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Wetland Restoration Mesocosm Studies:

Examination of Hematite-based Drilling Mud Phytotoxicity and Suitability as Wetland Substrate

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EXECUTIVE SUMMARY

Wetland loss, conservation, and restoration have become increasingly important environmental issues as the benefits of wetlands and their alarming rate of loss have been elucidated. Louisiana contains 40% of the coastal wetlands in the United States and accounts for 80% of the nation's coastal wetland loss. The need to protect and restore Louisiana's wetlands, which are an integral part of the state's and nation's productivity, is paramount.

The high rate of wetland loss in Louisiana has been largely attributed to high rates of subsidence (sinking of land) due to sediment deprivation from alteration of the hydrology of the Mississippi River, as well as canal dredging and saltwater intrusion. Diversions of freshwater and sediment from riverine sources and utilization of dredge material to create wetlands have proven to be some of the most successful methods of offsetting wetland loss caused by sediment deprivation. However, some deteriorating wetlands are isolated from potential substrate sources. Therefore, a need exists to find alternative substrates and methods for wetland restoration and creation.

This study builds upon earlier research conducted by Southeastern Louisiana University concerning the efficacy of utilizing processed drill cuttings as an alternative substrate source for wetland rehabilitation (wetland creation and restoration). Two different types of processed drill cuttings were utilized in these early experiments: one obtained from physical separation of the drilling fluids from the cuttings, the other from further processing and encapsulation of the cuttings in a silica matrix. Performance of plants grown in the first substrate was comparable to both dredge spoil, a common wetland restoration substrate, and topsoil. Performance of plants grown in the encapsulated substrate was greatly reduced compared to all other substrate types under fresh water conditions, but became equivalent under saline conditions. In all studies to

date, there appears to be little danger of migration of metals from drill cuttings into interstitial waters or of bioaccumulation of metals from these drill cuttings, with the exception of barium. Although barium accumulation in plant tissue was noted, the concentrations were determined to be within acceptable ranges for many plant species, including agricultural varieties.

As promising as these initial results were, it was decided that a more direct approach would be to engineer drilling fluids (i.e., drilling muds) that are environmentally safe and amenable for wetland rehabilitation, thereby minimizing the need for processing drill cuttings. This approach would explicitly minimize environmental impacts from the onset of the drilling process and improve the capacity of the cuttings to support wetland plant growth. Therefore, a research partnership was established between Southeastern Louisiana University (and later with the University of New Orleans) and MI Drilling Fluids, Inc. that was funded through the U.S. Department of Energy in association with Argonne National Laboratory to develop and assess the potential of environmentally-benign, hematite-based simulated drill cuttings to support vigorous wetland plant growth. The assessment of such a hematite-based drilling mud is the subject of this report.

The evaluations of new hematite-based drilling muds developed by MI Drilling Fluids, Inc. were very promising. Because actual drill cuttings that were produced with this new drilling mud formulation could not be obtained, the drilling mud was evaluated in mixtures with either river silt or topsoil. Evaluations were conducted through a series of four experiments. Three preliminary experiments investigated the ability of various mixtures of drilling muds and stabilizing agents to support the growth of a widespread coastal grass species, *Spartina patens* (marshhay cordgrass). A fourth, larger mesocosm evaluation assessed plant responses of three common coastal plant species (*Spartina patens*, *Sagittaria lancifolia* (bulltongue), and

Rhizophora mangle (red mangrove)) in terms of plant growth, plant photosynthesis, plant photosynthetic integrity, and plant tissue elemental composition (to detect potential metal accumulation). Substrate interstitial water (pore water) analyses, as well as complete substrate acid digestions, were conducted to determine availability of metals in solution and total potential metal availability.

Results from the three preliminary experiments indicate that the hematite-based drilling mud can support excellent growth of marshhay cordgrass under a variety of conditions. Of particular interest is that a comparison of plant performance in a hydroponic nutrient solution versus a hydroponic drilling mud solution showed no significant differences in plant growth response. Also, an experiment that assessed plant growth response in topsoil (control) and mixtures of drilling mud with topsoil (up to 30% drilling mud) showed no decrease in plant growth response with increasing concentration of drilling mud. There were some differences detected among various stabilizing amendments when mixed to equal consistency with pure drilling mud. Seven stabilizing amendments were assessed (Fiberlock, Phenoseal, Kwickseal mix, medium ground mica, fine-ground mica, pecan nut plug, and walnut nut plug). Fiberlock and Phenoseal, and to a lesser extent Kwikseal mix, tended to result in the poorest plant performance. However, when the seven stabilizing amendments were compared independently from the drilling mud in equal-volume mixtures with topsoil no significant differences between stabilizing amendments were detected in their ability to support plant growth. Therefore, although there may be some differences in plant growth response associated with stabilizing amendment mixtures under certain conditions, these preliminary studies clearly show that the hematite-based drilling mud can support vigorous growth of a widespread wetland plant, marshhay cordgrass.

Results from the mesocosm evaluation were similarly very promising. Importantly, the analyses of metal accumulation in the plant tissue showed that no significant metal accumulation had occurred as indicated by a lack of significant differences between the control substrate (river silt) and drilling mud mixtures of either river silt only, or river silt and bagasse (organic amendment that results from processed sugarcane). Analyses of the substrate pore water, as well as substrate acid-digestion extracts (to release all potentially-available metals) both showed that iron and chromium were significantly elevated in substrates that contained drilling mud. However, for both of these metals the detected concentrations were lower than USGS low-alert levels. Therefore, although elevated relative to river silt, these higher concentrations do not appear to pose an environmental threat. Although live aboveground biomass and total biomass were somewhat depressed in both substrates containing drilling mud, midpoint and final measures of instantaneous growth (net CO_2 assimilation and live to total stem ratio) and stress response (fluorescence characteristics) indicated essentially no differences between the substrates.

In summary, hematite-based drilling muds do show great promise for the resultant drill cuttings potentially used for wetland creation/restoration efforts. However, prior to a field demonstration we recommend that a long-term, controlled mesocosm study be conducted over a period of at least one year utilizing actual drill cuttings to verify that no bioaccumulation of metals is likely to occur. Additionally, responses of faunal components, such as aquatic invertebrates and fish, should also be assessed under a longer-term controlled study.

INTRODUCTION

The coastal wetlands of Louisiana have experienced an extraordinary loss of area over the last century, and continue to have the highest rates (35-45 km^2yr^{-1}) of coastal wetland loss in the United State (Barras et al. 1994, Dunbar et al. 1990). Major factors implicated in this loss include the leveeing of the Mississippi River for flood control, saltwater intrusion, and canal dredging for oil and gas extraction (Day et al. 2000, Turner 1997). These factors can reduce marsh elevation either by preventing the addition of allocthonous sediments, as in the leveeing of the Mississippi River, or in the case of canal dredging and saltwater intrusion, through the direct removal or impairment of the vegetation, which reduces belowground productivity and therefore marsh accretion (Nyman 1993, Mitsch and Gosselink 2000). This reduction in marsh elevation is further aggravated by the natural process of subsidence, which in Louisiana's deltaic plain is occurring at an accelerated rate of approximately one order of magnitude greater than other wetland systems (Stumpf and Haines 1998). Thus, the restoration of wetlands within the coastal environment of Louisiana often must focus on sediment addition and the enhancement of marsh elevation to be successful.

Drill cuttings are the waste products that result from drilling excavations, consisting of the parent substrate (earthen materials) and drillings fluids or "muds" (composed of a fluid component: oil, synthetic organic, or water and additives such as organic thinners, hydroxides, and barite, etc.) that are used in the drilling process (Scholten et al. 2000, Gray and Darley 1980). Water-based drilling muds have a greatly reduced environmental impact compared with oil-based muds, and are often employed in near-shore drilling activities (Rogier and Mulder 1996). The type of drilling mud used varies depending on the characteristics of the drilling vicinity (e.g., drilling depth, substrate composition, etc.; Carls and Rice 1984, Gray and Darley 1980). A variety of processes have been developed that allow for the majority of the drilling muds to be

separated from the drill cuttings and reused (recycled) in the drilling process. This is an economical measure in that it allows for the retention of drilling fluids for use in further drillings and also reduces the contaminant load and weight of the cuttings considerably. Excavation of gas or oil wells generally produces approximately 1,000 m^3 of drill cuttings per well (Scolten et al. 2000). These drill cuttings, which are considered oil-field waste, may be a viable source of sediment for the restoration of coastal marshes in Louisiana if they can be shown to be environmentally safe and support vigorous wetland plant growth.

The potential of processed drill cuttings to support vegetative growth has been examined for uses in both wetland restoration (Kelley and Mendelssohn 1995) and agriculture (Chaineau et al. 1996). Kelley and Mendelssohn (1995) determined that processed drill cuttings could support the growth of wetland macrophytes following amendment with organic material. Further studies examining the capacity of processed drill cuttings to serve as substrates for wetland rehabilitation have been performed at Southeastern Louisiana University (Shaffer et al. 1996, DesRoches 1998, Shaffer et al. 1998, Hester et al. 2000, Willis 2000). In these studies two types of processed drill cutting were assessed in terms of vegetation performance and contaminant release in mesocosm settings. The first processed drill cutting type (DC-A) results from centrifugal separation of the cuttings from the drilling mud, while the second (DC-B) results from further processing that encapsulates the cuttings and any remaining drilling fluids within a silica matrix (proprietary information, SWACO, New Orleans).

The studies conducted at Southeastern Louisiana University concerning the safety and suitability of processed drill cuttings for use in wetland restoration have been encouraging (Shaffer et al. 1996, DesRoches 1998, Shaffer et al. 1998, Hester et al. 2000, Willis 2000). Results from these previous studies indicated that both types of processed drill cuttings exhibited

low toxicity, both as tested by mysid shrimp seven-day chronic toxicity trials and a long-term mesocosm evaluation of phytotoxicity (Shaffer et al. 1996, DesRoches 1998, Shaffer et al. 1998, Hester et al. 2000, Willis 2000). These studies also indicated a low probability of heavy metal release to substrate interstitial water (pore water) under freshwater and saline conditions. Plant establishment and productivity under freshwater conditions was shown to be comparable among DC-A (drill cuttings centrifugally separated from drilling fluids), dredge spoil, and a control substrate (topsoil). The drill cuttings that underwent further processing to encapsulate the cuttings and remaining drilling fluids (DC-B) showed low levels of toxicity, but only supported very limited growth of wetland vegetation under freshwater conditions. The poor response of vegetation grown on DC-B under fresh water conditions was attributed to both the high pH of the interstitial waters of this substrate (mean pH=11.1) and its coarse texture (DesRoches 1998). However, under saline conditions (9 ppt, 18 ppt, and 36 ppt) the mean pH of the DC-B substrate became ameliorated (~8.5) and measures of plant instantaneous growth (net CO_2 assimilation) became comparable with all other substrates, including the control (Hester et al. 2000, Willis 2000). This again indicated that drill cuttings have the capacity to support vigorous growth of wetland plants once artifacts of processing are ameliorated.

Based upon these findings, we suggested that the development of more environmentally-benign drilling cuttings, by reducing the toxicity of the drilling mud and therefore the need for processing, may be the most efficacious approach to utilize drill cuttings in coastal restoration. This approach could simultaneously reduce the cost of implementing drill cuttings as a wetland substrate restoration technique and improve the likelihood for successful wetland creation. Many heavy metals tend to be associated with barite, an ore of barium that is often a major constituent of drilling muds (Olsgard and Gray 1995). Also, barite itself has been determined to

be toxic to several marine invertebrates (Barlow and Kingston 2001, Cranford et al. 1999). Thus, replacement of the barite with a compatible and more benign constituent could be a key to the reduction of the toxicity of drill cuttings.

Hematite-based drilling muds may provide a suitable alternative to barite-based drilling muds, especially when the resulting drill cuttings are intended to serve as a substrate for plant propagation (Curtis 2001). The addition of hematite, which is an ore of iron, could actually benefit vegetation in iron-deficient soils. Further, hematite may also have the capacity to reduce the concentration of available sulfides in flooded soils by forming insoluble compounds, and thus may also be an especially good choice in many coastal wetlands where sulfide production is enhanced by prolonged flooding and the presence of sulfate (Ponnamperuma 1972, Mitsch and Gosselink 2000). Sulfides are extremely potent phytotoxins (plant toxins) formed by the microbial reduction of sulfate when more metabolically-favorable electron acceptors, such as Fe^{3+}, have been exhausted (Havill et al. 1985, Mendelssohn and Mckee 1988, Bradley and Dunn 1989, Koch and Mendelssohn 1989). However, excessive concentrations of iron can result in iron toxicity in wetland grasses such as *Oryza sativa* (rice) (e.g., Medhi 1999, Sahrawat 2000) and *Glyceria fluitans* (Lucassen et al. 2000). Therefore, controlled mesocosm evaluations are critical prior to the initiation of any field trials.

The studies detailed herein were designed to elucidate the capacity of simulated drill cuttings created with a hematite-based drilling mud to support vigorous growth of several wetland plant species and to determine the potential benefits of organic matter addition.

Specifically, this study addressed the following questions:

1. Can hematite-based, simulated drill cuttings support vigorous growth of common wetland plant species?
2. Does the addition of organic amendments improve the growth responses of wetland plant species to hematite-based, simulated drill cuttings?
3. Do levels of metal concentrations in the interstitial waters of hematite-based, simulated drill cuttings and tissues of plants grown in them in fall within acceptable levels?

MATERIALS AND METHODS

Experimental Design

Overview

This report details three preliminary studies and one expanded mesocosm evaluation of the safety and suitability of a hematite-based drilling mud as substrate for wetland rehabilitation. The experimental design and specific aspects of each study are presented below, with method discussions that are common to multiple experiments reported thereafter. The drilling mud assessed was a freshly-manufactured, hematite-based drilling mud acquired from MI Drilling Fluids, Incorporated. Amendments for stabilization were also acquired from MI Drilling Fluids and included MIM (medium-ground, mica-based material), fine-ground mica (fine-ground mica), Fiberlock (mixture of recycled cellulose fibers), Phenoseal (mixture of recycled cellulose fibers), Kwickseal (mixture of recycled cellulose fibers), walnut nut plug (medium-ground walnut-nut plug), and pecan-nut plug (medium-ground pecan-nut plug). Plant species utilized in these studies were as follows: *Spartina patens* (marshhay cordgrass) for the three pilot studies and *Spartina patens*, *Sagittaria lancifolia* (bulltongue), and *Rhizophora mangle* (red mangrove) for the mesocosm evaluation. *Spartina patens* and *Rhizophora mangle* were acquired from a nursery supplier (Horticultural Systems, Inc.), whereas *S. lancifolia* was collected from intermediate marshes in the Lake Maurepas basin. Sugarcane bagasse was acquired from Raceland Sugar Corporation. All topsoil was acquired from the Southeastern Louisiana University Horticultural Center.

