

# Bioremediation of an Experimental Oil Spill in a Coastal Louisiana Salt Marsh

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**Abstract** The massive oil release from the Deep Water Horizon disaster has reemphasized the need to remediate oil impacted marshes. Due to the physically fragile nature of salt water marshes, bioremediation is often proposed as an appropriate technology and nutrient amendment is often proposed as a means of accelerating biodegradation of crude oil. However, no information is currently available concerning the efficacy of in situ nutrient amendments in Gulf Coast salt marshes. An experimental crude oil spill (142 l over 100 m<sup>2</sup>) was conducted to evaluate the efficacy of nitrogen amendment to stimulate bioremediation in a *Spartina alterniflora* dominated Louisiana salt

marsh. A randomized complete block design with replication ( $n=10$ ) was utilized to test the hypothesis that additions of fast-release ammonium nitrate (60 g N/m<sup>2</sup>) and slow-release urea (30 gN/m<sup>2</sup>) fertilizers could enhance biodegradation of selected crude oil components in the marsh. Crude oil degradation was monitored by analyzing sediment samples for branched and unbranched alkanes over the 180-day study period. The compound/hopane ratio was used to correct for nonbiological losses. No consistent statistically significant effect of fertilizer addition on degradation rates was observed, despite success in increasing the porewater ammonium and NaCl-extractable ammonium over the time frame of the trial. Intrinsic pseudo-first order degradation rates of alkanes in all plots were substantial (0.003–0.008 day<sup>-1</sup>). Existing, background levels of N did not appear to limit biodegradation rates in *Spartina*-dominated salt marshes. These results suggest that nutrient amendments will not be successful in stimulation biodegradation of crude oil in these systems.

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## 1 Introduction

Coastal salt marshes are at considerable risk of crude oil contamination, since major oil production, refining, and transport operations are located near these areas. The

efficacy of nutrient-stimulated bioremediation has been studied for crude oil spills in a variety of environments (Lee and Levy 1989, 1991; Leahy and Colwell 1990; Prince et al. 1994; Bragg et al. 1994; Venosa et al. 1996; Venosa et al. 2002; Venosa and Zhu 2003; Lindau et al. 2003; Garcia-Blanco et al. 2007; Li et al. 2007; Santos et al. 2011). Field-scale bioremediation studies of salt marshes are lacking, in particular those near the Gulf Coast. Salt marsh soils have an intrinsic potential to biodegrade oil spills (Jackson et al. 1996; Jackson and Pardue 1998) and increased degradation rates have been achieved by nutrient addition in laboratory microcosms (Jackson and Pardue 1999). Nitrogen addition increased biodegradation rate constants of both alkanes and PAHs several-fold in aerated slurries of salt marsh soil (Jackson and Pardue 1998). Using fully aerated microcosms no statistical difference was observed between N and N and P amended treatments, although all N-amended treatments were significantly higher than non-amended treatments (Jackson and Pardue 1999). Porewater nitrogen concentrations required to stimulate crude oil bioremediation in salt marshes were between 2 and 5 mg/l (Jackson and Pardue 1999) similar to concentrations necessary in other environments (Venosa et al. 1996) although maximum stimulation occurred at higher nitrogen concentrations. While N amendment stimulated crude oil degradation, the increase in degradation rates relative to controls was greatly reduced in intact core studies (~2×) compared to completely mixed mesocosms (~10×), which was attributed to oxygen limitations (Jackson and Pardue 1999). In a separate study, the mineralization enhancement of  $^{14}\text{C}$ -hexadecane and  $^{14}\text{C}$ -phenanthrene in crude oil spiked salt marsh slurries by N addition was substantial but varied seasonally (Jackson and Pardue 1997). The seasonal changes were only partially explained by available porewater nitrogen and background microbial populations. In a field study conducted in a Canadian salt marsh, a statistically significant increase in the alkane but not the PAH fraction was achieved by the addition of N and P (Garcia-Blanco et al. 2007). These studies suggest that fertilization may be a reasonable approach for stimulating crude oil degradation provided that oxygen limitations are removed.

