Alaska Crude Oil and Diesel Effects on California *Spartina foliosa* and *Spartina foliosa x alterniflora* Hybrid Cordgrass

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ABSTRACT

As industrial processes with oil continue to take place in sensitive ecosystems such as the San Francisco Bay Area, it is important to understand how potential petroleum accidents and spills would affect the local vegetation. In northern California, the exotic *Spartina alterniflora* cordgrass have been hybridizing with the native *Spartina foliosa* coastal marsh communities. The objectives of this ten-week greenhouse study were to determine (1) how Alaska crude oil and diesel affects survival and new plant growth, and (2) whether *Spartina* cordgrass accumulate polycyclic aromatic hydrocarbons and other oil constituents in their tissues. The control group yielded the highest survival rate of 75%, followed by 60% survival in the crude oil group, and only 15% survival for the diesel treatment, possibly because its refined nature and low-molecular weight make it more bioavailable to the plant. Of the plants that survived, those in the control and crude-oil group grew significantly more than those in the diesel group. I also found that the cordgrass uptake more petroleum constituents from the diesel than from the crude oil. The results, within the confines of this experiment, suggest that while both crude oil and diesel negatively affect survival and growth, diesel has a more detrimental effect, and the native *S.foliosa* cordgrass are more severely affected by the treatments than the hybrid genotype. Further studies on how *Spartina* deals with hydrocarbons will elucidate this cordgrass' potential use as a phytoremediation tool in oil-contaminated soils and marshes.

KEYWORDS

Phytoremediation, polycyclic aromatic hydrocarbons, greenhouse study, petroleum uptake, marsh restoration

INTRODUCTION

Coastal marshes provide valuable ecosystem services, including flood mitigation, wildlife habitat, waste assimilation and detoxification, nutrient cycling, and water quality enhancement (Zedler and Kercher 2005, Getter et al. 1984). However, oil spills pose a major threat to these shoreline ecosystems, especially in areas such as the San Francisco Bay Area, which houses the largest oil refinery in the West Coast (Chevron USA). Intensive cargo ship traffic and the transport of crude oil from Alaska to the Richmond Refinery everyday increase the risk of devastating oil spill accidents around industrial processes (Lin and Mendelssohn 2008, Chevron USA). Crude oil causes substantial damage to sensitive habitats and marsh plants. For example, the polycyclic aromatic hydrocarbons (PAH) contained in the crude oil are especially detrimental to marsh vegetation by disrupting plant metabolism and nutrient uptake and causes cell death and decreased root functioning (McCown and Denek 1972, Prendevolle and Warren 1977).

Traditional methods to remove petroleum hydrocarbons from marsh habitats often involve soil excavation and incineration or applying chemical agents to marshland (Lin et al. 1999). Because of damage frequently associated with oil spill clean-ups in marshes, many scientists have recommended phytoremediation, the direct use of living vegetation to treat contaminated soil and sediment, which provides a less intrusive method to remove, degrade or contain the petroleum-contaminated sediment (EPA 1999). Numerous studies have shown that plants may be able to bioremediate soils by sequestering and metabolizing components of petroleum hydrocarbons, resulting in their inactivation, degradation or immobilization (Reilley et al. 1996). Smooth cordgrass, the genus *Spartina*, is the dominant species in saltwater marshes and is therefore used as a primary restoration plant (Bergen et al. 2000).

Coastal marshland vegetation is predominantly smooth cordgrass. *Spartina alterniflora* is native to the Atlantic coast, but was introduced to California in 1973 by the Army Corps of Engineers in an attempt to reclaim marshland, and was subsequently replanted around the bay in further restoration projects (Callaway and Josselyn 1992). It out-competed the native species *Spartina foliosa*, and could potentially eliminate the native species from San Francisco Bay (Callaway and Josselyn 1992). In San Francisco

Bay, a rapid invasion of hybrid *Spartina* occurred after *S. alterniflora* from the Atlantic and Gulf coast of North America hybridized with the California native species, *S. foliosa* (Sloop et al, 2011). The hybrids produce larger numbers of fertile seeds than the native *Spartina*, and the hybrid population is increasing in numbers and rate of population growth (Ayres et al., 2004). Although studies and literature on *Spartina alterniflora* have been exhaustive, crude oil and diesel effects on California native *Spartina foliosa* and the *Spartina foliosa x alterniflora* hybrids, have not been explored.