Initial Evaluation of Drilling Mud and Stabilizing Amendments

Experimental Setup: A 10-level, one-way ANOVA with 4 true replicates was utilized to determine the growth response of *S. patens* to a hematite-based drilling mud stabilized with 7 commonly used amendments. Amendments consisted of medium-ground mica, fine-ground

mica, Fiberlock, Phenoseal, Kwickseal, walnut nut plug, and pecan-nut plug. A topsoil-only control was employed for comparison with these 7 mixes. Two additional treatments, a hydroponic drilling mud and a hydroponic control were included to elucidate the specific toxicity of the drilling muds. This experiment was performed in a Percival growth chamber (Model #AR-75L) in which the environmental conditions were maintained as follows: 14 hours light with 10 hours dark, daytime average PAR levels (photosynthetically active radiation) of 1,100 µmoles m^{-2} s^{-1}, day and night temperature levels set to 25º C, and humidity between 40% and 60%. A volume of 250 ml of drilling mud was mixed with each type of amendment until equal consistency was obtained (lower plastic limit: see McBride 1993 for details of method) and placed in acid washed 1,000-ml containers. The two hydroponic treatments consisted of containers planted with *S. patens* in acid-washed pea gravel, which were then filled with either drilling mud (hydroponic drilling mud) or distilled water (hydroponic control). Plants were allowed to acclimate to growth chamber conditions in test substrates for one week prior to the initiation of the experiment. Water levels were checked daily and maintained at the soil surface. The experimental duration was 5 weeks.

Data collection: Live and dead stem counts were performed weekly during the experiment. Final biomass was collected and partitioned into aboveground live, aboveground dead, and belowground biomass. Interstitial pH was determined at the conclusion of the experiment.

Evaluation of Stabilizing Amendments

Experimental Setup: An eight-level, one-way ANOVA with 4 true replicates was employed under greenhouse conditions to determine the growth response of *S. patens* to the seven stabilizing amendments in the absence of drilling mud. All amendments were mixed with topsoil in a 1:1 (v:v) ratio. The control consisted of an all-topsoil mixture. Substrates were prepared

and placed in 5-liter vessels, which in turn were placed in 20-liter reservoirs. *Spartina patens* plants were allowed to acclimate to greenhouse conditions in test substrates for one week prior to the initiation of the experiment. Water levels were checked daily and maintained to within 5 cm of the substrate surface. The experimental duration was 5 weeks.

Data collection: Live and dead stem counts were performed weekly during the experiment. Final biomass was collected and partitioned into aboveground live, aboveground dead, and belowground biomass. Interstitial pH was determined at the conclusion of the experiment.

Evaluation of Drilling Mud Concentration

Experimental Setup: A four-level, one-way ANOVA with 5 true replicates was employed under greenhouse conditions to determine if there was a critical concentration at which the hematite-based, drilling mud negatively impacted *S. patens* growth response. The four levels of concentration were 0% (control), 10% drilling mud, 20% drilling mud, and 30% drilling mud. Concentrations were created by mixing drilling mud with topsoil in volume to volume ratios. Substrates were prepared and placed in 5-liter vessels, which in turn were placed in 20-liter reservoirs. *Spartina patens* plants were allowed to acclimate to greenhouse conditions in test substrates for one week prior to the initiation of the experiment. Water levels were checked daily and maintained to within 5 cm of the substrate surface. The experimental duration was 5 weeks

Data collection: Live and dead stem counts were performed weekly during the experiment. Final biomass was collected and partitioned into aboveground live, aboveground dead, and belowground biomass. Interstitial pH was determined at the conclusion of the experiment.

Mesocosm-Scale Evaluation of Drilling Mud Suitability

Experimental Setup: Suitability of the hematite-based, drilling mud as a substrate for wetland plants was determined under greenhouse conditions using a 4 (vegetative condition) x 3 (substrate) factorial ANOVA with 4 true replicates. The four vegetative conditions consisted of *Sagittarria lancifolia* under fresh conditions, *Rhizophora mangle* under fresh conditions, *Spartina patens* under fresh conditions, and *Spartina patens* under saline conditions (9 ppt). Substrates consisted of 100% river silt (control), 10% drilling muds with 90% river silt, and 10% drilling muds with 45% river silt and 45% sugarcane bagasse. All concentrations were based on a volume to volume basis. Substrates were prepared and placed in 20-liter mesocosm vessels. Vegetative conditions were then established and the plants were given 2 weeks to acclimate prior to the initiation of the experiment. Water levels were checked daily and maintained to 2.5 cm above the soil surface. Salinity levels were checked monthly with a salinity meter (YSI Model # 30 salinity/conductivity meter) and adjusted as necessary.

Data collection: Halfway through the experiment live and dead stem counts, net CO_2 assimilation, stomatal conductance, and substrate redox potential at 1 cm and 10 cm depths were determined. At the conclusion of the experiment live and dead stem counts, net CO_2 assimilation, stomatal conductance, and leaf fluorescence characteristics (instantaneous indicators of photosynthetic integrity and capacity), biomass partitioning, leaf metal concentrations, interstitial metal concentrations, total-extractable substrate metal concentrations, interstitial pH, and substrate redox potential at 1 cm and 10 cm depths were determined.

Data Collection and Analysis

Net CO_2 Assimilation and Stomatal Conductance

Plant photosynthetic response (net CO_2 assimilation and stomatal conductance) was determined using a LICOR® 6400F Photosynthesis System with a built-in light source and CO_2 controller. This system provides instantaneous measurements of photosynthetic rate as net CO_2 assimilation under controlled levels of light and CO_2. Measurements were performed on two representative leaves selected from each test plant and then averaged. Reference settings for the photosynthesis system were light levels (Photosynthetically Active Radiation, hereafter PAR) of 1500 µmol per m^2 per second (at or near light-saturated conditions for selected species; see Dai and Wiegert 1997) and CO_2 levels of 370 ppm.

Leaf Fluorescence Characterization

To assess the health of the plant photosynthetic machinery steady-state, light-adapted fluorescence and maximum-yield, light-adapted fluorescence were determined, the latter by subjecting the leaf in the chamber to a pulse of 3,000 µmol m^{-2} s^{-1} PAR (modified from Scholes et al. 1997). Variable to maximum fluorescence, non-photochemical quenching, photochemical quenching, and electron transport rate of PSII were determined using the Li-6400F. Light settings for normal actinic light and saturating light were 1,500 µmol$m^{-2}s^{-1}$ PAR and 3,000 µmolm^{-2} s^{-1} PAR respectively (see Schreiber et al. 1998). Leaves were dark-adapted for 24 hours by placing light-excluding shields around the leaves. All fluorescence setting were adapted from the fluorescence autoprogram of the Li-6400F (Licor, Lincoln, NE; See Schrieber et al. 1998 for discussion of method).

Soil Reduction Potential

Soil redox potential (Eh) was monitored in all experimental vessels with a Hanna model HI 9025 pH/mV meter and brightened platinum redox electrodes. Probes were constructed as per Faulkner et al. (1989) and were calibrated and brightened prior to each measurement period. Redox probes were placed 1 cm below the soil surface in each vessel, allowed to equilibrate, and the measurement recorded. Redox probes were then inserted to 10 cm below the soil surface, allowed to equilibrate, and the measurement recorded.

Interstitial Water Analysis

Interstitial waters were collected using a 60-ml syringe connected by flexible aquarium tubing to a 20-cm length of rigid aquarium tubing that was perforated along the lower 5 cm. Interstitial water was collected after carefully inserting the perforated tip of the tubing to a depth of 15 cm below the soil surface according to the methods of Mckee et al. (1988). Interstitial water pH was then immediately monitored using a Hanna model Hi 9025 pH/mV meter calibrated with pH 7 and pH 4 buffers. The remaining interstitial water was then passed through a 0.45-µm filter, yielding the dissolved elemental and nutrient portion, which was partitioned into a 10-ml aliquot for elemental (ICP) analysis and a 40-ml aliquot for nutrient (NO_3-NO_2 and NH_4) analyses. Samples for nutrient analysis were divided into two, 20-ml aliquots and frozen until transported to the Louisiana State University Coastal Ecology Institute to be analyzed with a Latchat automated analyzer (Quickchem 8000). Aliquots of 10-ml samples were placed in acid-washed scintillation vials for elemental analysis and preserved by adding two drops of concentrated, reagent-grade nitric acid to each sample and then refrigerating. Samples were then transported to the Louisiana State University Wetland Biogeochemistry Laboratory for analysis. Elemental analysis of samples was performed with a Jarrel-Ash inductively coupled argon

plasma mass-optical emission (ICP-OES ATOM COMP SERIES 800) spectrophotometer, except for Ba, which was subjected to Cold Vapor Atomic Absorption (CVAA) with a Perkin-Elmer Model 5000 Atomic Absorption Spectrophotometer. Samples were analyzed for the following elements: P, cations (K, Na, Ca, Mg), micronutrients (Fe, Mn, Cu, Zn, Al), and heavy metals (Cd, Cr, Pb, As, Ba).

Biomass Measurements

Biomass was separated into aboveground live, aboveground dead, and belowground partitions and placed in paper bags at harvest. All biomass was dried in a convection oven (VWR 3050) at 65° C for a minimum of three days or until constant weight was achieved.

Plant Tissue Analysis

Subsequent to being weighed, a subsample of dried aboveground biomass (approximately 1 gram of stem and leaf) was obtained, ground with a porcelain mortar and pestle, and acid digested as described below. One gram of dry aboveground tissue was placed in 5 ml of concentrated, reagent-grade nitric acid and allowed to partially digest overnight in a fume hood. The samples were then placed in a block heater and heated to 130° C for 3 hours. After the samples were removed from the block heater and cooled, they were brought up to 70-ml total volume with distilled-deionized water. Samples were mixed by covering the digestion vials with parafilm and inverting 10 times. Samples were allowed to settle and 10 ml of the supernatant from each sample was collected and placed in an acid-washed scintillation vial. The 10-ml samples were then transported to the Louisiana State University Wetland Biochemistry Institute to undergo elemental analysis with a Jarrel-Ash inductively coupled argon plasma mass-optical emission spectrophotometer (ICP-OES ATOM COMP SERIES 800) or Cold Vapor Atomic

Absorption (CVAA). Target elements were the same as those chosen for interstitial water analysis (P, K, Na, Ca, Mg, Fe, Mn, Cu, Zn, Al, Cd, Cr, Pb, As, and Ba).

Sediment Metal Content

One gram of substrate was collected from each vessel and dried for acid digestion and subsequent elemental analysis. Sample analysis proceeded in an identical fashion to the plant tissue analysis stated above.

Statistical Analysis

All factor analyses were performed using the principle components analysis procedures of SYSTAT version 10.0 (SPSS Inc. 1998). Factor analyses employed a Varimax rotation and minimum eigenvalue of 1.0. All ANOVAs were performed using the general linear model procedures of SYSTAT version 10.0 (SPSS Inc. 1998). All univariate analyses were tested for departures from parametric assumptions. Acid-digested substrate Fe, acid-digested substrate Cr, interstitial Mg, Photosystem II variable to maximum fluorescence ratio, and Photosystem II electron transport rate violated parametric assumptions and were therefore transformed to their square root values, to correct these deviations. Dead aboveground biomass, belowground biomass, and total biomass also violated parametric assumptions and were therefore transformed to their logarithmic base 10 values, to correct these deviations. All data presented are raw values to allow for visual comparison of actual data. Repeated measures ANOVAs were adjusted for departures from sphericity using the Hunyh-Feldt technique, which compensates for these deviations (Girden 1992).

RESULTS

Initial Evaluation of Drilling Mud and Stabilizing Amendments

Time and amendment type interacted in a significant fashion in regard to live to total stem ratio, likely because of the consistently, high live to total stem ratios of the control soil compared to the downward trend of all other substrate types (Fig. 1-1; F= 1.953, P< 0.001). Interestingly, plants grown in the hydroponic drilling mud treatment had significantly greater live to dead stem ratios than plants grown in the hydroponic control treatment (Fig. 1-1; Contrast F= 2.539, P=0.047). The control substrate had greater aboveground live and belowground biomass compared to all other substrate types (Fig. 1-2; Contrast F= 211.142, P<0.001 and Contrast F= 177.448, P<0.001, respectively). Importantly, no significant differences were detected between the hydroponic control and the hydroponic drilling mud treatment in any biomass partitioning category.

