

PHYTOREMEDIATION OF OIL BY BRACKISH MARSH SPECIES: EFFECTS OF SOIL TEXTURE AND INUNDATION REGIME

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Phytoremediation of Oil by Brackish Marsh Species: Effects of Inundation Regimes and Soil Textures

Abstract

Because fragile coastal marshes are sensitive to disturbance by mechanical cleanup, cleaning up oil in wetland environments may do more damage than the oil spill itself. Phytoremediation with native marsh plants for the *in situ* treatment of oil contaminated soil promises an effective, inexpensive, low impact oil spill cleanup procedure that can restore oil contaminated marsh habitats.

The overall goal of this research was to determine how well phytoremediation enhanced oil degradation and habitat restoration in coastal wetlands. Our specific objective was to determine how effectively brackish marsh plants phytoremediated oil under common coastal marsh inundation environments and soil textures.

Artificially weathered South Louisiana Crude oil was well mixed with either a sandy soil or an alluvial silty soil by a powered cement mixer to produce a concentration of about 25 mg oil g⁻¹ dry soil. Three brackish marsh plant species, *Distichlis spicata*, *Juncus roemerianus*, and *Spartina patens*, were transplanted separately into either the oiled sandy soil or oiled alluvial silt soil. No transplants in either oiled sandy soil or oiled alluvial silt soil served as a control. In the greenhouse, the soil and plants in the experimental units were maintained under appropriate inundation conditions—one

simulating a daily tide inundation regime, and the other maintained with 5 cm of standing water over the soil surface.

Three brackish marsh plant species were successfully transplanted into the oil contaminated soils. Oil in the soil did not adversely affect the transplants, as evidenced by relatively high plant photosynthesis, increased stem density, and plant biomass. For example, live stem density of *D. spicata* increased by as much as 17 fold in a one year period. Phytoremediation significantly increased oil degradation for both the sandy soil and the alluvial silt. Oil degradation rates in the treatments receiving *Distichlis spicata*, *Juncus roemerianus*, and *Spartina patens* were 3.1, 2.9, and 2.6 fold higher respectively than rates without phytoremediation. However, the effectiveness of phytoremediation by brackish marsh plants depended upon the plant species and its growth environment. For example, the most effective phytoremediator among the three brackish species was *D. spicata* in a sandy soil under a daily tidal inundation regime. The oil degradation rate was significantly higher in sandy soil than in alluvial silt, a 29.5% reduction in the former versus 17.6% in the latter. Phytoremediation significantly increased oil degradation in both the daily tidal regime and the standing water regime. Thus, phytoremediation can effectively remediate oil contamination in most common wetland environments, such as intertidal streamside areas and inland waterlogged sites. The oil degradation rate in the treatment receiving the daily tidal inundation regime was significantly higher than the degradation rate in the standing water inundation regime; we observed a 29.1% reduction in the former versus 16.6% in the latter. The present study demonstrated that phytoremediation can accelerate oil degradation and site restoration in a wide range of common coastal environments.

1.0 Introduction

The risk of oil spills in the Gulf of Mexico coastal marshes is high because of intensive oil related activities in this area. Not only can spilled petroleum hydrocarbons harm coastal marshes, but mechanical cleanup methods may damage these highly sensitive ecosystems more than the oil itself (Alexander and Webb 1987; Baker *et al.* 1993). Therefore, it is important to develop less intrusive oil spill cleanup techniques that effectively accelerate the degradation of spilled oil.

Phytoremediation, the use of vegetation for the *in situ* treatment of contaminated soil and sediment, is an emerging technology that promises effective and inexpensive cleanup of certain hazardous wastes (Stomp *et al.* 1993; Schnoor *et al.* 1995; Lin and Mendelsohn 1998a and 1998b). Phytoremediation has increasingly attracted scientific attention and has already been shown to be effective for the removal of both inorganic and organic pollutants, including polycyclic aromatic hydrocarbons (PAHs) (Anderson Reilley *et al.* 1996; Huang *et al.* 1997; Burken and Schnoor 1997; Kling 1997; Lin and Mendelsohn 1997, 1998a and 1998b; Lin *et al.* 1999b and others).

Many factors, especially soil physico-chemical parameters, such as soil texture, soil oxidization status, soil fertility, soil pH, concentration of oil, and the presence of acclimated microbes (Alexander 1989; Blaba *et al.* 1991; Lin and Mendelssohn 1997 and 1998b; Lin *et al.* 1999b), may affect oil degradation. Oxygen and nutrients in wetland soils are generally not sufficient for maximum biological activity. Due to slow biodegradation in anaerobic sediments, oil that has penetrated into the anoxic substrate may persist in wetlands for decades (Hambrick *et al.* 1980; Getter *et al.* 1984; Baker *et al.* 1993). Baker *et al.* (1993) reported that an oil layer was clearly visible in core samples collected 22 years after a spill in a salt marsh in Wales; chemical analysis still recognized it as heavy fuel oil. Bioremediation attempts to accelerate the natural degradation process of contaminants, such as petroleum hydrocarbons in the coastal environment, by adding materials to overcome factors that limit bacterial hydrocarbon degradation. In the application of bioremediation, the most common agents shown to enhance oil degradation are inorganic nutrients and soil aeration where O₂ is deficient; microbial seeding has also been frequently used for bioremediation (Mikesell *et al.* 1991; Altas 1993; Bragg *et al.* 1993; Prince *et al.* 1993; Venosa *et al.* 1996; Lin *et al.* 1999b).

Although aerobic conditions are essential for the complete degradation of organic pollutants, aeration of marsh soils is difficult due to the slow diffusion of oxygen in the water that usually saturates marsh sediment. Additionally, mechanical aeration is logistically impractical due to the marshes' fragility and the remote locations of many wetland ecosystems. The inherent capacity of many wetland plant species to aerate the soil rhizosphere (Teal and Kanwisher 1966; Armstrong 1978; Smirnoff and Crawford 1983) may be used to overcome this oxygen limitation, thus enhancing aerobic biodegradation of spilled oil.

Hydrology and associated inundation regimes are major influences in wetlands. The effectiveness of phytoremediation for oil spill cleanup could be very different under different inundation conditions. The oil degradation rate may be much slower in anaerobic, water saturated soils compared to wetland soils that experience daily drainage and are less biochemically reduced (Hambrick *et al.* 1980, Lin and Mendelssohn 1998a, Lin *et al.* 1999c). Thus, field application of phytoremediation in coastal marshes may be suitable for one inundation environment, but not for others.

Brackish marshes extend over the coastal wetlands of the northern Gulf of Mexico. Since there are more plant species in brackish marshes than in salt marshes, brackish marshes contain a greater pool of species that may exhibit high phytoremediation potential. Our previous study (Lin *et al.* 1999c) indicated that fresh marsh species such as *Phragmites australis* and *Typha latifolia* had considerable potential to phytoremediate oil spills. Little research, however, has been conducted to assess the capability of brackish marsh plant species to enhance the biodegradation of residual oil under different marsh environments. The current study attempts to fill these data gaps.

and Walton 1992; Bell 1992; Brown *et al.* 1995; Salt *et al.* 1995; Schwab *et al.* 1995;

Objectives

The specific objectives of this study were to: (1) evaluate the oil phytoremediation effectiveness of common Louisiana brackish marsh plants, (2) determine the effectiveness of phytoremediation in common coastal marsh inundation regimes, and (3) evaluate the effectiveness of phytoremediation in soils with different textures.

The following hypotheses were tested by this research:

- Phytoremediation can be used to restore oil contaminated habitat.
- Phytoremediation accelerates natural rates of oil degradation.
- The natural oil degradation rate is higher in a tidal inundation regime than under constant standing water.
- Phytoremediation accelerates oil degradation more in a daily tidal inundation regime than in a standing water regime.
- The effectiveness of phytoremediation varies among brackish marsh plant species.
- Phytoremediation accelerates oil degradation more in coarse sandy soil than in fine alluvial silt soil.

2.0 Materials and Methods

2.1 Marsh Plants and Soil Material

Three brackish marsh plants, *Distichlis spicata*, *Juncus roemerianus*, and *Spartina patens*, were collected from Louisiana coastal marshes. The selected plants are important brackish marsh species in Louisiana and the southeastern United States. The soils used in this experiment were alluvial silt and sandy soil. We used these soils in order to determine the effect of soil texture (particle size) on oil phytoremediation. Both soils had less than 2% soil organic matter, thus excluding the differential effect of organic matter on phytoremediation. The particle size and organic matter content of the soils are shown in Table 1.

Table 1. The particle size and organic matter of the alluvial silt and sandy soil used in the experiment

Soil Type	%Clay	%Sand	%Silt	%Organic Matter	Class
Alluvial Silt	28.54	0.80	70.66	1.77	Silty Clay Loam
Sandy Soil	1.17	94.53	4.30	1.21	Sand

2.2 Experimental Design

In the greenhouse, the following treatments were randomly applied: (1) marsh plants (phytoremediation treatment), (2) soil with different textures, and (3) inundation regimes (hydrologic treatment). The experimental design was a completely randomized block with a 4 x 2 x 2 factorial treatment arrangement [four phytoremediation types (three marsh plant species and one control without plants), two soil types (either the sandy soil or alluvial silt soil), and two inundation regimes (one simulating a daily tide and the other maintaining 5 cm of standing water over the soil surface)]. Treatment-level combinations were replicated five times. A total of 80 experimental units were used.

2.3 Experimental Procedures

Artificially weathered (25% by volume) South Louisiana Crude oil was applied to either the sandy substrate or alluvial silt soil at the rate of about 25 mg per gram dry soil. Water soluble fertilizer (Peters 20-20-20 with micro-nutrients: N 20%, P₂O₅ 20%, K₂O 20%, Mg 0.5%, B 0.02%, Cu 0.05%, Fe 0.10%, Mn 0.05%, Mo 0.0005%, and Zn 0.05%, Spectrum Group, St. Louis, MO) was applied at rates of 100 kg N/ha and 44 kg P/ha during the mixing. The applied fertilizer can provide micro-nutrients since the sandy soil contains few macro- and micro-nutrients. The applied oil and fertilizer were each mixed into the sandy soil or the alluvial silt soil. A powered cement mixer was used to produce a homogeneous concentration of oil. Directly mixing the oil with the soil ensured the same oil concentration in each treatment-level combination. The mixed sandy soil or alluvial silt soil was then loaded into individual 8 liter buckets. Ten stems of the three selected brackish marsh plant species were transplanted separately into the oiled soils. The experimental units (soil plus plants) were kept under the appropriate inundation regimes: 40 experimental units were exposed to a simulated diurnal tide (12 hours of low tide and 12 hours of high tide with 5 cm of water over the soil surface). To create the tidal effect, water reservoirs, which were connected to the experimental units by rubber tubing, were either raised or lowered for the desired period. In the remaining 40 experimental units, the water level was kept 5 cm above the soil surface throughout the experimental period. In addition, slow release fertilizer (Osmocote 14-14-14) was applied to all experimental units six months after transplantation at rates of 100 kg N/ha and 30 kg P/ha. The fertilizer was spread on the soil surface using standard field application techniques.

