Comparison of replanting tactics after a simulated marine diesel fuel spill in estuarine mesocosms

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ABSTRACT

Coastal marshlands are ecologically critical areas that provide essential food, refuge, and nursery habitat. They are highly sensitive to oil spills and exceedingly difficult to clean up. Many of the techniques used to clean oiled shorelines can cause additional damage in marshlands and are not viable treatment options in these sensitive environments. During the *Deepwater Horizon* (DWH) oil spill, a wide variety of clean-up and primary restoration tactics were investigated in the most heavily impacted coastal marshes of Louisiana, USA. Subsequent monitoring revealed that one of the most beneficial tactics employed was to replant native grasses in the impacted areas. The objective of this study is to determine what combination of marsh grass (*Spartina alterniflora,* smooth cordgrass) replanting tactics produces the best outcome for a marine diesel fuel oiled marsh in a controlled setting. The study consisted of 20 oiled mesocosms (approximately 450 L tanks with simulated tidal flux) with four treatments (control mesocosms with no oil and no replanting, oiled mesocosms with no replanting, oiled mesocosms replanted with local field transplants, and oiled mesocosms replanted with nursery stock) with 5 replicates each. Replanting tactics tested also included containerized/plug plantings vs. bare-root plantings. The oiled and dead vegetation was cut and removed from the mesocosms that later received replanted *S. alterniflora*. Marsh replanting success was followed over 9 months. Data presented

include oil effects on the original marsh prior to replanting, hydrocarbon residues in water and sediments over time, and measurements of the structure and growth of the replanted grasses (stem density, shoot height, above-ground biomass, and below-ground biomass). An initial replanting effort was attempted 2 months after the oiling event. All the replanted *S. alterniflora* from the initial replanting event died, likely due to residual diesel that remained in the mesocosms and was still toxic. A successful replanting effort occurred 8 months after the oiling event and preliminary results indicate that plugs containing plants with sediment performed well and similarly between local field transplants and nursery-grown material, especially considering aboveground biomass at 9 months post-planting, although values were still well below reference conditions. Bare root nursery material failed overall, and bare root field transplants had intermediate results. Oiled marsh that was not replanted showed no aboveground recovery. This data will help inform future restoration efforts that are considering using replanting as a tactic for restoring an oil-contaminated saltmarsh.

INTRODUCTION

Coastal marshlands provide essential food, refuge and nursery habitat for many ecologically, recreationally, and commercially important species (Vernberg, 1993). Southeastern United States saltmarsh vegetation is frequently dominated by saltmarsh cordgrass, *Spartina alterniflora* which is vital to the marsh ecosystem (Day et al., 1989; Vernberg, 1993). Petroleum, in the form of oil spills, is a significant source of pollution in marine environments (NRC, 1985) and more specifically in coastal areas (Vikas and Dwarakish, 2015). Many of the techniques used to clean other types of oiled shorelines, such as manual or mechanical removal, can cause additional damage in marsh ecosystems and are often not viable treatment options in these

sensitive environments (Pezeshki et al., 2000; Pietroski et al., 2015). During the *Deepwater Horizon* oil spill, a wide variety of clean-up tactics in the most heavily impacted coastal marshes in Louisiana were investigated and implemented (Zengel et al., 2021). Subsequent monitoring and investigations revealed that one of the most beneficial tactics employed was to replant native marsh grasses in the impacted areas (Bernik et al., 2021; Zengel et al., 2021). While this tactic shows potential, standard methods for replanting as an oil spill response treatment have not been defined or optimized.

The current study utilized NOAA's marsh mesocosm facility in Charleston, SC to simulate an oiled coastal marsh. The oiled mesocosms were replanted with selected treatments and their recovery was followed over a 9-month period. This project sought to assess the recovery of structure and function of replanted marsh grasses and compare different planting treatments relative to unoiled reference conditions. The initial round of experiments compared the relative viability of replanting using local, field-collected transplants vs. commercially available grasses obtained from regional nurseries. The clean-up tactics employed were: (1) leaving the oiled marsh vegetation intact, without cleanup treatments or replanting (natural recovery), (2) cutting and removing the oiled and dead marsh vegetation and replanting with nursery or field-collected marsh grass.

Following oiling and the removal of dead plants, we quantified how replanting impacts the recovery of vegetation structure and function compared to oiled controls (no replanting) and reference/unoiled conditions. We examined how clean-up and replanting scenarios influenced weathering and degradation of oil in marsh surface waters and marsh sediments. Samples were collected and analyzed for residual PAHs (polycyclic aromatic hydrocarbons) and TEH (total

extractable hydrocarbons). The results of this study will provide input for the use of vegetative replanting as an oil spill response tactic. Contributing to best practices for marsh replanting will reduce the long-term loss or damage to salt marshes and accelerate recovery time for these critical environments.

METHODS

Mesocosm set up

Each mesocosm system consisted of two tanks, one upper and one lower in accordance with procedures outlined in Pennington et al. (2007) with further modifications (Key et al., 2014) to accommodate working with oil slicks. The 20 systems used in this study were enclosed in a greenhouse, which incorporated natural light and temperature conditions. The lower tank, or sump, provided tidal water to the upper tank via a pump set to a timer. Twice daily seawater (25 psu) was pumped into the upper tank (mesocosm) from the lower tank (sump) to simulate the typical SE USA diurnal ebb/flood tide. The seawater was dispensed into the mesocosm tanks (443 L each). A PVC pipe was installed in each tank to allow for water sample collection and water quality measurements to be taken without contact with the surface oil slick.