Evaluation of Stabilizing Amendments

Time and amendment type interacted significantly in regard to live to total stem ratio (Fig. 2-1; F= 2.743 P <0.001). This was likely driven by the tendency of the Fiberlock and medium mica mixes to have somewhat decreased live to total stem ratios with time, along with a low week 4 value for the Phenoseal treatment, compared with consistently high live to total stem ratios of all other substrates. The control treatment had significantly greater live aboveground biomass than all other treatments (Fig. 2-2; Contrast F=4.833 P=0.038). Graphical inspection indicated that this significant difference was overwhelming the result of low values in the medium mica treatment. Therefore, a contrast excluding this treatment was performed to evaluate the performance of all other amendment types. Analysis of control versus all other amendment types excluding medium mica failed to find a significant difference in live aboveground biomass (Fig. 2-2; Contrast F= 0.297, P= 0.551). Significant

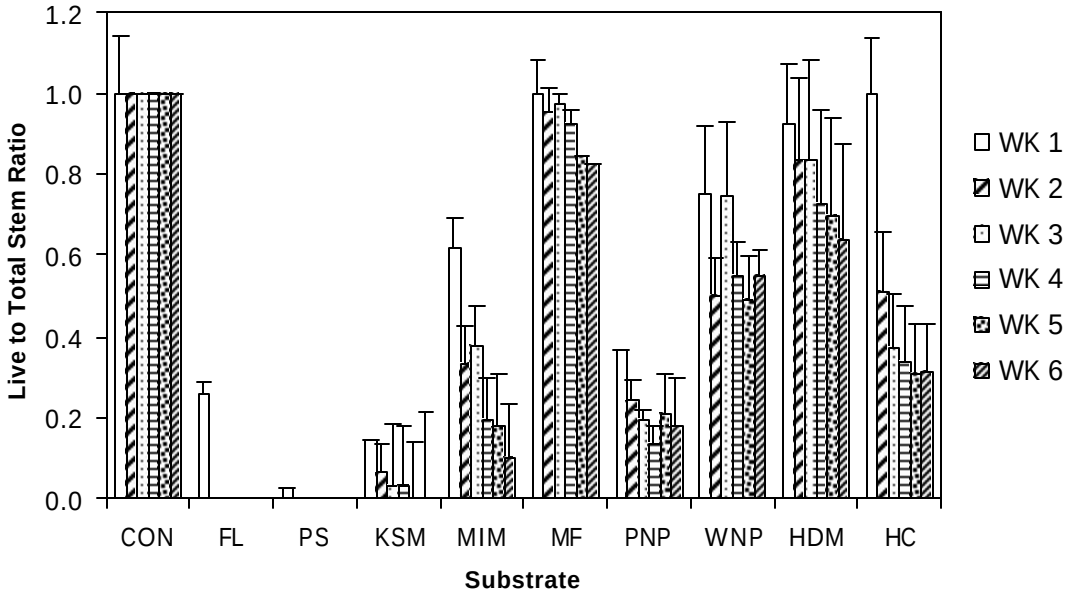

Fig. 1-1 The effect of time and stabilizing amendment treatment on live to total stem ratio (mean +/- SE). Treatment abbreviations are as follows: CON= Control, FL= Fiberlock, PS= Phenoseal, KSM= Kwickseal mix, MIM= Medium-ground mica, MF= Fine-ground mica, PNP= Pecan nut plug, WNP= Walnut plug, HC= Hydroponic control, HDM= Hydroponic drilling mud

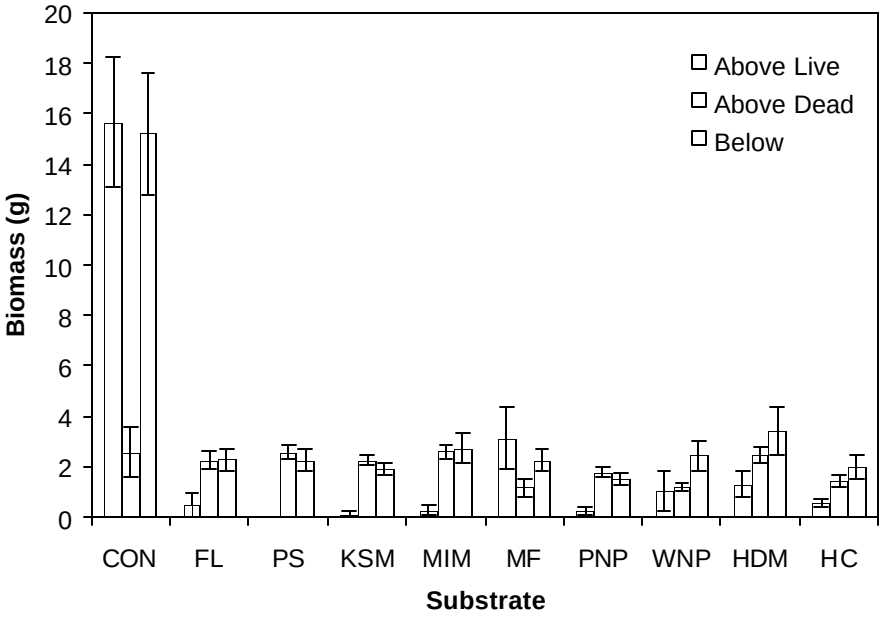

Fig. 1-2 The effect of stabilizing amendment treatment on biomass (mean +/- SE). Treatment abbreviations are as follows: CON= Control, FL= Fiberlock, PS= Phenoseal, KSM= Kwickseal mix, MIM= Medium-ground mica, MF= Fine-ground mica, PNP= Pecan nut plug, WNP= Walnut plug, HC= Hydroponic control, HDM= Hydroponic drilling mud

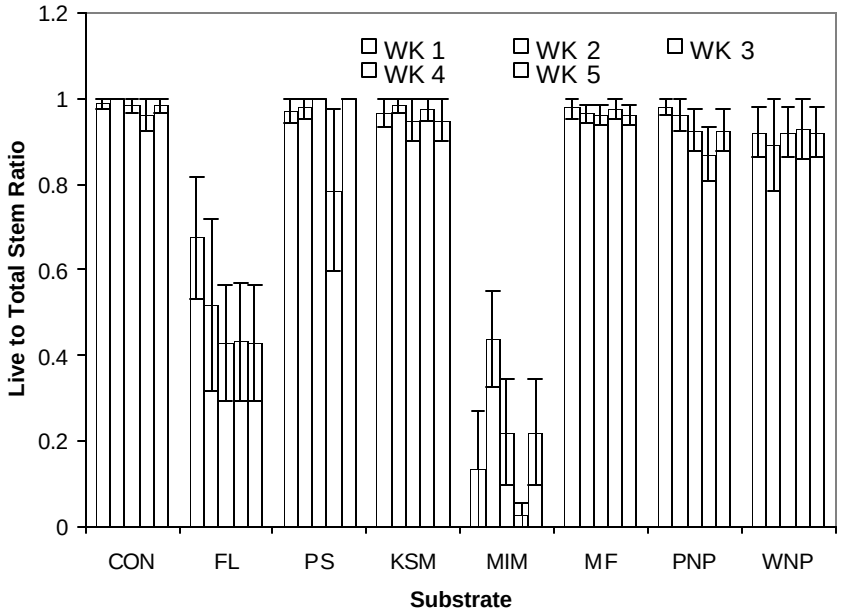

Fig. 2-1 The effect of stabilizing amendment-topsoil mixtures and time (week) on live to total stem ratio (mean +/- SE). Treatment abbreviations are as follows: CON= Control, FL= Fiberlock, PS= Phenoseal, KSM= Kwickseal mix, MIM= Medium-ground mica, MF= Fine-ground mica, PNP= Pecan nut plug, WNP= Walnut plug, HC= Hydroponic control, HDM= Hydroponic drilling mud

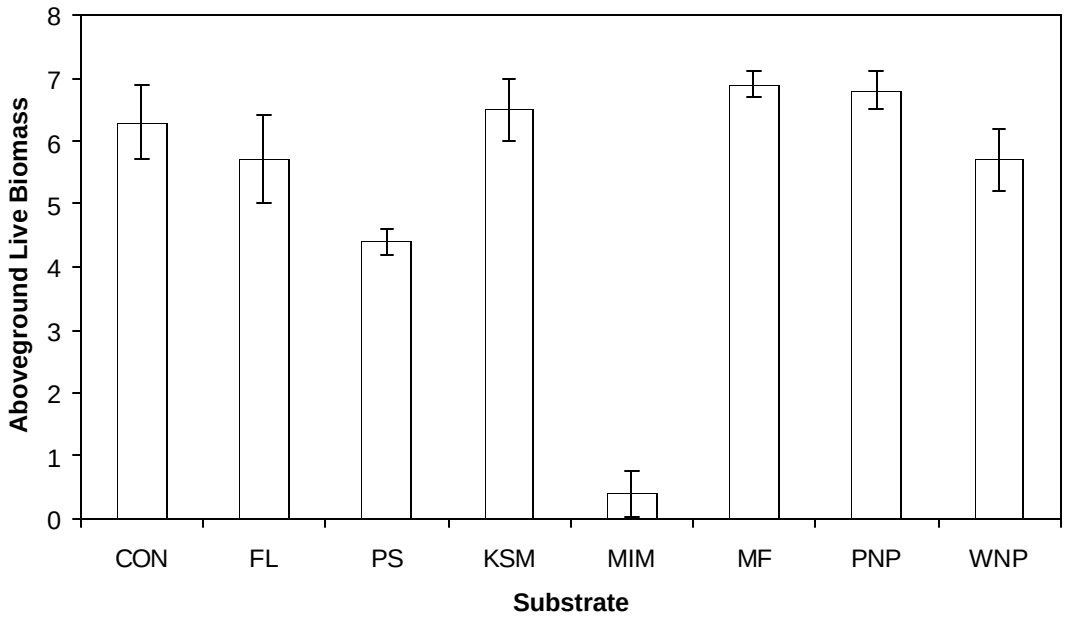

Fig. 2-2 The effect of stabilizing amendment-topsoil mixtures and time (week) on aboveground live biomass (mean +/- SE). Treatment abbreviations are as follows: CON= Control, FL= Fiberlock, PS= Phenoseal, KSM= Kwickseal mix, MIM= Medium-ground mica, MF= Fine-ground mica, PNP= Pecan nut plug, WNP= Walnut plug, HC= Hydroponic control, HDM= Hydroponic drilling mud

substrate differences were detected in final pH (Fig. 2-3; F= 4.602, P<0.001). However, all values fell within a range (5.5-6.5) that would be considered mildly acidic

Evaluation of Drilling Mud Concentration

Time and drilling mud concentration interacted significantly in regard to live to total stem ratios, likely due to a depression in the live to total stem ratio of the 10% and 20% drilling mud treatment in week 1 that was ameliorated by weeks 3, 4, and 5 (Fig. 3-1; F= 4.642, P=0.002). No significant differences were detected in any biomass partitioning category. Also, no significant pH differences were detected.

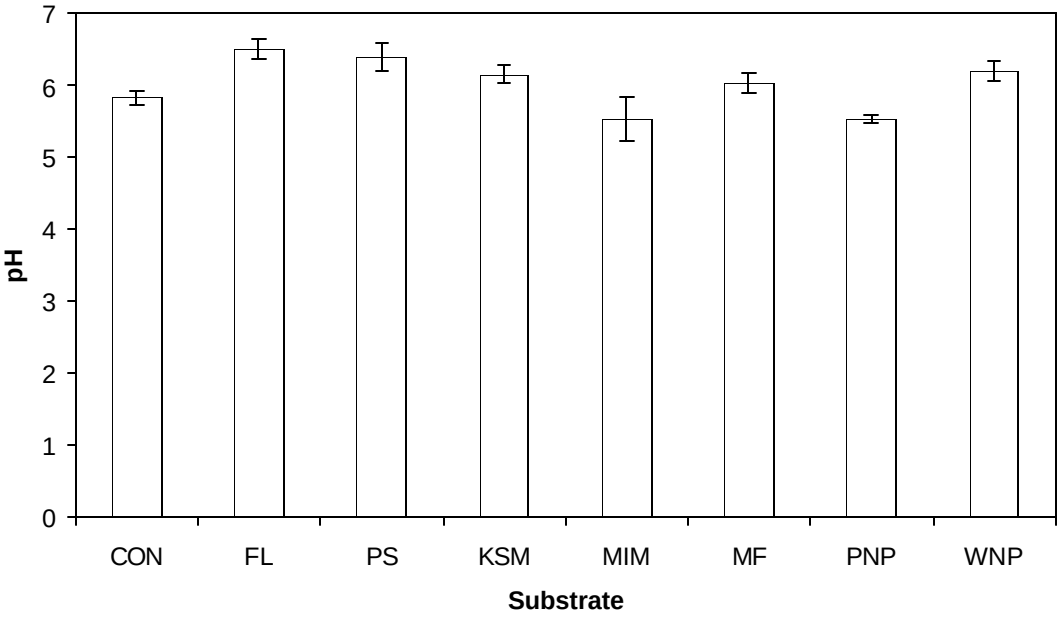

Fig. 2-3 The effect of stabilizing amendments-topsoil mixtures on interstitial soil pH (mean +/- SE). Treatment abbreviations are as follows: CON= Control, FL= Fiberlock, PS= Phenoseal, KSM= Kwickseal mix, MIM= Medium-ground mica, MF= Fine-ground mica, PNP= Pecan nut plug, WNP= Walnut plug, HC= Hydroponic control, HDM= Hydroponic drilling mud

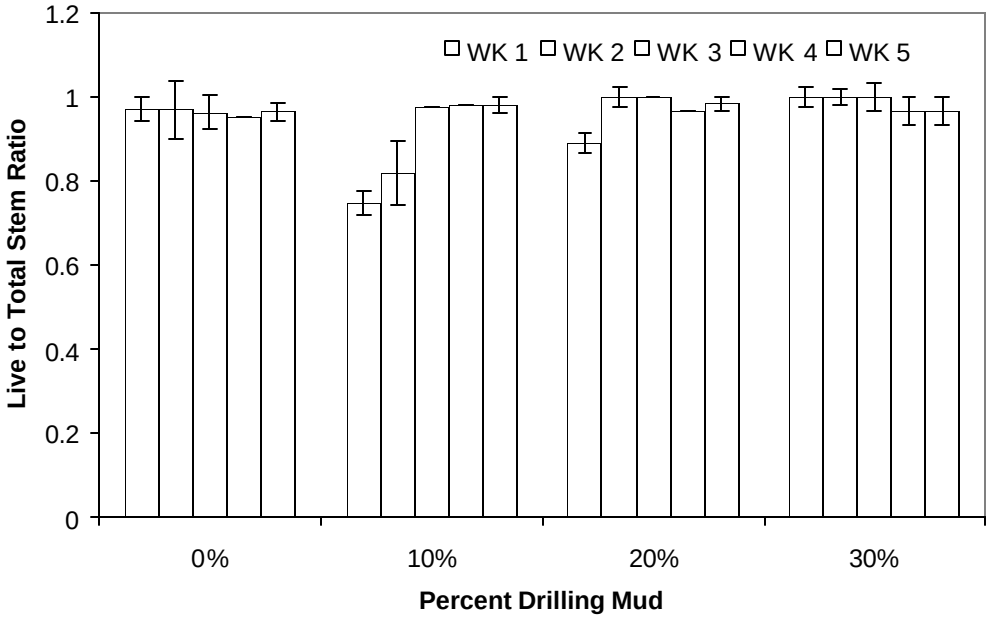

Fig. 3-1 The effect of drilling mud percentage in topsoil mix and time (week) on live to total stem ratio (mean +/- SE).