Nitrogen budgets of gulf coast salt marshes have been widely studied (e.g., DeLaune and Patrick 1990). Much of the nitrogen present is in the organic form and relatively unavailable (DeLaune et al. 1989,

1990; DeLaune and Smith 1985; Feijtel et al. 1985). Salt marsh vegetation is nitrogen-limited and large increases in *Spartina* biomass has been observed following fertilization (Smart and Barko 1980). Slow N mineralization kinetics may limit porewater  $\text{NH}_4^+$  concentrations and subsequently limit crude oil biodegradation kinetics following a spill.

To date, there have been no field studies evaluating the efficacy of nutrient addition to stimulate biodegradation in Gulf Coast salt marshes. The recent Deepwater Horizon oil release highlights the potential risk to these marshes and the need to understand the relative benefits of nutrient addition to stimulate oil biodegradation in these unique ecosystems. Addition of nutrients to estuarine systems can have negative impacts and therefore their use should only be utilized if they can clearly be shown to reduce the persistence of oil significantly. We conducted a field study to evaluate in situ nitrogen addition to evaluate the overall potential for nitrogen enhanced crude oil degradation in salt marshes. Different nitrogen sources (ammonium nitrate and a time release urea) were used to determine whether the critical nitrogen concentration can be maintained in situ and which formula is best suited for coastal salt marshes. Phosphorous was not elevated as previous lab studies have shown that it does not limit crude oil degradation (see previous discussion). While the preceding study was conducted prior to the Deepwater Horizon release, the results should provide valuable information to guide remediation decision makers.

## 2 Materials and Methods

### 2.1 Site Description

The study location for this field study is a salt marsh located in the Pointe au Chien Wildlife Management Area in Terrebonne Parish, LA. This marsh is located near the left descending edge of the Bayou Jean Charles natural levee. This site experiences approximately a 1-ft daily tidal variation daily. The site is heavily vegetated by *Spartina alterniflora*.