Sediments were also added to the mesocosms prior to dosing. Intertidal sediments were collected for each mesocosm from Leadenwah Creek, Wadmalaw Island, SC (32º 38.848’ N, 080º 13.283’ W). Specifically, the sediments were collected from the mud flat at low-tide within 2-3 m of the lower edge of the creek adjacent to marsh grass (*S. alterniflora*) stands. Using a shovel, the top 2-4 cm of sediment from the mud flat were removed and placed into clean five-gallon plastic buckets. The sediments were sieved, at the mesocosm facility, through a coarse sieve (3 mm) to remove larger benthic fauna and other debris and then placed into the plastic

mesocosm sediment trays (20 cm x 20 cm x 20 cm depth) until slightly overflowing (approximately 12.75 kg of mud per tray) according to Pennington et al. (2007). Sediment trays were filled and placed randomly into each of the 20 mesocosm systems (6 trays of sediment per system). Sediment tray surfaces were completely submerged at high tide and exposed at low tide. This allowed them to drain from the bottom at low tide to simulate tidal pumping and sediment drainage (Pennington et al., 2007).

Ten days following the sediment collections, *S. alterniflora* marsh grass plugs with sediment (7 cm in diameter) were collected from the same site using a common garden auger and planted randomly into the sediment trays in the mesocosms. Two plugs were placed into each of the six *S. alterniflora* sediment trays. *Spartina alterniflora* was allowed to grow in the tank system 45 d before the addition of other species. The 20 systems then remained in a pretesting phase for nearly 12 months.

The 20 mesocosms included 4 treatments, with 5 replicates each. The treatments were 1) CTL: Control (no oil, unaltered, reference), 2) TRT A: Oil (oiled, no cutting or replanting), 3) TRT B: Oil LT (oiled, cut and replanted with local field transplants), 4) TRT C: Oil NP (oiled, cut and replanted with nursery stock). Local field transplants were collected from the same site as the original source plants. One year old nursery plants were purchased from a commercial nursery located in Maryland that specializes in growing wetland plants for restoration projects. The nursery plants were grown from wild-collected seeds from the Chesapeake Bay area of Maryland. The seedlings were grown in commercially available potting soil and amended with slow-release fertilizer. They were allowed to grow for any entire year which included an over-winter period. For each replicate of TRT B & C, half of the trays were planted as whole plugs

(with intact sediments either from the field or from the nursery plugs) and the other half were bare root plantings. Bare root plants were obtained by washing away sediment or soil from field and nursery plugs with copious amounts of tap water.

Water quality parameters (temperature, salinity, pH, and dissolved oxygen) were taken daily using hand-held instruments. In addition, each mesocosm treatment had one tank containing a multi-parameter probe for continuous water quality measurements of the same parameters.

Oiling Event

A nominal 3-mm slick of marine diesel fuel oil was targeted based on the area of the upper tank. Marine diesel was added (three aliquots of 732 mL) to each system over a 24-h period, for a total of 2,196 mL. This dose was selected based on preliminary experiments that determined the amount of diesel needed to cause complete mortality of *S. alterniflora* in the mesocosms. The marine diesel was added to the mesocosms on June 7-8, 2021 (Figure 1). Simulated fiddler crab burrows were added to all treatments (4/tray) prior to the addition of oil, to enhance oil penetration into the sediment and root zone at low tide for the oiled treatments.

Vegetation Cutting and Marsh Replanting

Four weeks after dosing, the original vegetation in TRT B: Oil LT and TRT C: Oil NP was cut by hand using a razor blade at the <0.5 cm above the sediment surface and removed. No sediments or below-ground biomass were removed. An initial replanting occurred 8 weeks after dosing in those same treatments (Figure 1). CTL and TRT A were left unaltered. Replanted systems, TRTs B & C, received field transplants and nursery plants, respectively. Within each system, three of the trays received plug plantings and the other three received and bare-root

plantings, two per tray. That replanting effort was not successful and all of the replanted vegetation died because the oil levels were still too high in the systems (rainbow sheens were still present on the water surface). After actively removing the sheens from the water surface and allowing more time for oil degradation on and in the marsh sediments, a second replanting effort was attempted and was largely successful 8 months after dosing (Figure 1). The growth of marsh grass (*S. alterniflora*) was monitored for 9 months beyond the 2nd replanting effort. Replanting success was determined by measuring stem density (# of individual living stems/m^2), stem height (cm), above-ground biomass (g/cm^2) and below-ground biomass (g/cm^3) after 9 months.

Plant stem density was measured by directly counting the number of stems in each mesocosm, and converting these to m^2 basis. Stem height was determined by measuring the tallest blade from each stem using a meter stick to the nearest 1.0 cm. The living *S. alterniflora* in the mesocosms were measured for stem density and stem height prior to dosing, post oil-dose (5wk, 8wk, and 6mo), and post-2nd replanting (0h, 28d, 8wk, 3mo, 6mo, and 9mo). For the purposes of this report only the growth metrics at 9 months post-2nd replanting are presented.

At the end of the 9-month period (November 2022), above-ground and below-ground plant material was harvested, separated, weighed, dried in an oven at 70°C for 7 d, and reweighed to obtain above-ground dry-weight biomass (g/m^2) and below-ground dry-weight biomass (g/m^3), respectively. Only living material was used to determine above-ground biomass. Below-ground biomass consisted of both of live and dead material (rhizomes and roots).