Mesocosm Evaluation

Factor Analysis of Elemental Concentrations

Factor analysis was employed to determine if elements were behaving in similar fashion in substrate interstitial water (pore water), acid-digested substrate, and acid-digested plant tissue. Interstitial concentration of Mn, S, Na, Al, and Zn behaved in similar fashion. Also, Mo demonstrated a strong inverse relationship to Fe and K. Ca and Mn interstitial concentrations also varied in a comparable fashion. Strong similarities in behavior for the elements Ca, Mg, Mn, K, and Na were detected in regard to plant tissue concentration. Al and Zn plant tissue concentration also varied in a similar fashion. Again, a strong inverse relationship was discerned in regard to plant tissue concentrations of Mo and Fe, although no similarities with K were detected. Total substrate Mg, Na, Ca, and K concentration varied in like fashion. Total substrate Fe, Mn, and Zn were also comparable in behavior. Similarity was also noted for total substrate concentration of S and B.

Substrate Elemental Analysis

As expected in an iron-based drilling mud, acid-extractable substrate Fe concentrations were significantly greater in hematite-based, simulated drill cuttings and bagasse-amended hematite-based, simulated drilling cuttings treatments than in the control substrate (Fig. 4-1; Contrast F =6.593, P= 0.014) Chromium was also found to be significantly higher in the hematite-based, simulated drill cuttings and bagasse-amended hematite-based, simulated drilling cuttings treatments compared with the control substrate (Fig. 4-2; Contrast F=3.723, P=0.032 respectively). Interestingly, the acid-extractable Mn concentration was significantly greater in the control sediment (river silt) than in the other substrates (Fig. 4-3; F= 5.221, P= 0.028).

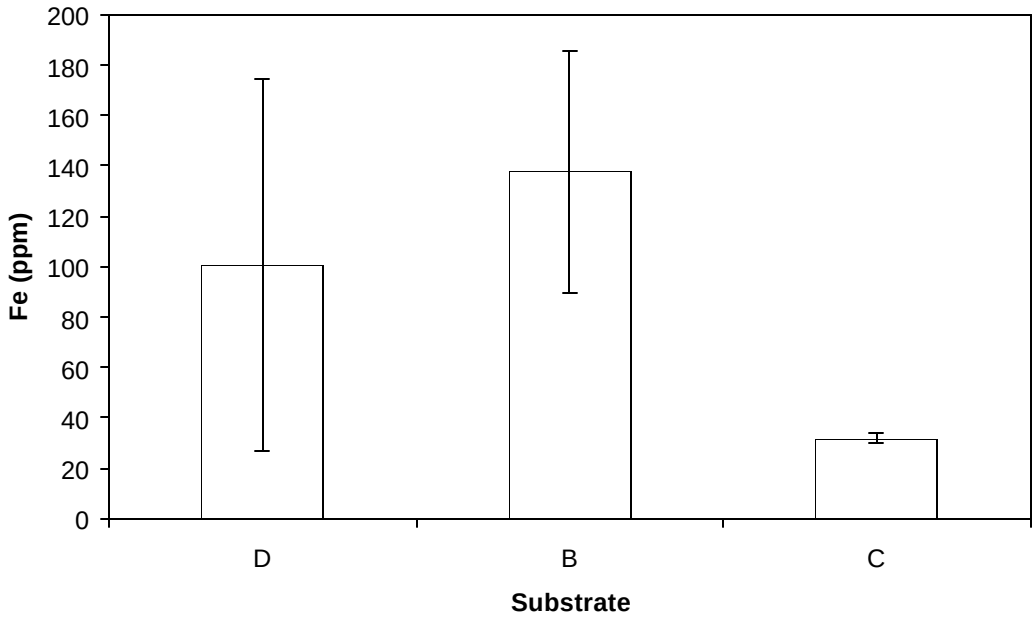

Fig. 4-1 The effect of substrate on acid-extractable substrate Fe concentration (mean +/- SE), averaged across vegetative condition. Treatment abbreviations are as follows: D= 10% hematite-based drilling mud, 90% river silt; B= 10% hematite-based drilling mud, 45% bagasse, 45% river silt; C= 0% hematite-based drilling mud, 100% river silt.

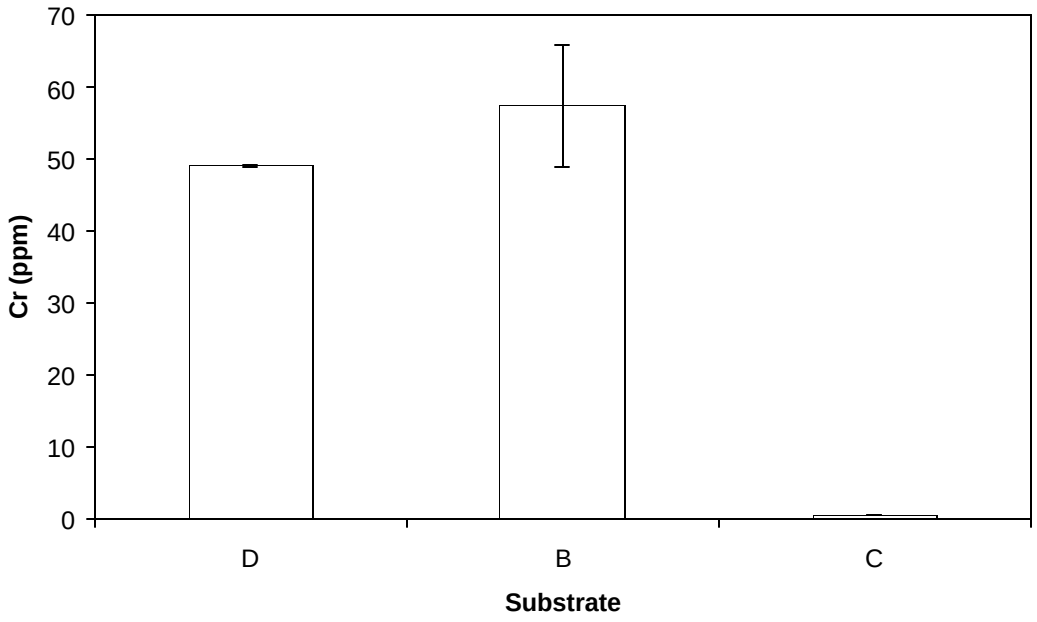

Fig. 4-2 The effect of substrate on acid-extractable substrate Cr concentration (mean +/- SE), averaged across vegetative condition. Treatment abbreviations are as follows: D= 10% hematite-based drilling mud, 90% river silt; B= 10% hematite-based drilling mud, 45% bagasse, 45% river silt; C= 0% hematite-based drilling mud, 100% river silt.

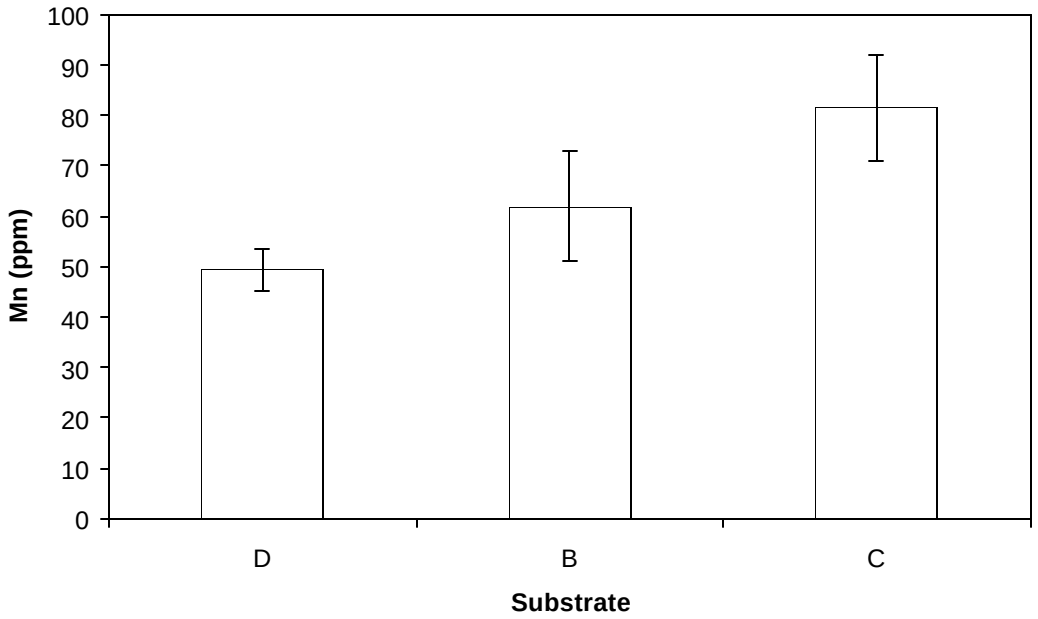

Fig. 4-3 The effect of substrate on acid-extractable substrate Mn concentration (mean +/- SE), averaged across vegetative condition. Treatment abbreviations are as follows: D= 10% hematite-based drilling mud, 90% river silt; B= 10% hematite-based drilling mud, 45% bagasse, 45% river silt; C= 0% hematite-based drilling mud, 100% river silt.

No other significant differences were detected in regard to substrate, species, or the interaction thereof. Therefore, in terms of total-extractable element makeup, the mixture of 10% hematite-based, drilling muds and 90% river silt was not significantly different than 100% river silt, except in regard to Fe, Cr, and Mn concentrations. Mean, maximum, and standard error values for substrate acid digestions are presented for each element by substrate (Table 1).

Interstitial Water Element and Nutrient Analysis

Interstitial water concentrations of both Fe and Cr were significantly higher in the hematite-based, simulated drill cuttings and bagasse-amended hematite-based, simulated drilling cuttings treatments than the control (Fig. 4-4; F= 4.686, P= 0.037 and Fig. 4-5; F= 3.615, P= 0.034 respectively). Interestingly, higher interstitial water concentrations of Al, B, Ca, Co, Fe, Mn, K, Na, S, and Z were consistently associated with *Sagittaria lancifolia* vegetative treatments (all P values < 0.05). *Rhizophora mangle* treatments had significantly elevated interstitial concentrations of Mg compared with other vegetative conditions (Fig. 4-6; Contrast F= 142.844, P< 0.001). Mean, maximum, and standard error values for interstitial water concentration are presented for each element by substrate (Table 2). No significant differences were detected in either interstitial nitrate-nitrite or ammonium concentrations for substrate type or vegetative condition.

Plant Tissue Elemental Analysis

No significant differences were detected in plant tissue elemental concentrations between substrate, species, or the interaction thereof. Mean, maximum, and standard error values for plant tissue digestions are presented for each element by substrate (Table 3).

Table 1. Results of elemental analysis of substrate interstitial water. Values are mean and maximum concentrations (µg/g) plus or minus standard error in parentheses (n=16).

Element	Substrate					
	Control		Drill cuttings		Bagasse-amended Drill cuttings	
	Mean	Max.	Mean	Max.	Mean	Max.
Ba	0.000	0.000	0.000	0.000	0.000	0.000
	(0.000)		(0.000)		(0.000)	
Cu	0.040	0.260	0.042	0.100	0.036	0.070
	(0.032)		(0.014)		(0.122)	
Zn	0.151	0.262	0.141	0.196	0.160	0.337
	(0.011)		(0.005)		(0.013)	
Cd	0.000	0.000	0.000	0.000	0.000	0.000
	(0.000)		(0.000)		(0.000)	
Pb	0.048	0.09	0.098	0.190	0.111	0.160
	(0.014)		(0.026)		(0.016)	
Cr	0.041	0.067	0.070	0.21	0.080	0.22
	(0.006)		(0.010)		(0.140)	
Ni	0.036	0.080	0.242	0.980	0.133	0.220
	(0.011)		(0.150)		(0.260)	
Fe	5.265	9.276	10.310	39.520	9.202	20.260
	(1.065)		(2.201)		(1.425)	
Mn	0.567	1.233	0.022	0.031	0.021	0.047
	(0.07)		(0.001)		(0.259)	
Ca	29.910	76.660	24.906	54.700	28.501	69.800
	(5.058)		(3.417)		(4.534)	
Mg	10.980	33.730	8.245	22.060	9.616	24.100
	(2.412)		(1.480)		(1.518)	
P	5.218	10.950	4.304	8.870	7.843	17.600
	(0.665)		(0.040)		(1.089)	
Al	0.550	1.174	0.563	1.447	0.739	2.137
	(0.054)		(0.060)		(0.104)	
K	52.699	185.800	34.158	83.800	57.814	175.500
	(12.546)		(4.370)		(13.160)	
Na	23.069	84.200	30.854	95.000	36.523	64.500
	(5.852)		(5.052)		(6.280)	
S	7.073	13.540	8.894	28.000	6.817	21.090
	(0.901)		(1.374)		(1.380)	

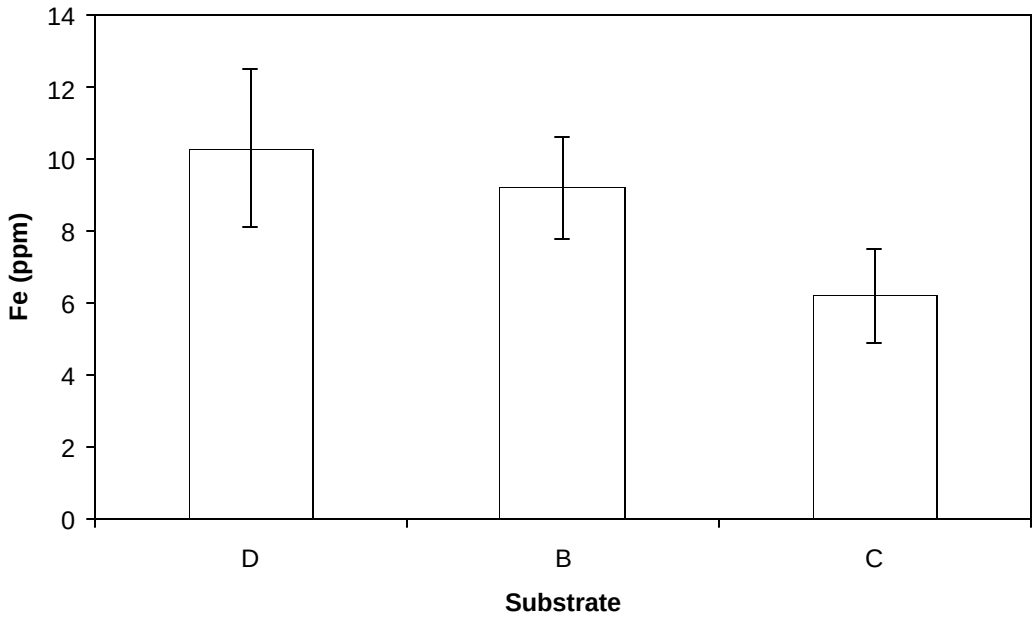

Fig. 4-4 The effect of substrate on interstitial Fe concentration (mean +/- SE), averaged across vegetative condition. Treatment abbreviations are as follows: D= 10% hematite-based drilling mud, 90% river silt; B= 10% hematite-based drilling mud, 45% bagasse, 45% river silt; C= 0% hematite-based drilling mud, 100% river silt.