### 2.2 Plot Design

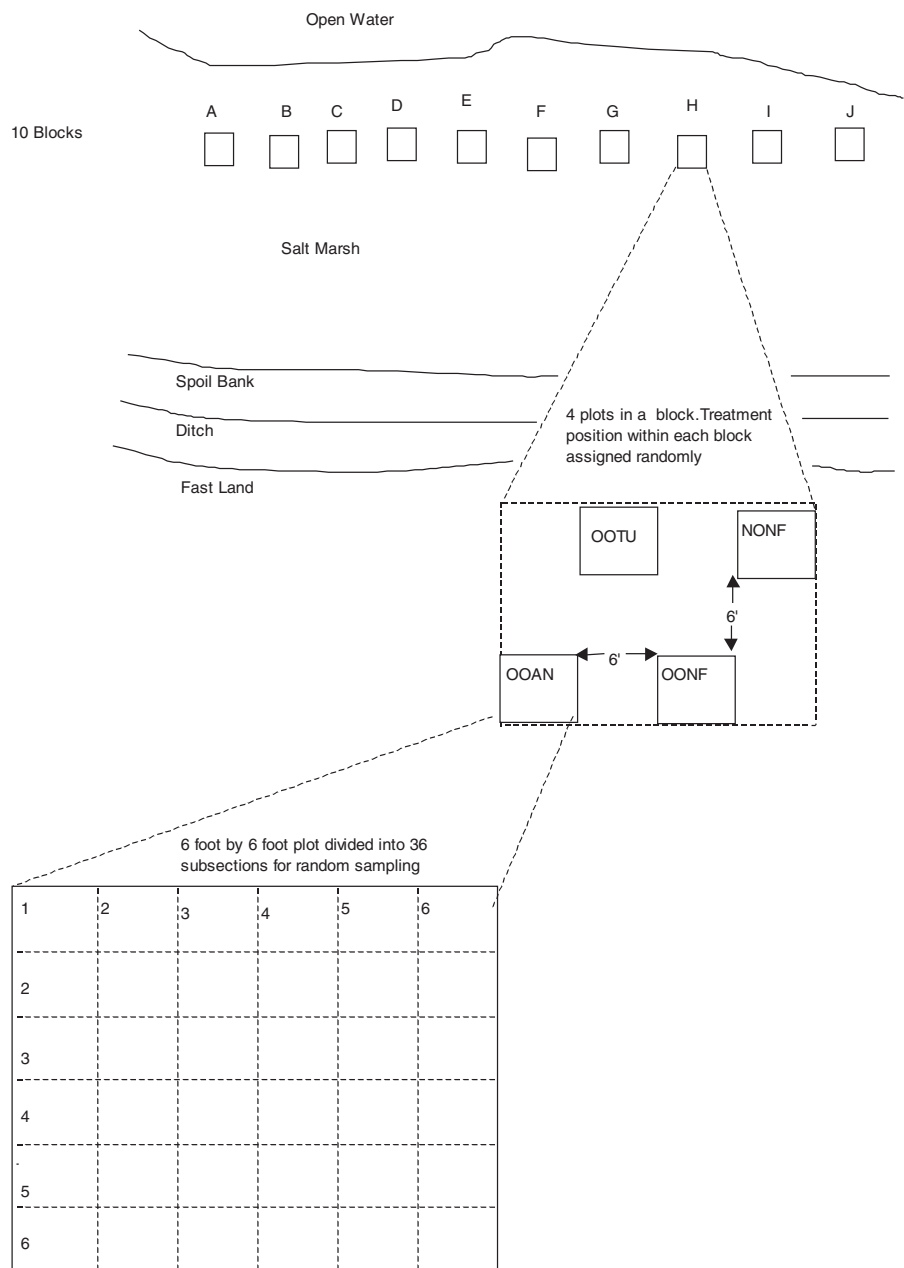
A completely randomized block design (RBD) was applied to evaluate treatment effects. Ten blocks of

four plots each (one treatment per plot) were established as shown in Fig. 1. The blocks (5.5×7.3 m or 18×24 ft) were located in one row in the salt marsh parallel to the water’s edge. Each plot was 1.8×1.8 m (6×6 ft) or 3.345 m<sup>2</sup> and was marked with four wooden corner stakes with supporting silt fence between stakes. The distance between plots within a block was 1.8 m (6 ft) and the distance between blocks varied but was at least 1.8 m (6 ft). Block

locations were chosen so that all plots in a block had similar topography and vegetation density. An oil-absorbent boom was placed on the open-water side of the experimental area.

Four treatments were tested: (1) NONF (no oil, no fertilizer), (2) OONF (oil no fertilizer), (3) OOAN (oiled and fertilized with ammonium nitrate), and (4) OOTU (oil and fertilized with a time-release urea). In addition to these treatments, locations outside the

**Fig. 1** Layout of plots and blocks in the salt marsh field trial, Pointe Au Chien Wildlife Management Area, Terrebonne Parish, LA



block area were used as background locations. The four treatments were randomized in each of the ten blocks. NONF was used as a control for spatial and temporal variations of nutrient levels in the marsh porewater; OONF was used to confirm intrinsic degradation rates, as well as the control against which to measure the efficacy of nitrogen applications on the biodegradation rate. OONF and OOTU were used to determine if nutrient levels could be enhanced and which formulation, if any, was optimal.

### 2.3 Application of Oil to Plots

The crude oil used in this study is a sweet Louisiana crude oil (SLCO) which is relatively nontoxic oil with high alkane, low polar, and low PAH concentrations. The 142 l of oil used was weathered prior to application by sparging 90 standard ft<sup>3</sup> of class D breathing air through the oil (while still in a drum) over an approximately 2-h period to simulate the loss of volatiles expected by floating in open water prior to landfall. The oil was applied uniformly on the surface of marsh soils using a pressurized spray bottle at the same loading rate of previous laboratory studies (1.42 l/m<sup>2</sup> or 1.16 kg/m<sup>2</sup>) (Tate 1998). Care was taken to spray onto the ground at the base of the vegetation rather than the upper portion of the vegetation itself.

