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Marsh plants mediate the influence of nitrogen fertilization on degradation of oil from the Deepwater Horizon spill

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Abstract. Coastal wetlands are commonly exposed to hydrocarbon pollutants derived from extraction disasters like the Deepwater Horizon oil spill. Naturally occurring microbes can degrade oil, but the rate of oil degradation depends heavily on the key chemical and biological factors. The goal of this study was to determine the influence of interactions between marsh plants and nitrogen (N) on the degradation of oil from the Deepwater Horizon oil spill. Oil disappearance was measured with gas chromatography (GC) focusing on the change in C18 n-alkane-to-phytane ratio of oil, and instantaneous oil degradation rates were measured using an instantaneous carbon isotopic partitioning method. N addition often stimulates oil decomposition in soil slurries, but the effect of N in our mesocosms depended on plant species. N addition accelerated oil degradation in Spartina alterniflora mesocosms but slowed oil degradation in Spartina patens mesocosms. Across all plant and N treatments, oil degradation related to plant root growth. In many ecosystems including marshes, N addition has been shown to diminish root growth by reducing the need for nutrient foraging. Where N addition reduces root growth, N may ultimately exacerbate oxygen scarcity in marsh soils possibly negating or reversing the positive, direct effects that N has on oil degradation. Based on these findings, fertilization strategies that maximize marsh plant root growth will be the most effective at increasing the microbial degradation of oil and will have the greatest potential to mitigate the impacts of oil in marsh ecosystems.

Key words: cavity ring-down spectroscopy; isotopic partitioning; priming; soil respiration; Spartina alterniflora; Spartina patens.

INTRODUCTION

Following the Deepwater Horizon oil spill of 2010, oil was deposited on large stretches of marsh-dominated shorelines in the Gulf Coast region of the USA (Kokaly et al. 2013). Oil-driven declines in wetland biodiversity, productivity, or resilience in these marshes have important consequences for the sustainability of these ecosystems (Silliman et al. 2012), for storm surge abatement, and for the health of fisheries in this entire region (Mendelssohn et al. 2012, Incardona et al. 2014). Without remediation, oil can persist in marsh ecosystems for decades (Reddy et al. 2002, Peacock et al. 2007) and have long-lasting impacts on vegetation and wildlife (Culbertson et
al. 2007). Physical means of removing oil from soils creates another disturbance that may further jeopardize marsh sustainability, especially in the Gulf region (Pezeshki et al. 2000) where coastal regions have suffered rapid marsh collapse in recent decades (Dahl 2005) and are subject to high rates of relative sea-level rise (Coleman et al. 1998).

Oil is composed of a mixture of energy-rich hydrocarbons but contains little total nitrogen (N) to support microbial growth and enzyme production (Atlas 1981). Laboratory incubations of marsh slurries have shown that nutrient addition can stimulate the microbial breakdown of oil (Jackson and Pardue 1999, Nyman 1999). Accordingly, nutrient fertilization is commonly employed as a method to accelerate the natural breakdown of oil along shorelines (Atlas 1981). Previous work has shown that application of N fertilizer may accelerate oil degradation on contaminated beaches (Atlas and Bartha 1972, Bragg et al. 1994, Swannell et al. 1999, Röling et al. 2002, Snedden et al. 2007; see preliminary study in Appendix), and inland soils (Westlake et al. 1978); however, nutrient addition was found to have no effect on oil degradation in a field study in a Gulf Coast salt marsh (Tate et al. 2012). In this latter study, the authors suggested that instead of N, oxygen availability was the dominant factor limiting oil degradation. Assessments of nutrient effects on oil degradation in situ have been confounded by the difficulty of randomly interspersing treatments and replicating adequately (Venosa et al. 1996). Mesocosm studies, allowing for controlled manipulation of key variables, have found that nutrient addition generally stimulates oil breakdown but also interacts with other factors such as soil type (Lin et al. 1999), plant growth (Lin and Mendelssohn 1998) and soil aeration (Dowty et al. 2001). It is therefore important to explore the role that these other interacting factors together may have on the degradation of oil.