Objectives

The objectives of this study are to determine (1) how Alaska crude oil and diesel affects survival and plant growth, and (2) whether *Spartina* cordgrass accumulate polycyclic aromatic hydrocarbons and other crude oil constituents in their tissues. I will examine both of these questions, by comparing results between the native *Spartina foliosa* and the native-exotic hybrid *Spartina foliosa x alterniflora*.

METHODS

This experiment was conducted in a greenhouse at the Richmond Field Station. I obtained sixty *Spartina* plants – thirty *S. foliosa* and thirty *S. foliosa x alterniflora* hybrids – from the University of California, Davis. Prior to treatment, I allowed the transplants to grow for two weeks in order to allow them to acclimate to the greenhouse environment. Chevron supplied the Alaska crude oil, which I applied without weathering beforehand because prior research has shown that there is no significant difference in weathered vs. unweathered crude oil effects on plants (Ferrell 1984).

Control and Treatment

I separated out one stem from each transplant and trimmed all plants at 12 inches above the base. After standardizing starting height for all specimens, I planted each stem in a one-gallon trade pot of Metro Mix 200 potting mix, with ten transplants per genotype (native or hybrid) per sediment-oil concentration treatment (control, crude oil, diesel), leading to a total of 10*2*3 = 60 plant subjects. To sub-irrigate plants and maintain moist soil conditions to simulate marsh conditions, I partially immersed pots in tanks containing enough water to reach about eight centimeters below the potted soil line (Pearcy and Ustin 1984). I grouped ten pots of each of the two genotypes together in each tank, so that the control, crude oil and diesel treatments were confined to the twenty pots in their specific tank. This reduced the likelihood of cross-contamination of treatments through seepage from the bottom of the pots to the shared water in each tank.

I applied the crude oil and diesel to the ten hybrid and ten native *Spartina* in their respective treatment tanks with a spray mister. I applied two layers of the treatment to the surface of the soil to mimic a light coating from an oil spill. To allow for significant plant growth, I ran the experiment for ten weeks in the greenhouse, watering plants every two weeks to facilitate oil or diesel absorption.

Data Collection

To evaluate survival between treatment groups and genotypes, I recorded the number of dead and alive plants every two weeks. I considered a plant to be dead if it did not exhibit any new growth and its original stem and leaves were brown and dry. To understand plant growth responses to the treatments, I measured new above-ground growth per plant every two weeks with a ruler to the nearest centimeter, and noted where new growth was occurring (new shoots vs. at the top of the plant where I initially trimmed it).

To evaluate the effectiveness of both native vs. hybrid species to potentially uptake petroleum hydrocarbons, I measured the polycyclic aromatic hydrocarbons (PAH) content in above-ground (stem and leaf) and below-ground (root) plant tissue. I tested twelve plant tissue samples at the end of the growing season – an above- and below-ground sample from each of the two genotypes (native and hybrid) from the control and two treatment groups (2x2x3). I sent these samples to Lawrence Berkeley Laboratory, where they used methylene chloride to extract the hydrocarbons from the plant tissue, and then ran Gas Chromatography – Mass Spectrometry (GC-MS) tests to quantify concentrations of PAHs in each tissue sample. The tissue samples from the control group were used as a standard baseline.

Statistical analysis

To compare crude oil effects between treatments and control, and between genotypes of *Spartina* cordgrass, I used R-Commander programs (R Development Core Team 2011). I conducted Shipiro-Wilk tests to check for normality in the survival and growth data, and then performed a Kruskal-Wallace and Tukey HSD test to check for significant differences between the treatment groups and between the genotypes. Because I did not have sufficient replicates for the TPH testing in plant tissue, I did not test for significant differences in how much and where plants store the hydrocarbons in their tissues (roots vs. aboveground).

