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Long-term consequences of residual petroleum on salt marsh grass

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Summary

1. Remnants from oil spills can persist for decades within anoxic coastal sediments affecting local flora and fauna, but few studies have examined the long-term impacts of the residual petroleum on these valuable coastal ecosystems.
2. The Wild Harbor salt marsh (Buzzards Bay, Massachusetts, USA) still harbours residual petroleum from a 1969 oil spill, which released 700 000 L of No. 2 fuel oil. Previous effects have been noted in fiddler crabs and ribbed mussels inhabiting the oiled marsh.
3. *Spartina alterniflora* biomass was sampled above- and below-ground in elevational transects through areas with different total petroleum hydrocarbon (TPH) content. Further, elevational differences were mapped in oiled and non-oiled locations.
4. Stem density and above- and below-ground biomass decreased in oiled areas. The decreased vegetation biomass led to unconsolidated sediments, increased topographical variation and, ultimately, loss of salt marsh habitat.
5. Four decades after the *Florida* spill, with only 100 kg of the original 595 000 kg of spilt oil persisting in salt marsh sediment, the effects on large-scale ecosystem functions are still evident.
6. *Synthesis and applications*. Our results demonstrate specific impacts of residual petroleum on *S. alterniflora* biomass and coastal erosion. Further, we have defined TPH content thresholds at which to expect long-term ecosystem impacts. The recalcitrant nature of these contaminants and their chronic large-scale ecosystem effects leads to the potential demand for alternative compensation, such as preservation of coastal systems at risk elsewhere.

Key-words: *Florida*, No. 2 fuel oil, oil pollution, oil restoration, petroleum hydrocarbons, *Spartina alterniflora*

Introduction

Salt marsh ecosystems are highly valued for the many important functions they provide, including the export of energy-rich material to deeper waters, shoreline stabilization, nursery and stock habitats, and the interception of land-derived nutrients and contaminants (Valiela, Rutecki & Fox 2004). Unfortunately, their coastal locations make them highly susceptible to oil spills.

There is extensive information on acute effects of spilled oil on marine ecosystems and populations but little on the possible lingering effect of long-term exposure to petroleum hydrocarbons (Burns & Teal 1971; Sanders, Grassle & Hampson 1972; Sanders *et al*. 1980; Teal *et al*. 1992). A recent report highlighted priority areas for oil spill research (NRC 2003). Cited as a ‘high priority’ was the study of ‘chronic biological effects resulting from the persistence of medium and high molecular weight aromatic hydrocarbons and heterocyclic compounds and their degradation products in sediments’ (NRC 2003). In September 1969, the barge *Florida* ran aground in Buzzards Bay, Massachusetts, USA, spilling 700 000 L of No. 2 fuel oil. Spilt oil entered Wild Harbor and impacted the salt marsh ecosystem, killing large quantities of fish, invertebrates and *Spartina* grasses (Blumer *et al*. 1970; Burns & Teal 1971; Blumer & Sass 1972a,b; Sanders, Grassle & Hampson 1972; Sanders *et al*. 1980; Teal *et al*. 1992).

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Many studies over the past 39 years have shown the continued presence and effects of residual oil in Wild Harbor marsh sediments (Teal, Burns & Farrington 1978; Sanders *et al.* 1980; Teal *et al.* 1992; Reddy *et al.* 2002; Frysinger *et al.* 2003; Peacock *et al.* 2005; Slater *et al.* 2005; White *et al.* 2005; White, Reddy & Eglinton 2005; Culbertson *et al.* 2007a,b). Recent work found that a substantial residue, approximately 100 kg, of the No. 2 fuel oil was still present 8–20 cm below the salt marsh surface across Wild Harbor (Reddy *et al.* 2002; Frysinger *et al.* 2003; Peacock *et al.* 2005; Slater *et al.* 2005; White, Reddy & Eglinton 2005; White *et al.* 2005). The oiled sediments are now overlain by sediment devoid of oil sediment that has accreted at a rate of approximately 0·35 cm $year^{-1}$ (White, Reddy & Eglinton 2005). Despite slight differences in analytical methods, the concentrations of residual total petroleum hydrocarbons (TPH) within the 8–20 cm layer are in roughly the same range as those measured in 1973 in surface sediments (Reddy *et al.* 2002). The petroleum hydrocarbons present at depth are moderately degraded: volatile and water-soluble compounds and *n*-alkanes have been removed, concentrations of acyclic isoprenoids have been reduced, but alkyl cyclohexanes, alkylbenzenes and polycyclic aromatic hydrocarbons (PAH) as well as many other unidentified hydrocarbons still persist (Reddy *et al.* 2002; Frysinger *et al.* 2003; White *et al.* 2005). In particular, total PAH (mostly alkylated naphthalenes and phenanthrenes/anthracenes) in a core collected in 2000 were 134 $\mu g\ g^{-1}$ in the 14–16 cm horizon (White *et al.* 2005); such levels are very similar to concentrations of PAH found in the 1970s (Teal, Burns & Farrington 1978; Teal *et al.* 1992). This current PAH content is 40× greater than thresholds thought to be associated with the incidence of biological effects (Long *et al.* 1995), assuming that the biological response for parent and alkylated PAH is the same. Therefore petroleum hydrocarbons from the *Florida* spill persist, at remarkable concentrations, at depth in the anoxic sediments in some areas of Wild Harbor (Teal *et al.* 1992; Reddy *et al.* 2002; Peacock *et al.* 2005; White *et al.* 2005).