Water and sediment sampling

Water samples for chemistry (polycyclic aromatic hydrocarbons; PAHs and total extractable hydrocarbons; TEH) were collected prior to dosing (n=4; t=-24h), post oil-dose

(t=1h, 12h, 24h, 48h, 72h, 96h, 7d, 14d, 5wk, 8wk, 3mo and 6mo), and post-2nd replanting (0h, 28d, 8wk, 3mo and 6mo). Samples were taken from each replicate mesocosm (n=20) on timepoints 1h – 7d post oil-dose, while composite samples (across replicates) were collected pre-oil dose and post-replanting. The composited samples were necessary to reduce analytical chemistry costs and instrument time. Water samples were collected during the high tide cycle in the upper mesocosm tank through the PVC standpoint to avoid disrupting the oil slick. Samples for chemistry were collected into 1 L glass amber bottles and acidified to pH<2 using hydrochloric acid. Additionally, samples for microbial and nutrient analysis were collected into autoclaved 500 mL Nalgene bottles.

Sediment samples were collected for the same analyses and time points described above during the low tide cycle following water collection while the sediment trays were exposed. The top 1-2 cm of sediment was scraped from one of the sediment trays. The location in the trays for sampling was rotated so that the same areas were not repeatedly sampled during the experiment. Chemistry samples were collected into 4 oz glass jars and samples for microbial analysis were collected into 15 mL polypropylene centrifuge tubes; samples were stored at -80°C until analysis. Hydrocarbons (as polycyclic aromatic hydrocarbons [PAH] and total extractable hydrocarbons [TEH]) were analyzed according to DeLorenzo et al. (2017). PAH data for water and sediment samples are reported as total PAH50 (tPAH50), which is the sum of 50 2-6 ring PAHs and their C1-C4 alkylated derivatives.

Other samples collected

Additional samples and measurements collected through the study included water column chlorophyll a, nutrients (NH_3, NO_2/NO_3, and PO_4), effects on fauna (shrimp and snails), and

microbial community abundance and diversity (analyzed using 16S metagenomic sequencing techniques). The results of those samples are not included in these Proceedings and are still under analysis to be submitted for publication at a later date.

Statistical analysis

Mean *S. alterniflora* measures of stem density, stem height, and biomass at 9 months post-2nd replanting were compared using a split-plot design mixed model ANOVA in SAS 9.4 (PROC MIXED) with post-hoc Dunnett's and all pair-wise comparisons. The alpha for all tests was 0.05.

RESULTS AND DISCISSION

Initial Post-oiling Observations

At low tide the marine diesel contacted and penetrated the marsh sediments and pooled in the simulated fiddler crab burrows. The 3 mm diesel slick resulted in approximately 50% plant chlorosis after 4 days and 90% plant chlorosis after 2 weeks. After 14 days, there was approximately 50% dead stems, and approximately 90% dead stems after 21 days. The measured water column tPAH50 concentration immediately after dosing ranged from 10 to >500 µg/L across the oiled treatments, and remained in the 30-40 ng/mL range after 21 days. The tPAH50 concentrations decreased to 20 ng/mL after 56 days, and continued to decline until stabilizing at <5 ng/mL after 175 days. See below for initial and peak oil concentrations in the sediments.

Oil Weathering and Degradation

Maximum tPAH50 concentrations in the water column, in all oiled treatments, were reached at 96 h, after which tPAH50 concentrations decreased (Figure 2). Average maximum tPAH50 concentrations at 96 h for TRT A, TRT B and TRT C were 39.9 ± 7.4, 42.6 ± 4.0 and

48.9 ± 5.2 ng/mL, respectively. There was a slight increase in tPAH50 in all oiled treatments at 28 d which corresponds with the first *S. alterniflora* harvesting. It was also noted that there was another spike in tPAH50 concentrations after the second replanting. Both of these small increases are likely related to entrained oil in the sediment being released as the harvesting or replanting occurred. Water column TEH (Figure 3) did not follow similar trends. In many of the early timepoints (1 h -96 h), TEH water concentrations were either at or below the method detection limit (MDL=0.005 mg/L). The maximum observed TEH water concentration in all oiled treatments was observed at three months. Maximum TEH concentrations for TRT A, TRT B and TRT C were 0.0067, 0.0076 and 0.151 mg/mL, respectively. This is likely due to the disturbance of the sediments during the first attempt at replanting at 2 months.

For sediment chemistry (Figure 4), maximum tPAH50 concentrations were observed within 24-72 hours with no substantial differences between oiling treatments. In all treatments, tPAH50 declined steeply between 72 and 96 h. As noted with the water tPAH50 concentrations, a spike in sediment concentrations was observed at 28 d, likely related to the initial harvest that was performed. Additionally, there was another tPAH50 spike observed after the second replanting, followed by a gradual reduction in tPAH50 concentration. For TEH sediment concentrations, sediment TEH increased between the initial dose and 48 h, after which TEH concentrations remained stable through 28 d, followed by a gradual decline (Figure 5). As observed with tPAH50, a spike in TEH sediment concentration was observed after the second replanting, likely a result of a disturbance in the sediments during replanting and subsequent re-release of oil that was trapped in the sediment. Observationally, TEH and tPAH50 sediment concentrations were higher in TRT A when compared to TRT B and TRT C (Figures 4 and 5)

after the second replanting. This suggests that replanting after an oil spill can aid in hydrocarbon degradation.

Replanting Success

The first attempt at replanting (8-weeks post-dose) was not successful. All of the plants died likely due to tPAH50 concentrations that were still quite high in the systems (Figure 2 and 4). The second replanting effort (8-months post-dose) was successful and the tPAH50 concentrations were considerably lower (Figure 2 and 4) likely due to natural weathering and partitioning processes.