### 2.4 Nutrient Application

The nutrient loading rate was adopted from the results of our previous laboratory studies (Tate 1998). Ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) was applied as 60 g N/m<sup>2</sup> (573 g ammonium nitrate/plot) that includes 30 g NH<sub>4</sub><sup>+</sup>-N/m<sup>2</sup> and 30 g NO<sub>3</sub><sup>-</sup>-N/m<sup>2</sup>. This loading rate exhibited the highest biodegradation rate in previous core studies. Meister 70 (Helena Chemical, Tampa, FL), a time-release urea designed to release nitrogen over a 70-day period, was applied at the loading rate of 30 gN/m<sup>2</sup> (251 g urea/plot). Pelletized nutrients were sprinkled by hand onto the plots. Both nitrogen applications were reapplied at the same loading rate when the porewater nutrient concentration dropped significantly (after 139 days).

### 2.5 Field Sampling

Soil and porewater samples were collected from the plots every 3 to 6 weeks. Each plot (Fig. 1) was

divided into 36 equal 0.3 × 0.3 m (1 × 1 ft) subsections. Subsamples were collected from the top 5 cm of four randomly selected subsections of each plot at each sampling event. The soil samples were placed into glass jars and transported to the laboratory. Approximately 60 ml porewater was collected from just below the marsh surface in each plot, stored in gas-tight plastic bottles, and placed on ice. Homogenized soil samples were analyzed for microbial populations (total heterotrophs and oil degraders), NH<sub>4</sub><sup>+</sup>, and oil compounds. Porewater samples were analyzed for ammonia and nitrate.

### 2.6 Oil Analysis

Oil was extracted from the soil samples using hexane/acetone (50:50, v/v). After cleanup of the extracts, samples were analyzed using an HP 5890 GC-MS operating in the SIM mode as described previously (Jackson et al. 1996). Each sample was analyzed for *n*-alkanes (C10 through C36), pristane, phytane, and a stable biomarker C<sub>30</sub>-17 $\alpha$ (H), 21 $\beta$ (H)-hopane (Prince et al. 1994). Very low PAH concentrations prevented their routine analysis during the field trial.

### 2.7 Nutrient Analysis

The porewater ammonia nitrogen concentration (NH<sub>4</sub><sup>+</sup>-N) was measured using an ammonium ion selective electrode (Hach Co., Loveland, CO) following standard methods (APHA 1992). Nitrate concentration in the porewater was determined using a cadmium reduction method. Ammonium nitrogen in soil was measured by extracting the ammonia from soil with 1 N NaCl. The centrifuge tube was then shaken for 3 h and centrifuged. The supernatant was analyzed as described above.

### 2.8 Microbiological Analysis

The presence of oil-degrading bacteria was determined by the Sheen Screen method as described elsewhere (Brown and Braddock 1990). Approximately 1–2 g of soil (wet wt.) was placed in a dilution bottle containing 9 ml of sterile detachment solution (8 g/l NaCl) and shaken. Serially diluted solution was transferred into screen plate (columns of six wells) and mixed with 1 ml of the Buschnell–Haas broth. Crude oil (50  $\mu$ l) filtered through a microfilter

(Nalgene sterilization filter unit, pore size: 0.2  $\mu\text{m}$ ) was added in each cell of the sheen screen. The sheen screen was placed in the dark and counted after a 2-week incubation. Results were expressed as a most probable number per gram dry weight of soil. Total heterotroph counts were also made by counting colonies on HB pour plates. The initial soil detachment was the same as for the Sheen Screen testing, and then subsequent serial dilutions were prepared. Dilutions were used to make pour plates using Bushnell Hass broth and agar. The covered pour plates were stored in the dark and colony forming units (CFUs) counted after 1 to 2 weeks. Results were expressed as CFUs per gram dry weight of soil.