Plant photosynthetic rate, plant stem density, and average plant canopy height were measured six and 12 months after treatment application to determine phytoremediation effects and habitat restoration success. At the termination of the experiment in November 2000, plant above-ground biomass was measured to determine the treatment effects and their relationship to oil degradation rates. This measurement was taken one full year after the initiation of the phytoremediation treatment. Soil redox potential (an indicator of soil aeration status) and evapo-transpirational rates were measured six and 12 months after treatment to determine their response to the treatments and their relationship to oil degradation rates. Soil heterotrophic microbes and oil degrading microbes were analyzed 12 months after treatment to determine treatment effects and their relationship to oil degradation. Interstitial NH₄, NO₃, and PO₄ concentrations were analyzed 12 months

after treatment to determine their relationship to plant biomass, microbial activity, and residual oil degradation. Residual oil concentrations in the soil at depths of 0.5 to 3.5 cm below the soil surface were analyzed 12 months after treatment to determine the effects of phytoremediation and fertilization on oil degradation under the different inundation environments.

2.4 Methods

Photosynthetic Rate

The leaf photosynthetic rate was measured at the times specified previously. A portable photosynthesis system, including an infrared gas analyzer (ADC model LCA-2), an ADC air flow control unit, and an ADC Parkinson leaf chamber, were used. Sample air, taken 5 m above ground to obtain relatively stable CO₂ concentrations, was led through an ADC air flow control unit at a flow rate of 5 ml s⁻¹ during photosynthetic rate measurements. Measurements were conducted at a quantum flux density of 2000 mmol m⁻² s⁻¹ provided by a Kodak projector lamp. An intact, attached, and fully expanded young leaf was enclosed in the leaf chamber, and the difference in CO₂ and air humidity concentration between inlet and outlet air was measured. Photosynthetic rate (CO₂ exchange) was calculated in accordance with the von Caemmerer and Farquhar method (1981) and expressed as μmol CO₂ m⁻² s⁻¹.

Evapo-transpiration Rate

Evapo-transpiration, water loss by transpiration through plant leaves and by evaporation from the water surface, was determined for each experimental unit by measuring the decrease in water volume in a 24 hour period. The evapo-transpiration rate was expressed as grams H₂O per pot per day. Pots without transplants were used to estimate evaporational water losses alone.

Plant Stem Density

Plant stem density was measured by direct counting of stem numbers in each experimental unit and expressed as the number of stems per pot.

Average Plant Canopy Height

The average shoot height of the transplants was measured to the nearest centimeter.

Above-ground Biomass

Above-ground biomass was harvested at the termination of the experiment in November 2000, and live and dead plant tissues will be separated and dried at 65 °C to a constant weight. The relationship between above-ground biomass and the oil degradation rate will be evaluated.

Soil Redox Potential

Soil redox potential was measured at the times specified previously at 3 cm below the soil surface. Measurements were taken with bright platinum electrodes, a calomel reference electrode, and a pH/mV meter.

Interstitial Nitrogen (NH₄ and NO₃) and Phosphate

Interstitial water was drawn from the soils with a syringe, and flexible tubing was attached to a rigid tube with 1 mm holes (Mckee *et al.* 1988). The interstitial water was filtered with a syringe filter (0.45 µm). NH₄ and NO₃ were analyzed with an autoanalyzer (QuickChem 800, Lachat Instruments Division, Zellweger Analytics, Inc.). Phosphate was measured by the Murphy-Riley procedure (Persons *et al.* 1984).

Soil Heterotrophic Microbes and Oil-Degrading Microbes

Analyses of heterotrophic microbes and oil degrading microbes were conducted to determine treatment effects and their relationship to oil degradation. The top 3.5 cm of the soil in each experimental unit were sampled with sterile plastic 3 ml syringes, which were modified by cutting the hub end off the barrel. From each soil sample, one gram of soil was retrieved and delivered to 100 ml of phosphate-buffered saline, (PBS, pH 7.3) contained in a sterile 250 ml beaker holding a magnetic stir bar. The PBS soil suspension (1:100 dilution, wt/vol, 10⁻²) was stirred on magnetic stirrer at high speed for two minutes.

Estimation of Total Bacterial Numbers Per Gram of Soil Sample

Total colony-forming units (cfu) were determined for each soil sample. Sequential 10-fold dilutions of each soil sample were prepared in PBS, starting at 10⁻³ through 10⁻⁶. From each dilution, 0.1 ml was removed and deposited onto an agar surface Petri dish that contained Plate Count Agar (PCA, see Table A1 of Appendix). The soil-dilution was spread over the surface using sterile glass hockey sticks on a turntable. Each dilution sample was plated in triplicate. The PCA plates were incubated for five days at 30°C (which approximates the temperature in the greenhouse at harvest). Incubation colonies (cfu) were counted under magnification on a lighted colony counter. Soil dilutions, which produced 30 to 300 colonies per plate, were recorded. An average was computed from each of the three plates prepared at that dilution.

Oil-Degraders cfu

These bacterial numbers were also estimated by spread plates on Basal Medium (Table A2 of Appendix) using the same prepared soil-dilution suspensions prepared for total bacterial counts. For each dilution, 0.1 ml was deposited onto Basal Medium supplemented with weathered oil (BM-WO, pH 7). For each liter of basal medium, 10 ml of each of the four solutions (Table A2 of Appendix) were added.

Residual Oil in the Soil

Residual oil in the sediment was analyzed gravimetrically using a modification of EPA method 9071 (EPA 1986). Soil cores (2 cm in diameter and the depth from soil surface to the bottom of the bucket) were taken from each pot. The 0.5 to 3.5 cm soil depth was sampled. About 8 grams of soil were extracted with three successive 20 ml volumes of methylene chloride and sodium sulfate as chemical drying agents (Lin *et al.* 1999b) The soil and methylene chloride were vigorously mixed with a stainless steel spatula. The extract was concentrated by evaporation. The concentrated extract was then transferred to a preweighed dish, the remaining extraction solvent evaporated, and the unevaporated oil remaining in the dish weighed. After extraction, the sediment was dried at 65 °C and

weighed. The total hydrocarbon concentrations in the sediment and percentage degradation of oil were calculated.

2.5 Statistical Analysis

Statistical analysis was conducted with the Statistical Analysis System (SAS 1985). Plant parameters, soil variables, microbial populations, and oil concentrations were analyzed with analysis of variance (ANOVA) as a 4 X 2 X 2 factorial arrangement of treatments in a completely randomized block design. Duncan's test was used to evaluate statistical differences of the main factors when no interaction occurred. The least square means test was used to evaluate statistical differences between treatment level combinations. Significant differences were reported at the 0.05 probability level, unless otherwise stated.

3.0 Results

3.1 Transplant Growth Status

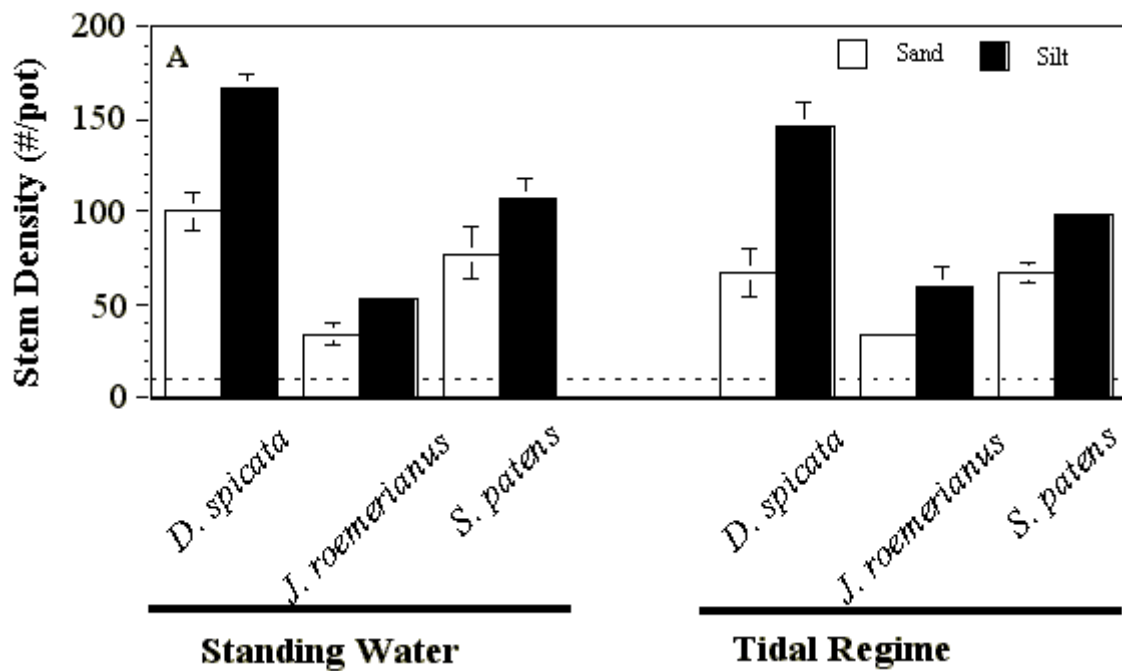
Restoration of oil contaminated soil by vegetative transplantation of brackish marsh plant species was successful based on the growth status of the transplants. Six and 12 months after transplantation, stem densities of all three transplants (Figs. 1A and B) increased three to 17 fold from the initial stem densities (10 stems per pot at the beginning of the experiment), indicating that the selected brackish plants can grow in the soil with oil concentrations of 25 mg of weathered Louisiana crude oil per gram dry soil. The stem density was highest for *Distichlis spicata* and lowest for *Juncus roemerianus*. Plant stem densities in the silt soil were significantly higher ($p < 0.0001$) than in the sandy soil (Fig. 1A and B, Table 2). In addition, plant stem densities in the treatments with the standing water regime were significantly higher than plant stem densities in the tidal inundation regime. (Fig. 1A and B, Table 2).

As with plant stem density, canopy height and maximum plant shoot height were significantly greater ($p < 0.0001$) in the silty soil than in the sandy soil 12 months after treatment (Figs. 2 A and B). Furthermore, canopy height and maximum plant shoot height were significantly higher ($p < 0.0001$) in the treatment with the standing water inundation regime than in the tidal inundation regime 12 months after treatment (Figs. 2 A and B, Table 2). Significant interactions between phytoremediation and soil type indicated that responses of plant shoot height to soil type differed with plant species. For example, the difference in the canopy height between the treatments with silt and sand was greater for *J. roemerianus* than for the other two species (Figs. 2A and 2B, Table 2).

