor fall 2013. No significant impact of plant oiling on *J. roemerianus* live aboveground biomass was detected in any zone or season.

3.3. Vegetation health index

Plant oiling (i.e., contrast of reference vs. all oiling categories combined) resulted in significant reductions in zone 1 vegetation

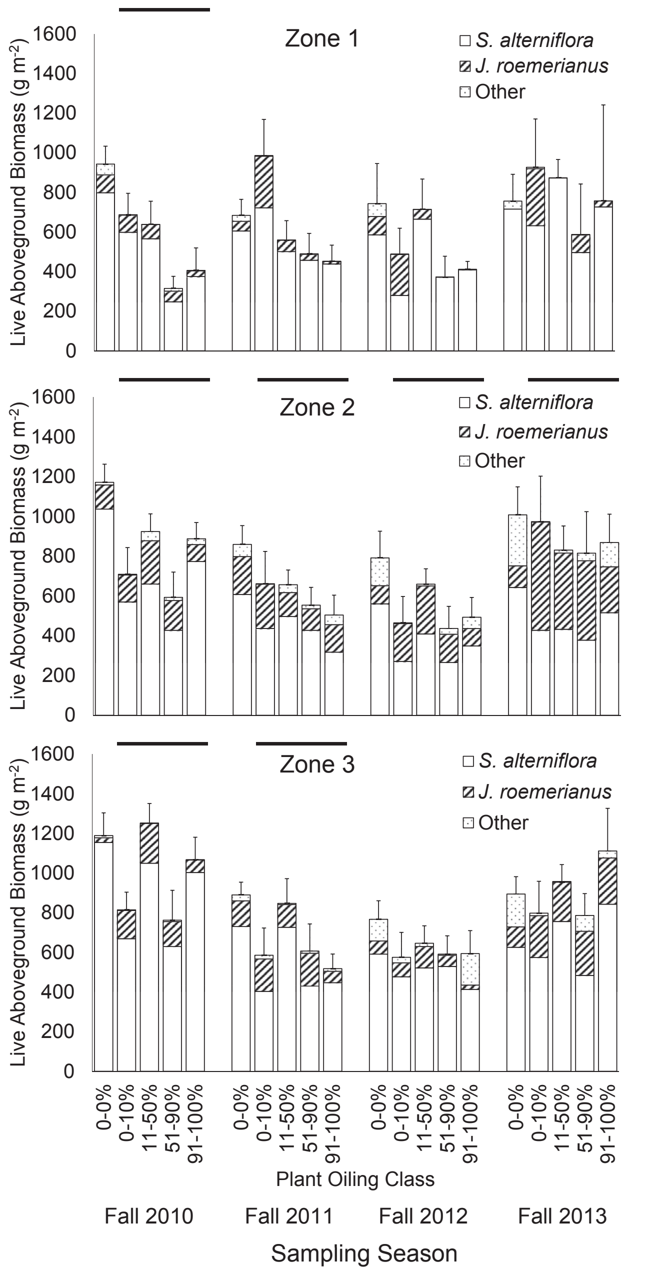

Fig. 4. The effect of plant oiling class and zone within a sampling season on live aboveground biomass by species (*S. alterniflora*, *J. roemerianus*, and all other species; mean ± 1 standard error for total live aboveground biomass). Solid horizontal bars represent combined groupings significantly ($p \leq 0.1$) different from reference in live *S. alterniflora* aboveground biomass by contrast. No significant effect was detected for live *Juncus roemerianus* aboveground biomass.