### 2.9 Degradation Rate Determination

The compound to hopane ratio C/H was determined for each monitored compound, in each plot, for each of eight sampling events. Each compound/hopane ratio was further divided by the original weathered compound/hopane ratio of the oil and multiplied by 100 to obtain a percent of compound/hopane ratio remaining versus time. Data were fit using first-order kinetics. Based on the reciprocal of the standard error for the curve fit for  $k$  (first order rate constant) in each plot, a weight was assigned to  $k$  determined from that plot. A weighted average  $k$  and weighted standard deviation was then determined for each compound/treatment based upon the ten replicate plots of a treatment.

## 3 Results

### 3.1 Nutrient Persistence in Marsh Soils

Application of the fertilizers did not significantly maintain an elevated concentration of nitrate ( $>5 \text{ mg/l NO}_3^- \text{-N}$ ) in the porewater (Fig. 2). In only two of the eight sampling events was the nitrate concentration in the OOAN plots significantly greater (at the 0.05 significance level) than the nitrate concentration in the NONF plots. In only one of the eight sampling events was the nitrate concentration in the OOAN plots significantly greater than the nitrate concentration in the OONF. In no cases did application of the time-release urea (OOTU) result in a significant increase in nitrate concentration through a nitrification mechanism.

Application of ammonium nitrate was successful in maintaining elevated concentrations of ammonium ( $>20 \text{ mg/l NH}_4^+ \text{-N}$ ) (Fig. 2). In seven of the eight sampling events, OOAN plots had significantly greater concentrations of ammonia in the porewater than the NONF plots. In six of the eight events, the ammonium concentration was elevated significantly in OOAN plots compared to OONF plots. The only sampling events when the concentration of ammonium was not elevated with respect to OONF plots were the last two events before reapplication (44 and 99 days after the initial and reapplication of ammonium nitrate, respectively). The OOTU treatment did not result in significantly increased ammonium porewater concentration in a single sampling event.

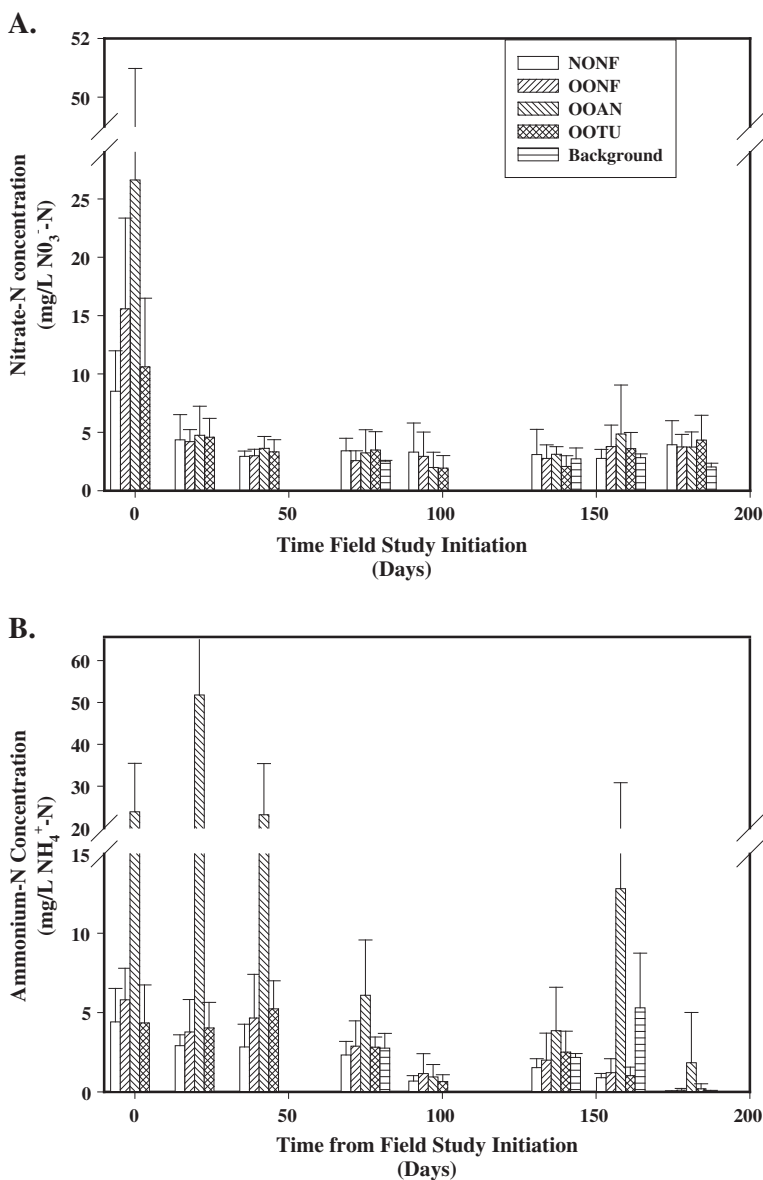
A large pool of ammonium exists in an exchangeable form in these marsh soils (Fig. 3). The OOAN treatment in comparison to OONF had significantly elevated extractable ammonium concentrations in four of eight sampling events. The elevated concentrations fell within background levels within 44 days after each application. Concentrations of extractable ammonium were also significantly higher in the OOTU compared to the OONF in six of eight sampling events.