The plant photosynthetic rate also demonstrated that these plants could grow in soils with high oil concentrations. Photosynthetic rates of all three species were relatively high 12 months after transplantation (Figs. 3), ranging between 11 and 22 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Photosynthetic rates were highest for *J. roemerianus* and lowest for *D. spicata*, documenting the difference of species characteristics. Inundation environment and soil type did not significantly affect the photosynthetic rates of the transplants.

Plant above-ground biomass (Fig. 4), a comprehensive index of plant growth status, was analyzed 12 months after phytoremediation application to determine the treatment's effect and phytoremediation potential. Plant above-ground biomass was significantly ($p < 0.0001$) different among the three plant species, with *S. patens* the highest and *J. roemerianus* the lowest. Plant above-ground biomass was significantly ($p < 0.0001$) higher in the treatment with silt soil than in the sandy soil. In addition, plant above-ground biomass was significantly higher ($p < 0.0001$) in the treatments receiving standing water than in those receiving the daily tidal inundation regime.



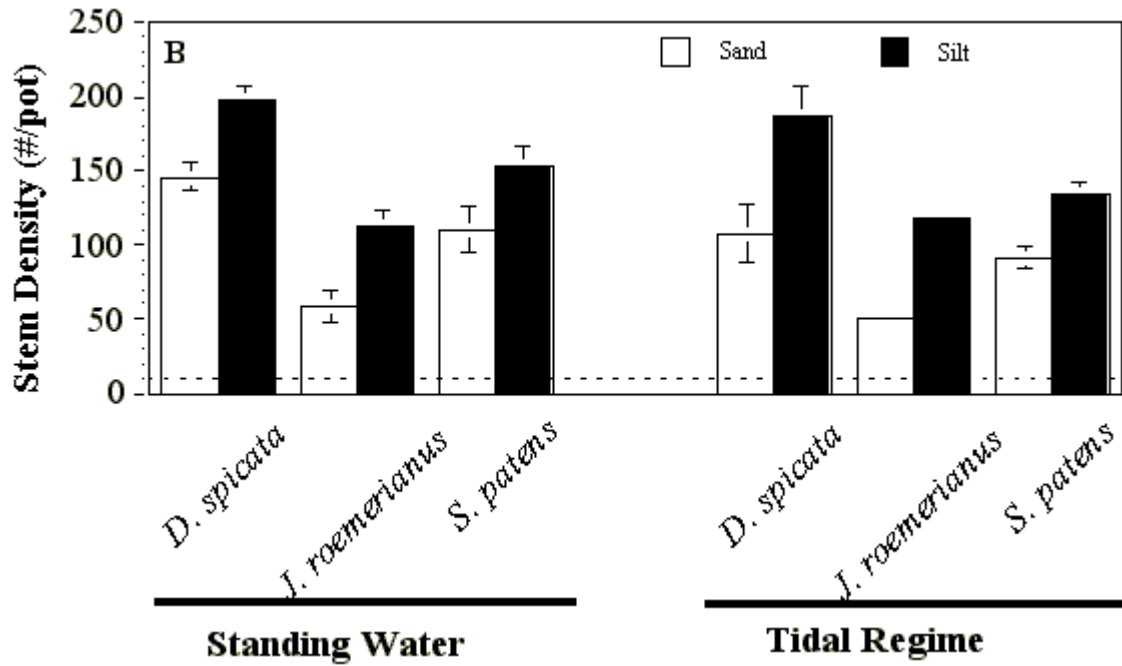
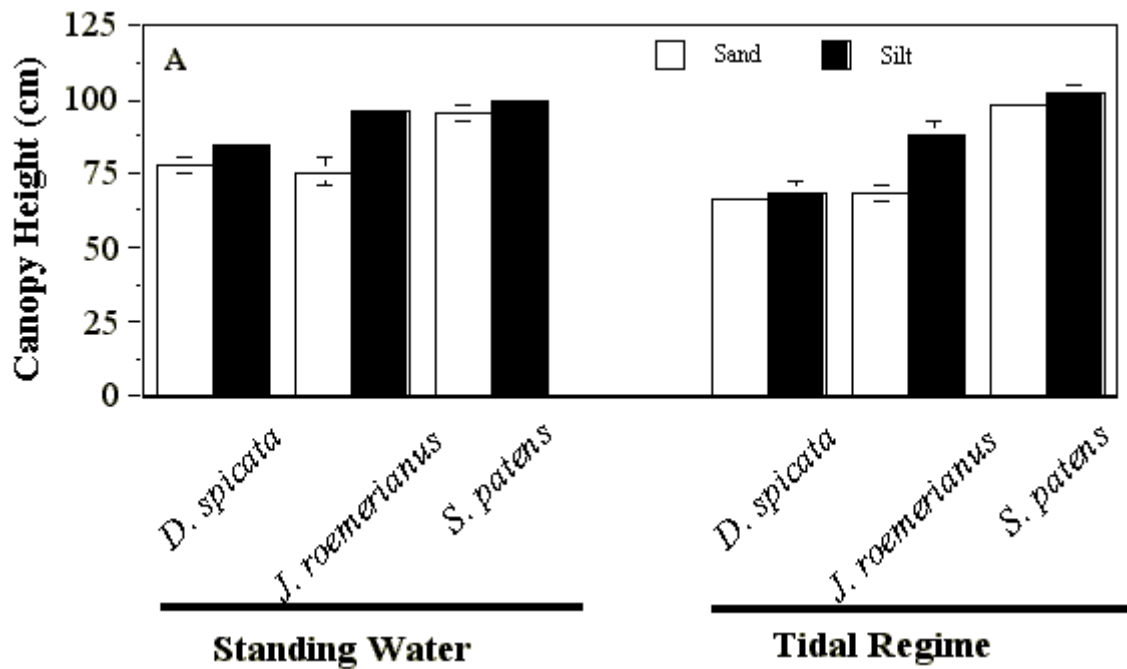


Fig. 1 Effect of soil texture and inundation regime on transplant stem density six (A) and 12 (B) months after the phytoremediation treatment. The horizontal dashed line represents the initial stem density. Values are means ($n=5$) with standard errors.



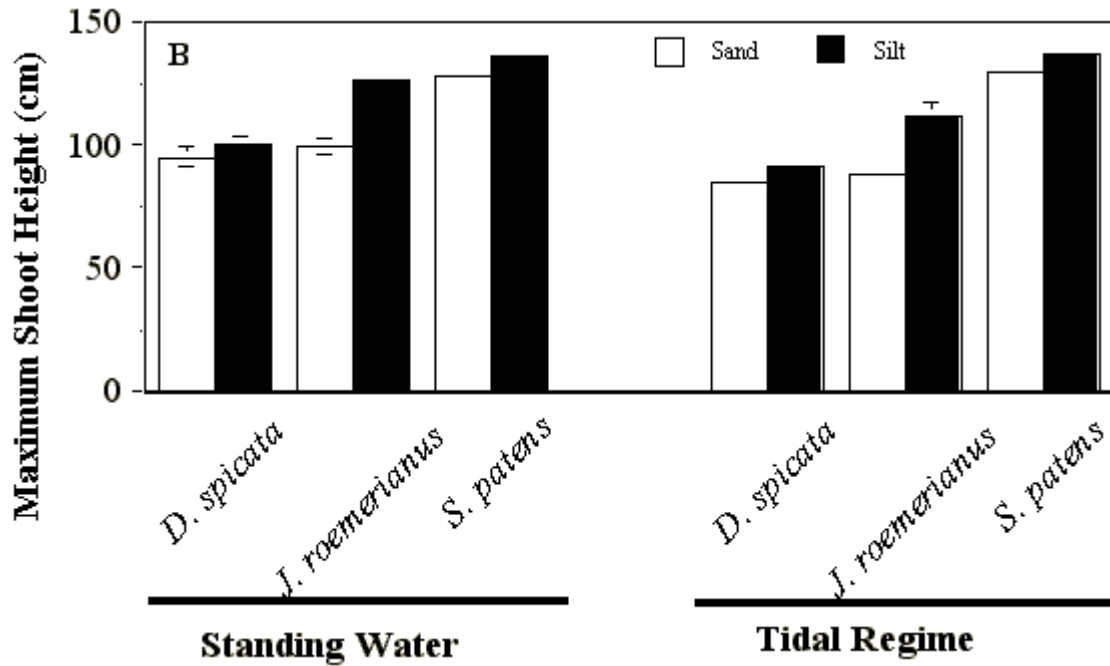
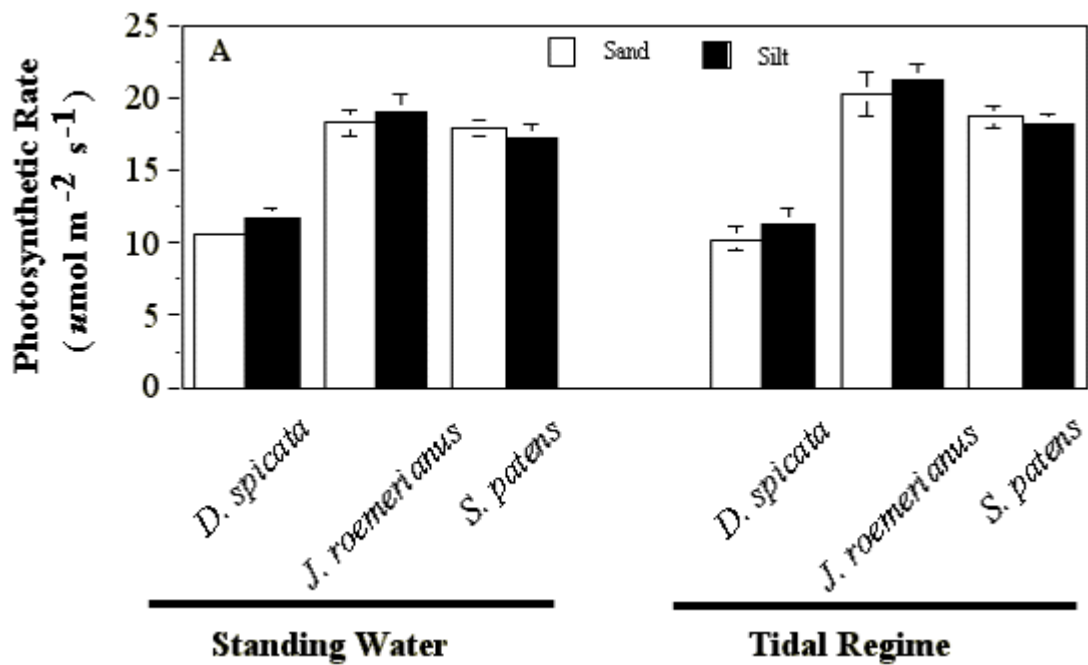


Figure. 2 Effect of soil texture and inundation regime on transplant average shoot height (A) and maximum shoot height (B) six months after the phytoremediation treatment. Values are means ($n=5$) with standard errors.