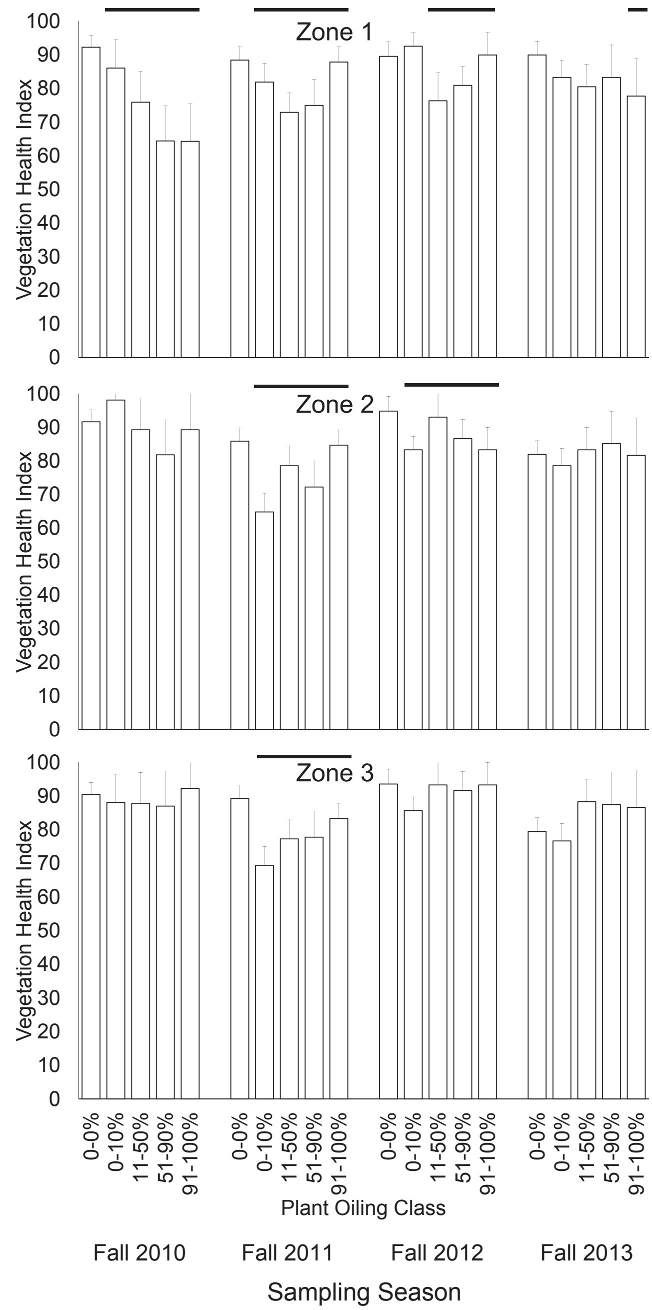

Fig. 5. The effect of plant oiling class and zone within a sampling season on vegetation health index (mean ± 1 standard error). Horizontal bars represent combined oil class groupings that are significantly ($p \leq 0.1$) different from reference.

health index of 22% in fall 2010 (Fig. 5; $F_{1,182} = 6.813$, $p < 0.01$) and 11% in fall 2011 (Fig. 5; $F_{1,171} = 2.147$, $p = 0.07$). In fall 2012, >10% plant oiling significantly diminished vegetation health index in zone 1 by 10% (Fig. 5; $F_{1,113} = 2.152$ $p = 0.07$). In fall 2013, >90% plant oiling significantly diminished vegetation health index in zone 1 by 14% (Fig. 5; $F_{1,47} = 2.195$, $p = 0.07$). No significant effect of plant oiling on vegetation health index was detected in zone 2 in fall 2010 or 2013. However, vegetation health index in zone 2 of oiled marshes was significantly reduced by 12% in fall 2011 (Fig. 5; $F_{1,171} = 2.165$, $p = 0.07$) and 8% in fall 2012 (Fig. 5; $F_{1,137} = 2.596$, $p = 0.05$). No significant effect of plant oiling on vegetation health index was detected in zone 3 in fall 2010, fall 2012, or fall 2013; however, plant oiling did significantly reduce vegetation health index in zone 3 by 13% in fall 2011 (Fig. 5; $F_{1,171} = 2.780$, $p = 0.05$).

3.4. Environmental setting and soil tPAH

Importantly, the environmental setting was generally consistent across oiling classes at the outset of the study, with no significant differences among plant oiling class detected for extractable soil salinity, marsh elevation, or the percentage of time the marsh surface was flooded in fall 2010 (Table 1). The only significant effect of plant oiling was detected in soil bulk density in fall 2010 (Table 1;

Table 1
Effect of plant oiling class and zone on percentage of time flooded, marsh surface elevation, soil bulk density, and soil salinity in fall 2010 (mean ± 1 standard error in parenthesis).