Plants, N and the interactions thereof can each influence the overall degradation of oil. First, plant oxygenation of waterlogged wetland soils has been shown to increase microbial activity, eliciting a “priming effect,” a plant-mediated stimulation of decomposition, on carbon substrates (Wolf et al. 2007). Priming effects can dominate other factors in controlling decomposition rates in soil (Cheng and Kuzyakov 2005), and the same may be true for oil deposited in soil. Second, N addition can directly stimulate microbial degradation of oil but may dampen plant-mediated priming effects, by indirectly reducing soil aeration (Darby and Turner 2008b). Though N addition typically increases aboveground plant productivity, it often decreases root production belowground (Darby and Turner 2008a, Langley et al. 2009, Turner 2011, Deegan et al. 2012) because alleviating nutrient limitation can reduce the need for root foraging for nutrients (White et al. 2012). In order to obtain a fuller understanding of controls on oil degradation in coastal ecosystems, it is essential to know how N affects oil decomposition in a broader ecological context that incorporates plant responses.

The primary goal of our study was to determine how added N influences the degradation of oil, particularly in the presence of plants that occur in Gulf Coast marshes. We used two common dominant Gulf coast marsh grasses, Spartina patens and Spartina alterniflora, to determine how each may influence oil degradation. To fully assess oil processing, we used two independent methods of measuring oil decomposition: one that characterized oil remaining chromatographically and one that assayed complete respiration of oil-derived carbon to CO2. Oil degradation was measured directly by examining oil residue in soil by gas chromatography (GC-FID) and by a novel carbon isotopic partitioning method to instantaneously measure oil-derived CO2 respired from mesocosms using the difference in stable carbon isotopic composition (δ13C) signature of oil compared to C4-plant-derived carbon. We hypothesized that plants would stimulate the degradation of oil in salt marsh mesocosms due to increased soil oxygenation by roots. We also hypothesized that N addition would stimulate oil degradation in both the presence and absence of plants and that this stimulation would occur for both species of marsh plants.

**Methods**

**Experimental design**

Marsh mesocosms were constructed from 7.3 cm diameter PVC pipes cut in 50-cm segments
that were capped on the bottom. Drain holes were drilled in the side of each pipe at 10 cm from the bottom to allow for exchange of water between soil and surrounding water, and a threaded elbow fitting was placed in the hole to allow for periodic porewater sampling through tygon tubing (Fig. 1). A total of 144 greenhouse mesocosms with either *Spartina alterniflora*, *S. patens* or no plants were established. Plants were obtained from the Gulf Coast area (Green Seasons Nursery, Parish, Florida, USA) and six mesocosms (two of each plant type) were placed in each of 24 reservoirs. Gulf Coast soil was extracted from several points at a site on the Marques Canal east of the Chef Menteur pass between lakes Pontchartrain and Borgne in southeast Louisiana (30°4'26" N, 89°47'31" W). The collection site is situated within an expansive, high brackish marsh dominated by near-monospecific meadows of *Spartina patens* with *S. alterniflora* demarcating the marsh-water interface. The site is representative of the inland brackish marshes affected by the Deepwater Horizon spill but was itself unaffected. The region experiences an average annual temperature of 20°C, an average rainfall of 1570 mm, an average tidal flux of <1 m, and a salinity range of 1–10 ppt. Soil was extracted at the physical interfaces of *S. patens* and *S. alterniflora*. Collected soil was cut 1:2 with carbon-free sand in order to reduce the contribution to respiration from soil organic matter while maintaining a realistic soil microbial community. The plants were allowed to establish and grow under the same conditions for 8 months, at which time stem densities approximated those in Gulf Coast marshes (Hopkinson et al. 1980). Surface porewater wells were constructed from 20-ml syringes, that were perforated, covered with window screen and implanted 10 cm deep in the soil, so that porewater from 0 to 10 cm could be examined to provide information about the geochemistry of the microenvironment where the oil was deposited. Plungers were left in place between sampling to minimize the artificial introduction of oxygen into the soil from the atmosphere.