							Soil
Stem Density (six months)	0.0001	0.00365	0.0001	0.0689	0.0005	0.5165	0.9124
Stem Density (12 months)	0.0001	0.0330	0.0001	0.3961	0.3996	0.3032	0.7427
Canopy Height	0.0001	0.0010	0.0001	0.0046	0.0001	0.7129	0.6627
Maximum Shoot Height	0.0001	0.0004	0.0001	0.0130	0.0001	0.8387	0.8349
Photosynthetic Rate	0.0001	0.1321	0.4333	0.2223	0.3973	0.8371	0.9828
Above ground Biomass	0.0001	0.0019	0.0001	0.4605	0.058	0.9840	0.8580
Evapo-transpiration Rate	0.0001	0.0001	0.0001	0.5336	0.0001	0.6735	0.1835

3.2 Effect of Phytoremediation, Inundation Regime, and Soil Texture on Soil Parameters

The water evapo-transpiration rate was measured to determine the effect of the transplants, inundation regime, and soil type on the water loss rate. Evapo-transpiration rates in the treatments with transplants were significantly higher ($p < 0.0001$) than in those without transplants (Fig. 5, Table 2), especially for *J. roemerianus*. Eleven months after transplantation, water evapo-transpiration rates in the silt soil containing *J. roemerianus* were more than eight times higher than in soil without transplants (Fig. 5). The water evapo-transpiration rate in the treatments receiving the daily tidal regime was significantly higher ($p < 0.0001$) than in the standing water regime (Table 2), suggesting that soil exposure during low tide increased evaporational water loss. In addition, the water evapo-transpiration rate in the treatments with silt soil were significantly higher ($p < 0.001$) than in treatments with sand soil (Table 2). A significant ($p < 0.0001$) interaction between phytoremediation and soil type indicated that the higher evapo-transpiration rate in the treatments with silt soil occurred only in the presence of transplants.

Plants may affect the soil environments through their rhizosphere. Soil redox potential is a measure of the degree to which a soil is oxidized or reduced. Higher redox potentials signify more aerated soils. As expected, soil redox potentials in the daily tidal regime were significantly higher than redox potentials in the standing water regime (Table 3) six and 12 months after the treatment (Fig 6). More importantly, soil redox potential was significantly higher ($p < 0.001$) in the treatments receiving transplants than in treatments without transplants, indicating that transplants aerated the soil by transporting atmospheric air to their rhizosphere. For example, average redox potential was +68 mV for the treatment receiving *J. roemerianus* and -78 mV for the treatment without

transplants 12 months after the treatment (Fig 6B).

3.3 Interstitial Nutrient Status

Interstitial inorganic nitrogen and phosphorus were analyzed to determine the available inorganic nutrient status of the experimental units and their effect on phytoremediation. Twelve months after treatment, inorganic nitrogen concentrations (Fig. 7A, Table 3) were significantly higher ($p=0.0023$) in the treatment with the sandy soil than in treatments with alluvial silt. In addition, inorganic nitrogen concentrations were significantly higher ($p=0.0010$) in the treatments without plants than in the treatments with plants, suggesting plant uptake of nitrogen from the soil. Furthermore, phytoremediation, inundation regime, and soil texture significantly affected interstitial phosphate concentrations (Fig. 7B, Table 3). Twelve months after treatment, the concentration of the interstitial phosphate in the standing water regime was significantly higher ($p<0.0001$) than in the daily tidal regime. Also, the concentration of interstitial phosphate in the treatment with sandy soil was significantly higher ($p<0.0001$) than in alluvial silt. The concentration of interstitial phosphate in the treatment without plants was significantly higher than in the treatment receiving transplants of *D. spicata* and *S. patens*.

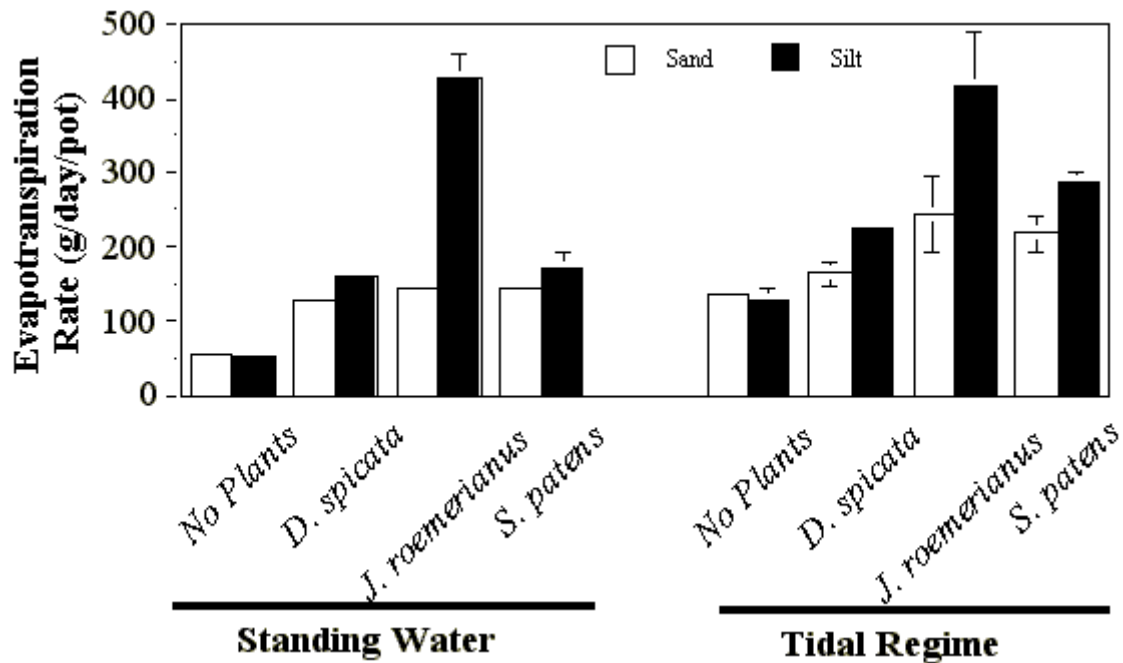


Fig. 5 Effects of phytoremediation, soil texture, and inundation regime on evapotranspiration rates 12 months after the phytoremediation treatment. Values are means ($n=5$) with standard errors.

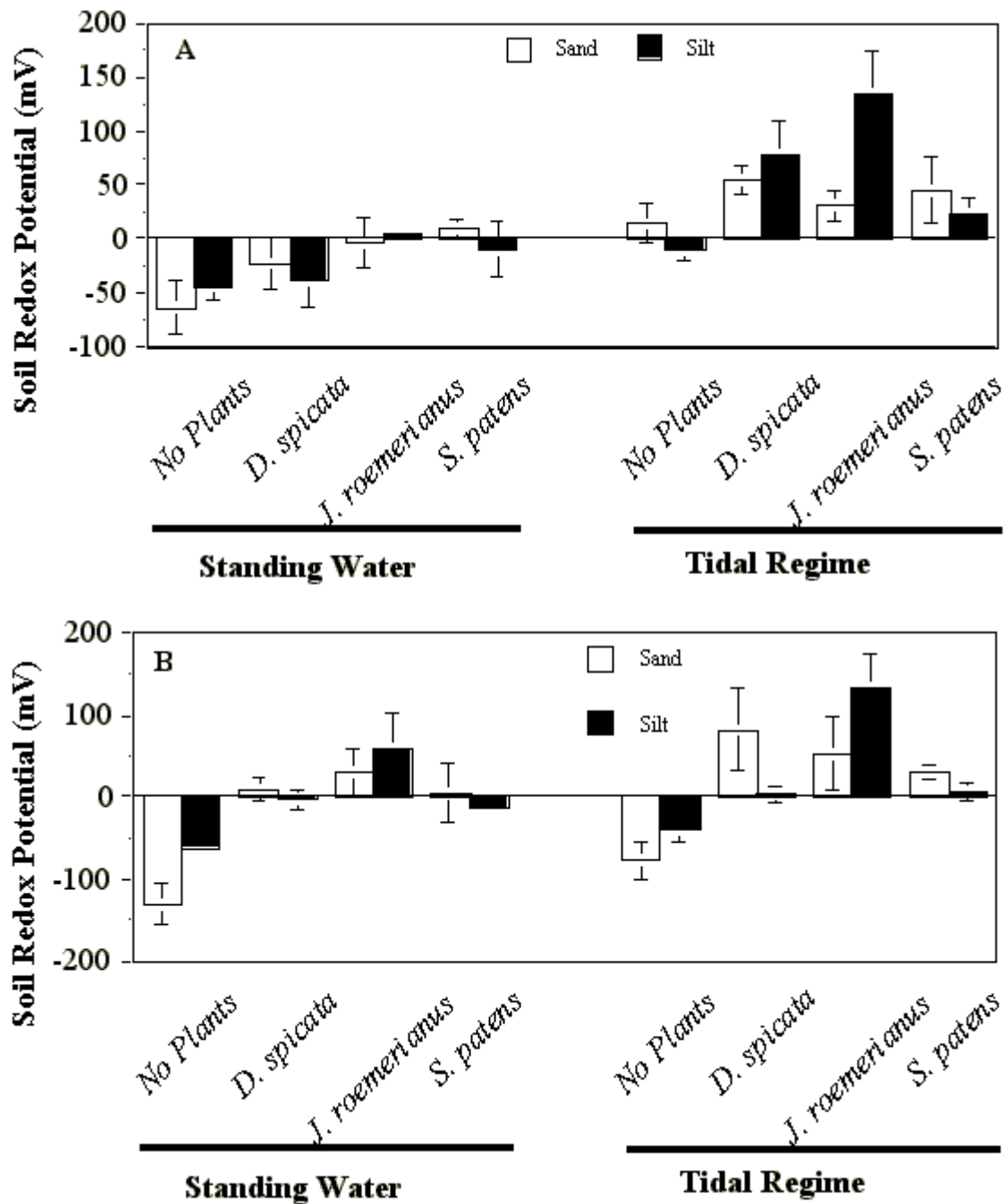


Fig. 6 Effect of phytoremediation, soil texture, and inundation regime on soil redox potential at 3 cm below the soil surface six (A) and 12 (B) months after the phytoremediation treatment. Values are means (n=5) with standard errors.