Plant oiling class	Zone	Percentage of time flooded (%)	Marsh surface elevation (cm NAVD88)	Soil bulk density (g cm^{-3})	Extracted soil salinity (psu)
0–0%	1	55 (6)	17.9 (2.7)	0.31 (0.03)	3.3 (0.4)
	2	54 (7)	21.8 (3.2)	0.30 (0.04)	3.5 (0.4)
	3	50 (6)	23.5 (1.9)	0.31 (0.03)	3.7 (0.4)
0–10%	1	64 (7)	11.9 (6.5)	0.39 (0.05)	3.4 (0.4)
	2	55 (8)	23.4 (4.4)	0.40 (0.09)	3.6 (0.5)
	3	48 (9)	28.4 (4.7)	0.46 (0.10)	3.8 (0.5)
11–50%	1	63 (4)	18.0 (2.7)	0.34 (0.04)	3.0 (0.3)
	2	44 (5)	28.1 (3.0)	0.32 (0.04)	3.6 (0.4)
	3	38 (4)	31.2 (3.1)	0.33 (0.04)	3.9 (0.5)
51–90%	1	59 (5)	16.6 (3.7)	0.39 (0.06)	3.4 (0.3)
	2	43 (5)	27.6 (2.9)	0.49 (0.09)	3.5 (0.5)
	3	39 (6)	29.7 (2.8)	0.39 (0.09)	4.2 (0.6)
91–100%	1	53 (5)	17.6 (4.1)	0.39 (0.03)	3.2 (0.4)
	2	41 (6)	26.7 (3.2)	0.41 (0.05)	3.7 (0.4)
	3	43 (5)	26.6 (3.6)	0.35 (0.04)	4.0 (0.3)

$F_{4,176} = 2.90$, $p = 0.023$), which was significantly higher for all plant oiling categories combined than the reference (Contrast $F_{1,191} = 1.82$, $p = 0.09$). Soil tPAH concentrations in zone 1 were significantly higher at oiled than reference sites (i.e., contrast of reference vs. all oiling categories combined) in fall 2010 (Table 2; Contrast $F_{1,190} = 5.6$, $p = 0.02$) and fall 2011 (Table 2; Contrast $F_{1,173} = 4.8$, $p = 0.03$), and significantly elevated with >10% plant oiling compared to the reference in fall 2012 (Table 2; Contrast $F_{1,108} = 4.06$, $p = 0.05$) and fall 2013 (Table 2; Contrast $F_{1,94} = 7.23$, $p = 0.01$). Only fall 2013 with >50% plant oiling exhibited significantly greater soil tPAH than the reference in zone 2 (Table 2; Contrast $F_{1,66} = 5.02$, $p = 0.03$) and no significant differences in soil tPAH were detected in zone 3.

4. Discussion

Louisiana's salt marshes are well recognized for their provision of valuable ecosystem goods and services (Batker et al., 2010). Many of these services are inextricably linked to the vigor and health of the dominant macrophytes in these ecosystems (Mitsch and Gosselink, 2000). As documented by Shoreline Cleanup Assessment Technique (SCAT) observations, the Louisiana coastline received some of the *DWH* spill's heaviest oiling (Michel et al., 2013). Several geographically and temporally-focused studies have reported impacts of *DWH* oiling on Louisiana salt marsh vegetation and structure (Lin and Mendelssohn, 2012; Silliman et al., 2012; Zengel et al., 2015, 2016a; Lin et al., 2016) and associated ecosystem attributes, including macroinvertebrates (Zengel et al., 2014, 2015, 2016a,b) and benthic microalgae and meiofauna (Fleeger et al., 2015). The assessment reported herein was designed to be geographically and temporally extensive and documented that key indicators representing vegetation health and production in Louisiana salt marshes (e.g., total live cover, live standing crop, vegetation health index) were significantly impacted after being exposed to oil from the *DWH* spill.

In observational studies, an important aspect of the experimental design is to be able to effectively apportion effects to the intended independent variable, in this case the extent of plant oiling, and minimize the effects of likely confounding factors, such as the inherent environmental setting. At the onset of this study, the majority of variables representing environmental setting displayed no significant differences between plant oiling classes, including soil salinity, plot elevation, and percentage of time the marsh surface was flooded. Soil bulk density was actually significantly higher in oiled classes, which has been reported to be associated with better growth of *S. alterniflora* (DeLaune et al., 1979). As such, higher soil bulk densities in some areas that received oiling would not be expected to have resulted in decreased vegetation health and productivity prior to oiling. These findings indicate that the plant oiling classes, including the reference marsh category, are largely similar regarding the potential confounding factors in this assessment, specifically soil type, elevation and hydrologic regime.

Impacts of plant oiling on salt marsh plant production were evident from reductions in live cover and live aboveground biomass, particularly in zone 1, which were detected from fall 2010

Table 2
Effect of plant oiling class, zone and sampling season on soil tPAH (ppb; mean ± 1 standard error in parenthesis).