Historically, studies of oiled salt marshes have used laboratory (DeLaune, Patrick & Buresh 1979; Li, Morris & Yoch 1990; Lin & Mendelssohn 1996; Lin *et al.* 2002; Lin *et al.* 2005) and field experiments (DeLaune, Patrick & Buresh 1979; Hershner & Lake 1980; Alexander & Webb 1987; Bergen *et al.* 2000) on a short-term basis (< 5 years). In general, the laboratory studies found that presence of oil decreased marsh grass biomass stem density and shoot height (Alexander & Webb 1985; Webb & Alexander 1985; Pezeshki *et al.* 2000).

The effects of oil extend to lowering below-ground biomass of salt marsh grasses (Lin *et al.* 2002). These reductions have further ecosystem-level effects. Roots and rhizomes of macrophytes stabilize salt marsh sediments (Chmura & Hung 2004) and their disappearance may therefore lead to less stable sediment within salt marshes.

The degradation of salt marsh ecosystems subject to oil has prompted efforts at remediation. Nutrient amendment of oil-impacted salt marshes has had some success in increasing oil biodegradation (Lee & Levy 1991; Mills *et al.* 1997). In particular, nitrogen additions have received attention, as increased nitrogen increases marsh grass growth (Valiela & Teal 1974; Valiela, Teal & Sass 1975; Valiela *et al.* 1978). Other remediation strategies have included the stimulation of nitrogen-fixing bacteria present in salt marsh sediments as an alternative to direct fertilization (Piehler *et al.* 1999; Musat, Harder & Widdel 2006). Increasing plant growth in oiled areas may not only restore vegetative cover and prevent erosion of sediment but could also enhance oil degradation through increased oxygen availability (Atlas 1995; Burns *et al.* 1999).

The continued presence of the residual oil in Wild Harbor presents an unusual opportunity to assess the multiple possible ecological effects of chronic exposure in field conditions within salt marsh ecosystems, as well as explore some remediation methods. We report the effects of residual oil in Wild Harbor sediments on above-ground biomass, stem density and below-ground biomass of *Spartina alterniflora* (Loisel.). We assessed the use of fertilization of oiled plots to increase below-ground plant growth and encourage the biodegradation of the residual oil, and we investigated the effect of the reduced below-ground biomass on sediment infiltration rates and stability.

Methods

STUDY AREA

In summers of 2005 and 2006, we studied salt marsh vegetation from Wild Harbor and a reference site at Great Sippewissett salt marsh (see Appendix S1 in the Supplementary material). The latter is a neighbouring salt marsh that has similar flora, fauna, sediments and geological history (Krebs & Burns 1977) and contains no detectable oil residues in sediment cores (Culbertson *et al.* 2007). Comparisons between these sites have been ongoing for the past 38 years (Blumer *et al.* 1970; Burns & Teal 1971; Blumer & Sass 1972a,b; Sanders, Grassle & Hampson 1972; Sanders *et al.* 1980; Teal *et al.* 1992; Reddy *et al.* 2002; Peacock *et al.* 2005; Culbertson *et al.* 2007a,b). A non-oiled comparison within Wild Harbor was performed at a site adjacent to the oiled locations of the same area (300 m^2), sediment particle type and hydrology.

Wild Harbor and Great Sippewissett marshes show vegetative zonation relative to elevation below mean high tide (MHT): high marsh (< 25 cm), low marsh (25–45 cm) and creek bank (> 45 cm). The tide has a maximum excursion of 1·5 m. The high marsh is dominated by *Spartina patens* (Aitch.) Muhl., *Distichlis spicata* (L.) Greene and the short form of *Spartina alterniflora*. The low marsh is typically dominated by *Salicornia virginica* L. and the short form of *S. alterniflora*. Creek bank locations are monotypic stands of tall *S. alterniflora*. In this study, we focused on *S. alterniflora* because it was present throughout the vertical range of the marsh, particularly the area affected by oil from the *Florida*.