Stem Density

Nine months post-replanting, CTL systems (no oil, no replanting) had significantly higher stem densities than all other treatments p=0.0004) and TRT A: OIL systems (no replanting) had no recovery of originally planted material (p=0.0001) when compare to controls. TRT B: OIL LT and TRT C: OIL NP were both significantly lower than controls (p=0.003 and p-0.043, respectively). In all pair-wise comparisons, TRT C: OIL NP plug plantings had significantly higher stem densities than bare root plantings in TRTs B or C (p=0.023 and p=0.001, respectively). There was an overall significant difference (p=0.0058) between bare root and plug plantings (Figure 6) with plug plantings having greater stem densities.

Stem Height

In terms of mean stem height, CTL systems (no oil, no replanting) had significantly greater mean stem heights than all other treatments p<0.0001) after nine months. Again, TRT A: OIL systems (no replanting) had no recovery of originally planted material. TRT B: OIL LT and TRT C: OIL NP were both significantly lower than controls (p<0.0001, for each). In all pair-wise

comparisons, TRT B: OIL LT bare root and plug plantings had significantly higher ($p<0.001$, in each case) mean stem heights than bare root and plug plantings in TRT C (Figure 7).

Above-Ground Biomass

For above-ground biomass, CTL systems (no oil, no replanting) had significantly greater mean dry weight biomass (of live material only) than all other treatments $p<0.0001$) after nine months. Again, TRT A: OIL systems (no replanting) had no recovery of originally planted material. TRT B: OIL LT and TRT C: OIL NP were both significantly lower than controls ($p<0.0001$, for each). In all pair-wise comparisons, only TRT C: OIL NP plug plantings had significantly higher ($p=0.0004$) mean dry weight biomass than bare root plantings in TRT C (Figure 8).

Below-Ground Biomass

While CTL systems had the highest dry weight biomass for, there were no significant difference between any of the treatments. This is likely because it was very difficult to distinguish between living versus dead material. Additionally, there was a high degree of variability within and between treatments (Figure 9).

CONCLUSIONS

Salt marshes are threatened by many stressors, including disease, climate change, non-point source runoff, sewage treatment discharges and industrial point sources, and oil spills. The loss of salt marshes endangers entire coastal ecosystems, and establishing effective restoration techniques is essential to coastal resource management. This study compared the response of nursery attained vs. field collected *S. alterniflora* in oiled-marsh restoration. Oiled systems did not recover on their own. In the replanted systems, bare root plantings were not successful for

nursery plants, with plug plantings producing significantly more live stems and greater above-ground biomass. Using local transplants, there was moderate success with the bare root plantings as shown in stem height, but the plug plantings were more successful with regard to greater stem densities and above-ground biomass. Bare root plantings from nursery-sourced plants largely failed. When comparing the plug plantings, nursery-sourced plants had greater stem density and above-ground biomass after nine months of growth. Within the context of this study, local transplants and nursery plugs performed similarly, especially when considering above-ground biomass and that stem density and growth form may have differed from the onset. Bare root local transplants appear to have been less beneficial but perhaps still acceptable, particularly in cases where bare root plantings may be preferred (such as in higher energy settings likely to dislodge plugs or potted material with lower bulk density). The bare root nursery material, at least as supplied and prepped, did not perform well.

This study demonstrated that after a salt marsh ecosystem is heavily oiled, the vegetation can undergo a complete mortality event. Given the transport of oil from water to marsh sediments, toxic levels can persist for weeks to months and impair restoration attempts. This study quantified sediment concentrations of diesel oil that remained toxic to marsh grass and underlines the importance of monitoring sediment concentrations during oil spills and prior to subsequent restoration events. Oiled treatments where the vegetation was left in place had no aboveground recovery or regrowth 19 months post-oiling. Based on the results of this study, the response tactic of replanting would be recommended. Replanting using both nursery plants and field transplants with sediment plugs resulted in new growth with nursery plants producing a somewhat better outcome. The choice of where to obtain plants for a restoration project may

depend on a number of factors, including availability and sensitivity of nearby healthy marsh, time of year, geographic location, amount of planting material needed, permitting considerations, nursery availability and productivity, concerns with introducing differing regional plant genotypes and phenotypes, along with personnel and transport costs.

ACKNOWLEDGEMENTS

The authors would like to thank the following for their assistance with this project: Pete Key, Joe Wade, Tom Greig (NOAA/NOS/NCCOS), Blaine West, Grant Burdine, Jonathan Stewart, Brian Shaddrix, Elizabeth Harris, Allisan Beck (CSS Inc. contracted to NOAA/NOS/NCCOS), Nicolle Rutherford (NOAA/NOS/ORR), and Jacqui Michel (RPI contracted to NOAA/NOS/ORR). Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