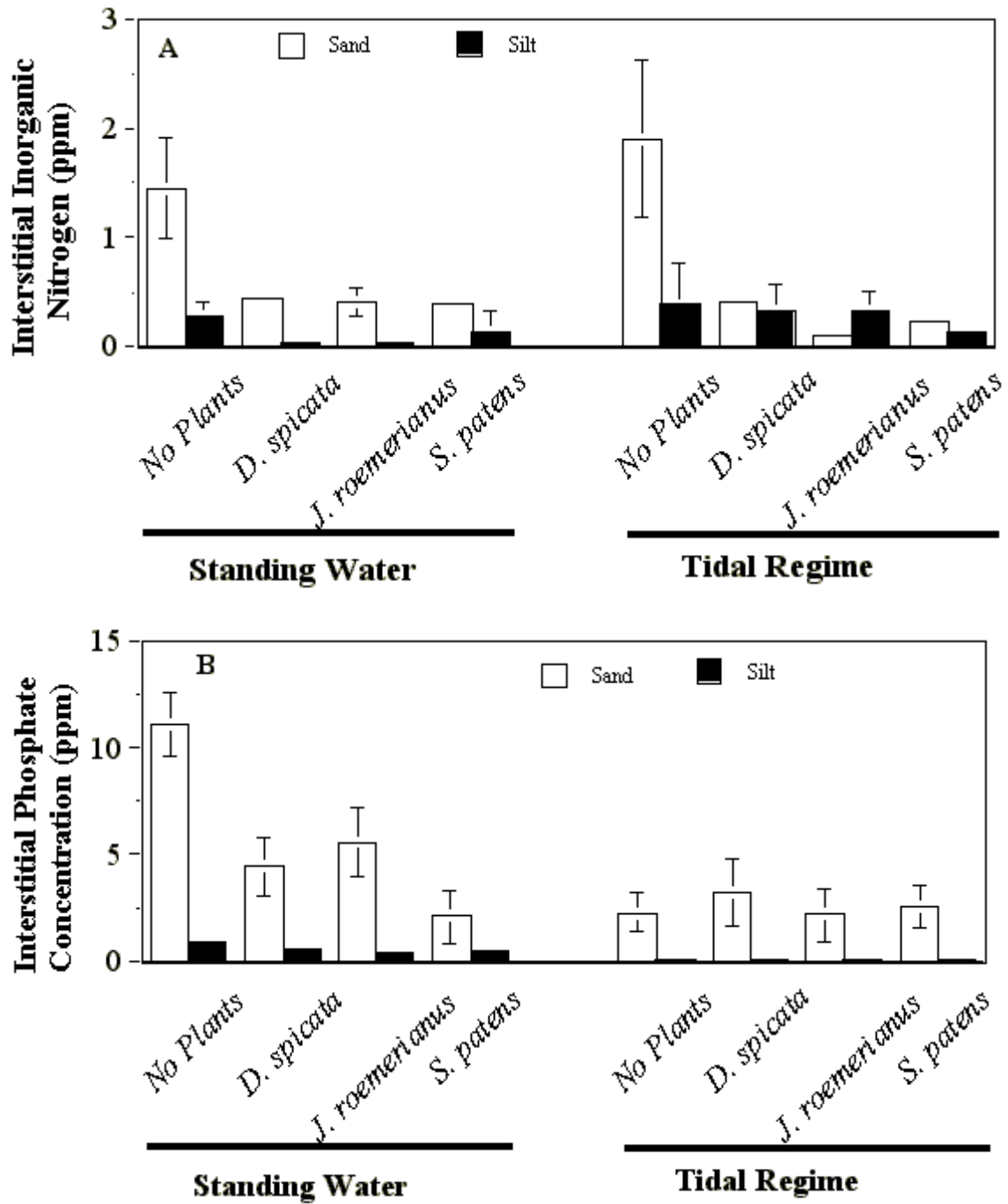


Fig. 7 Effect of phytoremediation, soil texture, and inundation regime on inorganic nitrogen (A) and phosphate (B) in the interstitial water 12 months after the phytoremediation treatment. Values are means (n=5) with standard errors.

Table 3 ANOVA table for the soil and oil degradation rate variables with p-values provided for main factors and their interactions. Phyto: phytoremediation; Fert: fertilization; Inund: inundation; Phyto*Fert: phytoremediation by fertilization, etc.

Parameter	Main Factors			Interaction			
	Phyto	Inund	Soil	Phyto* Inund	Phyto* Soil	Inund* Soil	Phyto* Inund* Soil
Eh at 3 cm (6 months)	0.0006	0.0001	0.4045	0.1985	0.0832	0.3473	0.1656
Eh at 3 cm (12 months)	0.0001	0.0168	0.5741	0.8583	0.0279	0.5897	0.5449
Interstitial Nitrogen	0.0010	0.5588	0.0023	0.5937	0.0040	0.5432	0.6919
Interstitial Phosphate	0.0118	0.0001	0.0001	0.0023	0.0406	0.0030	0.0051
Soil Respiration (6 months)	0.0001	0.9829	0.0001	0.8004	0.0233	0.3287	0.3954
Soil Respiration (12 months)	0.0001	0.0084	0.6835	0.3380	0.3187	0.7479	0.0938
Heterotrophic Microbes	0.0003	0.4420	0.0001	0.4420	0.0523	0.1611	0.1467
Oil-degrading Microbes	0.0653	0.0261	0.0111	0.1143	0.0520	0.0213	0.0534
Oil degradation rate	0.0001	0.0001	0.0001	0.0031	0.0002	0.0001	0.0066

3.4 Soil Respiration and Microbial Populations

The soil respiration rate was analyzed to determine the treatment effect on soil microbial activity. The soil respiration rate in all treatments with transplants was significantly higher ($p < 0.0001$) than the rate in treatments without transplants (Fig. 8, Table 3) six and 12 months after phytoremediation application. For instance, the soil respiration rate in the treatment receiving *S. patens* was more than 40 fold higher than in treatments without transplants 12 months after transplantation (Fig. 8B). After 12 months, soil respiration rates were significantly higher in the treatments receiving the daily tidal inundation regime than rates in the standing water regime (Fig. 8B). In

addition, soil respiration rates were significantly higher in the treatments with silt soil than in treatments with sand after six months (Fig. 8A).

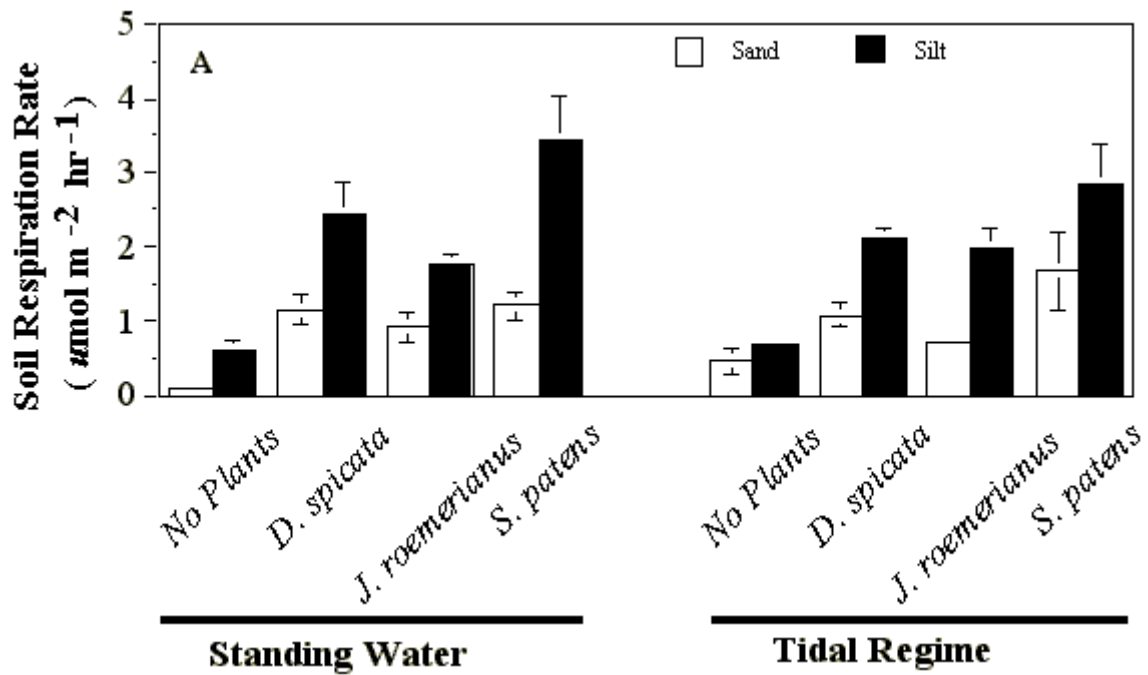
Phytoremediation with transplants significantly ($p < 0.0005$) increased heterotrophic microbial populations in the soil. For example, soil heterotrophic microbial populations in the treatments receiving *S. patens* and *J. roemerianus* were about 14 and 11 fold higher, respectively, than populations in treatments without transplants 12 months after transplantation (Fig. 9). Treatments with transplants had the highest soil respiration rates. In addition, soil heterotrophic microbial populations in the treatments with alluvial silt soil were significantly (Table 3) higher than populations in treatments with sandy soil. The former had $2.04E+07$ counts/g soil versus $6.23E+06$ in the latter six months after treatment (Figs. 9A and 9B).

Phytoremediation significantly ($p = 0.055$) increased oil degrading microbial populations 12 months after the treatments (Figs. 10, Table 3). The oil degrading microbial populations were 19 and 11 fold higher in the treatments receiving *S. patens* and *J. roemerianus* than the populations in treatments without plants, respectively. The oil degrading microbial populations were significantly higher in the treatments receiving the standing water regime compared to the treatments receiving the tidal regime, with $1.37E+06$ counts/g soil in the former versus $3.42E+05$ in the latter. In addition, the oil degrading microbial populations were significantly higher in the treatments with alluvial silt compared to treatments with sandy soil (Fig. 10A and 10B), with $1.45E+06$ counts/g soil in the former versus $2.67E+05$ in the latter. A significant ($p = 0.0213$) interaction between phytoremediation application and soil type indicated that the higher oil degrading microbial populations in the treatments with silt soil occurred only under the standing water regime.

3.5 Oil degradation in the Soil

The main factors, including phytoremediation with transplants, inundation treatment, and soil texture, all affected the oil degradation rate. Oil degradation rates in the treatments with phytoremediation were significantly higher ($p < 0.0001$) than rates in treatments without phytoremediation (Fig. 11A and B, Table 3). Oil degradation rates in the treatments receiving *Distichlis spicata*, *Juncus roemerianus*, and *Spartina patens* were 3.1, 2.9 and 2.6 fold higher than rates in treatments without phytoremediation, respectively. Oil degradation rates in the treatments receiving the daily tidal inundation regime were significantly higher ($p < 0.0001$) than rates in the standing water inundation regime; we observed a 29.1% reduction in the former versus 16.6% in the latter. Oil degradation rates in treatments with sandy soil were significantly higher ($p < 0.0001$) than rates in treatments with alluvial silt; we observed a 29.5% reduction in the former versus 17.6% in the latter. A significant ($p = 0.0031$) interaction between phytoremediation and inundation condition indicated that the effect of phytoremediation on the oil degradation rate was greater under the daily tidal inundation regime than under the standing water inundation regime. A significant ($p = 0.0002$) interaction between phytoremediation and soil type indicated that the effect of phytoremediation on the oil degradation rate was

greater in the treatments with the sandy soil than in treatments with the alluvial silt. In addition, a significant ($p < 0.0001$) interaction between inundation condition and soil texture indicated that effect of the tidal inundation regime on the oil degradation rate was greater in the treatment with sandy soil than in the treatment with alluvial silt. Furthermore, a significant ($p = 0.0066$) interaction among phytoremediation, inundation, and soil texture indicated that the oil degradation rate varied with specific combinations of treatments. For example, phytoremediation of oil by *D. spicata* was greatest in the treatment with sandy soil, but not in the treatments with the alluvial silt. At the same time, the phytoremediation effectiveness by *J. roemerianus* was greater in the treatments with the alluvial silt than in the treatments with the sandy soil.