TPH CONTENT

Sedimentary oil contents have previously been measured and modelled over a *c.* 300 m^2 area (Reddy *et al.* 2002; Peacock *et al.* 2005). The hydrocarbons in our operationally defined TPH consisted mainly of an unresolved complex mixture (UCM) in the boiling range from *n*-C_{10} to *n*-C_{26} alkanes. Our estimated method detection limit was 0·015 mg of TPH g^{-1} of dry sediment (Reddy *et al.* 2002).

Peacock *et al.* (2005) analysed TPH distributions from multiple sediment cores at Wild Harbor. These data were used in a three-dimensional, isotropic linear interpolation model (MATLAB; The Mathworks Inc.) that calculated the oil content at each point in a three-dimensional grid for which sediment data were unavailable and allowed interpolation of oil content over the area. Unfortunately, this method does not readily provide estimates of uncertainty. From the model results, we could map oil content in Wild Harbor across our study area. This initial model followed similar trends as a second statistical model presented by Peacock *et al.* (2005) that assumed that TPH content was homogeneous within 1 m^{-1} and found that lnTPH content declined linearly from the most contaminated region. The model extrapolation meant that we could relate our data on vegetation directly to a pinpointed location within the marsh and the location's modelled oil content.

ABOVE-GROUND BIOMASS

To assess whether oiled and non-oiled sites differed in above-ground biomass of *S. alterniflora*, we harvested 0·04-m^2 quadrats at the end of the growing season (late September–early October) in 2005 and 2006. Each year, replicate quadrats ($n = 4$) were randomly selected from areas with varying TPH content [low (< 50 g m^{-2}), intermediate (50–200 g m^{-2}) and high (> 200 g m^{-2})] within three zones below MHT [high marsh (< 25 cm), low marsh (25–45 cm) and creek bank (> 45 cm)] in oiled areas on the west side of Wild Harbor. The high marsh sediments in the 'oiled' portion of Wild Harbor did not contain high amounts of oil (Peacock *et al.* 2005) but allowed comparisons within Wild Harbor. Replicate quadrats ($n = 4$) were also randomly sampled within each of the three zones in non-oiled areas of Wild Harbor and within Great Sippewissett salt marsh. The material collected was separated into live material and dead matter and sorted into species. The number of stems of each species was counted per quadrat. All dead and live material was then dried at 60 °C for 5–7 days and weighed.

BELOW-GROUND BIOMASS

We measured live root and rhizome biomass in oiled and non-oiled sites. Below-ground plant tissues were sampled in July (2005 and 2006) by collecting sediments with a plastic corer (6·0-cm diameter). The cores extended 30+ cm deep, about the extent of root penetration (Valiela & Teal 1974). Replicate cores were taken from areas of varying oil content in low marsh areas on the west side of Wild Harbor: low, intermediate, high and none. The cores were split in half vertically and then sectioned horizontally at 5-cm intervals. The sections were thoroughly salt water-washed in a 0·5-mm mesh sieve to remove inorganic matter, and the roots, rhizomes and dead vegetation were separated (Valiela, Teal & Persson 1976). Roots and rhizomes were considered 'live' if their tissues were pearl-white, translucent and fairly rigid. Dead material was flaccid and grey in colour. Once separated, tissues were dried at 60 °C for 5–7 days and weighed.

BIOSTIMULATION EXPERIMENT

Plots of salt marsh vegetation in areas oiled to an intermediate degree ($n = 3$) on the east side of Wild Harbor, along with a control plot in a non-oiled area, were fertilized with N-P-K fertilizer (Espoma Plant-tone, Millville, New Jersey; 5% N, 3% P_2O_5, 3% K_2O) to establish if we could stimulate the growth of below-ground biomass (Valiela, Teal & Persson 1976) into oiled sediments. Fertilizer was broadcast during low tide every 2 weeks from June 2004 until September 2006 at rates of 150 g m^{-2}. Plots were sampled for below-ground plant tissue at the end of the growing season (late September 2005 and 2006).

INFILTRATION RATE

To define whether oiled sediments led to increased salt marsh erosion, we examined infiltration rates as well as topography and elevation of the marsh surface. We considered whether sediments that allow fast infiltration might have less of a matrix of stabilizing roots and rhizomes. To do this we examined differences in a modified water infiltration rate within sediment cores from areas with different oil content (high, intermediate, low and none), as predicted from the Peacock *et al.* (2005) model. We took cores with a 6-cm diameter plastic corer to a depth of 20-cm, during low tides in July 2006. And immediately transported them to the laboratory, where we placed the cores upright and recorded the time required for 250 mL of DI water to pass through them.

To see if the addition of nitrogen increased biomass and hence decreased water exchange, we also obtained cores from oiled (intermediate content) and non-oiled fertilized plots and measured biomass and infiltration rate as described above.

ELEVATION MEASUREMENTS

To account for elevational influences on *S. alterniflora* biomass, we measured the elevation of each quadrat and core location using water as a levelling device (Evgenidou & Valiela 2002). This method provides detailed surveys of topography and provides data accurate to within 1 cm. Specific elevations for each data point were related to measurements of tide height relative to a tide stake. The tide stakes were used to measure tides at the new and full moons to determine the MHT for each location during the study period.