RESULTS

Survival and growth results

Between treatments

After the ten-week growing season, the treatment groups showed diverse survival rates. The control group yielded the highest survival rate of 75%, followed by a 60% survival rate in the crude oil treatment group, and only 15% survival for the diesel treatment (Figure 1). I ran a Tukey HSD test and found that survival rates were statistically significant between diesel and control groups ($p<0.001$) and between diesel and crude oil groups ($p = 0.006$), but not when comparing crude oil and control ($p = 0.533$).

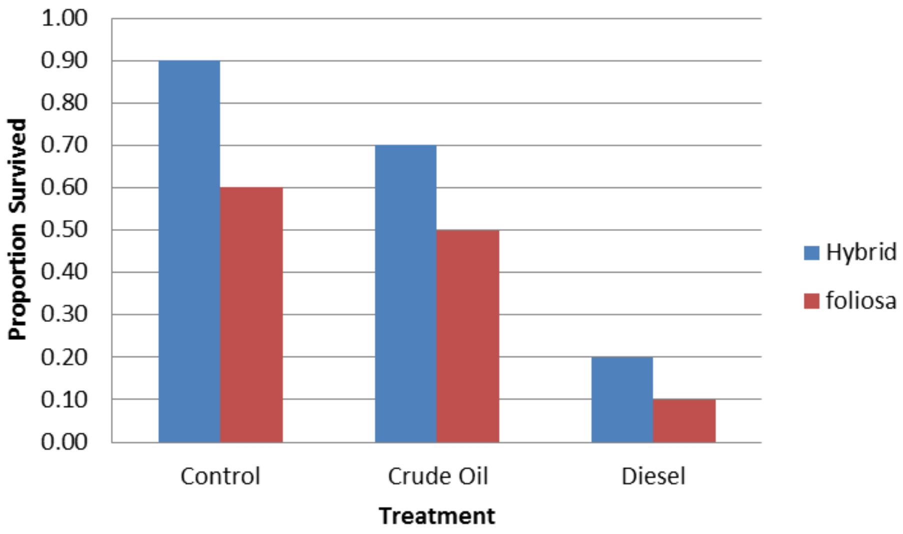

Figure 1. Survival rates categorized by genotype and treatment. I considered a plant as "not surviving" if it was brown and dry, and did not exhibit any new growth or foliage. Each treatment and genotype pairing started with ten plants before treatment application and growing season.

In addition to quantifying survival rates, I also measured new growth from the top of the plant as well as from new shoots at the base, and found that of those that survived, the *Spartina* that did not come into contact with crude oil or diesel grew an average total of 60.6 centimeters after ten weeks, while those in the crude oil treatment grew about 29.0 centimeters, and the diesel treatment group grew an average of only 17.5 centimeters per plant (Figure 2). A Kruskal-Wallis test reveals a significant difference between treatments and the average new growth per plant (p=0.04, H = 0.04, 1 d.f., p=0.04). To determine which pairs of treatments were significantly different, I ran a Tukey HSD test and found that plant growth in the crude oil treatment group was significantly lower than that of the control group (p = 0.018) but the difference in growth between diesel and control plants was on the threshold of being significant (p = 0.051), and plant growth between the crude oil and diesel treatment groups did not differ significantly (p = 0.796). When comparing the genotype variable to new growth, the calculated p-value of 0.001 shows that native *S.foliosa* growth was significantly lower than that of the hybrid *S.foliosa x alterniflora*.