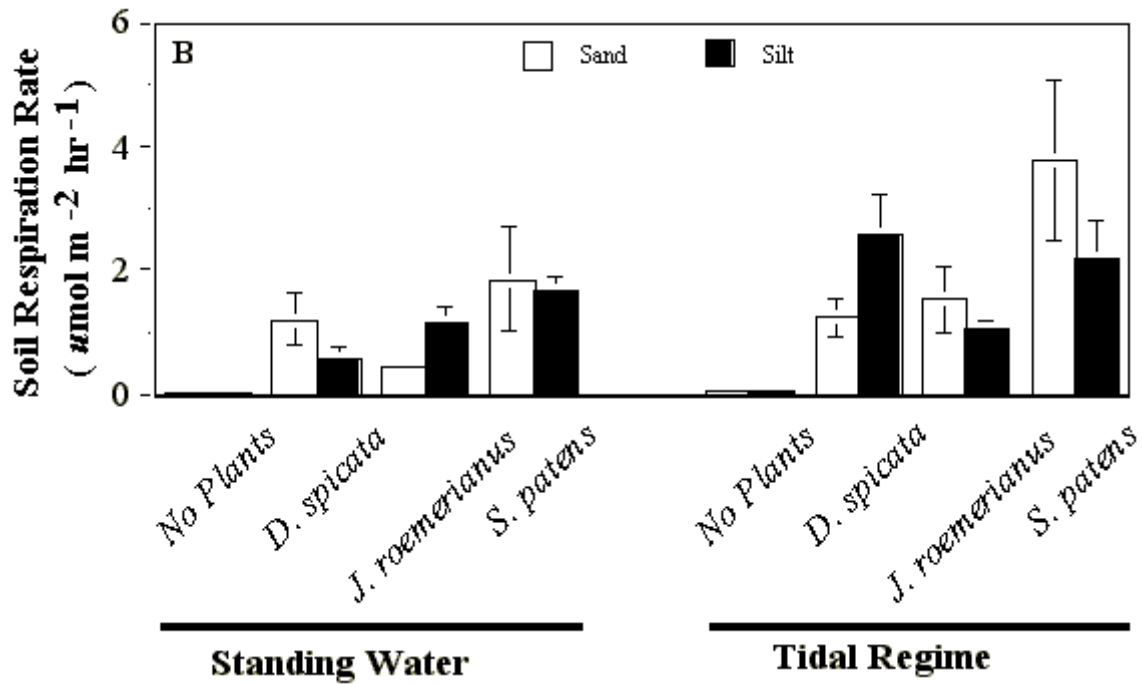
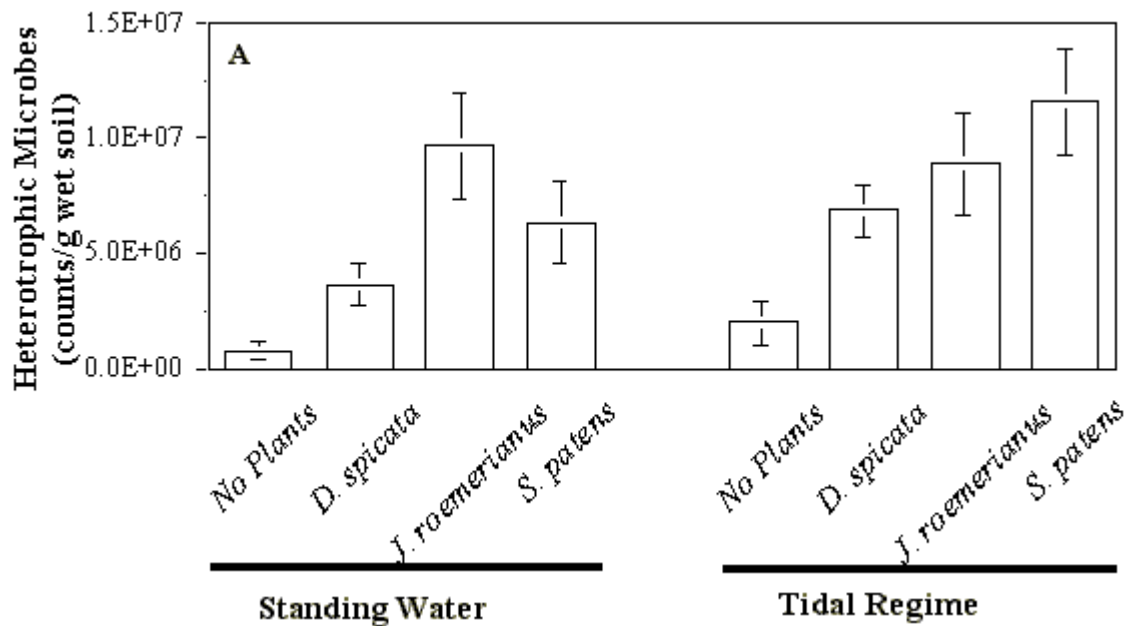


Fig. 8 Effect of phytoremediation, soil texture, and inundation regime on soil respiration rates six (A) and 12 (B) months after the phytoremediation treatment. Values are means ($n=5$) with standard errors.



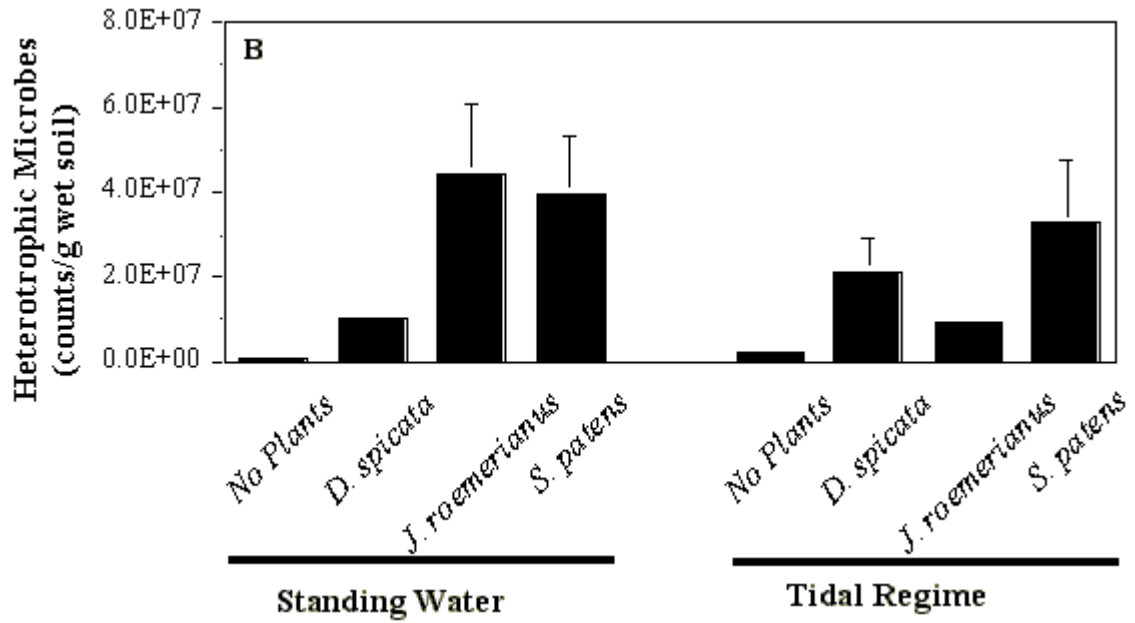
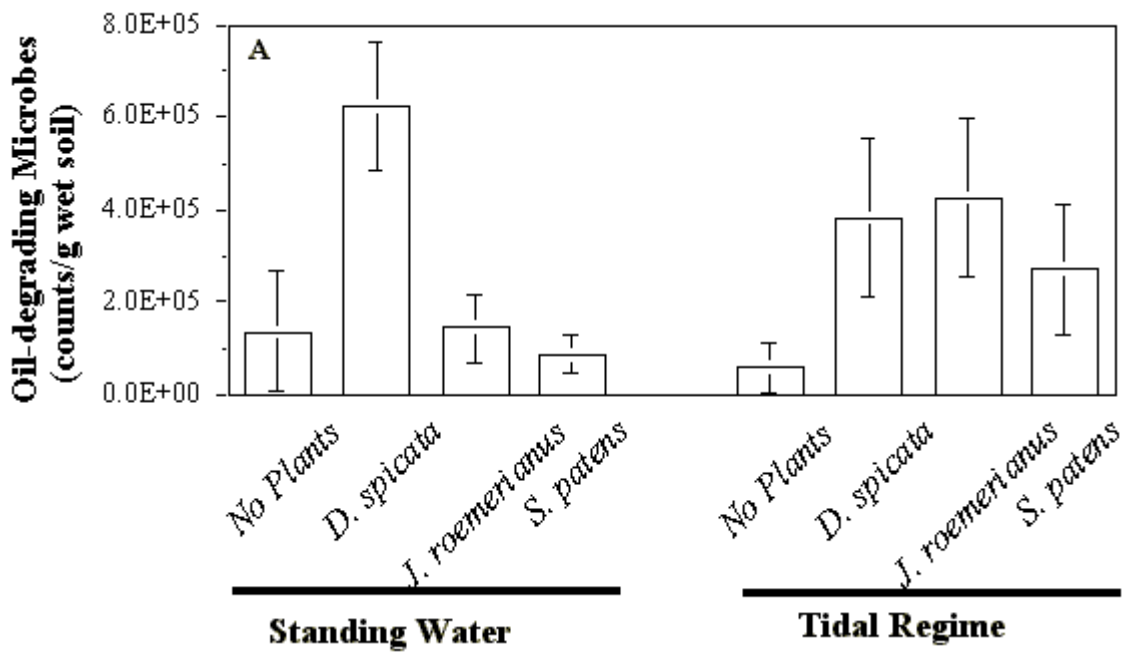


Fig. 9 Effect of phytoremediation, soil texture, and inundation regime on soil heterotrophic microbial populations in the sandy soil (A) and alluvial silt (B) 12 months after the phytoremediation treatment. Values are means ($n=5$) with standard errors.