To determine if the changes in below-ground biomass affected the elevation of the marsh sediment surface, we produced and compared detailed topographic maps of oiled (west side) and non-oiled areas of Wild Harbor using MATLAB software, with detailed elevational data obtained over a period of 3 months (November 2006–January 2007).

DATA ANALYSES

Analysis of covariance (ANCOVA), *t*-tests and regression (Microsoft Excel X and SPSS version 11·0·4) were used to test the hypotheses related to changes in biomass with changes in oil content. All data were examined to see if they met underlying assumptions. F_{reg} indicates the *F*-statistic from the regression analysis while F_s indicates the *F*-statistic from the ANCOVA analysis. In Figs 2 and 4 the non-linear regression used was the best fit for the data. In Fig. 3a,b, biomass measurements were compared by treatment within each depth using a *t*-test following examination for normality and equality of variances. Comparison of relationships of below-ground biomass in fertilized and non-fertilized plots (S2) were done by using a *t*-test to compare the slope of the two calculated regression lines to the 1 : 1 slope (Steel & Torrie 1960; Zar 1984; Sokal & Rohlf 1995). Total below-ground biomass means were compared (Fig. 4 inset) using a *t*-test and infiltration rate data were analysed using a regression analysis.

The Wild Harbor site is unique in being subject to a spill of a particular oil and in having a 39-year history of repeated study. There is no possible similarly treated site to use as a replicate. We must add that in our view there is great value in the comparisons

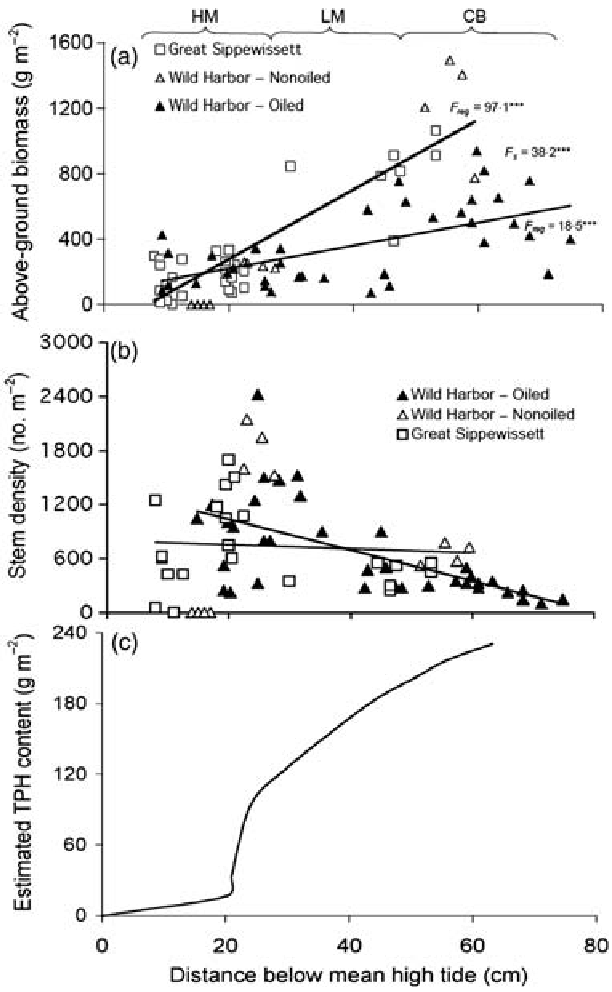

Fig. 1. *Spartina alterniflora* biomass (g m^{-2}) in Wild Harbor and Great Sippewissett marshes in relation to elevation below mean high tide (MHT) in three zones: high marsh (HM), low marsh (LM) and creek bank (CB). (a) Above-ground biomass of *S. alterniflora*; (b) *S. alterniflora* stem density; (c) estimated mean oil content (g m^{-2}) in the oiled Wild Harbor area, within the top 24 cm of sediment as modelled by Peacock *et al.* (2005). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

made. In spite of statistical issues based on unreplicated contrasts, such comparisons often provide powerful results, for example the Rothamsted plots (Leigh & Johnston 1994), the Hubbard Brook deforestation experiments (Trimble 1955) and the iron-fertilization experiments of IronEx (Martin *et al.* 2002), all of which were unreplicated. We have estimates of uncertainty in this study that permit comparisons within the site we studied and, we believe, are of interest as these sites are representative of many other circumstances.

Results

ABOVE-GROUND MEASUREMENTS

Biomass

Spartina alterniflora predominated in the low marsh and creek bank areas. There was a mixture of *S. alterniflora*, *S. patens* and *D. spicata* in the high marsh and upper reaches of the low marsh. Oil content in these areas, was very low (Peacock *et al.* 2005) therefore we did not compare these other species for oil effects.