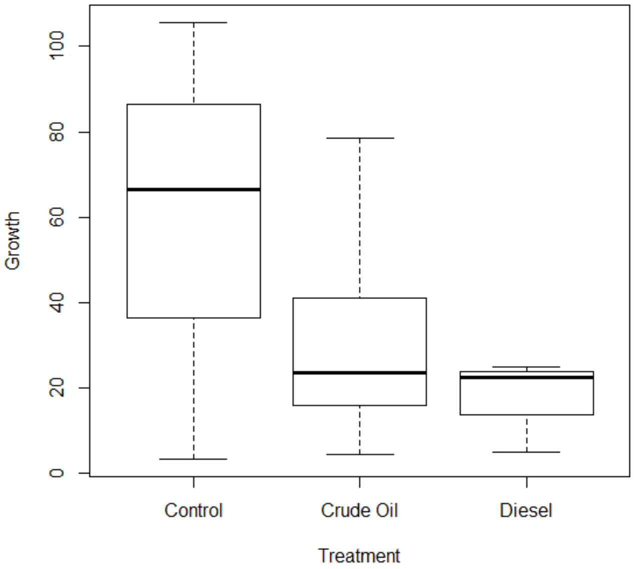

Figure 2. New growth in surviving *Spartina*. This box plot is based on total new growth measurements, in centimeters, that I recorded at the end of the ten-week growing season.

Between Genotypes

The *S. foliosa x alterniflora* hybrid plants yielded the highest survival rate of 60% initial survival after treatment, while only 40% of the natives survived the ten week growing season (Table 1). However, the Kruskal-Wallis test did not reveal any statistically significant differences in survival rate between the native and hybrid genotypes (H = 2.36, d.f. = 1, p = 0.125).

In all three treatment groups, the hybrid’s average new growth was higher than that of the *S.foliosa*, with the greatest difference in the control group, with a slightly less drastic difference as the survival rate decreased in the crude oil and diesel group (Figure 1). I found that the total new growth average per plant of the hybrid genotype was significantly higher than for that of the native genotype with p-value = 0.00123.

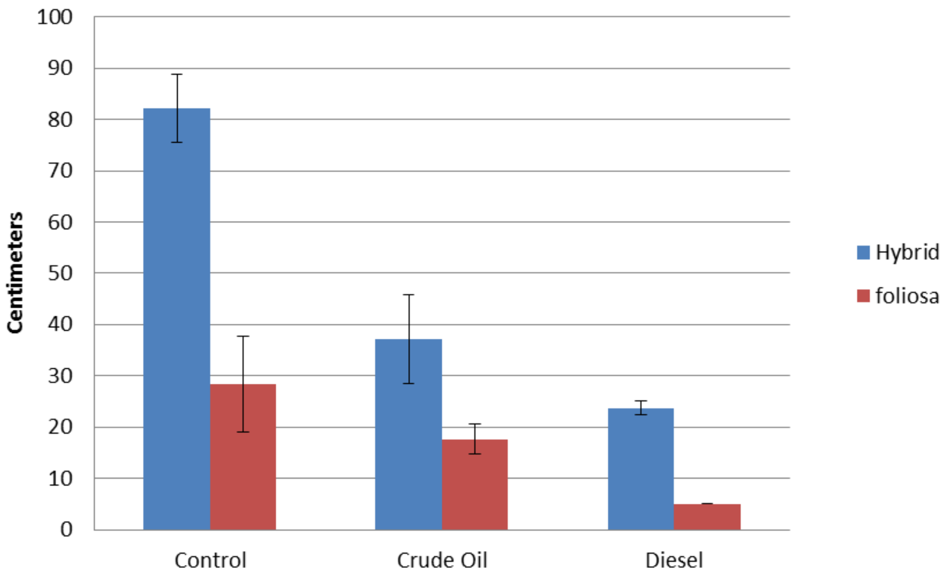

Figure 2. Average Growth per Plant. New growth included both growth extending from the top of the plant beyond the 12-inch initial trimmed height, as well as new shoots from the plant base. Measurements were recorded in centimeters. Error bars represent Standard Errors. *S. foliosa* impacted by diesel does not have an error bar because the sample only included one surviving individual.