**Oil and N treatment applications**

Weathered oil recovered from the surface of the Gulf of Mexico in the months following the Deepwater Horizon oil spill was supplied by British Petroleum and was applied to the mesocosms in February 2012. Each of the six mesocosms in each reservoir received the same one of six combinations of oil (added oil or no oil) and N (zero added, low N or high N). The weathered oil was mixed with sand in the volumetric ratio of 1:10 and shaken for 48 hours to achieve a homogenous mixture of oil and sand. The resulting mixture was passed through a 1 cm sieve to break up clumps. Six pretreatment samples of this mixture were immediately frozen at −20°C. Aliquots of 200 ± 0.1 g of the mixture were added to the surface to each of the 72 oiled mesocosms, creating an oil horizon ~5 cm deep, simulating oil deposition patterns observed in Gulf marshes. Starting on 12 March 2012 at biweekly intervals, the low N and high N mesocosms were watered with 30 ml of 1.4 mg N L⁻¹ and 5.6 mg N L⁻¹ ammonium nitrate solution, respectively. The control mesocosms with no added N received 30 ml of water.

**Plant and soil measurements**

Plant growth measurements were made at approximately biweekly intervals to examine the
effects of the treatments on aboveground plant growth. The height and width of the tallest green stem and the total number of green stems were used as indices of plant growth in each mesocosm. On two dates, both aboveground and belowground plants were destructively harvested from subsets of mesocosms. Shoots were clipped, dried and weighed. Soil monoliths were extruded, sliced horizontally to separate the top 10 cm from the bottom 40 cm, washed over a 1-cm sieve, and refrigerated for a maximum of 2 weeks until further processing. At each harvest period, soil cores (1 cm diameter) were taken from the top 10 cm and transferred to clean vials and frozen at −20°C until further analysis of the oil remaining by GC (see details below). Soil porewater redox potential was measured monthly during respiration measurements in porewater wells using a redox probe and meter (YSI, Yellow Springs, Ohio, USA).

Respiration rates and isotopic partitioning

At four approximately monthly intervals, week-long campaigns were undertaken to assess the instantaneous rates of oil respiration from the mesocosms. This process required over 3000 individual $^{13}$C-CO$_2$ measurements. On each afternoon of the campaign, a subset of 8 mesocosms (three of each plant type across each N addition level and two unplanted controls) was capped with airtight PVC chambers, approximately 2 L in headspace volume for planted mesocosms and 1-L volume for unplanted mesocosms. Precise volumes were estimated for each mesocosm accounting for differences in soil level among mesocosms. Chambers were covered with aluminum foil to ensure opacity. An outlet line from each chamber was affixed to an automated sampling manifold (Picarro A0311, Picarro, Santa Clara, California, USA). Sampling lines were automatically evacuated prior to sampling. Headspace atmosphere samples of ~25 ml were drawn in sequence from each of eight mesocosms and from one standard and delivered to a cavity ring-down spectroscopic (CRDS) carbon isotope analyzer (Picarro G2101-i) using a small-sample module (Picarro SSIM). The analyzer cavity was flushed with CO$_2$-free air between each sample, so accounting for buffer time, each sample required 15 minutes for a 10-minute peak average. Therefore, mesocosms were sampled every 2.25 hours overnight yielding 7–8 measurements on each. To avoid creating negative pressure inside the chamber as gas samples were drawn, a 500-ml collapsible gas bag (Calibrated Instruments, Hawthorne, New York, USA) was filled with CO$_2$-free air (Zero Grade, Keen Compressed Gas, Wilmington, Delaware, USA) and attached to a second outlet on each chamber. Keeling plots were used to eliminate variability in atmospheric contribution to initial chamber headspace [CO$_2$] and isolate the $^{13}$C composition of respired CO$_2$ (Keeling 1961) by fitting $^{13}$C-CO$_2$ values of each measurement to the inverse of the corresponding [CO$_2$]; the y-intercept represented the $^{13}$C composition of respired CO$_2$ (Fig. 2). $R^2$ values > 0.90 were
considered acceptable. Outliers, fewer than 3% of measurements, were removed, and average $R^2$ for Keeling determinations was 0.96. To calculate respiration rates, linear relationships of $[\text{CO}_2]$ by time were estimated and multiplied by the chamber headspace volume (average $r^2 > 0.99$; Fig. 2). Between campaigns, three $[\text{CO}_2]$ standards spanning the range of measurements were run to estimate a conversion between analyzer reported values and actual $[\text{CO}_2]$ because the small sample module slightly but consistently dilutes the samples with $\text{CO}_2$-free air but does not affect $^{13}\text{C}$ values.