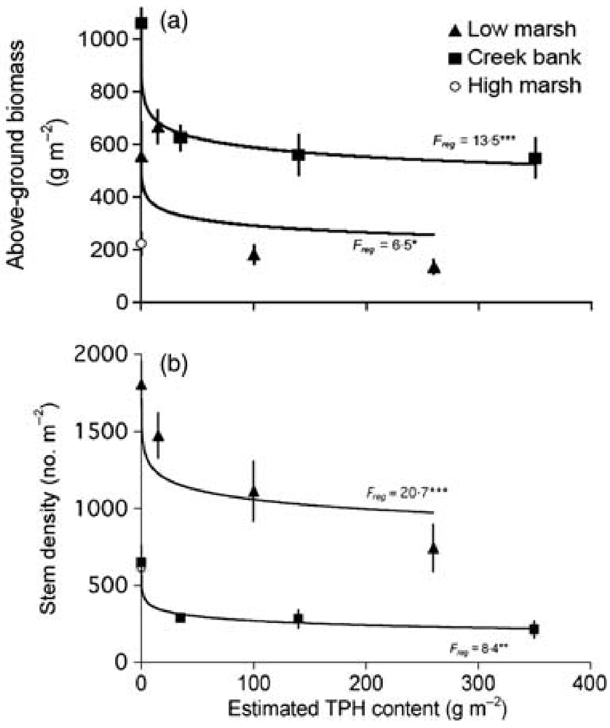

Fig. 2. *Spartina alterniflora* biomass (g m^{-2}) in Wild Harbor and Great Sippewissett marshes compared with estimated content of TPH (g oil m^{-2}) using modelled data (Peacock *et al.* 2005). (a) Above-ground biomass of *S. alterniflora* and (b) stem density of *S. alterniflora*. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

The above-ground biomass of *S. alterniflora* increased from high to low elevations in both marshes (Fig. 1a). The oiled site supported a significantly lower biomass of *S. alterniflora* than non-oiled sites, whether in Wild Harbor itself or in Great Sippewissett marsh (ANCOVA, $F_s = 38.2$, $P < 0.001$; Fig. 1a). The difference in biomass of *S. alterniflora* was far greater between oiled and non-oiled sites lower in the intertidal, where there was greater TPH content (Fig. 1c). As there was little or no oil in the sediment within the high marsh zone, the effect of oil on above-ground biomass was imperceptible (Fig. 1a). The effect of oil on above-ground biomass was related to increases in TPH content (Fig. 2a) and was evident even at relatively low TPH content (*c.* 25 g m^{-2} TPH).

Stem density

The stem density of *S. alterniflora* did not decrease significantly from high to low elevations in both marshes (Fig. 1b). The difference in stem densities between oiled (mean ± SE, 297 ± 27·3 m^{-2}) and non-oiled (523 ± 54·2 m^{-2}) sites was far greater along the creek bank, which had the greater TPH content (t-stat = −4·31, $P < 0.001$; Fig. 1c). No effect on stem density was seen in higher elevations in Wild Harbor (Fig. 1b). The decreased stem densities were associated with increased TPH content (Fig. 2b) and were evident even at relatively low TPH content (< 50 g m^{-2}).