Tissue TPH results

Between Treatments

Due to resource and time constraints, I was only able to test one above-ground tissue sample (grass) and one below-ground tissue sample (root) from each genotype and treatment. From this limited sample size, I determined that the plants (one hybrid + one native) in the crude oil treatment group accumulated less polycyclic aromatic hydrocarbons (PAH) from the Alaska crude oil (0.00075ppm) than the plant in the diesel contaminated soil (0.1314 ppm), in the below-ground root tissue (Figure 3 and 4). However, the plants in the crude oil contaminated soil were able to uptake more PAH (0.00936 ppm) than the plants in the diesel (0.0045 ppm). In both treatments, I found that the concentrations of total hydrocarbons in plant tissue above- and below-ground were orders of magnitude higher than the concentrations of just the polycyclic aromatic hydrocarbons found in petroleum products.

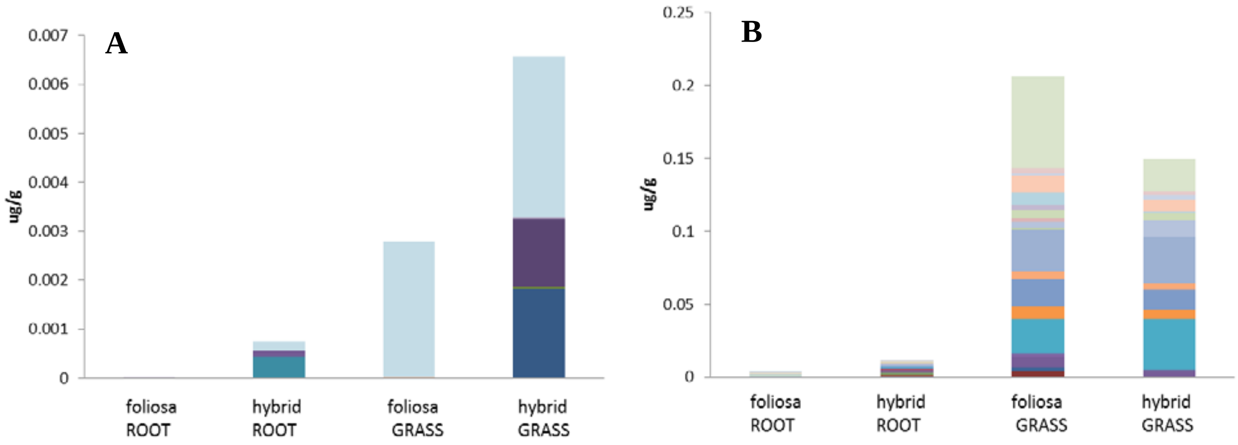

Figure 3. Alaska crude oil uptake in plant tissue. Polycyclic aromatic hydrocarbon (A) and total hydrocarbon (B) concentrations found in native and hybrid plant tissues below ground (roots) and above ground (grass). The colors represent specific hydrocarbon compounds identified, but the identity is not of interest in this particular study.