We used a mixing model to partition respired $\text{CO}_2$ between two sources: oil or plants/soil (both with $\text{C}_4$ signal). The endpoint for oil was determined for each campaign by plant-free, sand-only mesocosms which contained no other organic matter (~28 to ~29%). The plant endpoint was determined separately for each species for each campaign from mesocosms containing grasses and soil organic matter arising from $\text{C}_4$-dominated marshes, so that all respired $\text{CO}_2$ originated from $\text{C}_4$ photosynthesis (~14.5 to ~15.5%). The fraction of respiration derived from carbon in oil ($F_{\text{oil}}$) was calculated as

$$F_{\text{oil}} = \frac{\delta^{13}\text{C}_\text{sample} - \delta^{13}\text{C}_\text{plant}}{\delta^{13}\text{C}_\text{oil} - \delta^{13}\text{C}_\text{plant}}.$$  

The total respiration rate was multiplied by $F_{\text{oil}}$ to estimate the instantaneous oil respiration rate.

**Gas chromatographic analysis of oil residues**

Soil samples taken in May 2012 were solvent-extracted with 20 mL of methanol:dichloromethane (DCM:MeOH, 9:1) using a Microwave Accelerated Reaction System (MARS) at 100°C and 800 W for 30 min. Extracts were analyzed on an Agilent 7890 series gas chromatograph interfaced to a flame ionization detector (FID). Compounds were separated on a J&W DB-XLB capillary column (30 m, 0.25 mm internal diameter (I.D.), 0.25-µm film) with helium carrier gas at a constant flow of 1 mL min$^{-1}$. The GC oven had an initial temperature of 40°C (1 min hold) and was ramped at 5°C min$^{-1}$ until 320°C (15 min hold). To assess the extent of oil degradation in each sample, the abundance of $\text{C}_{17}$ alkane-to-pristane and $\text{C}_{18}$ alkane-to-phytane was determined. A decrease in the ratios of $\text{C}_{17}$ alkane-to-pristane and $\text{C}_{18}$ alkane-to-phytane indicates the onset of the biodegradation of oil because pristine and phytane are relatively recalcitrant to enzymatic degradation compared to the corresponding alkanes (Miget et al. 1969). These compounds were quantified by integrating the total FID area for each compound and using response factors determined from pure standards. Laboratory blanks of combusted sand were free of oil compounds.

**Statistical analyses**

To address our hypotheses about N and plant effects on oil degradation, we analyzed the two metrics of oil degradation separately. Oil respiration measurements were made on the same mesocosms in multiple campaigns. Therefore, we tested for plant and N treatment effects on sequential instantaneous oil respiration rates using a two-way ($N \times \text{Plant}$) repeated measures MANOVA through time (Table 1). Oil remaining (GC) was measured once in May and was analyzed using a two-way ANOVA ($N \times \text{Plant}$).

To further explore the influence of plants on oil degradation (respiration and GC), we used correlations between plant growth variables and metrics of oil disappearance. Because it is possible that our treatments could affect plants in a way that would affect oil degradation, we analyzed the impact of oil and N on plant above- and belowground biomass using a two-way ANOVA ($oil \times N$). However, because oil had no impact on plant biomass, we collapsed the design to examine the impacts of N addition on plant. To identify which effects of plant and N could mediate effects on oil decomposition, we analyzed the impact of oil and N on plant above- and belowground biomass using a two-way ANOVA ($oil \times N$). However, because oil had no impact on plant biomass, we collapsed the design to examine the impacts of N addition on plant. However, because oil had no impact on plant biomass, we collapsed the design to examine the impacts of N addition on plant above- and belowground biomass using a two-way ANOVA ($oil \times N$). However, because oil had no impact on plant biomass, we collapsed the design to examine the impacts of N addition on plant. However, because oil had no impact on plant biomass, we collapsed the design to examine the impacts of N addition on plant. However, because oil had no impact on plant biomass, we collapsed the design to examine the impacts of N addition on plant. However, because oil had no impact on plant biomass, we collapsed the design to examine the impacts of N addition on plant.
Greenhouse mesocosms: plant and N influence on oil degradation