The possibility that application of fertilizer bled into the nonfertilized plots was evaluated by comparing nitrate and ammonia porewater concentrations in the NONF and OONF treatments to background concentrations. In only one of the 16 possible comparisons was the concentration of either ammonia or nitrate within the NONF and OONF treatments greater than background ammonia or nitrate concentrations at the 0.05 significance level.

### 3.2 Oil Degradation Kinetics

First order rate constants for the measured alkanes ranged from 0.003 to 0.008  $\text{day}^{-1}$ , with the exception of the branched alkanes (pristine and phytane) which were substantially lower (0.001–0.002  $\text{day}^{-1}$ ) (Fig. 4). Based on the average and standard deviation from the replicates of a given compound/treatment and the average and standard deviation of the OONF treatment a Student's  $t$ -test determined which given compound/treatment was significantly different than the control (i.e., greater  $k$  at the 0.05 level of significance). Statistically significant greater biodegradation rates were found for five alkanes out of a possible 18 for the OOTU treatments. Only one compound, phytane, showed

**Fig. 2** Porewater nitrate-N (a) and ammonium ion (b) concentrations over time in field plots. *NONF* no oil, no fertilizer; *OONF* oil no fertilizer; *OOAN* oiled and fertilized with ammonium nitrate; *OOTU* oil and fertilized with a time-release urea



significantly greater degradation rate constants than the control for the OOAN treatments (Fig. 4). Since only six compound/treatment pairs were found significantly different out of 36 possible, there is no overall significance at the 0.05 level based on Bonferroni's inequality. Therefore, for the conditions of this research, the addition of nitrogen had no beneficial effect on the rate of biodegradation of the crude oil.

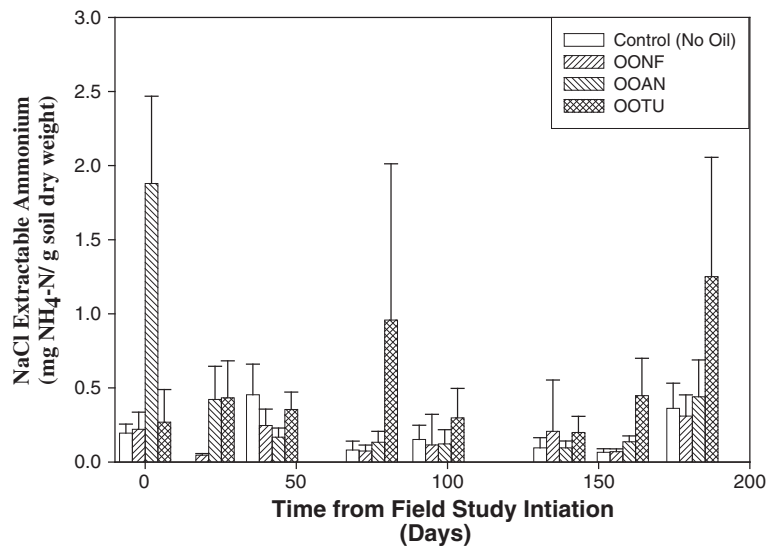
Microbial populations measured through the field study period were variable, but generally bacterial population in oil-contaminated marsh soils was higher than non-oiled soils (Table 1). No significant difference