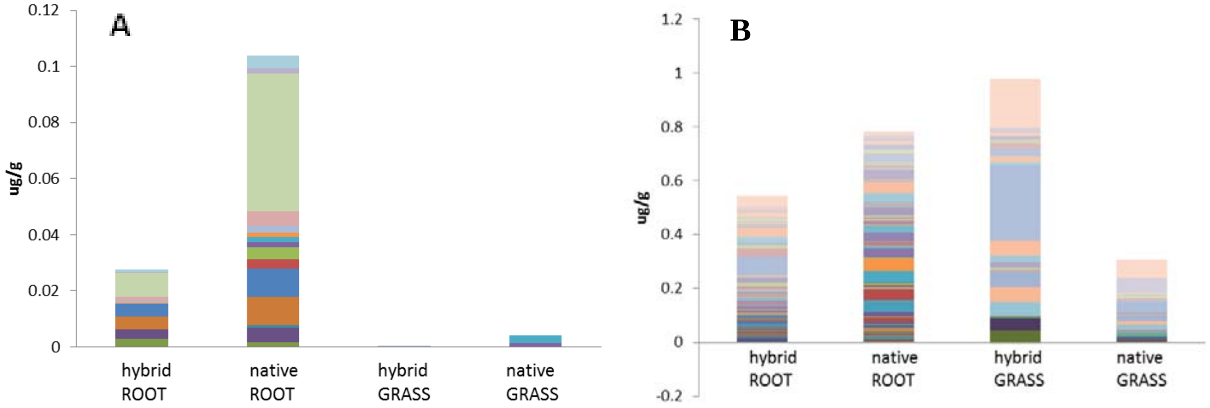

Figure 4. Diesel uptake in plant tissue. Polycyclic aromatic hydrocarbon (A) and other hydrocarbon (B) concentrations found in native and hybrid tissues below- and above-ground. The colors represent specific hydrocarbon compounds identified, but the identity is not of interest in this particular study.

I found differences between treatment types and PAH concentrations in above- and below-ground plant tissue. Both genotypes growing in the diesel contaminated soil stored proportionally more of the PAHs in their root tissue (0.1314 ppm) than in their above-ground grass tissue (0.0045 ppm). However, the plants growing in the crude oil contaminated soil stored proportionally more of the PAHs in their grass tissue than in their root tissue, though both of these concentrations were still lower than the total sequestered amount in the diesel group.

Between Genotypes

I found mixed results when comparing between genotype and whether the plant stored the PAHs above-ground or below-ground, depending on whether it was growing in crude oil or diesel contaminated soils (Figure 3 and 4).

DISCUSSION

Survival and growth

Between treatments

At the completion of the ten-week growing period, the control group had a significantly higher survival rate than the crude oil or diesel, and also grew the most total centimeters per plant post-treatment. Plant survival and new growth was not inhibited in the control group due to lack of any treatment that would have inhibited natural processes. Both the crude oil and diesel treatment groups experienced higher mortality rates and growth rates, likely because oil 1) reduces transpiration by blockage of stomata and intercellular spaces, 2) reduces photosynthesis because interference with carbon dioxide diffusion and light absorption, and 3) decreases diffusion of oxygen through aerial tissues to roots (Baker 1971). In addition, petroleum pollution has many indirect effects. For example, the presence of petroleum hydrocarbons may stimulate and promote hydrocarbon-degrading microbial activity, but the microorganisms compete with plants for oxygen and mineral nutrients (Stevenson 1966).

Plants in the crude oil treatment displayed higher survival rates and an increased total new growth compared to the diesel treatment group. This may be due to the differences in chemical and physical structure of diesel compared to crude oil. Diesel typically consists of compounds with 10 to 24 carbon atoms, whereas crude oil is the unrefined substance that includes the lighter diesel compounds as well as longer and heavier hydrocarbon constituents (Wilkinson et al. 2002). Previous studies have shown that lighter hydrocarbon components degrade more readily, and are generally more toxic, than heavier ones (Salminem 2004; Alexander and Webb 1985). In addition, heavy crude oils, because of their high viscosity, tend to move horizontally, while gasoline and diesel penetrate more easily into deeper soil strata (McGill and Rowell, 1977). Other studies have also confirmed the impact of oil penetration depth and increased mortality in Spartina cordgrass (Ferrell et al. 1984). Thus, diesel had a more detrimental effect than crude oil in this study likely because it is lighter, more bioavailable, and more easily taken up by plants or absorbed elsewhere.

Between genotypes

Results show that the native cordgrass was more negatively affected by both crude oil and diesel treatments, compared to its hybrid relative. However, this is not statistically significant, though a larger sample size may have resulted in a different finding. The hybrid may be more vigorous because it is a genetic cross between the smaller *S. foliosa* and the aggressive and larger *S. alterniflora x foliosa* from the Atlantic Coast (Callaway and Josselyn 1992). The hybrid cordgrass shares many characteristics with its invasive parent, which produces almost 10-fold the aboveground and twofold the belowground biomass as the native *S. foliosa*, and spreads laterally 1.5 times faster (Callaway and Josselyn 1992). The overall vigor of the pure invasive *S.alterniflora* likely contributes to the hybrid offspring's ability to better deal with contamination and thus out-survive and out-grow the more delicate native. We could not test the effects of contamination treatments on a pure *S. alterniflora* group due to invasive species eradication efforts, which made obtaining these pure non-natives very difficult. Although the survival and total new growth results were straight-forward to evaluate, the plants' internal uptake and metabolization of the petroleum hydrocarbons was more complex.