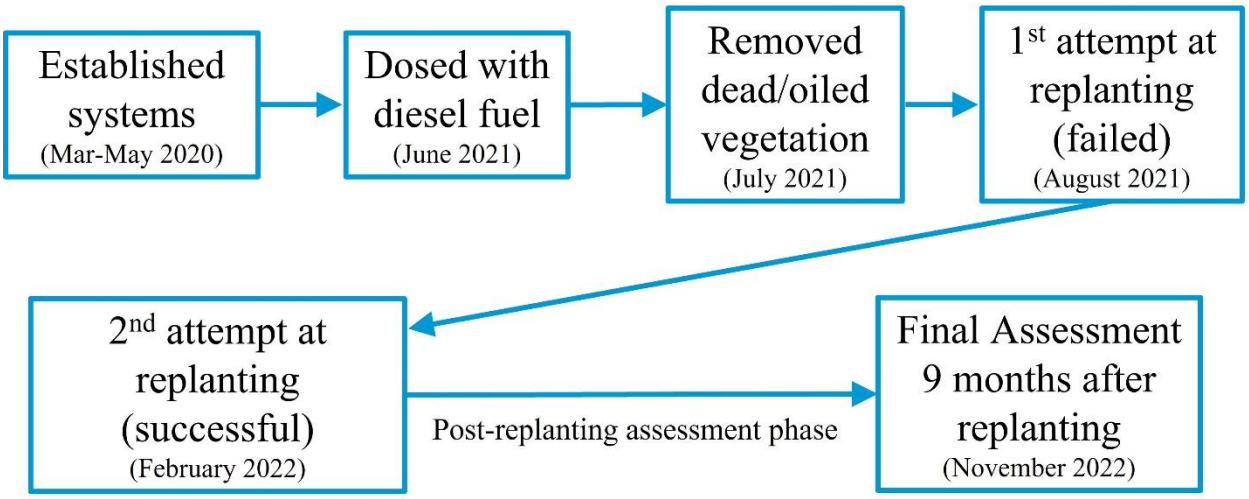

Figure 1. Mesocosm study timeline from March of 2020 to November of 2022

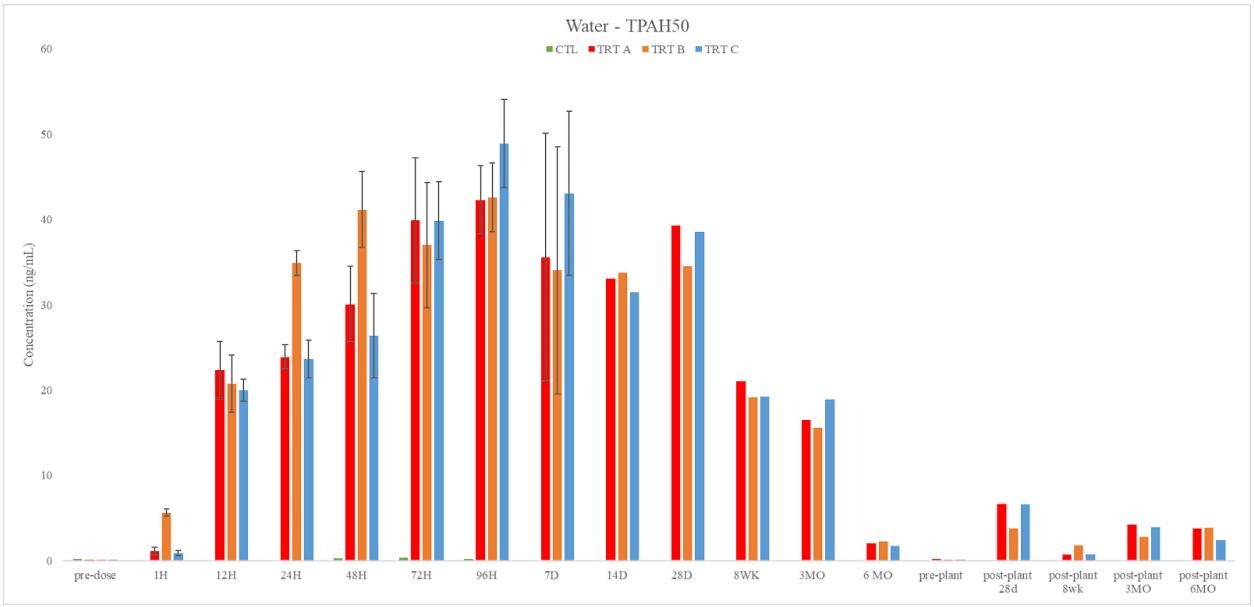

Figure 2. tPAH50 water concentrations (in ng/mL); (mean + SD). Single, composited samples were used after the 7-day timepoint.