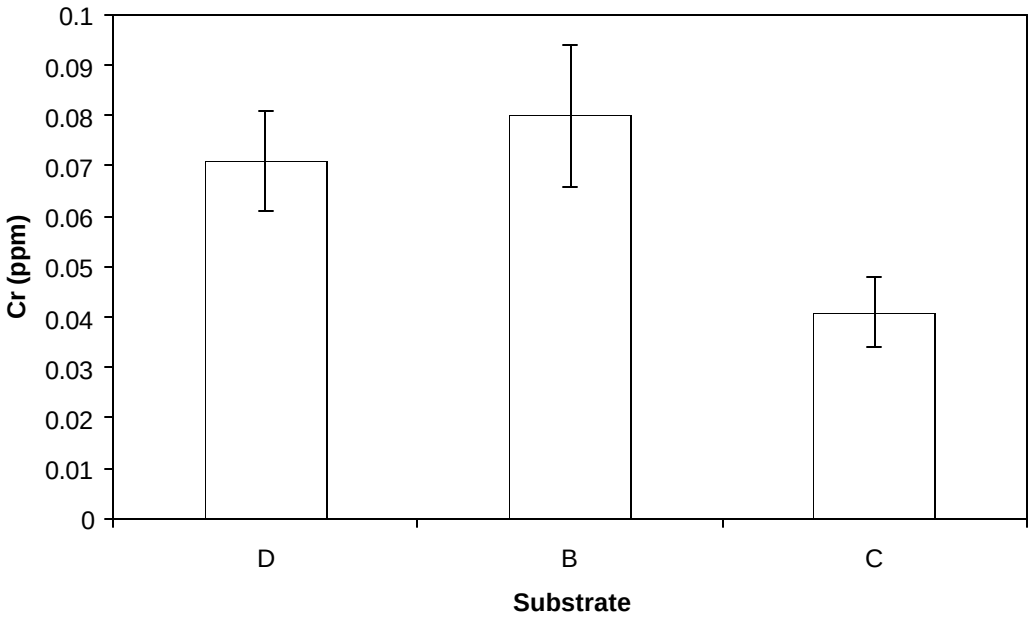

Fig. 4-5 The effect of substrate on interstitial Cr concentration (mean +/- SE), averaged across vegetative condition. Treatment abbreviations are as follows: D= 10% hematite-based drilling mud, 90% river silt; B= 10% hematite-based drilling mud, 45% bagasse, 45% river silt; C= 0% hematite-based drilling mud, 100% river silt.

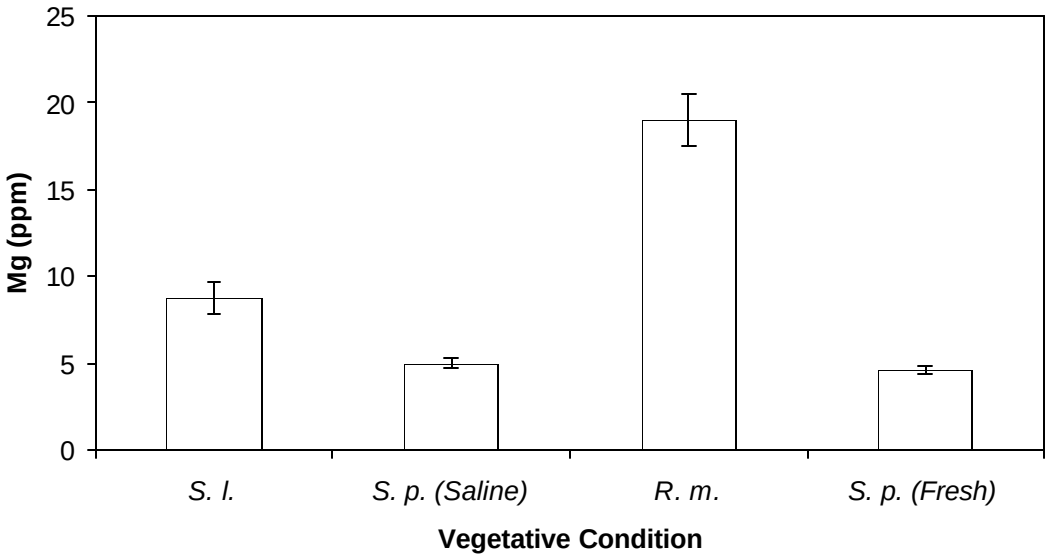

Fig. 4-6 The effect of vegetative condition on interstitial Mg concentration (mean +/- SE), averaged across substrate. Treatment abbreviations are as follows: *S.l.* =*Sagitarria lancifolia*, *S.p.* (saline)= *Spartina patens* in 9 ppt, *R.m.* = *Rhizophora mangle*, *S.p.* (fresh)= *Spartina patens* in 0 ppt.

Table 2. Results of elemental analysis of acid-digested yielding total potentially extractable element concentration. Values are mean and maximum concentrations (µg/g) plus or minus standard error in parentheses (n=16).

Element	Substrate					
	Control		Drill cuttings		Bagasse-amended Drill cuttings	
	Mean	Max.	Mean	Max.	Mean	Max.
Ba	0.000	0.000	0.000	0.000	0.000	0.000
	(0.000)		(0.000)		(0.000)	
Cu	0.000	0.000	0.000	0.000	0.000	0.000
	(0.000)		(0.000)		(0.000)	
Zn	9.634	65.325	5.880	19.815	5.370	19.642
	(4.108)		(1.717)		(1.743)	
Cd	0.000	0.000	0.000	0.000	0.000	0.000
	(0.000)		(0.000)		(0.000)	
Pb	0.000	0.000	0.000	0.000	0.000	0.000
	(0.000)		(0.000)		(0.000)	
Cr	0.62	0.94	40.94	48.37	52.46	218.29
	(0.03)		(1.70)		(11.18)	
Ni	0.000	0.000	0.000	0.000	0.000	0.000
	(0.000)		(0.000)		(0.000)	
Fe	90.21	194.93	65.30	98.33	116.49	853.50
	(10.97)		(7.54)		(49.71)	
Mn	81.640	167.625	49.360	82.593	62.408	207.075
	(10.488)		(4.074)		(10.920)	
Ca	0.000	0.000	0.000	0.000	0.000	0.000
	(0.000)		(0.000)		(0.000)	
Mg	5857	8287	7080	10965	6848	14520
	(540.9)		(527.1)		(738.2)	
P	0.0338	0.0675	0.580	8.813	0.694	10.695
	(0.009)		(0.549)		(0.667)	
Al	0.000	0.000	0.000	0.000	0.000	0.000
	(0.000)		(0.000)		(0.000)	
K	4185.723	5362.500	4469.845	5782.500	4399.583	6097.500
	(4169.673)		(167.218)		(5180.348)	
Na	41700	64050	53075	87825	48858	95175
	4359		4169		5180	
S	25052	37035	22689	35692	22173	27799
	(1344)		(1752)		(1054)	

Table 3. Results of elemental analysis of acid-digested plant tissue. Values are mean and maximum concentrations (μg/g) plus or minus standard error in parentheses (n=16).

Element	Substrate					
	Control		Drill cuttings		Bagasse-amended Drill cuttings	
	Mean	Max.	Mean	Max.	Mean	Max.
Ba	0.000	0.000	0.000	0.000	0.000	0.000
	(0.000)		(0.000)		(0.000)	
Cu	0.000	0.000	0.000	0.000	0.000	0.000
	(0.000)		(0.000)		(0.000)	
Zn	12.782	25.920	11.251	20.452	12.503	36.938
	(1.130)		(0.685)		(1.752)	
Cd	0.000	0.000	0.000	0.000	0.000	0.000
	(0.000)		(0.000)		(0.000)	
Pb	0.000	0.000	0.000	0.000	0.000	0.000
	(0.000)		(0.000)		(0.000)	
Cr	0.000	0.000	0.000	0.000	0.000	0.000
	(0.000)		(0.000)		(0.000)	
Ni	0.000	0.000	0.000	0.000	0.000	0.000
	(0.000)		(0.000)		(0.000)	
Fe	0.000	0.000	0.000	0.000	0.000	0.000
	(0.000)		(0.000)		(0.000)	
Mn	31.423	105.300	18.363	56.850	20.745	48.600
	(5.758)		(3.415)		(2.999)	
Ca	2537.20	4507.50	2265.89	3591.75	2394.42	4882.50
	(168.157)		(118.159)		(188.939)	
Mg	4565.95	11002.5	3650.81	9330	3811.27	12412.5
	(618.544)		(432.212)		(614.055)	
P	9.846	156.525	3.452	54.225	0.0675	0.607
	(9.779)		(3.395)		(0.070)	
Al	55.349	206.025	44.646	2.705	52.901	189.825
	(10.252)		(2.705)		(9.653)	
K	3360.38	6945	2851.265	6382.5	2727.515	7425.0
	(5150.99)		(301.624)		(350.478)	
Na	30.438.3	83700.0	22913.4	72375.0	23953.6	93750
	(5150.99)		(3706.89)		(5016.36)	
S	25993	60300	20512	30120	23055	41850
	(3297.611)		(1833.860)		(2387.062)	

Interstitial pH and Soil Redox Potential

No significant differences were detected in interstitial pH as a function of substrate type or vegetative condition. The bagasse-amended, hematite-based, simulated drill cuttings treatment was more reduced than either the hematite-based, simulated drill cuttings or control (river silt) substrate (Fig. 4-7; F= 12.405, P= 0.001)

Net CO_2 Assimilation and Stomatal Conductance

A significant interaction between time, species and substrate was noted for net CO_2 assimilation rate, which was probably driven by the higher assimilation rate of *S. patens* (saline) in the control substrate at time 2 compared with time 1, whereas all other species had reduced or equivocal assimilation rates in the control substrate (Fig. 4-8; F= 2.864, P= 0.022). The interaction between time and species approached significance (Fig. 4-9; F=2.659, P=0.063). This is likely due to the *S. patens* saline (9 ppt) treatment demonstrating higher rates of net CO_2 assimilation at the conclusion of the experiment compared with the midpoint and the *R. mangle* treatments experiencing a decrease in net CO_2 assimilation at the conclusion compared with the midpoint of the experiment. Because of the interaction with time, net CO_2 assimilation rates were also examined within each time period. No significant main effects or interactions were detected for net CO_2 assimilation rates measured during the midpoint of the experiment. However, *R. mangle* had significantly lower net CO_2 assimilation rates in the control and bagasse-amended sediments compared to the drilling mud, whereas all other species performed equivocally in all substrates (Fig. 4-8; F= 3.325, P=0.016). Overall, *R. mangle* demonstrated a significantly lower net CO_2 assimilation rate than all other species at time 2 (Fig. 4-8; F= 3.175, P=0.042). No significant effects of substrate, species, time, or the interactions thereof on either stomatal conductance or water-use efficiency were detected (all $P > 0.05$).

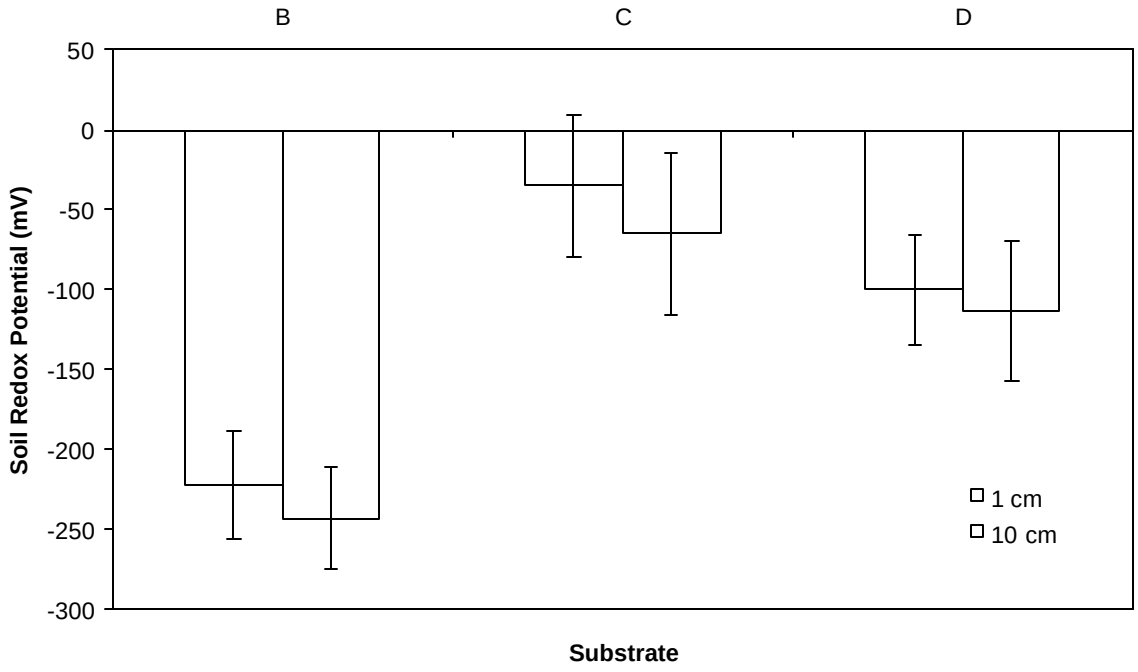

Fig. 4-7 The effect of substrate on soil redox potential at 1 cm and 10 cm depths (mean +/- SE), averaged across vegetative condition. Treatment abbreviations are as follows: D= 10% hematite-based drilling mud, 90% river silt; B= 10% hematite-based drilling mud, 45% bagasse, 45% river silt; C= 0% hematite-based drilling mud, 100% river silt.