was observed between only oiled and oiled/fertilized soils. Populations of oil degraders were in the range of those found in a seasonal study of oil degrading population in a nearby Louisiana salt marsh which found populations seasonally dependent ranging from 1.9 to 4.8 log CFU/g sediment (Jackson and Pardue 1997).

#### 4 Discussion

The ammonia concentration in the marsh was significantly enhanced by fertilizer application, with the

**Fig. 3** NaCl-extractable ammonium concentrations over time in field plots. *NONF* no oil, no fertilizer; *OONF* oil no fertilizer; *OOAN* oiled and fertilized with ammonium nitrate; *OOTU* oil and fertilized with a time-release urea

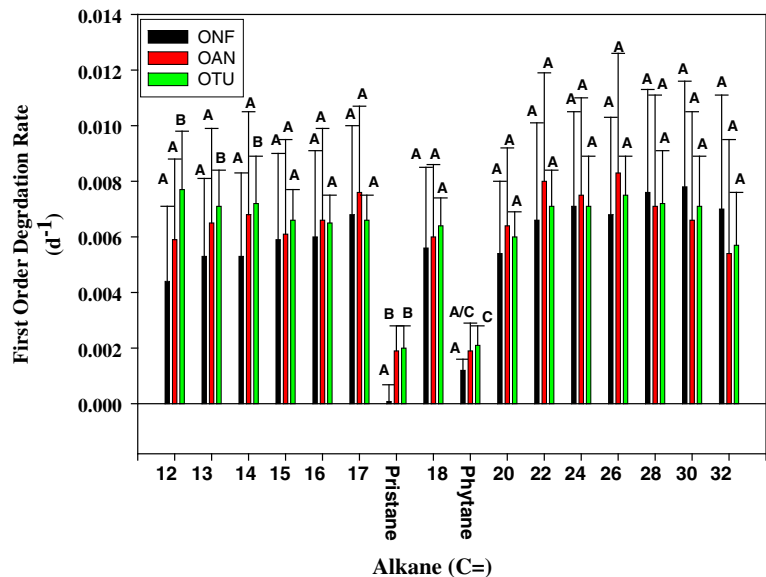


increase lasting 2 to 3 months. In contrast, the nitrate concentration of porewater in the marsh was not significantly increased by fertilizer addition. Ammonium nitrate was most effective for increasing the ammonia in the porewater, while time-release urea was most effective for increasing NaCl-extractable ammonium concentration in the soil.

Porewater concentrations of ammonia were several milligrams per liter in control plots lower than those reported for a Canadian salt marsh (Garcia-Blanco et al. 2007). The source of this N is likely the diagenesis of

recently deposited organic matter present in salt marsh soils (Gambrell and Patrick 1978). Fertilizer treatments increased ammonia concentrations several fold. Despite the increased ammonia concentrations, alkane degradation rates were not enhanced demonstrating that nutrient limitations did not exist or some other limitation controlled degradation rates. The lack of a fertilizer effect should be interpreted in light of spatial and temporal changes that have been documented in salt marshes (Jackson and Pardue 1997; Buresh et al. 1980). A spatial N gradient exists in salt marshes with