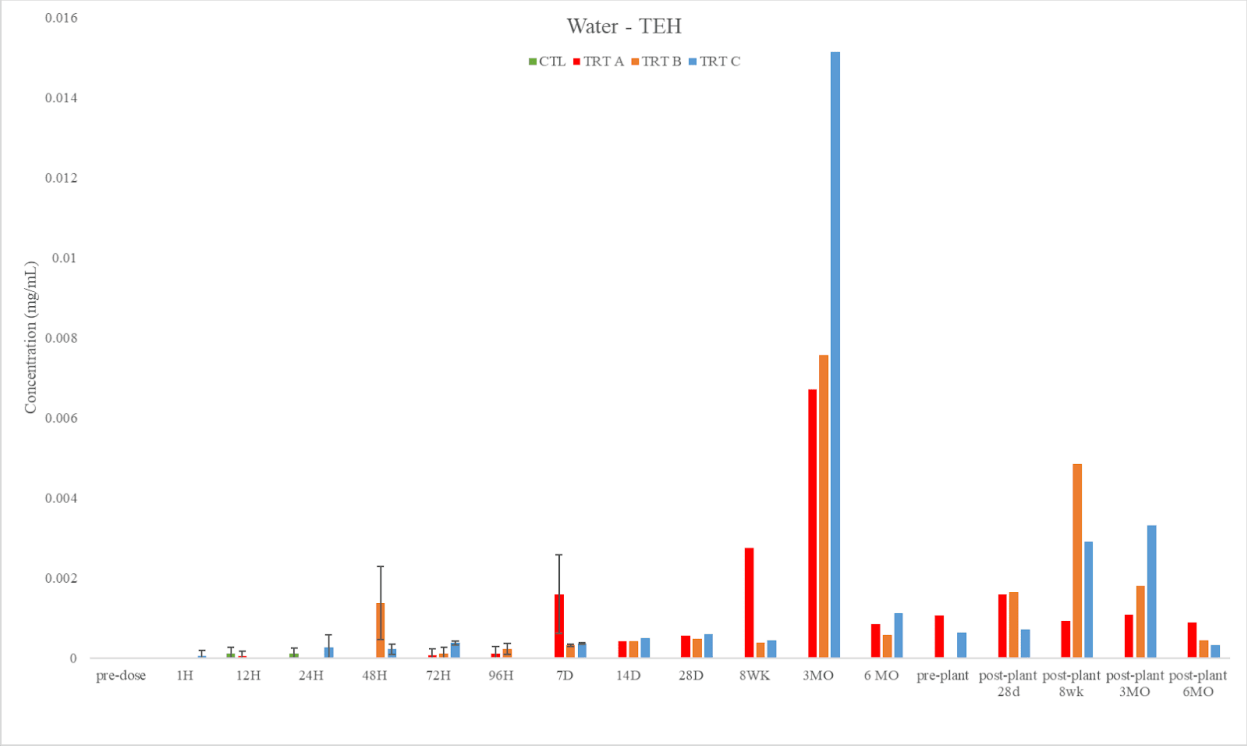

Figure 3. TEH water concentrations (in mg/mL); (mean + SD). Single, composited samples were used after the 7-day timepoint.

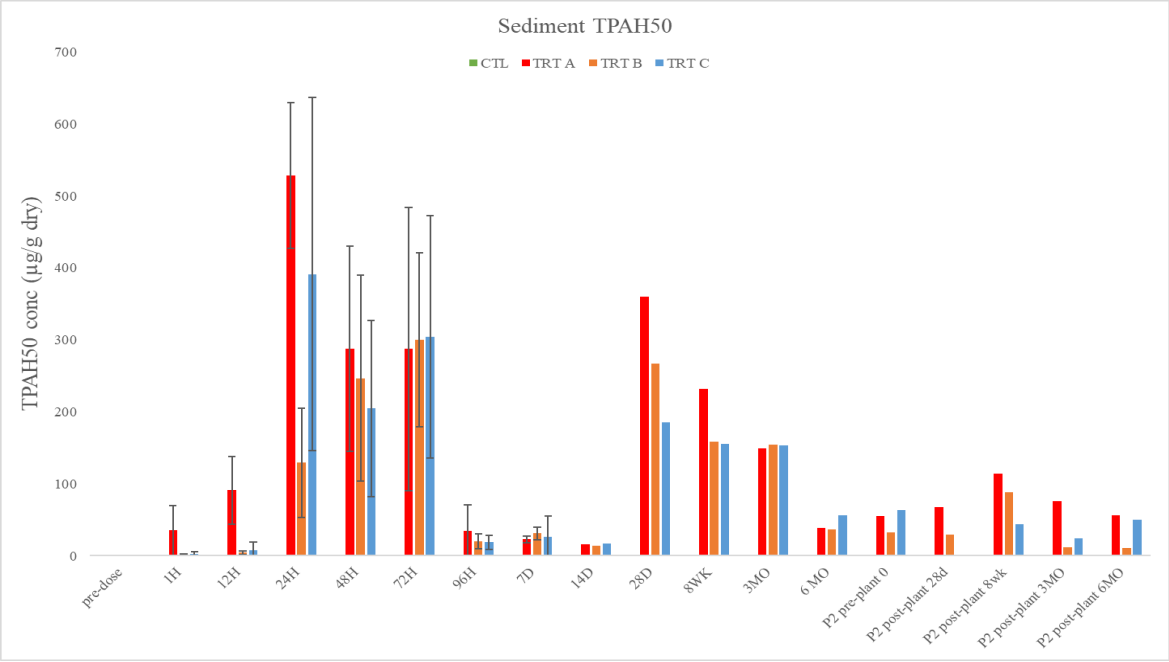

Figure 4. tPAH50 sediment concentrations (in µg/g); (mean + SD). Single, composited samples were used after the 7-day timepoint.