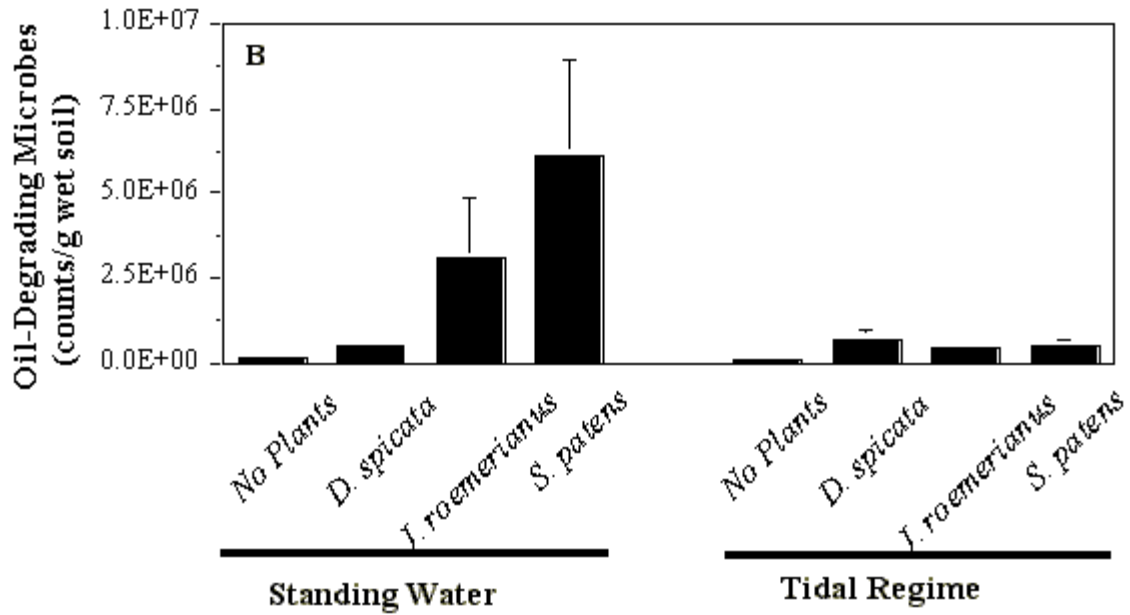
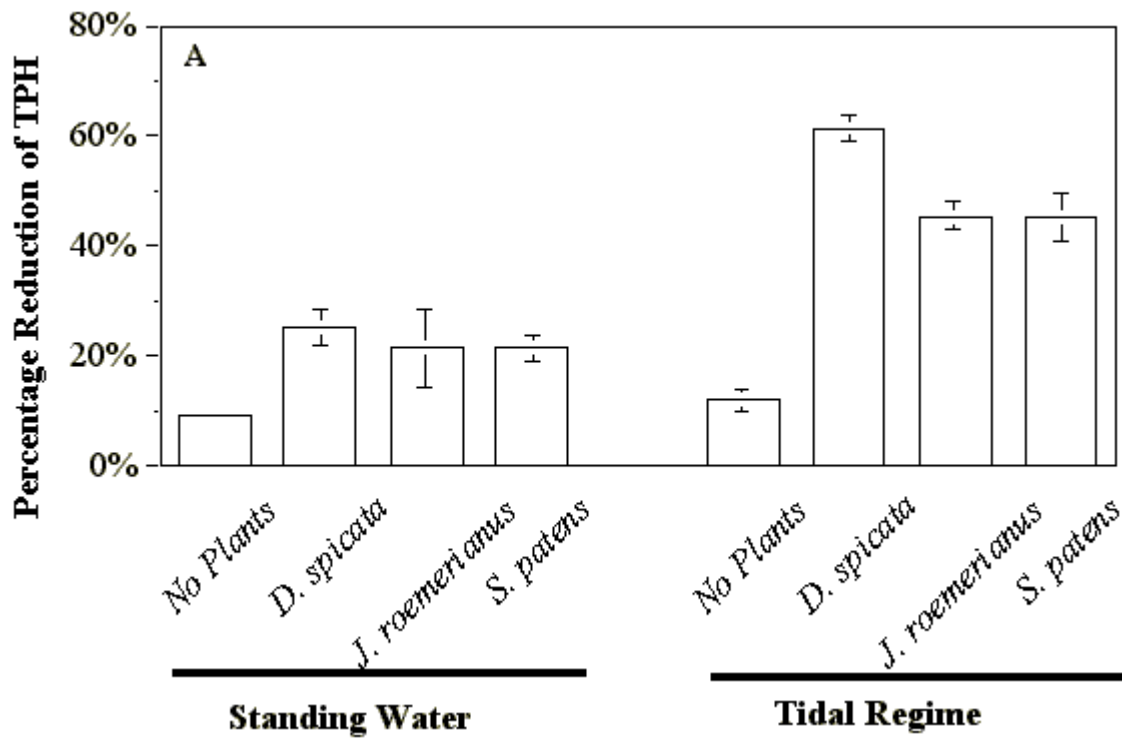


Fig. 10 Effect of phytoremediation, soil texture, and inundation regime on oil degrading microbial populations in the sandy soil (A) and alluvial silt (B) 12 months after the phytoremediation treatment. Values are means ($n=5$) with standard errors.



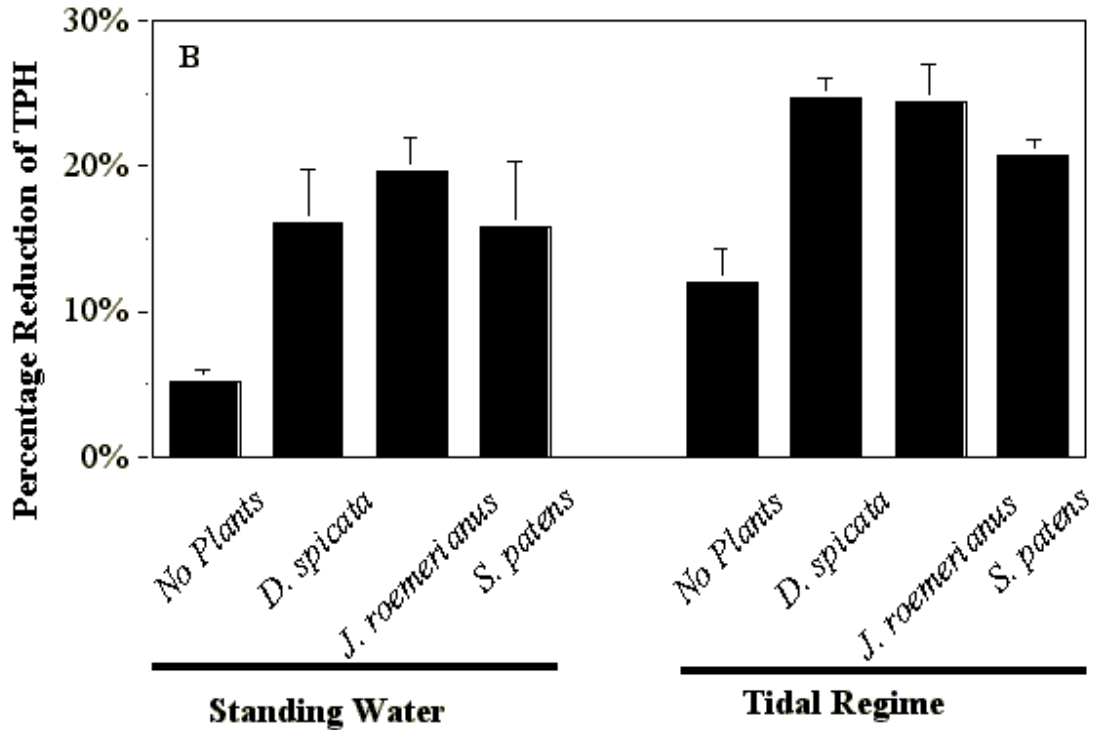


Fig. 11 Effect of phytoremediation, soil texture, and inundation regime on percentage reduction of total petroleum hydrocarbons in the sandy soil (A) and alluvial silt soil (B) 0.5-3.5 cm below the soil surface 12 months after the phytoremediation treatment. Values are means ($n=5$) with standard errors.

4.0 Discussion

The present study further supports our previous findings (Lin and Mendelssohn 1997, 1998a; and Lin *et al.* 1999) that phytoremediation has considerable potential for restoring habitat and accelerating oil degradation in coastal wetlands. A number of common brackish and fresh water plant species can be successfully established in soils contaminated with weathered crude oil. Phytoremediation of oil by brackish marsh species significantly reduced the residual oil concentration in both sand and alluvial silt. In addition, phytoremediation of oil by brackish marsh species significantly reduced the residual oil concentration under both standing water and daily tidal inundation conditions.

Three brackish marsh species established and grew well in the soil with high concentrations of weathered South Louisiana Crude oil. Successful transplantation of marsh plant species into oil contaminated soil is a first and important stage for phytoremediation. Plant stem density increased by as much as 17 fold compared to the initial stem density. Plant photosynthetic rate, plant shoot height, and live above-ground biomass were high. All these responses indicated successful transplantation and subsequent growth. Oil spills can have a wide range of negative impacts on marsh

vegetation, affecting growth, photosynthetic rate, stem height, density, live percent cover, and biomass. If the oil toxicity or volume is high, mortality may result (Krebs and Tanner 1981; Alexander and Webb 1987; Mendelssohn *et al.* 1990, Li *et al.* 1990; Lin and Mendelssohn 1996). Previous studies (Lin and Mendelssohn 1998a) have shown that marsh plants can be transplanted into soils contained 57 mg g⁻¹ of weathered Louisiana crude oil. In the present study, the oil concentration in the soil was about 25 mg g⁻¹ of oil. This oil volume almost saturated the sandy soil because of low soil organic matter and the much higher bulk density of sandy soil and alluvial silt used in this study. These results suggest that phytoremediation can likely be used for habitat restoration in most oil spills, especially for crude oil spills.

Generally, higher stem density, canopy height, and above-ground biomass occurred under the standing water regime compared to the daily tidal inundation regime. All plants used in this experiment were brackish marsh species, which are well adapted to inundated environments. These plants have well developed aerenchyma (air space tissues) that allow aerobic root metabolism (Teal and Kanwisher 1966; Armstrong 1978; Smirnoff and Crawford 1983) and growth under flooded conditions. Higher stem density, canopy height, and above-ground biomass were also documented in the alluvial silt compared to sand in the present study. Inorganic nutrient concentrations in the alluvial silt were more stable over the whole experiment compared to concentrations in the sandy soil. The small soil particles in alluvial silt have much larger surface areas than sand, and can thus better absorb inorganic nutrients. For example, the availability of dissolved inorganic phosphate in wetlands primarily depends on the capacity of the soil to release orthophosphate-P to a solution low in P and to sorb it from a solution high in P (Patrick and Khalid 1974). Soils of a coarse texture with higher sand content usually have a lower capacity to sorb phosphorus, and thus they release less phosphorus than more fine textured soils containing silt and clay (Poach and Faulkner 1998; Syers *et al.* 1969; Lin *et al.* 1999b). Thus, adsorption and de-adsorption tend to moderate large variations in interstitial phosphate. The relatively constant supply of inorganic nutrients is mostly likely responsible for the better growth of the plants in the alluvial silt compared to the sand.