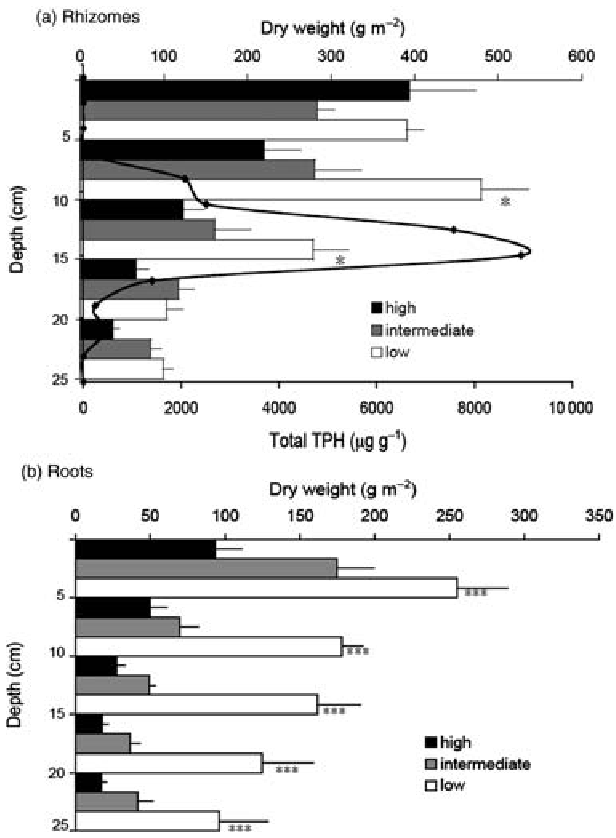

Fig. 3. Below-ground biomass in Wild Harbor. All collections were completed on the west side of Wild Harbor. (a) Total live rhizome biomass in Wild Harbor (g m^{-2}) in oiled areas with different TPH content: high (> 200 g m^{-2}), intermediate (50–200 g m^{-2}) and low (< 50 g m^{-2}). Inset, TPH content (mg g^{-1}) at different depths in a core of sediment from the highest oiled area of Wild Harbor (Reddy *et al.* 2002). (b) Total live root biomass in Wild Harbor (g m^{-2}) in oiled areas of varying TPH content: high, intermediate and low. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$.

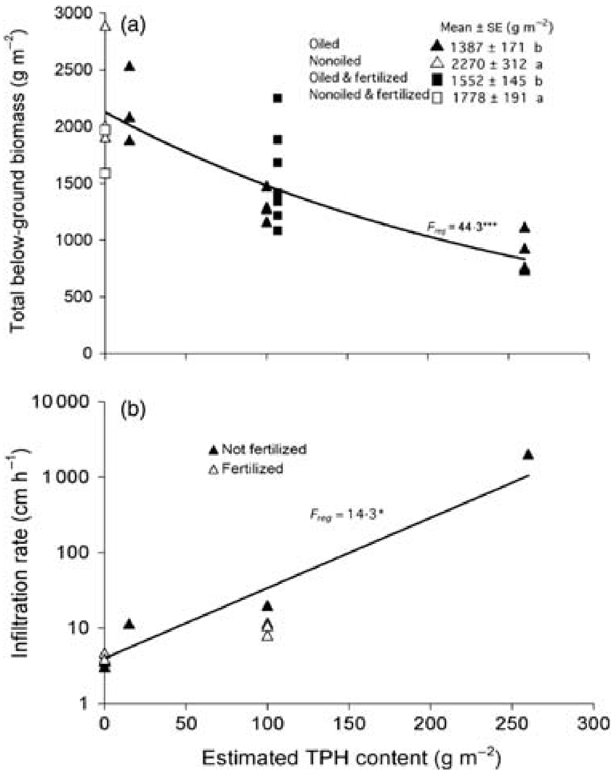

Fig. 4. (a) Total below-ground biomass (roots and rhizomes) (g m^{-2}) compared with estimated TPH content (g oil^{-2}) using modelled data (Peacock *et al.* 2005). Regression was done using all data (F_{reg} = 44·3***); means bearing the same letter (a,b) were not significantly different (*t*-test, *t*-stat = 1·35, $P > 0{\cdot}05$). (b) Infiltration rates (cm h^{-1}) compared with estimated TPH content (g oil m^{-2}) using modelled data (Peacock *et al.* 2005). Regression analysis was done on all non-fertilized data (F_{reg} = 14·3*). *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$.

BELOW-GROUND MEASUREMENTS

Root and rhizome biomass

Rhizome and root biomass decreased with depth in all sites (Fig. 3). We grouped the root and rhizome data into strata of oil content: high (> 200 g m^{-2}), intermediate (50–200 g m^{-2}) and low (< 50 g m^{-2}; Fig. 3) to assess more readily the effects of oil within the depth gradient. Rhizome biomass was significantly lower in the 5–10- and 10–15-cm layer in oiled sites (Fig. 3a), depths at which residual oil was still present (Fig. 3a) (Reddy *et al.* 2002). Fertilization experiments in areas of intermediate oil content significantly increased rhizome growth in the 0–5- and 5–10-cm strata (Fig. 4a; *t*-stat = −2·72 and −2·32, $P < 0{\cdot}01$, $P < 0{\cdot}05$, respectively) (see Appendix S2 in the supplementary material).

Root biomass, in contrast, was lower at all depths in sites that contained oil residues (ANOVA, $F = 3{\cdot}13$, $P < 0{\cdot}01$) (Fig. 3b), including areas oiled to an intermediate degree (Tukey's test, $P < 0{\cdot}001$; Fig. 3b). Fertilization of areas of intermediate oil content did not significantly increase root growth except at a depth of 5–10 cm (*t*-stat = −2·47, $P < 0{\cdot}05$) (see Appendix S2 in the supplementary material).

Below-ground biomass vs. TPH content

Increased TPH content was associated with decreased below-ground biomass even at relatively low levels (100 g m^{-2}; Fig. 4a). Mean total below-ground biomass in all oiled locations (mean ± SE = 1390 ± 171 g m^{-2}) was significantly lower than mean total below-ground biomass in all non-oiled locations (2270 ± 312 g m^{-2}; *t*-stat = 2·2, $P < 0{\cdot}05$). Fertilization did not lead to a significant change in mean biomass within each treatment (Fig. 4a, inset).