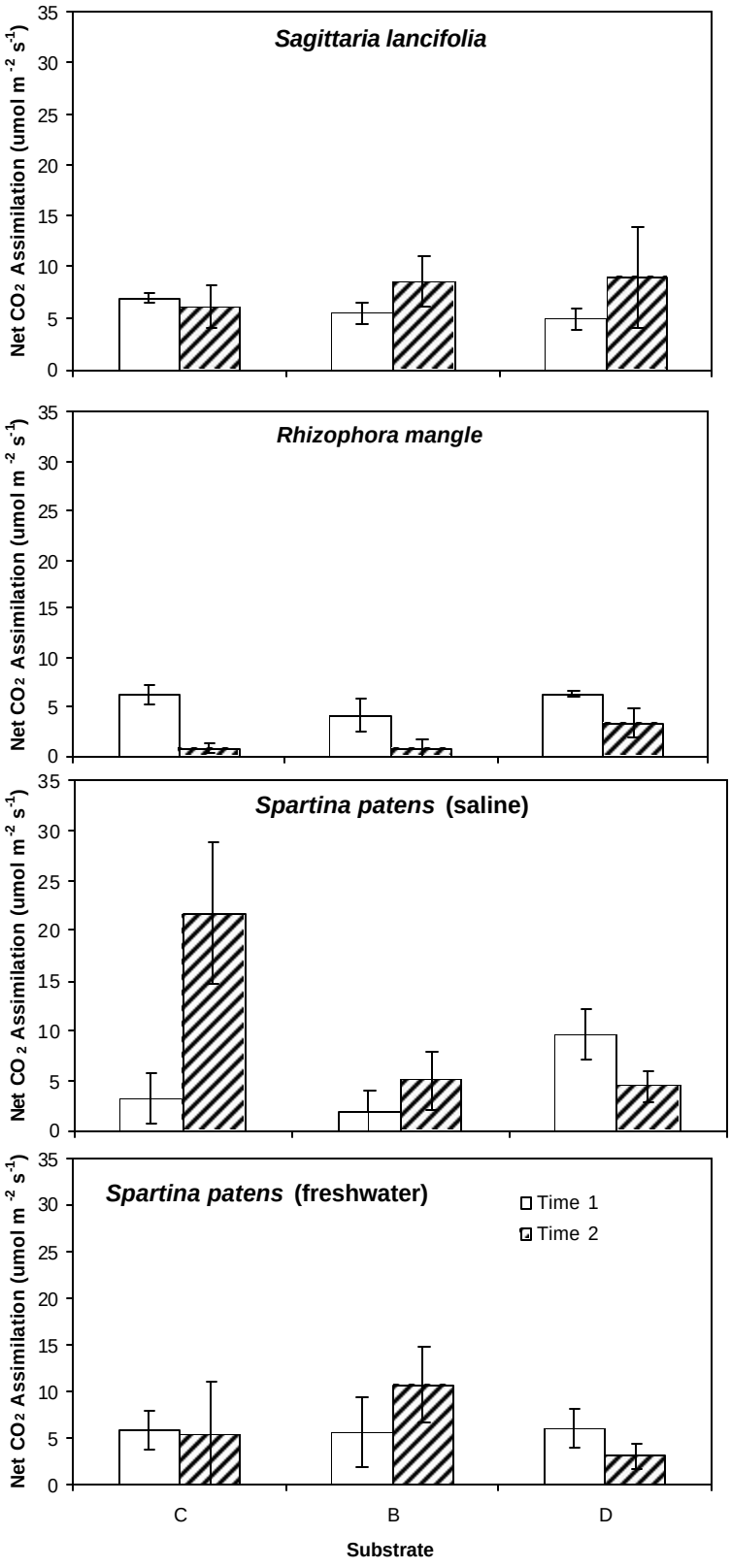

Fig. 4-8 The effect of substrate and vegetative condition on net CO_2 assimilation (mean +/- SE). Treatment abbreviations are as follows: D= 10% hematite-based drilling mud, 90% river silt; B= 10% hematite-based drilling mud, 45% bagasse, 45% river silt; C= 0% hematite-based drilling mud, 100% river silt.

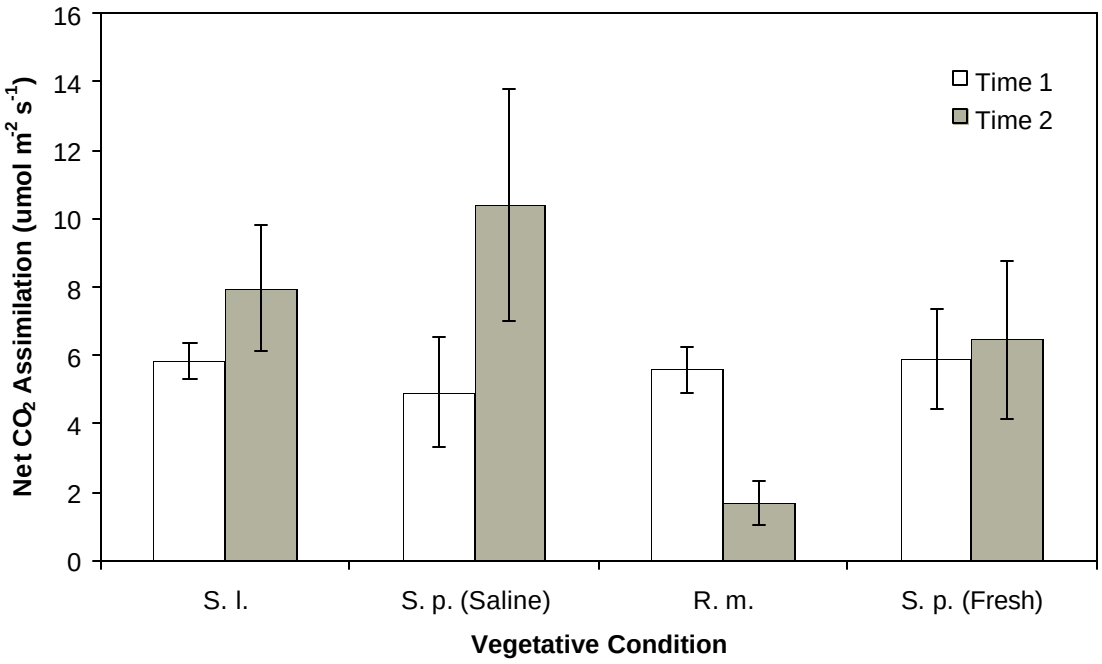

Fig. 4-9 The effect of vegetative condition on net CO_2 assimilation (mean +/- SE), averaged across substrate. Treatment abbreviations are as follows: *S.l.* =*Sagittaria lancifolia*, *S.p.* (saline)= *Spartina patens* in 9 ppt, *R.m.* = *Rhizophora mangle*, *S.p.* (fresh)= *Spartina patens* in 0 ppt.

Chlorophyll Fluorescence Characteristics

A highly significant effect of species was detected in variable to maximum fluorescence ratio (Fig. 4-10; F= 69.791, P< 0.001). This can best be explained by the high, similar Fv/Fm ratios of *S. lancifolia* and *R. mangle* compared with the lower, similar ratios for the two groups of *S. patens* (fresh and saline). No significant effects of substrate, species or the interactions thereof were detected in either photochemical quenching or non-photochemical quenching (all P > 0.05). A significant interaction was detected between substrate and species in electron transport rate (Fig. 4-11; F= 3.393, P= 0.014). This was likely driven by *R. mangle* and the saline *S. patens* having greater electron transport rates in the drilling mud treatment, whereas the *S. lancifolia* and fresh *S. patens* treatments experienced either reduced or equivocal electron transport rates in the drilling mud treatment than in the control and bagasse-amended substrate. Overall *S. lancifolia* and *R. mangle* had significantly higher rates of electron transport than either treatment of *S. patens* (Fig. 4-11; F= 16.596, P<0.001).

Live to Total Stem Ratios

There was a significant interaction of time and species in live to total stem ratios (Fig. 4-13; F= 19.645, P<0.001). This was likely due to *S. lancifolia* having a lower live to total stem ratio at time 2 than time 1, while neither treatment of *S. patens* differed significantly between time 1 and 2. Live to total stem ratios were significantly lower at time 2 than time 1 (Fig. 4-13; F=3.966, P= 0.054). Because there was a significant interaction within time, each measurement period was subjected to univariate analyses. No significant effects of substrate, species, or the interactions thereof were detected for live to total stem ratios measured at the midpoint of the experiment (all P > 0.05). *Spartina patens* under fresh conditions had a higher final live to total

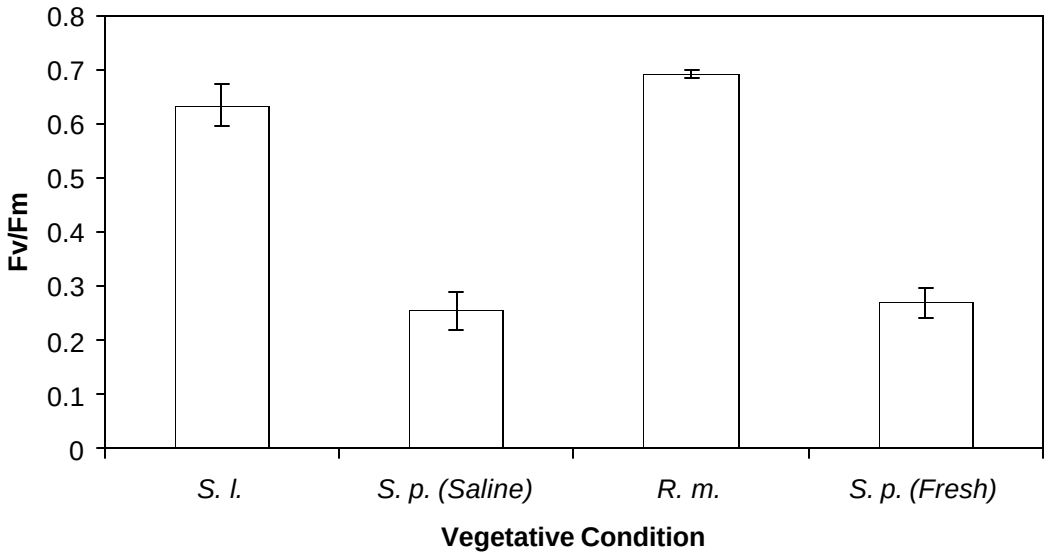

Fig. 4-10 The effect of vegetative condition on variable fluorescence to maximal fluorescence ratio (mean +/- SE), averaged across substrate. Treatment abbreviations are as follows: *S.l.* =*Sagittaria lancifolia*, *S.p.* (saline)= *Spartina patens* in 9 ppt, *R.m.* = *Rhizophora mangle*, *S.p.* (fresh)= *Spartina patens* in 0 ppt.

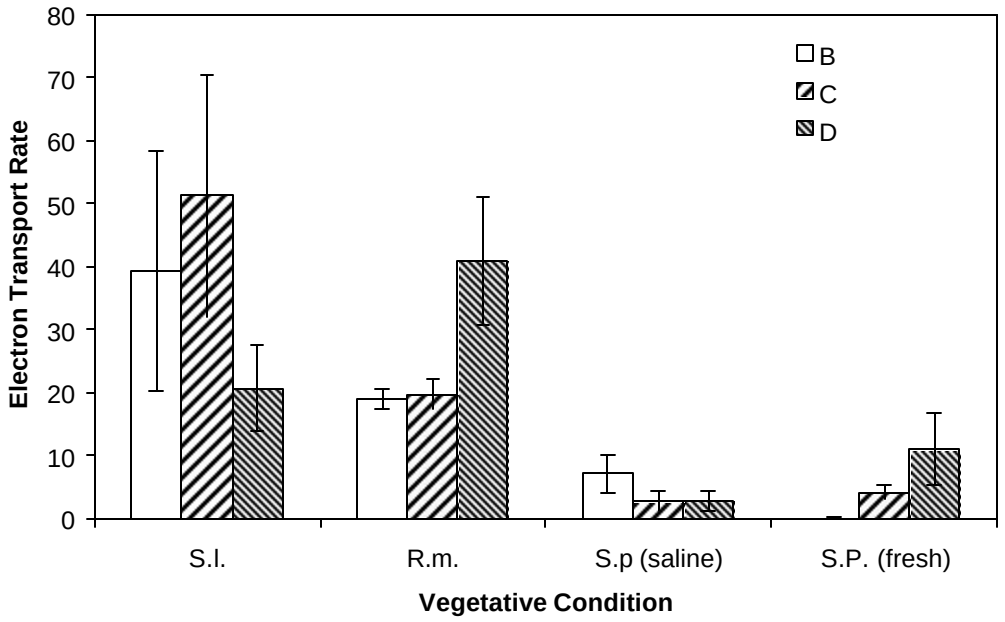

Fig. 4-11 The effect of substrate and vegetative condition on photosystem II electon transport rate (mean +/- SE). Treatment abbreviations are as follows: *S.l.* =*Sagittaria lancifolia*, *S.p.* (saline)= *Spartina patens* in 9 ppt, *R.m.* = *Rhizophora mangle*, *S.p.* (fresh)= *Spartina patens* in 0 ppt, C= 0% hematite-based drilling mud, 100% top soil; D= 10% hematite-based drilling mud, 90% river silt; B= 10% hematite-based drilling mud, 45% bagasse, 45% river silt

stem ratio in hematite-based, simulated drill cuttings treatment than in the control, whereas all other species treatments were not significantly different (Fig. 4-13; F= 3.647, P= 0.006). Also, *S. lancifolia* had a significantly lower live to total stem ratio at the conclusion of the experiment (Fig. 4-13; F= 86.019, P< 0.001). A nearly significant substrate effect was detected at the conclusion of the experiment, likely resulting from a trend of the drilling mud treatment to have a greater live to total stem ratio than the bagasse-amended substrate (Fig. 4-12; F= 3.044, P= 0.060).