In the present study, phytoremediation by brackish marsh plant species significantly reduced the residual oil concentration in the soil, supporting previous findings that phytoremediation can clean up various contaminants, such as petroleum hydrocarbons and polycyclic aromatic hydrocarbons (Lytle and Lytle 1987; Banks and Schwab 1993; Lin and Mendelssohn 1998, Salmon *et al.* 1998; Lin *et al.* 1999a and 1999b; Liste and Alexander 2000). The oil degradation rate was significantly higher in the treatment with brackish plants compared to the treatment without plants in the present study. A number of studies have found that phytoremediation enhances hydrocarbon degradation. Liste and Alexander (2000) reported that more pyrene was degraded in the presence of roots of all nine plant species tested than was degraded in unplanted soil. In approximately eight weeks, as much as 74% of the pyrene disappeared from vegetated soil compared to 40% or less from unplanted soil. Phytoremediation by *Typha latifolia* reduced 90% of hydrocarbons in an artificial wetland over a 360 day period (Salmon *et al.* 1998). Wright *et al.* reported that *Spartina alterniflora*, with the addition of phosphorus and nitrogen,

significantly increased oil degradation in mesocosms. Lin *et al.* (1999b) reported that fertilization significantly enhanced *Spartina alterniflora* growth and reduced total normal hydrocarbons and total targeted aromatic hydrocarbons in salt marsh mesocosms, which likely resulted from a combination of phytoremediation and bioremediation.

Several possible mechanisms of enhanced oil degradation via phytoremediation have been cited, although mechanisms of action are poorly understood. The soil physico-chemical environment can affect soil microbial activity, thus affecting the microbial degradation rate. Oxygen is an important electron acceptor for microbial oil degradation (Mikesell *et al.* 1991). The oil degradation rate is generally much slower in a water saturated anaerobic condition than in an aerobic environment (Hambrick *et al.* 1980, Lin and Mendelssohn 1998). Therefore, increased oxygen supply in the rhizosphere may enhance oil degradation. In the present study, phytoremediation by transplants significantly increased soil redox potential, indicating a more oxidized soil environment in the treatments receiving transplants. It is well known that wetland plants can transport oxygen from the atmosphere through plant air-space tissue to the soil rhizosphere (Teal and Kanwisher 1966; Armstrong 1978; Smirnoff and Crawford 1983), thus increasing soil oxidation status. In addition, evapo-transpirational water losses from the treatments with phytoremediation was up to eight fold greater than losses from treatments without transplants, especially in the treatment receiving *J. roemerianus*. This high water loss in the daily tidal regime increased water removal from the soil during low tide. This likely allowed for greater air entry into the soil, thereby increasing aerobic oil degradation in the treatments with phytoremediation.

Phytoremediation by plants may change the soil environment to favor soil microbes in other ways. In the present study, the soil microbial respiration rate and populations of soil heterotrophic and oil degrading microbes were greater in the phytoremediation treatments. Nichols *et al.* (1997) reported that organic chemical degrader populations were significantly higher in rhizosphere soils than in bulk soils. This result was found after 14 weeks of phytoremediation by *Medicago sativa* and *Poa alpina* in a soil containing a mixture of PAHs. This finding suggests the potential stimulation of bioremediation around plant roots. Phytoremediation may enhance microbial populations by providing organic leachates (such as amino acids, simple sugars, and complex carbohydrates) from plant roots to the soil to create a more favorable rhizosphere for microbial activity (Walton and Anderson 1990; Hegde and Fletcher 1996). In addition, the plant roots in the rhizosphere may provide a physical structure that favors microbial attachment. Hou *et al.* (1999) suggested that because grasses have fibrous root systems, they can provide high surface areas for soil microbial populations, which increases bioremediation in the rhizosphere. Grasses have been identified as having great potential as phytoremediators of petroleum contaminated soils. In the present study, the inorganic nutrient concentration was higher in the treatment without phytoremediation than in treatments with phytoremediation. However, soil microbial populations and activity were lower in the former than in the latter. Thus, something other than inorganic nutrients, e.g. soil aeration or root organic leachates, may play a more important role in enhancing microbial growth.

Plants used in phytoremediation may uptake contaminants from the soil and metabolize them. It was reported (Lytle and Lytle 1987) that *Juncus roemerianus* uptakes petroleum hydrocarbons from marsh sediments, thus reducing oil concentration. Schwab *et al.* (1998) suggested that one possible step in the phytoremediation process is adsorption of the organic contaminant onto the surface of the roots, followed by uptake and/or degradation. Their results indicated that root lipid content is a controlling factor in the adsorption of naphthalene onto plant roots. In a phytoremediation trial with hybrid poplar trees, Burken and Schnoor (1998) reported that translocation and the subsequent transpiration of volatile organic compounds from the leaves to the atmosphere constituted a significant pathway. Therefore, plant uptake, translocation, and metabolism of soil contaminants, such as hydrocarbons, may play an important role in phytoremediation. These processes need further study.

In the present study, phytoremediation induced oil degradation depended on the particular marsh plant species. *Distichlis spicata* and *J. roemerianus* showed a greater reduction in residual oil concentration in the soil, although all three brackish marsh plant species significantly enhanced oil degradation compared to the unplanted treatment. However, the live above-ground biomass of *S. patens* was highest among the three plant species. This result suggests that oil degradation is not necessarily related to plant above-ground biomass. For example, *J. roemerianus* had the highest evapo-transpiration rate, although its biomass was not the highest. A greater water transpiration stream may also carry larger amounts of petroleum hydrocarbons, especially more water soluble components such as PAHs, into the plant, thus increasing uptake of hydrocarbons. Carman *et al.* (1998) reported that 40 and 90% reductions in the concentration of diesel range hydrocarbons within the rhizospheres of trees were observed in a 24 week phytoremediation study. The chromatograms for those analyses exhibited a relative decrease in the proportion of the more water soluble and available shorter chained or lower molecular weight hydrocarbons compared to their higher molecular weight counterparts in the fuel. This suggests that plants may take up water soluble components more readily. In addition, the present study found that oil degrading microbes were not completely related to oil degradation rates, although they were significantly higher in the soil with transplants than in soil without plants. For example, oil degrading microbial populations in the treatment with *D. spicata* were lowest among all phytoremediation treatments, although the populations were significantly higher than in the unplanted treatment. However, the oil degradation rate was highest in the treatment with *D. spicata*. These results suggest that the effectiveness of phytoremediation was a combination of several processes, such as creating more aerobic conditions; providing organic leachates to the rhizosphere; providing more fibrous root systems, which can provide high surface area; having a higher transpirational stream that can carry more contaminants; and/or having roots with more lipid content favoring adsorption of hydrocarbons onto plant roots and uptake. The effective combination of pathways may determine the overall efficiency of phytoremediation.

In the present study, the marsh inundation environment affected oil degradation. The oil degradation rate was higher in the daily tidal inundation regime than in the standing water inundation regime. The oil degradation rate is generally much slower in a water

saturated anaerobic condition than in an aerobic environment (Hambrick *et al.* 1980, Lin and Mendelssohn 1998). The surface soil in the daily tidal regime had 12 hours of exposure to atmospheric air compared to no exposure in the standing water regime. The air could directly diffuse from the soil surface into the soil during the low tidal exposure. Thus, air may aerate the soil and increase aerobic oil degradation in a daily tidal regime. However, under a standing water regime, the air diffusion rate through the surface water is slow. Air transported by the plants may play a key role in aerating soil under standing water conditions. In addition, plants may provide a conduit for hydrocarbons from soil to air, especially for volatile components. Furthermore, the oil degradation rate was higher in the sandy soil than in the alluvial silt, especially under the tidal inundation regime. This was most likely due to transplants removing water from the coarse soil particles during low tidal exposure, thus letting air enter the pore space to create a more oxidized soil environment. In addition, water-free pores during low tidal exposure may let more volatile components release from the soil to the air, thus reducing oil concentration in the soil.

5.0 Conclusions

Phytoremediation by brackish marsh plants showed great potential as a means of restoring habitat restoration and cleaning up oil spills. All three dominant brackish marsh plants were successfully transplanted to crude oil contaminated soil. Oil in the soil did not adversely affect transplants, further suggesting that phytoremediation can be used for habitat restoration after most oil spills. Phytoremediation significantly increased oil degradation in both the sandy soil and the alluvial silt, indicating that phytoremediation can remediate oil contamination for various soil types, such as a sandy backbarrier marsh or silt and clay enriched inland marshes. Hydrology and associated inundation regimes are major-forcing functions in wetlands. Phytoremediation significantly increased oil degradation both in the daily tidal regime and in the standing water regime, indicating that phytoremediation can effectively remediate oil contamination under different inundation environments. This is especially true for most common wetland environments, such as intertidal streamside marshes and soil waterlogged inland marshes. The effectiveness of phytoremediation by brackish marsh plants depends upon the plant species and its environmental scenario. For example, the effectiveness of phytoremediation by *D. spicata* was greatest in the coarse textured sandy soil under the daily tidal inundation regime. However, the effectiveness of phytoremediation by *J. roemerianus* was greatest in fine particle alluvial soil under the standing water inundation regime. Overall, the effectiveness of phytoremediation by all plants was greatest in the sandy soil with daily tidal inundation. The present study demonstrated that phytoremediation should accelerate both oil degradation and site restoration in a wide range of common coastal environments.

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Appendix

Table A1. Plate count agar (Difco) formulated

Pancreatic digest of casein	5.0 g
yeast extract	2.5 g
dextrose	1.0 g
agar	15.0 g
	1000 ml, pH of 7.0

Table A2. Preparation of Basal medium: Trace Elements

solution #1

$(\text{NH}_4)_2\text{SO}_4$ at 15 g / 100 ml

solution #2

KH_2PO_4 at 3.6 g, and
 Na_2HPO_4 at 1.2 g in 100 ml

solution #3 trace elements

CuSO_4 at 100 ug / 100 ml
 MgCl_2 at 1.5 g / 100 ml
 ZnSO_4 at 800 mg / 100 ml
 MnCl_2 at 20 mg / 100 ml
 CaCl_2 at 10 g / 100 ml
 CoCl_2 at 20 mg / 100 ml

solution #4

$\text{FeSO}_4 \cdot 5\text{H}_2\text{O}$ at 250 mg / 100 ml

Add 10.0 ml of stock solution 1, 2, 3 and 4 to 1000 ml of water and dissolve. Next, add 15 g agar granules for each liter of basal medium, melt agar at 100°C and sterilize the agar base (basal medium, BM) with stirring bar in flask. Temper to 50 to 55°C for one hour in a water bath. The tempered medium was placed on a magnetic stirrer and 1.0 ml of weathered oil was added to each 1000 ml of BM and stirred for two to three minutes to emulsify oil. The medium was stirred following every 20 plates poured.