INFILTRATION RATE

As might be expected from the lower biomass of roots and rhizomes in oiled sediment layers, the rate of infiltration of water through sediment cores increased in cores with greater oil content (Fig. 4b). These rates differed, from less than 1 min in an area with high oil content to 1 h 45 min in a sediment core from an area with low oil content. While the slope of the line in Fig. 4b appears to be driven by the 260-g m^{-2}

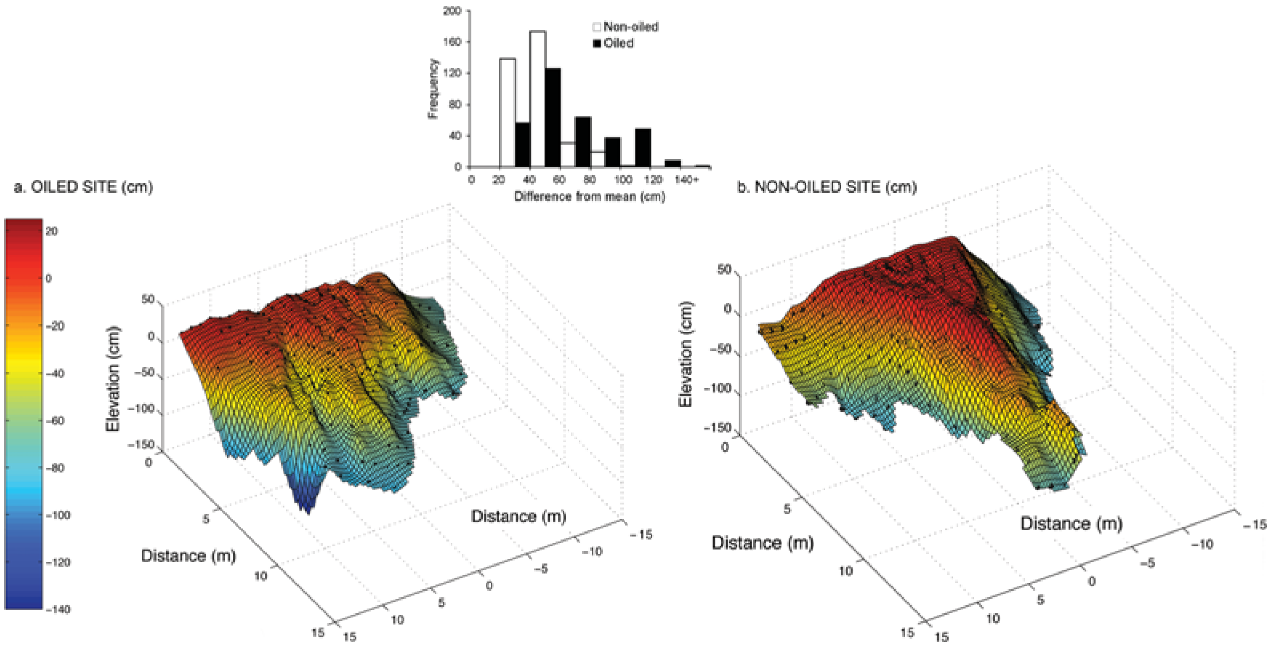

Fig. 5. Topography mapping indicating differences in surface topography in (a) oiled and (b) non-oiled areas of Wild Harbor. Inset, frequency distribution of the deviations from the mean elevation for the oiled and non-oiled sites.

data point, this was not the case. The regression was highly significant (F_{reg} = 99·03***) even if the 260 g m^{-2} data point was excluded.

TOPOGRAPHY

The topographic maps drawn from the elevation measurements in oiled and non-oiled areas of Wild Harbor showed contrasting surface features. The surface of the oiled marsh was far more irregular than the non-oiled marsh (Fig. 5), with evidence of peaks and troughs suggesting erosion and slumping. This was also evident from the distribution of the differences from mean marsh elevation: the mean deviation of the oiled data (46·4 cm) was higher than that of the non-oiled marsh (24·8 cm; Fig. 5, inset).

In addition, the profiles of the mean elevation across transects from the upper low marsh to the tidal channel in oiled and non-oiled areas (Fig. 6) showed that the surface of the marsh in the oiled site was lower than in the non-oiled site. Further, in oiled areas that were lower in the tidal range, slumping modified the creek bank slope, as evident by the increased variability and sediment accretion along the tidal channel (Fig. 6).