The presence of plants significantly increased total mesocosm respiration, and oil respiration \((p < 0.0001, p = 0.004, \text{respectively; Figs. 3 and 4). The fraction of respiration derived from oil was highest in the plant-free mesocosms because this respiration was not diluted by plant respiration (Table 1). The response of oil-derived respiration to N treatment depended on plant type and time of year in the experiment (two-way repeated measures ANOVA, Plant \times N; Fig. 3). The significant Plant \times N \times time interaction \((p = 0.025) was characterized wherein N addition tended to accelerate oil decomposition in mesocosms planted with \textit{S. alterniflora}, but surprisingly suppressed oil decomposition in mesocosms planted with \textit{S. patens}. In order to show instantaneous measures of oil respiration in graphical form, we highlight the May measurements (Fig. 4). N treatments did not affect the chromatographic indices of oil degradation (two-way ANOVA, all \(p > 0.05; \text{Table 1). Plant treatment (across both species and unplanted control) tended to decrease C}_{18}/\text{Phytane ratio} \((p = 0.109), and a post hoc pairwise contrast indicated that \textit{S. patens} mesocosms had a lower ratio than unplanted mesocosms indicating greater oil biodegradation \((p = 0.043). The two biomarker ratios correlated strongly \((r = 0.718, p < 0.0001); we focus hereafter on C}_{18}/\text{Phytane because preliminary analyses showed that it agreed better with respiration rate in closed microcosms (Appendix). C}_{18}/\text{Phytane did not relate strongly to instantaneous oil respiration rate in the mesocosm experiment \((r = 0.271, p = 0.115, N = 35). However, across treatment groups (Plant \times N) oil respiration rate correlated strongly with C}_{18}/\text{Phytane \((r = -0.83, p = 0.005, N = 9; \text{Fig. 5). C}_{18}/\text{Phytane was negatively correlated with mesocosm root mass \((r = -0.797, p = 0.010; \text{Fig. 6).}

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|}
\hline
\textbf{Factor} & \textbf{Fraction from oil} & \textbf{Total respiration} & \textbf{Oil Respiration} \\
\hline
N & 0.532 & 0.137 & 0.930 \\
Plant & <0.0001 & <0.0001 & 0.004 \\
N \times Plant & 0.084 & 0.093 & 0.784 \\
Time & <0.0001 & <0.0001 & <0.0001 \\
Time \times N & 0.251 & 0.940 & 0.774 \\
Time \times Plant & <0.0001 & 0.005 & 0.094 \\
Time \times N \times Plant & 0.375 & 0.887 & 0.025 \\
\hline
\end{tabular}
\caption{Responses of oil parameters to plant and N treatments reported as \(p\) values from two-way repeated-measures MANOVAs.}
\end{table}

Plant biomass responses to oil and N

In the greenhouse mesocosms, \textit{S. patens} produced about three times as much above and belowground biomass as \textit{S. alterniflora}. Oil addition had no significant effects on any plant parameters (two-way ANOVA, Oil \times N), so oil treatments were collapsed to examine the influence of N. Added N tended to increase shoot mass of \textit{S. alterniflora} though the pattern was not significant (one-way ANOVA, \(p = 0.146). N addition reduced root production in the top 10 cm for \textit{S. patens \((p = 0.018; \text{Fig. 6)} and tended to reduce total root production \((p = 0.091; \text{Fig. 7).}

Soil redox response to oil, plants, and N

Soil redox potential (Eh) decreased with oil addition (three-way, factorial ANOVA, \(p = 0.020), responded to N treatment \((p = 0.019), and marginally to plant treatments \((p = 0.097, \text{no significant interactions). Planted mesocosms (\textit{S. patens and S. alterniflora combined) had higher redox potential than unplanted controls (post

Fig. 3. Oil respiration rates for mesocosms containing \textit{S. alterniflora, S. patens} and the unplanted control over the three-month sampling period. Values are averaged across N treatments to show plant-mediated effects clearly.