Plant tissue uptake

Based on my limited sample, the plants absorbed more petroleum hydrocarbons from the diesel than they did from the crude oil. This is likely due to the increased bioavailability of the lighter diesel compared to the heavier crude oil to the plants. The Gas Chromatography – Mass Spectrometry tests also showed that the crude oil constituents that were absorbed were sequestered in the plants' above-ground leaf and stem tissue, whereas the hydrocarbons absorbed in the diesel treatment group's sample was mostly stored in the root biomass. Because crude oil is heavier and less bioavailable, it may not be actively taken up by the plant unless in direct contact with the tissue cells, which may have occurred during the application of the oils to each potted plant at the beginning of the experiment (McGill and Rowell 1980). While the lighter diesel hydrocarbons are more likely to be absorbed by the roots after it travels and degrades through the soil matrix, the denser crude oil may only have access to the plant tissue through physical contact, such as accidental application to the stem while I was spraying the oils. Results also showed that the diesel group plant tissue had higher concentrations of other hydrocarbons compared to the crude oil group, which again, is likely due to the fact that crude oil contains lower proportions of the lighter compounds and hydrocarbons.

Limitations

I designed this experiment as a greenhouse study, which poses some limitations on my ability to draw scientific inference based on the results. During the ten-week growing season, greenhouse conditions created an unrealistically warm climate and higher humidity than what the *Spartina* would normally have faced in its natural environment in the San Francisco marshes in winter. The intensity of oil damage depends on a range of abiotic and biotic factors, including the season of the spill, prevailing weather conditions and soil conditions (Alexander and Webb 1987). Though a field experiment may have provided a more realistic growth and hydrocarbon uptake response from the cordgrass, the volatile and potentially carcinogenic nature of the polycyclic aromatic hydrocarbons in the crude oil and diesel would have made an outdoor field study out of the question.

In addition, orientation of the treatment pools within the greenhouse may have provided more sunlight favorably to the treatment pool (control group) closest to the corners of the greenhouse and less light to the treatment pool (diesel group) closer to the potting shed. However, the treatment pools were still within three feet of each other so variation in natural lighting should not have a significant effect on the results. I could not randomize plants between the different pools because that would have introduced the possibility of cross contamination of treatment through soil or water exchange from the bottom of the pots to the holding pools of water. Also, I was limited in time and the number of samples I could test, but a longer growing season and an increased sample size may show different results. I also did not collect below-ground data such as root biomass or vertical or horizontal spread. However, Lin and Mendelssohn state that measurements of oil effects with biological end-points based solely on aboveground responses may underestimate the potential impacts of petroleum hydrocarbon spills, especially when the oil has migrated below the soil surface (2008). Although this study has some limitations in scope and method design due to practicality, my findings represent important preliminary information that will be useful in guiding future research.

Future directions

I tested my research questions through this small greenhouse study, but my findings prompt further studies that look into the specific biological processes that result in the differences I saw in growth and hydrocarbon uptake. For example, future research may look at various mechanisms of plant uptake and metabolism of hydrocarbons including aerobic oil degradation in the rhizosphere (Lin and Mendelssohn 2008), root-produced exudates that promote microbial numbers and increase their activity (Hedge and Fletcher 1996), and tissue metabolization of hydrocarbons from the soil. Further studies on how *Spartina* deals with hydrocarbons will elucidate this cordgrass' potential use as a phytoremediation tool in oil-contaminated soils and marshes.

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