Biomass Partitioning

The control substrate had significantly greater live aboveground biomass than the drilling mud treatment, which in turn had significantly greater live aboveground biomass than the bagasse-amended treatment (Fig. 4-14; Contrast F=6.755, P=0.013; Contrast F=4.809, P=0.035, respectively). Also, the control substrate had significantly greater total biomass compared to the hematite-based, simulated drill cutting treatment (Fig. 4-15; Contrast F=5.020, P=0.031). *Rhizophora mangle* had significantly greater live aboveground biomass at the conclusion of the experiment than the other species treatments (Fig. 4-16; F= 35.495, P<0.001). *Sagittaria lancifolia* had significantly greater dead aboveground (Fig. 4-17; F= 26.739, P<0.001), belowground (Fig. 4-18; F= 12.504, P<0.001), and total biomass (Fig. 4-19; F= 13.221, P< 0.001). No other significant main effects or interactions were detected in regard to any biomass partitioning.

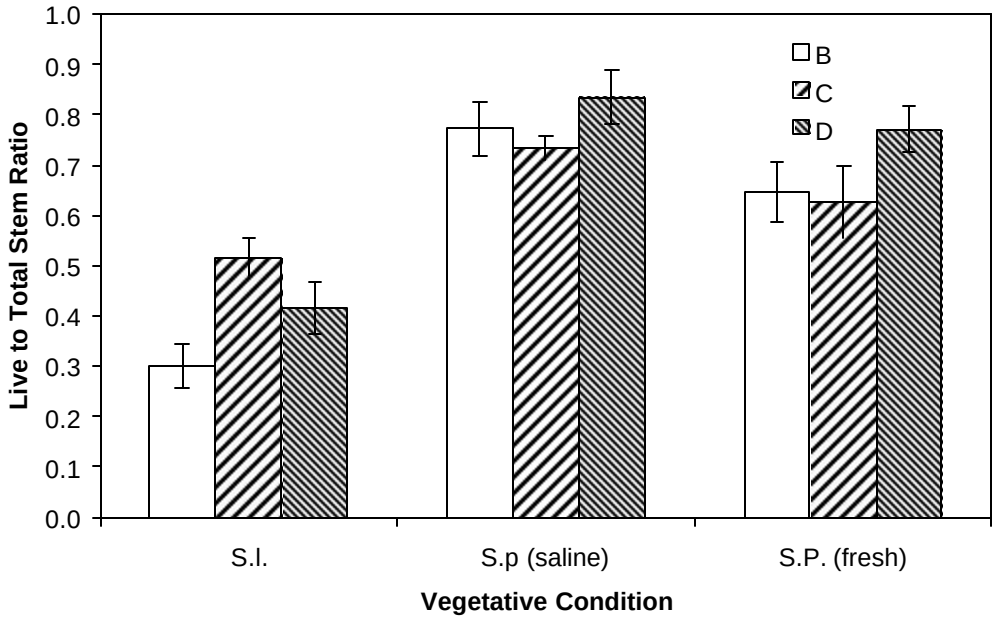

Fig. 4-12 The effect of substrate and vegetative condition on final live to total stem ratio (mean +/- SE). Treatment abbreviations are as follows: *S.l.* =*Sagittaria lancifolia*, *S.p.* (saline)= *Spartina patens* in 9 ppt, *S.p.* (fresh)= *Spartina patens* in 0 ppt, C= 0% hematite-based drilling mud, 100% river silt; D= 10% hematite-based drilling mud, 90% river silt; B= 10% hematite-based drilling mud, 45% bagasse, 45% river silt

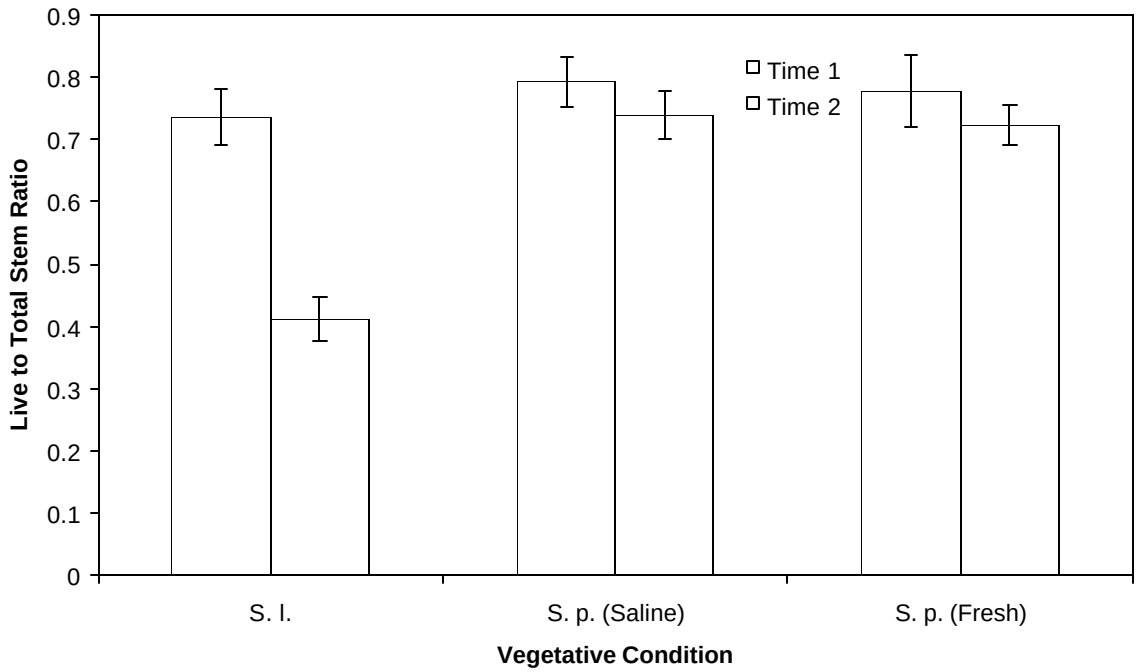

Fig. 4-13 The effect of vegetative condition on final live to total stem ratio (mean +/- SE), averaged across substrate. Treatment abbreviations are as follows: *S.l.* = *Sagittaria lancifolia*, *S.p.* (saline)= *Spartina patens* in 9 ppt, *S.p.* (fresh)= *Spartina patens* in 0 ppt.

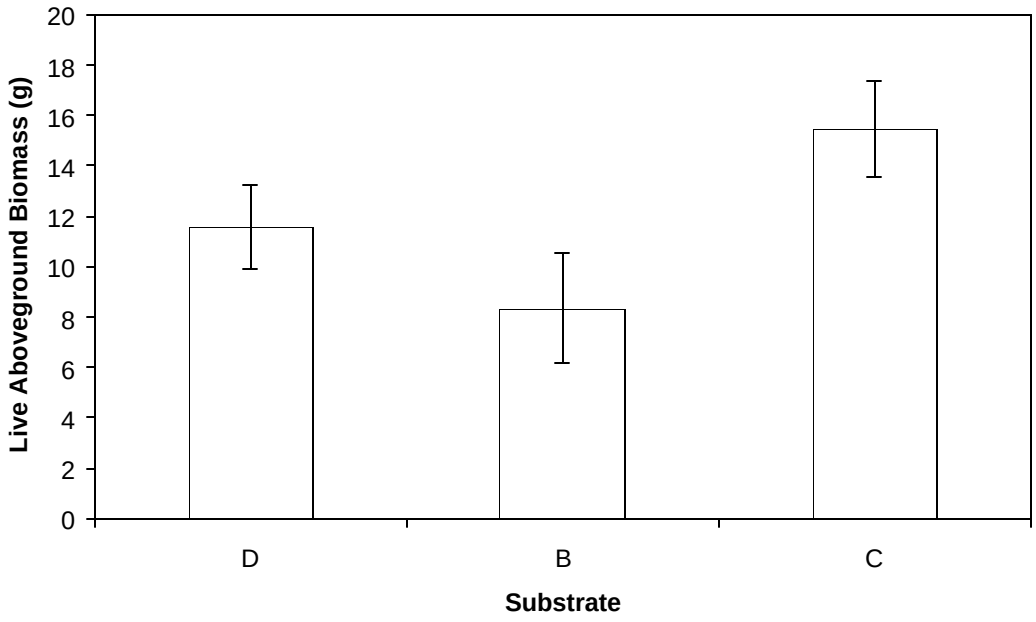

Fig. 4-14. The effect of substrate on live aboveground biomass (mean +/- SE), averaged across vegetative condition. Treatment abbreviations are as follows: D= 10% hematite-based drilling mud, 90% river silt; B= 10% hematite-based drilling mud, 45% bagasse, 45% river silt; C= 0% hematite-based drilling mud, 100%river silt.

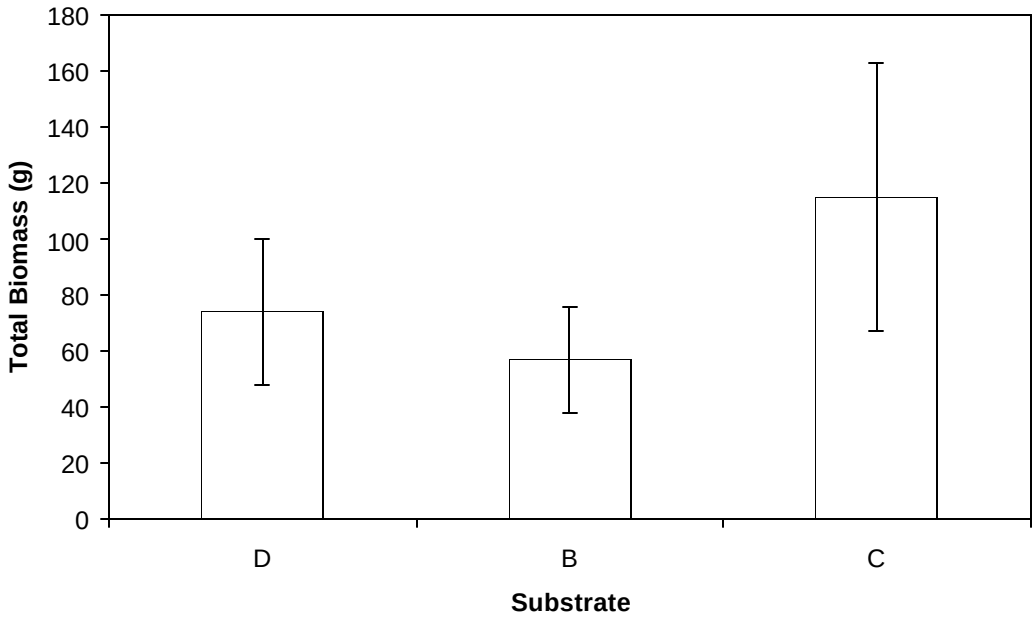

Fig. 4-15. The effect of substrate on total biomass (mean +/- SE), averaged across vegetative condition. Treatment abbreviations are as follows: D= 10% hematite-based drilling mud, 90% river silt; B= 10% hematite-based drilling mud, 45% bagasse, 45% river silt; C= 0% hematite-based drilling mud, 100%river silt.

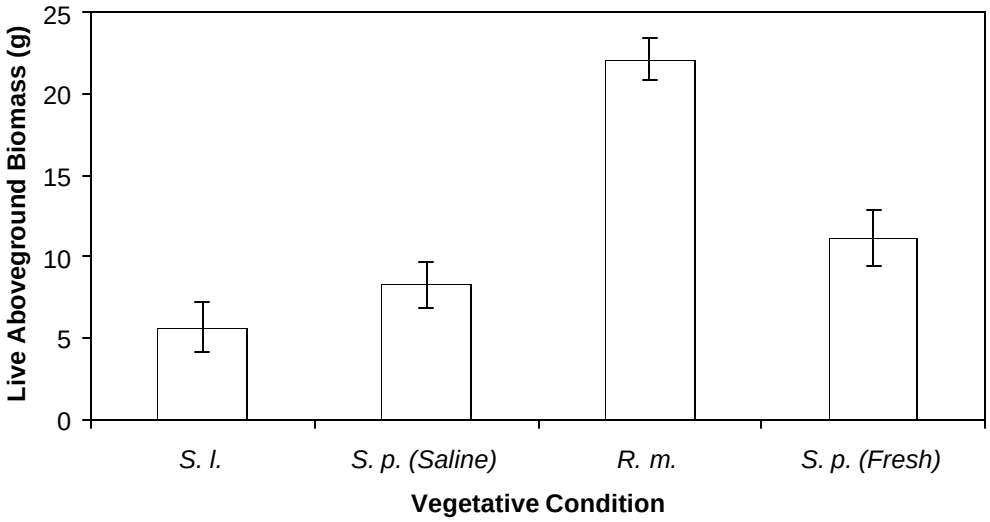

Fig. 4-16. The effect of vegetative condition on live aboveground biomass (mean +/- SE), averaged across substrate. Treatment abbreviations are as follows: *S.l.* = *Sagittaria lancifolia*, *S.p.* (saline)= *Spartina patens* in 9 ppt, *R.m.*= *Rhizophora mangle*, *S.p.* (fresh)= *Spartina patens* in 0 ppt.

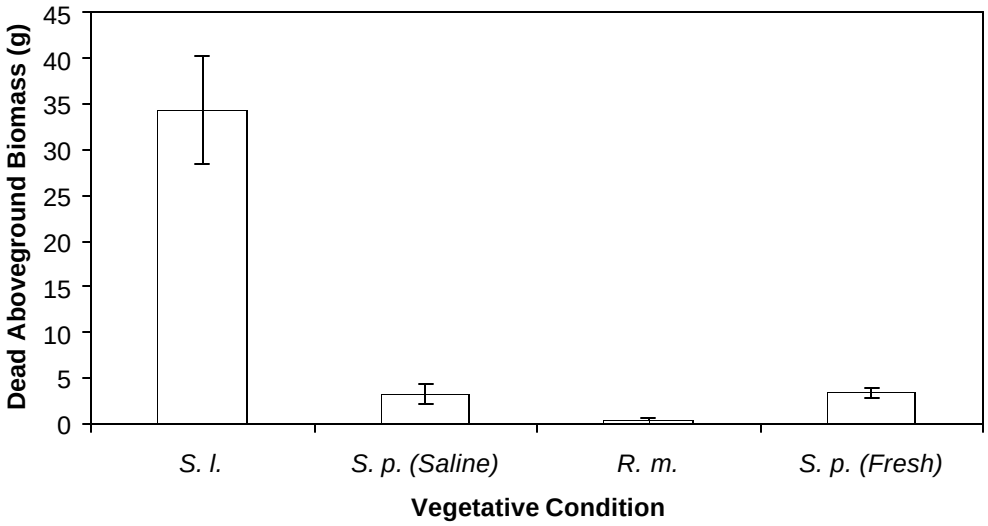

Fig. 4-17. The effect of vegetative condition on dead aboveground biomass (mean +/- SE), averaged across substrate. Treatment abbreviations are as follows: *S.l.* = *Sagittaria lancifolia*, *S.p.* (saline)= *Spartina patens* in 9 ppt, *R.m.*= *Rhizophora mangle*, *S.p.* (fresh)= *Spartina patens* in 0 ppt.