Fig. 4. A graph showing the monthly average oil respiration for each treatment group. The values for April, May, and June are plotted, with error bars indicating the standard error. The graph shows that the respiration rates vary depending on the treatment group, with the unplanted control having the lowest values. The y-axis represents the respiration rate in milligrams of carbon dioxide per day (mg CO₂ d⁻¹), while the x-axis represents the time of the year (April, May, June).
hoc contrast, $p = 0.018$). Because redox measurements were performed on a separate subset of mesocosms from oil measurements, relationships between redox and oil disappearance could not be explicitly tested.

Fig. 4. Instantaneous total respiration rate, the fraction of respiration derived from oil, oil-derived respiration rate, and C$_{18}$ n-alkane-to-phytane ratio, an indicator of oil degradation, all measured in May 2012. Significant effects from two-way ANOVAs (Plant × N) are indicated in each panel. Values represent mean ± SE.

Fig. 5. Relationship between oil respiration rate and C$_{18}$ n-alkane-to-phytane ratio across treatment groups ($N = 9$). Values represent mean ± SE.

Fig. 6. C$_{18}$ n-alkane-to-phytane ratio versus root mass in the top 10 cm of soil. Values represent mean ± SE for each treatment group of oiled mesocosms. The line represents the linear trend across the mean value for all treatments ($r = -0.797$, $p = 0.010$, C$_{18}$/Phy = 1.960 − 0.016 × Root mass).
DISCUSSION

We used a novel combination of analytical chemistry and gas flux techniques coupled with a laser-based isotope analyzer to assess oil degradation in a mesocosm experiment. A highly simplified preliminary incubation in which oil was the only carbon source indicated that CO₂ production from oil should correlate with chromatographic indices of remaining oil (Appendix). Across all individual mesocosms the two measures were poorly related, likely owing to the increased variability within a treatment group incurred by inclusion of plants. However, the results from the two methods correlated strongly across treatment groups (Fig. 5), indicating that replication can overcome error in either measurement and capture treatment effects. Each technique yielded valuable information about how oil was processed, and each has advantages in different scenarios. The respiration measurements provided instantaneous, nondestructive estimates of complete oxidation of oil-carbon to CO₂ throughout the entire mesocosm soil profile. The GC technique, on the other hand, provided a temporally integrated measurement of total oil disappearance and chemically characterized remaining oil. One shortcoming of our particular approach is that oil could have leached down the soil column below our sampling depth (10 cm) and would require more extensive sampling to detect (White et al. 2005). The GC technique is generally more amenable to field sampling, because instantaneous oil respiration rate measurement requires carbon endpoints are carefully determined, that there are only two isotopic pools of CO₂, and that oil ¹³C differs substantially (>10‰) from other pools. Moreover, frequent sampling would be required to establish a precise long-term budget. However, instantaneous respiration rates provide a more sensitive assessment particularly when short-term relative response to a given treatment is desired.

Plant priming of oil degradation

We hypothesized that plants would accelerate the degradation of oil. Oil decomposed 58% faster in plant-containing mesocosms than in plant-free mesocosms (Fig. 3). The large differences in planted vs. plant-free mesocosm oil degradation were most likely due to rhizosphere effects. We found a negative relationship between root biomass and C₁₈:Phytane ratio, showing that across all mesocosms, more oil was degraded where root biomass was greater (Fig. 6). Furthermore, mesocosms with plants had higher soil redox potential (Fig. 8). These data support the idea that soil aeration, via plant conduction of oxygen through stems and roots, exerts strong control over oil degradation. In a freshwater marsh mesocosm study in which aeration was manipulated, oil remaining was lowest in aerated soils (Dowty et al. 2001). Further, Lin and Mendelssohn (1996) suggested that the plant S. lancifolia is an attractive target species for oil bioremediation due to the presence of extensive aerenchyma (gas-space structures) in root tissue (Dowty et al. 2001). Accordingly, mesocosms with plants exhibited higher redox potential in the present study (Fig. 8).