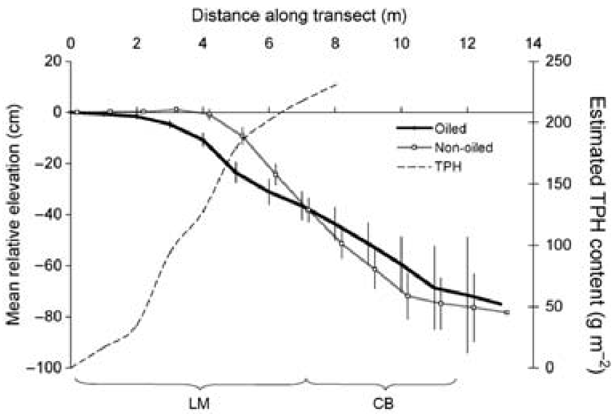

Fig. 6. Elevation and variation along transects from low marsh (LM) to creek bank (CB) in oiled and non-oiled areas of Wild Harbor compared with estimated TPH content (g oil m^{-2}) using modelled data (Peacock *et al.* 2005).

Discussion

The results presented here show decreased above- and below-ground *S. alterniflora* biomass at oiled vs. non-oiled sites, and consequent instability and erosion of sediment related to location and content of residual petroleum. These effects were demonstrable in Wild Harbor after almost 40 years. The effects of the residual oil were quite similar to those reported in previous studies performed over shorter time scales (several months to 5 years; Alexander & Webb 1987; Bergen *et al.* 2000; Lin *et al.* 2002). The effects we present follow the results reported from Wild Harbor following the spill, which found very little living plant matter in areas with greater than 1000–2000 mg oil g^{-1} sediment (Burns & Teal 1979). Our study also provides field confirmation of the effects of fuel oil on salt marsh grass previously demonstrated in laboratory studies (Lin *et al.* 2002).

Examination of total below-ground biomass indicated a significant response in plant tissue to the residual oil. Root tissue showed a more dramatic negative growth response to oil than rhizome tissue. This difference is to be expected, to some extent, as root tissue is involved in active uptake with the surrounding environment, unlike that of rhizomes, which are primarily for vegetative reproduction (Stern 2006). Biostimulation of root and rhizome tissues with fertilization was not successful at depth. However, in other spill areas with a shallower oiled layer, a decrease in rhizome biomass may be offset by the application of fertilizer (Lin & Mendelssohn 1998). Increases in rhizome biomass may lead to increased aeration of the salt marsh sediments, thereby facilitating increased bioremediation of the oiled sediments through aerobic microbial activity. The bioremediated decrease in oil content may then facilitate increased root growth; root growth is more sensitive to oil residues in salt marsh sediments.

The natural accretion of salt marsh sediments may be altered by a number of factors: alteration of sediment supply, hydrological characteristics and organic matter input (Chmura & Hung 2004). The first two factors have not occurred at the Wild Harbor study site. There is a minimum threshold of organic matter accumulation that is critical in order for a salt marsh to maintain vertical accretion rates in step with relative sea level rise (McCaffrey & Thomson 1980; Callaway, DeLaune & Patrick 1997; Turner, Swenson & Milan 2000). This threshold may not be met in the wild harbor oiled salt marsh sediments.

In Wild Harbor, salt marsh erosion was evident along the creek banks in oiled areas, and our data suggest that the reduction of below-ground biomass led to increased topographical variation and a lower marsh surface. These results are supported by the work of Peacock (2007) that showed that the loss of grass cover and increased erosion found at another marsh area subsequent to a 33-year-old spill yielded results similar to those reported here. Loss of salt marsh grass biomass results in the alteration of sediment accretion rates (led by sediment trapping of *S. alterniflora*). These decreases in sediment accretion may lead to an inability of the marsh to maintain critical elevations with the increasing pressure of relative sea level rise. Furthermore, the loss of vegetation may lead to a decrease in the interception of land-derived nutrients and in the export of carbon to coastal systems. Four decades after the *Florida* spill, with only 100 kg of the original 595 000 kg of oil spilt still persisting in marsh sediment, the effects on large-scale ecosystem functions are still evident. Similar long-term effects have been reported in other sites exposed to residual petroleum (Peterson *et al*. 2003).

Recovery from oil spills depends on the spill type, on the post-spill history and on characteristics of the specific environment (Baker 1999). In anoxic accreting salt marshes that have been exposed to spilt oil, the residual undegraded biologically active oil may last for many decades, with the potential for chronic lingering effects. The results presented here illustrate some potential for the rehabilitation of oiled coastal areas with addition of surface nutrients. These nutrient additions may lead to increased soil aeration and facilitate increases in plant biomass and sediment stabilization. Further, our examination of above- and below-ground biomass vs. TPH content provide a useful threshold for evaluating potential biological impacts for *S. alterniflora*. Overall, our results provide potential indications for salt marsh health following long-term exposure to spilt petroleum that may be used to determine the consequences for the parties involved. We have shown the long-term effects of these recalcitrant contaminants on salt marsh grasses and sediments. These long-term effects need to be included in considerations of the overall consequences and management of petroleum spills.

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Supplementary material

The following supplementary material is available for this article.

Figure S1. Map of study sites.

Figure S2. Below-ground growth response of salt marsh vegetation.

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