Plant oiling class	Zone	Fall 2010	Fall 2011	Fall 2012	Fall 2013
0–0%	1	973 (494)	457 (96)	578 (188)	744 (230)
	2	482 (114)	428 (81)	406 (87)	541 (185)
	3	569 (156)	550 (121)	473 (110)	452 (72)
0–10%	1	3352 (1513)	4381 (2601)	1106 (569)	669 (246)
	2	5493 (4970)	1660 (871)	672 (234)	372 (70)
	3	1205 (758)	1051 (488)	791 (347)	423 (174)
11–50%	1	55,099 (28,902)	39,754 (22,857)	81,396 (57,079)	24,145 (23,244)
	2	14,785 (6568)	9462 (3661)	3522 (1631)	3107 (1135)
	3	8316 (5963)	8360 (4705)	4764 (2504)	2369 (1470)
51–90%	1	14,108 (9116)	693 (163)	1675 (1139)	1169 (290)
	2	1474 (401)	1901 (623)	1517 (555)	1721 (554)
	3	5396 (1843)	2846 (1102)	1280 (435)	726 (169)
91–100%	1	65,335 (38,416)	66,746 (37,748)	6775 (5255)	4500 (4098)
	2	7416 (2813)	8266 (4074)	1719 (819)	1610 (864)
	3	6049 (2796)	4451 (1869)	1415 (566)	745 (210)

through fall 2012. Although these impacts lessened by fall 2013, it should be noted that by this point substantial erosion of CWV plots had occurred, which reduced the statistical power of the study design because once a plot was lost due to erosion it was treated statistically as a missing value. We do not consider vegetation impacts associated with marsh erosion (shoreline retreat) in this analysis. If oiling and subsequent vegetation impacts also caused accelerated marsh erosion, as has been reported in some studies (Silliman et al., 2012; McClenachan et al., 2013; Zengel et al., 2015; Lin et al., 2016), then our estimates of vegetation impacts would be considered conservative. Although effects over time were not statistically assessed in this study, a trend towards lower total and live cover from fall 2010 through fall 2013 can be visually discerned, which likely reflects the substantial tropical storm activity in the region in fall of 2011 and 2012 (National Hurricane Center). Similarly, some shifts in plant community composition can be visually noted, but these are generally inconsistent and also likely reflect factors such as tropical storm activity. Importantly, *Spartina alterniflora* was the dominant species in study plots throughout the assessment.

Lin et al. (2016) investigated *DWH*-associated oiling on salt marshes in Northern Barataria Bay that were composed of a mix of *Spartina alterniflora* and *Juncus roemerianus* from January 2011 to November 2013. Relative to their reference category, total live aboveground biomass was significantly reduced in their heavily oiled category throughout the 42 months of the study. However, species-specific differences were detected, with *Spartina alterniflora* live aboveground biomass in the heavily oiled category recovering to reference levels after 36 months and *Juncus roemerianus* live aboveground biomass remaining significantly reduced for the duration of the study. In moderately oiled marshes, total live biomass was significantly lower than the reference at the 9 and 18 month sampling periods, but not at any sampling periods thereafter. No significant decrease in *Spartina alterniflora* live aboveground biomass was detected in any sampling period in the moderately oiled category, whereas *Juncus roemarianus* initially displayed significant reductions in live aboveground biomass, but returned to reference levels after 30 months. This differs from what was observed in our study, in which total aboveground biomass was significantly reduced by any oiling relative to reference across all three zones in fall 2010, fall 2011, and fall 2012. Further, live aboveground biomass displayed this same pattern of significant reductions with oiling with the exception of zone 3 in fall 2010. There are several possible reasons why the results of these two field studies differ in the degree and extent of oil impact. The Lin and Mendelssohn (2012) study utilized a smaller sample size (21 total plots) that was conducted in a more limited geographic area (~40 square kilometers sampling area), and was sampled in one marsh zone (shoreline), which therefore does not represent trends of oil impact over the entire *DWH*-affected region as fully as the NRDA evaluation. Further, our study included a wider range of degree of plant stem oiling, and soil tPAH concentrations were elevated across a wide range of stem oiling categories and zones.