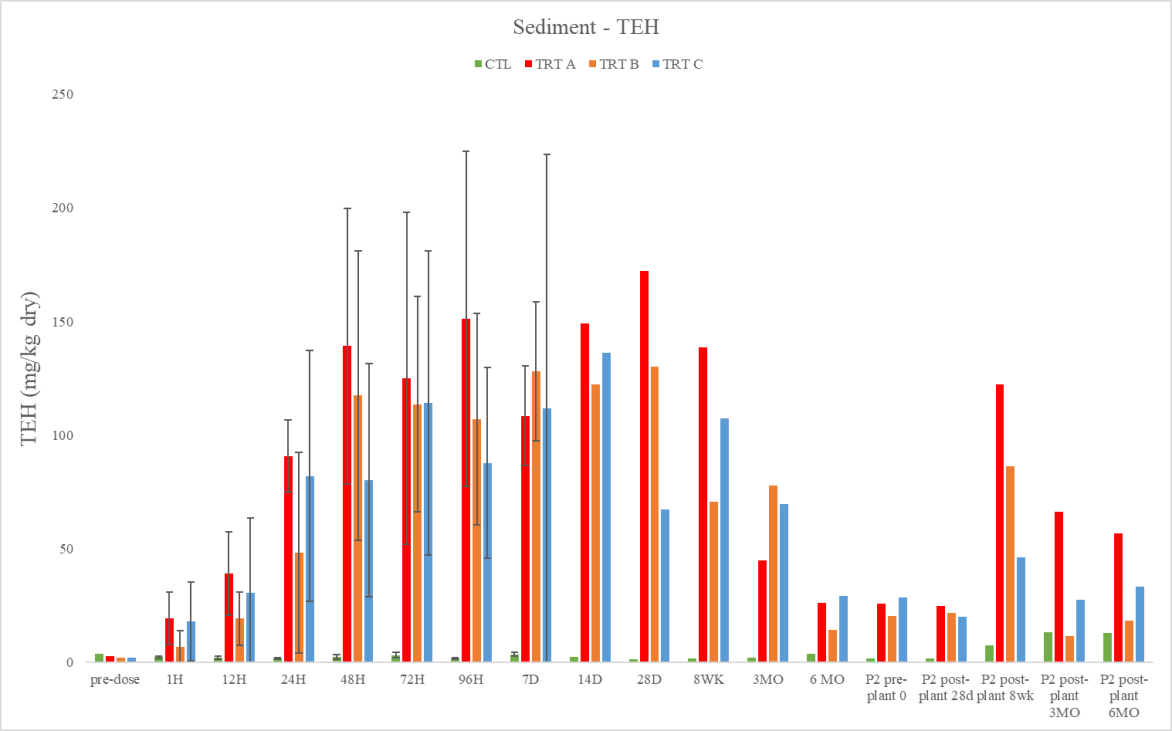

Figure 5. TEH sediment concentrations (in mg/kg); (mean + SD). Single, composited samples were used after the 7-day timepoint.

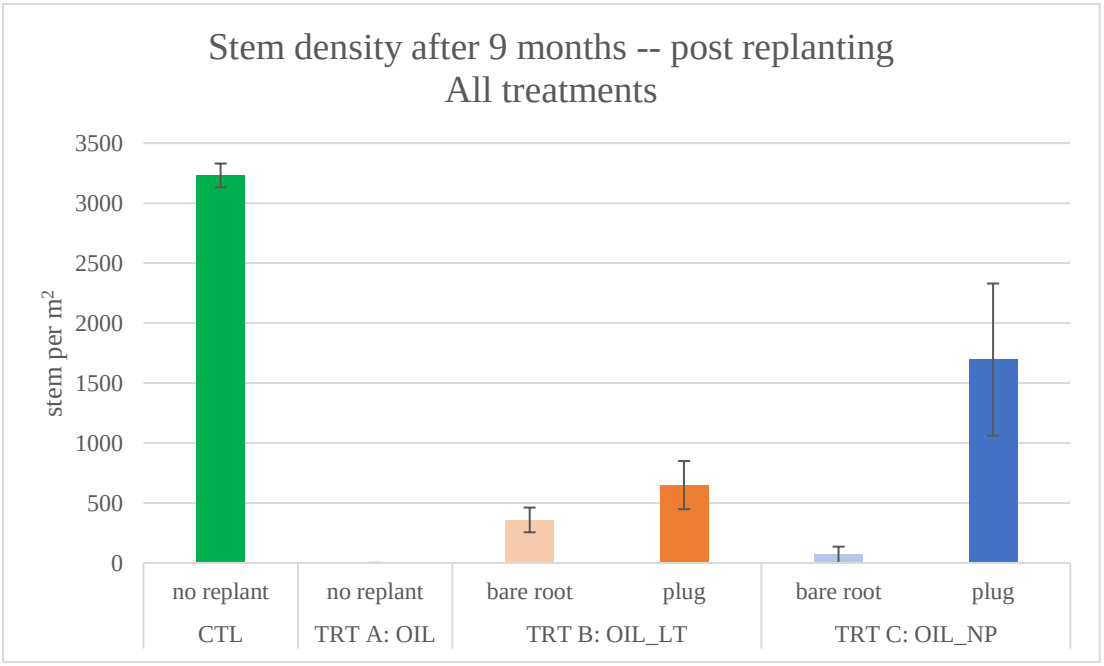

Figure 6. *Spartina alterniflora* stem density 9 months post-replanting (mean ± SD)

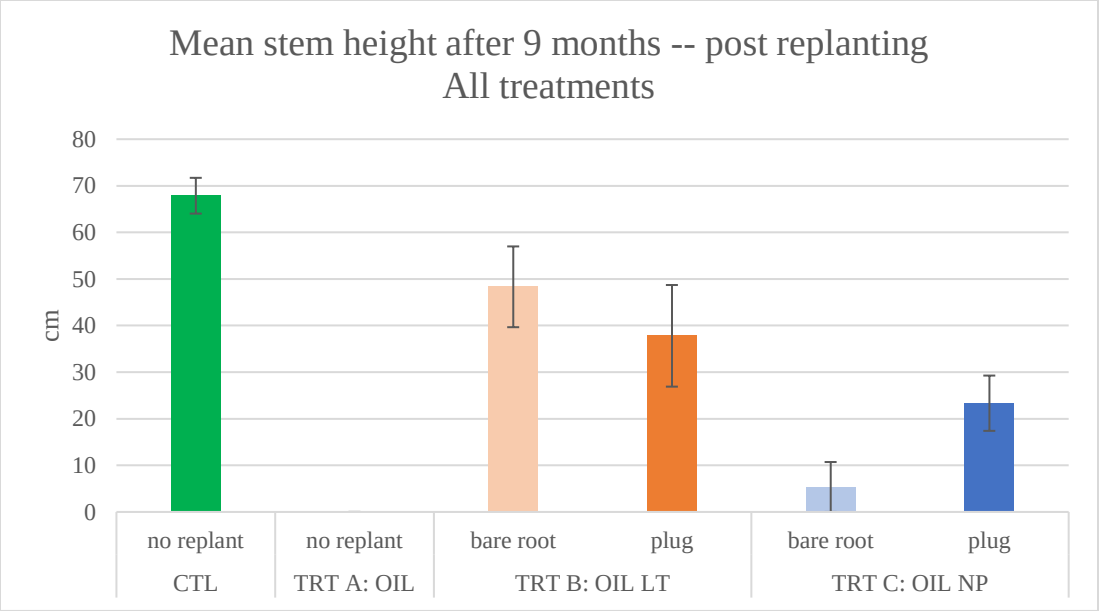

Figure 7. *Spartina alterniflora* mean stem height 9 months post-replanting (mean ± SD)