Though aeration of soil is one possible explanation for the plant-induced increase in oil degradation, other plant-mediated changes in the soil environment could also increase oil breakdown. For example, plant production of labile carbon compounds have been shown to increase more recalcitrant soil organic matter processing in terrestrial soils (Cheng and Kuzyakov 2005). Alternatively, soil organisms in mutualistic relationships with plants may receive resource subsidies that could facilitate oil pro-
cessing. Given the relatively high availability of organic C in marsh soils and low availability of oxygen, the oxygen-priming pathway is likely more important.

**N influence on oil degradation**

Previous lab incubation studies (Lin and Mendelsohn 1998, Jackson and Pardue 1999) and our preliminary plant-free incubation (Appendix) have shown that N addition can accelerate the degradation of oil. However, we show that in simulating more realistic marsh conditions by including plants in the mesocosm experiment, controls on oil degradation became more complex. We hypothesized that N addition would stimulate oil degradation irrespective of plant type. However, we found that the influence of N on oil breakdown depended on species identity. Specifically, N addition tended to accelerate oil decomposition in mesocosms planted with *S. alterniflora* but surprisingly suppressed oil decomposition in mesocosms planted with *S. patens* (Fig. 6). N fertilization tended to reduce root biomass in *S. patens* but not *S. alterniflora*. Microbes responsible for oil degradation require nutrients such as N to break down oil, which is a relatively N-poor substrate but can be even more strongly limited by oxygen scarcity (Tate et al. 2012). Interestingly, GC-derived oil degradation converged for both plant species at high N levels (Fig. 4), indicating that differences in oil breakdown rates between plant species may be most important at low N levels.

Patterns in porewater redox potential partly explain the higher rates of oil degradation in planted mesocosm, as planted mesocosms generally had higher redox potentials and greater oil degradation, but did not clearly explain the plant × N interactions we observed. We found that the low-N addition mesocosms had lower redox potential than the control, and the high-N treatment mesocosms tended to have higher redox potential than the low-N treatment (Fig. 8). This perplexing but consistent pattern in redox may result from two opposing influences of the N additions on redox, one indirect and one direct. N addition can reduce root growth and activity (Darby and Turner 2008a), potentially reducing rhizospheric oxygen delivery. However, the fertilizer itself, added as NH$_4$NO$_3$ may have directly increased redox status by supplying nitrate, thereby overwhelming the indirect effects of reduced root oxygenation when added at high rates. More importantly, these results point to reasons why redox may not be the best indicator of the oil decomposition environment. Oil degradation is carried out primarily by aerobic microbes in environments where oxygen is available, not only because O$_2$ is the most favorable terminal electron acceptor, but because O$_2$ is required to activate the hydrocarbon through oxygenase reactions (Widdel and Rabus 2001). Although added nitrate may have increased redox potential and intermittently stimulated some microbes such as denitrifiers, it would not have necessarily increased oxygen availability to the dominant oil-degrading microbes, which were likely aerobic. Therefore, redox potential could be an incomplete indicator of the oil decomposition environment in this or other field-based studies.

Another variable that may explain some of the differences between the differential plant species impacts on oil degradation is relative water level.
The *S. patens* mesocosms tended to maintain a high elevation while the *S. alterniflora* mesocosms tended to sink by 5–10 cm (data not shown). *S. patens* produced a great deal more root biomass that likely helped sustain the starting surface elevation and provide a more oxygenated environment by allowing more direct diffusion of oxygen from the atmosphere and through the more extensive root system. Interestingly, the relative elevations each species established mimic the different elevation ranges each species inhabits in the actual marsh, with *S. patens* occurring ~10 cm higher than *S. alterniflora* (Broome et al. 1995).