In another assessment that was focused on heavily oiled areas of Northern Barataria Bay, Silliman et al. (2012) reported substantial decreases in live plant cover, live stem density and live rhizomes in heavily oiled areas five months post-spill in marsh up to 10 m from shore, and indicated that the most severe plant impact (<20% live stems) occurred when approximately 65% of plant tissue was oiled. Similarly, a greenhouse study by Lin and Mendelssohn (2012) in which stems of *S. alterniflora* and *Juncus roemarianus* were experimentally oiled revealed that, as observed in the field component of their study, *J. roemerianus* was more sensitive to oiling than *S alterniflora* with significant reductions in live stem density occurring at stem oiling levels of 30% and greater. Significant reductions of *S. alterniflora* live stem density occurred when the stems were 100% coated with oil or had 70% of the stems repeatedly oiled, which is in general agreement with the observations of Silliman et al. (2012) that severe injury to *S. alterniflora* occurred at stem oiling of greater than 65%. Our data also indicated a tendency for stem oiling levels of 50%–90% to result in severe injury; however, the observed reductions in total live plant cover and live aboveground biomass reflect a general plant community response to oiling and do not appear to be substantially influenced by species-specific differences in oil tolerance.

Evaluations of visual chlorosis (vegetation health index) revealed that the health of live vegetation in oiled Louisiana mainland herbaceous marshes was decreased compared to reference marsh sites from the time of the first sampling (fall 2010) and, in some marshes, through fall 2012. Reductions in vegetation health index are most analogous to plant stress and impacts on plant photosynthetic processes, which have been documented in other *DWH*-focused studies. RamanaRao et al. (2011), for example, detected a 21% reduction in chlorophyll content index after exposure of two month-old *S. alterniflora* transplants to soil oiling levels of 20% and 40% (volume:weight; oil/soil). The oil mixture employed for their study was a blend (1:4; v/v) of weathered *DWH* oil collected from Queen Bess Island, LA and an unweathered light crude oil collected from an active production well in Louisiana. Biber et al. (2015) documented reductions in both effective and potential quantum yield of *S. alterniflora* in Mississippi salt marshes that experienced heavy *DWH* oiling relative to their reference site 13 days after oiling, but reported that fluorescence properties were equivalent to that of reference 88 days post-oiling. However, Biber et al. (2015) also conducted a focused field investigation of *DWH* oiling impacts on chlorophyll fluorescence and net CO_2 assimilation, and noted that although chlorophyll fluorescence recovered from acute oiling stress within two months, net CO_2 assimilation displayed chronic depression for up to four months.

Plant spectral signatures have been used to not only detect stress directly on individual plants, but also remotely at the landscape level. Kokaly et al. (2013) utilized spectroscopic analysis of AVIRIS (Airborne Visible/Infrared Imaging Spectrometer) data collected at low and medium altitudes along 40 km of salt marsh shoreline in Barataria Bay, Louisiana, in summer through fall 2010 to map the distribution and persistence of *DWH* oil. They reported zones of oiled plant canopies that extended an average of 11 m interior from the shoreline, with a maximum penetration of 21 m. Khanna et al. (2013) similarly utilized AVIRIS data collected in fall 2010 and August 2011 in conjunction with regression analyses and reported varying degrees of re-vegetation in 2011, with the poorest recovery being in the first three pixels (3.5 m pixel) from the shoreline. Although the data generated from these types of landscape-level analyses is very informative, the results from our study demonstrate the importance of exposure and injury determination conducted at ground level. In this NRDA study, significant injury (expressed as decreased total aboveground biomass) was documented beyond the shoreline into the marsh interior zones from fall 2010 through fall 2012. Similarly, Lin et al. (2016) reported that in their heavily oiled sites (which experienced near complete plant mortality) 42 months after the *DWH* spill live aboveground biomass was only 50% of reference values, and belowground biomass was still reduced by 76% relative to reference values.

5. Conclusion

This study documents significant reductions in metrics representing vegetation production and health for several years in Louisiana mainland salt marshes dominated by *S. alterniflora* due to plant oiling that occurred as a result of the *DWH* oil spill. These impacts were typically greatest in the heavier plant oiling classes and in the marsh edge zone (zone 1); however, significant impacts were also detected in the oiled marsh interior (zones 2 and 3). Sites were generally similar across plant oiling classes in regard to key aspects of the environmental setting that can influence plant growth, such as hydrologic regime and soil characteristics. Oil impacts were observed over three years (2010–2012) and a decrease in detectable impacts to indicators of coastal marsh production and health in fall 2013 likely resulted from erosion (shoreline retreat) of sampling plots, which reduced statistical power, and should not necessarily be considered as indicative of ecosystem recovery.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.05.065.

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