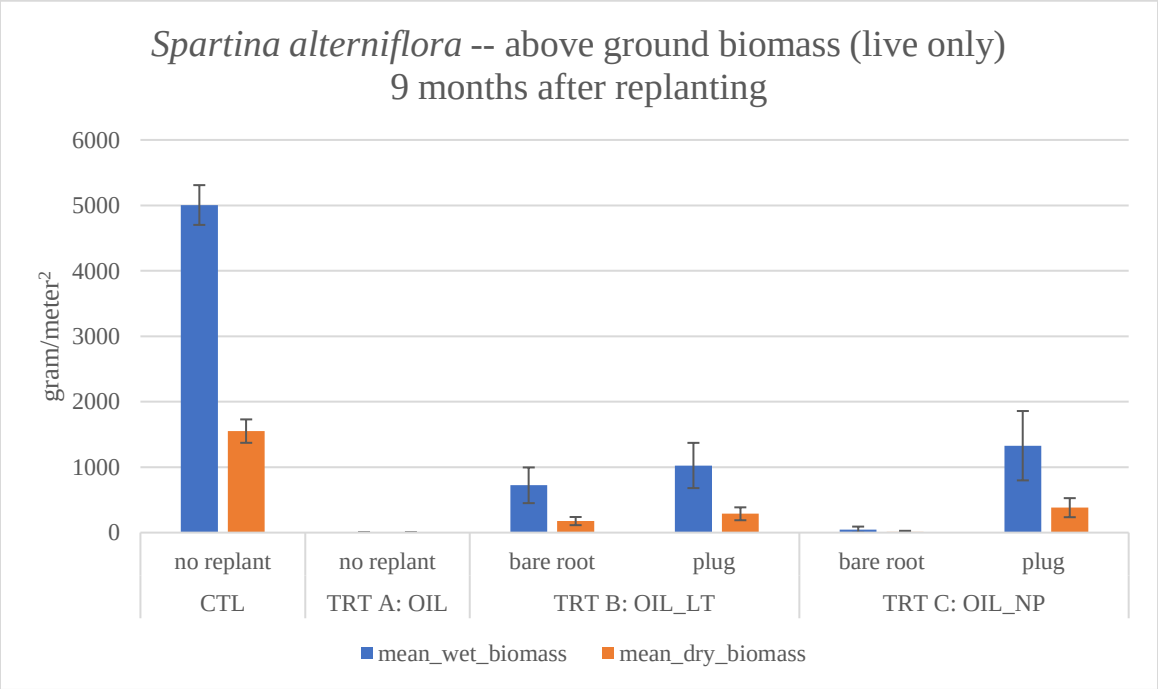

Figure 8. *Spartina alterniflora* above-ground biomass (live only) 9 months post-replanting (mean ± SD)

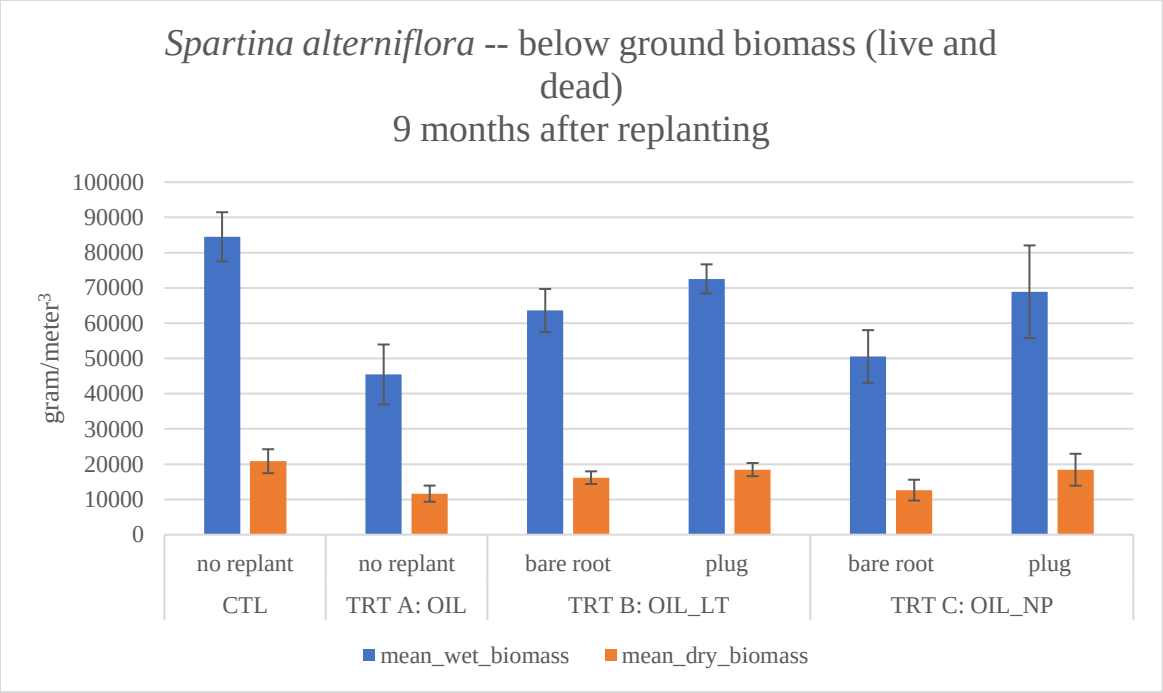

Figure 9. *Spartina alterniflora* below-ground biomass (live+dead) 9 months post-replanting (mean ± SD)

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