**Importance for natural marshes**

For decades, nutrient addition has been a common method for accelerating oil degradation in marshes (Atlas 1981, Jackson and Pardue 1999, Pezeshki et al. 2000). In this mesocosm study, we assessed effects of N addition and plant presence on oil degradation. N addition at times accelerated oil degradation in *S. alterniflora* mesocosms but not in *S. patens* mesocosms, indicating that the effects of N fertilization may depend on dominant plant species. We found that, although N additions tend to stimulate plant root growth in mesocosms with *S. alterniflora*, yielding more rapid oil degradation, the opposite tendency to occur in mesocosms planted with *S. patens*. We conclude that though N addition can directly stimulate oil-degrading microbes, the plant-mediated controls on oil degradation may be ultimately more important and should be considered in mitigation efforts. Where nutrient addition reduces root production, as is commonly observed (e.g., Turner 2011, Deegan et al. 2012) we expect that it will negatively affect oil degradation in the long term. This effect could partly explain the observation from previous studies that the N stimulation of oil degradation is greater in microcosms than in mesocosms that include plants and intact soil profiles (Jackson and Pardue 1999) and could explain the absence of an N effect on Deepwater Horizon oil in situ (Tate et al. 2012).

The effects of marsh plants on oil degradation will be nullified if the deposition of oil itself jeopardizes the viability of the marsh. The most pronounced impacts following the Deepwater Horizon oil spill, including marsh erosion, occurred at the marsh edges (<15 m), where *S. alterniflora* tends to dominate, and thus this species suffered more oil-driven mortality (Silliman et al. 2012). The sustainability of a given marsh is influenced by a complex combination of factors including marsh elevation, nutrient inputs, plant productivity, and sediment loads. Understanding the contribution of each of these factors in marshes dominated by different plant species is essential for predicting marsh responses to anthropogenic disasters such as oil spills. Our findings that marsh plants accelerate oil degradation, and that species responses to N additions can mediate this response (Fig. 6) provide mechanistic explanations of plant and nutrient influences that can be tested in field settings.

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To assess the degradation of oil in our experimental manipulations, preliminary plant-free incubations were constructed. Light, sweet crude oil from a proxy well in the Gulf of Mexico was provided by British Petroleum. Aliquots (30 ml) were added to carbon-free sand (750 ml), homogenized and then 40 ml of the mixture were added to individual 1-L glass jars. N treatments made from solutions of ammonium nitrate at 1.4 mg N L\(^{-1}\) (low N) and 14 mg N L\(^{-1}\) (high N) concentrations were added (30 ml) to wet the soil. For the zero N treatment water only (30 ml) was added to the soil. All jars received inoculum containing negligible C mass made from a slurry of marsh soils. Jars were kept capped and sampled on days 1, 2, 4, 8, 16 and 28.

Total respiration was measured over the course of 28 days by sampling 1 ml of headspace and injecting on an infrared gas analyzer (LI7000, LI-COR, Lincoln, Nebraska, USA) configured for small-volume measurements. To avoid impacts of negative pressure on the incubations, 1 ml CO\(_2\)-free air was pre-injected into the each jar before sampling. At the end of the 28-day period, the remaining sand was homogenized and frozen at \(-20^\circ\text{C}\) for future analysis of the remaining oil by GC (details provided below). Because oil was the only carbon source, all respiration was assumed to have arisen from oil degradation.

**Laboratory incubation: N influence on oil degradation**

N addition strongly accelerated the breakdown of oil as assessed by respiration rate (one-way ANOVA, \(p < 0.0001\); Appendix: Fig. A1) and key biomarker ratios, \(C_{17}\)-pristane (\(p = 0.0033\)) and \(C_{18}\)-phytane (\(p = 0.0033\); Appendix: Fig. A1). We
found strong negative relationships between oil respiration rates and oil remaining assessed by GC after only four weeks of decomposition for both biomarker ratios, C\textsubscript{17}:pristane ($r = -0.751$, $p = 0.0003$; not shown) and C\textsubscript{18}:phytane ($r = -0.877$, $p < 0.0001$; Appendix: Fig. A1), and very high agreement between the two ratios ($r = 0.950$, $p < 0.0001$; not shown).

Fig. A1. Two independent measures of oil degradation, lab incubation respiration rates and C\textsubscript{18} \textit{n}-alkane-to-phytane ratios (left), and the relationship (right) between the two indicators of oil degradation (C\textsubscript{18}/Phytane = 0.0710 $\times$ respiration rate + 2.334; $r^2 = 0.768$, $p < 0.0001$).