**Fig. 4** In situ first order degradation rate constants for Louisiana “sweet crude” in a salt marsh. Letters indicate treatment significance at 0.05 level over ONF. *ONF* oil no fertilizer, *OAN* oiled and fertilized with ammonium nitrate, *OTU* oil and fertilized with a time-release urea



**Table 1** Oil degrading population in sediments (log MPN of oil degraders per gram dry weight sediment)

| Treatment | Day 0   | Day 75  | Day 181 |
|-----------|---------|---------|---------|
| NONF      | 4.5±4.7 | 3.8±3.8 | 4.0±4.3 |
| OONF      | 5.2±5.6 | 5.5±5.7 | 4.4±4.6 |
| OOAN      | 4.8±5.0 | 5.7±6.1 | 5.4±5.6 |
| OOTU      | 4.5±4.8 | 5.9±6.0 | 5.9±5.9 |

ammonia concentrations increasing from the streamside to the inland marsh (Buresh et al. 1980). The field study was set up perpendicular to the likely N gradient nearer to the inland side of the marsh. Results may not be applicable to areas nearer the streamside where N concentrations are potentially much lower. Temporal changes in ammonia concentrations are also observed in salt marshes and concentrations were lower in spring and summer (Jackson and Pardue 1997).

Despite the lack of a measurable effect from nitrogen addition, overall rates of alkane biodegradation were significant with half-lives on the order of 100–200 days. Degradation rate constants for the control OONF treatment plots are very similar to rate constants reported in mesocosm experiments by Jackson and Pardue (1998) for salt marsh soils (0.001–0.008 day<sup>-1</sup>). However, nutrient enhanced rate constants (OOAN, OOTU treatments) in this study were approximately one order of magnitude lower than those observed in well-mixed, aerated microcosms (Jackson et al. 1996). Rate constants in our study were approximately one order of magnitude smaller than in a field trial conducted on a Delaware beach and a Canadian Salt Marsh (Venosa et al. 1996; Garcia-Blanco et al., 2007). The similarities in the study design lead to a straightforward comparison of results. Summary rate constants for measured alkanes were 0.026 and 0.022 day<sup>-1</sup> (beach and marsh, respectively) for the nonfertilized control versus 0.056 and 0.039 day<sup>-1</sup> for the plots receiving nutrients. These results were significantly different at  $p < 0.05$ . This is in contrast to the summary rate constants for alkanes in this study (0.0054 day<sup>-1</sup> for the control; 0.0059 day<sup>-1</sup> for the ammonium nitrogen addition and 0.0058 day<sup>-1</sup> for the time-release urea addition) that were not statistically significant. Rate constants for alkanes from the Delaware beach and Canadian marsh are remarkably similar to those observed in aerated Gulf Coast salt marsh mesocosms

(0.021 day<sup>-1</sup> for ammonium addition, 0.067 day<sup>-1</sup> for ammonium and phosphate addition; Jackson and Pardue 1999) for which fertilization effects were also significant. These results suggest that oxygen rather than nitrogen limits crude oil biodegradation in Gulf Coast salt marshes. Elevation and tidal range differences between the two marshes might account for the lack of impact in the Louisiana marsh if these led to a longer duration flooding pattern. The lower degradation rates observed in this study may be due to the higher oil loading (1.4 l/m<sup>2</sup>) compared to the Canadian marsh study (0.9 l/m<sup>2</sup>). Crude oil represents a large carbon source that increases the demand of oxygen in the marsh soils (Shin et al. 2000). This may have further contributed to redox difference between the two marshes. This is further supported by a separate study on Canadian salt marshes in which at higher loading rates greatly reduced rates of biodegradation were observed and there was no impact from nutrient addition (Lee and Levy 1991).

In summary, the apparent lack of stimulation from nitrogen addition is most likely due to either sufficient nitrogen being available in the marsh soils or another environmental factor controlling the rate of oil degradation. The most likely environmental factor is oxygen availability. Based on these results, there is no current scientific basis to recommend fertilizer addition as a method of accelerating crude oil biodegradation in salt marshes.

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