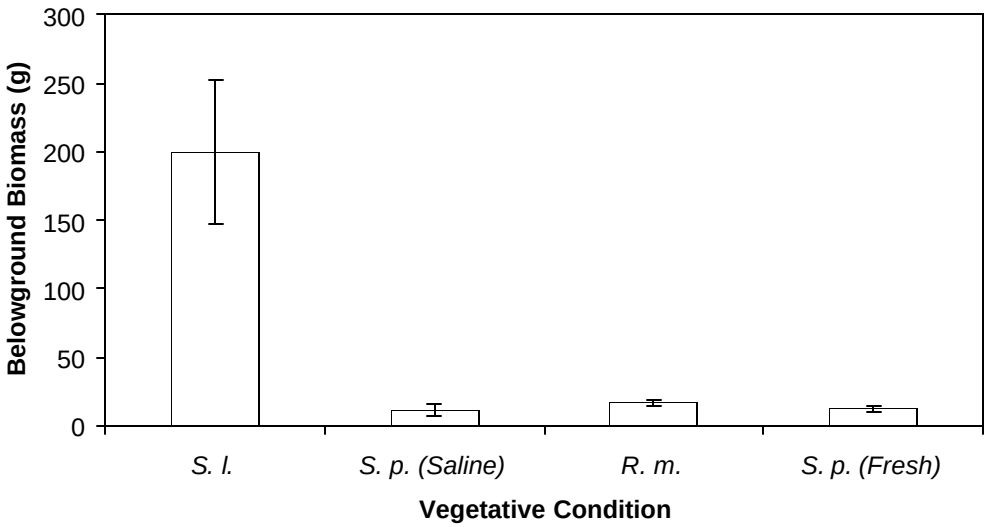

Fig. 4-18 The effect of vegetative condition on belowground biomass (mean +/- SE), averaged across substrate. Treatment abbreviations are as follows: *S.l.* = *Sagittaria lancifolia*, *S.p.* (saline)= *Spartina patens* in 9 ppt, *R.m.*= *Rhizophora mangle*, *S.p.* (fresh)= *Spartina patens* in 0 ppt.

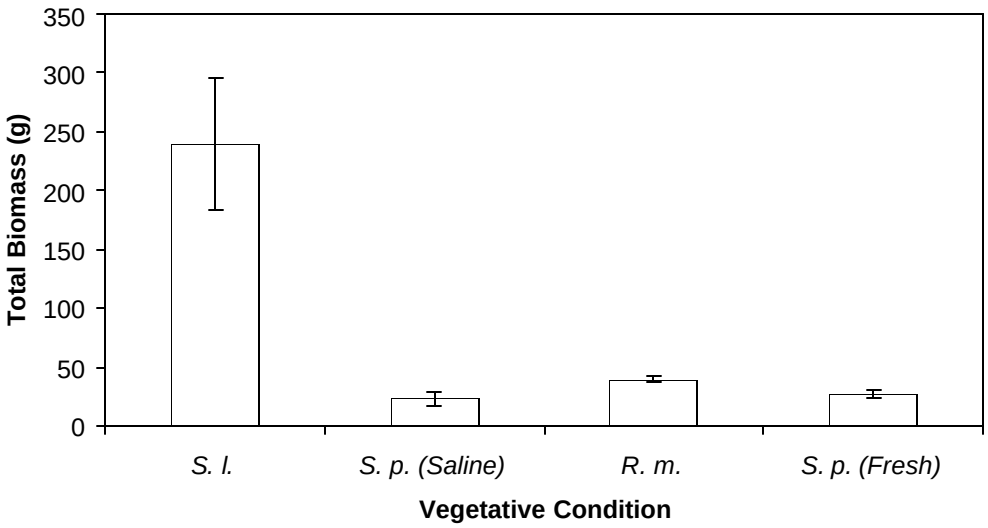

Fig. 4-19 The effect of vegetative condition on total biomass (mean +/- SE), averaged across substrate. Treatment abbreviations are as follows: *S.l.* = *Sagittaria lancifolia*, *S.p.* (saline)= *Spartina patens* in 9 ppt, *R.m.*= *Rhizophora mangle*, *S.p.* (fresh)= *Spartina patens* in 0 ppt.

DISCUSSION

Preliminary Studies

The initial evaluation of drilling mud and stabilizing amendments indicated that overall, *S. patens* displayed lower growth in the various hematite-based drilling mud-stabilizing amendment mixes than in the control (topsoil). However, plants in the hydroponic drilling mud treatment demonstrated greater growth than plants in the true hydroponic control treatment, indicating that some aspect other than the drilling muds themselves was likely the driving force of the reduced growth. It should be noted that the substrates generated in the initial examination of drilling mud-stabilizing amendments tended to be much less consolidated than the stabilizing amendment-topsoil mixtures generated for the evaluation of stabilizing amendments. Thus, the substrate consistency may have been the primary factor reducing the growth of *S. patens* rather than the drilling mud themselves. This is supported by the evaluations of stabilizing amendments in which reduced growth of *S. patens* occurred in only three stabilizing amendment-topsoil combinations (Fiberlock, Phenoseal, and to a lesser extent Kwickseal), with all other combinations demonstrating vigorous growth comparable to the topsoil control. Of great interest is that there appears to be no decrease in the growth response of *S. patens* as the percentage of hematite-based drilling muds in mixture with topsoil increased from 0 to 30%.

Mesocosm Study

Edaphic Variables

Soils were characterized in terms of, interstitial substrate pH, substrate redox potential, acid-digested substrate metal concentration, interstitial metal concentration, and acid-digested plant tissue metal concentration. Interstitial substrate pH and substrate redox potential, in addition to having major impacts on determining plant distributions in wetland systems, are also major controlling factors of metal solubility and bioavailability (Fergusson 1990, Gambrell et al.

1991, Bourg and Loch 1995). Acid-digested substrate concentration is the total concentration of potentially available metals in a substrate (both unavailable and available). Interstitial metal concentration is the available, dissolved concentration of metals available in pore water. Acid-digested plant tissue metal concentration is the total concentration of metals in plant tissue, and therefore can be used to indicate potential phytoaccumulation (bioaccumulation by plant species).

Soil redox potentials became most reduced in the bagasse-amended, hematite-based simulated drill cuttings, most likely due to the higher amount of labile carbon available for microbial metabolism. Interestingly, the higher available Fe in the hematite-based drill cuttings and bagasse-amended, hematite-based drill cuttings did not result in these soils having soil redox potentials poised at values indicative of Fe reduction (approximately +100 mV). Instead, the hematite-based, simulated drill cuttings and bagasse-amended, hematite-based, simulated drill cuttings demonstrated soil redox potentials more in the range of sulfate reduction (approximately -80 to -200 mV; Mitsch and Gosselink 2000). This is noteworthy because it does indicate that these hematite-based, simulated drill cuttings were experiencing conditions commonly found in coastal wetland environments (soil reduction in the range of sulfide formation), but the plants growing in them still demonstrated growth comparable to the control sediment. No significant differences were detected in pH, indicating that at least in the short term these simulated cuttings provided a substrate with an ecologically stable pH. Substrate pH has been a major impediment in the ability of processed drill cuttings to support the growth of wetland plant species in prior studies (Shaffer et al. 1996, DesRoches 1998, Shaffer et al. 1998, Hester et al. 2000, Willis 2000). Therefore, one essential characteristic of drill cuttings that are potentially to be used for

wetlands restoration is a pH within an ecologically stable range as was achieved in the present assessment of hematite-based, simulated drill cuttings.

Of great interest are the overall low levels of potential contamination demonstrated by the hematite-based simulated drill cuttings as indicated by acid-digested substrate metal concentrations, interstitial water metal concentrations, and acid-digested plant tissue metal concentrations. Acid-digested metal concentrations (total available metals) revealed that total substrate concentrations of Fe and Cr were indeed significantly higher in the hematite-based drill cuttings and the bagasse-amended, hematite-based drill cuttings than in the control substrate (river silt). However, for both of these metals detected concentrations were lower than the USGS low-alert levels for these metals (Manheim and Hayes 1999). In similar fashion, significantly higher levels of Fe and Cr were also detected in interstitial waters of the hematite-based drill cuttings and the bagasse-amended, hematite-based drill cuttings than in the control sediment (river silt). Potentially of greatest note is that no significant differences were detected in acid-digested plant tissue metal concentration, indicating that no bioaccumulation was occurring. Although results from all metal analyses indicated an extremely low level of metal availability and therefore contamination, it must be noted that these results are from a short-duration experiment utilizing simulated drill cuttings. Therefore it is critical that prior to employing drill cuttings in a wetland field trial, actual drill cuttings obtained with a hematite-based mud are subjected to a long-term, closed-system evaluation in order to be truly confident of their environmentally-benign nature.

Vegetative Performance

Both instantaneous and long-term indicators of plant growth response were employed in the mesocosm evaluation of hematite-based, simulated drill cuttings. Instantaneous

indicators included net CO_2 assimilation rate (plant photosynthetic rate as an indicator of short-term growth) and leaf fluorescence characteristics (evaluation of photosystem II integrity as an instantaneous indicator of plant metabolic health). Fluorescence characteristics are of particular interest because they have a high sensitivity and rapid response time to stresses such as metal uptake (Pereira et al. 2000). Plant biomass (i.e., dry weight production) is a standard metric for the integrated growth response of plants to their collective environment through time (Larcher 1995).

Overall, instantaneous growth indicators indicated fairly comparable performance of plants grown in the different substrates. Net CO_2 assimilation rates were comparable between all substrate and species combinations at the midpoint of the experiment. At the conclusion of the experiment the only difference detected was an increase in net CO_2 assimilation in the saline *S. patens* grown in the control (river silt). In general, the net CO_2 assimilation rates indicated that hematite-based, simulated drill cuttings can support vigorous wetland plant growth that is comparable to river silt. No significant differences were detected in any substrate and species combination in leaf fluorescence characteristics. As mentioned above, leaf fluorescence characteristics are extremely rapid and sensitive indicators of damage to photosynthetic machinery stemming from such stresses as plant metal uptake. Therefore, even with the short duration of this experiment, any significant damage to the photosynthetic machinery would have been detected. These results, along with those of the acid-digested plant-tissue metal analyses and the net CO_2 assimilation rates further confirm that there is no evidence of significant amounts of metals moving from the simulated drill cuttings and into the vegetation.

Live to total stem counts midway through the experiment indicated that hematite-based, simulated drill cuttings, and bagasse-amended, hematite-based, drill cuttings are comparable to

river silt in supporting wetland plant growth. Live to total stem counts at the conclusion of the experiment indicated that *S. lancifolia* performed better in river silt than either simulated, drill-cutting substrate. However, this relationship was opposite than what was observed for *S. patens* under fresh conditions, where final live to total stem counts actually tended to be greater in the two simulated drill-cuttings substrates than in river silt.

Plants grown in river silt had significantly greater live aboveground biomass and total biomass than plants grown in the hematite-based, simulated drill cuttings, which in turn had greater aboveground live biomass than plants grown in bagasse-amended, hematite-based drill cuttings. Although this was clearly evident for *S. patens* under saline conditions and for *S. lancifolia*, this trend was not as apparent with either *R. mangle* or with *S. patens* grown under fresh conditions. However, it should be noted that no significant differences between substrates were detected in dead aboveground biomass, indicating that the substrates containing drilling mud were generating no more dead tissue than the control substrate. Thus, in the short-term these hematite-based, simulated drill cuttings may support slightly less vigorous growth of wetland plants, but these differences may disappear over a larger time frame. This is corroborated by the instantaneous evaluations of growth and stress (i.e., net CO_2 assimilation, live to total stem count, and fluorescence characteristics), which indicate little if any difference between substrates at the midpoint and conclusion of the experiment. Further, our results concerning organic amendments suggest that hematite-based, simulated drill cuttings may be most beneficial in more mineral wetlands, such as coastal salt marshes and mangrove forests, and that organic amendments may be of little benefit in enhancing wetland restoration efforts involving these types of drill cuttings.

The results of these studies indicate that drill cuttings generated with hematite-based drilling fluids have a great potential to serve as an environmentally-benign sediment source for restoring and creating wetlands. Our previous research suggested that drill cuttings created with an initially environmentally-innocuous drilling mud would likely prove superior for use in wetland restoration events than drill cuttings that have undergone substantial processing to render them more environmentally safe. This pilot study, in which hematite-based, simulated drill cuttings were examined for their potential as a safe, nontoxic wetland substrate has corroborated this premise. The hematite-based, simulated drill cuttings have demonstrated extremely low potential toxicity both in regard to available metals and in overall plant performance. However, as promising as these initial results are, it must be noted that due to limitations there are two major constraints on our conclusions that prevent extrapolation to field scenarios. We utilized simulated drill cuttings because of the unavailability of actual, hematite-based drill cuttings. Thus, although our finding are extremely encouraging, further research employing actual cuttings resulting from a hematite-based drilling mud must be performed in a closed system in order to accurately evaluate all aspects of the actual drill cuttings that we could not emulate (e.g., metal concentration of parent rock material, texture, etc). Also, our series of studies were not of long enough duration to elucidate vegetation seasonal response or long-term bioaccumulation potential. Thus, although we have answered key questions as to the potential use and configuration of hematite-based cuttings for the restoration and creation of wetlands, key, long-term data is necessary before a field trial should be implemented.

Therefore, we recommend that that a long-term, closed-system evaluation is performed using actual drill cuttings prior to the initiation of field trials. This approach will allow for greater understanding and prediction of contaminant responses over an annual cycle. Also, the

seasonal responses of vegetation and the responses of faunal components, such as aquatic invertebrates and fish, to these substrates should be investigated. Thus, although hematite-based drill cuttings appear to have tremendous potential as a substrate for wetland restoration and creation, further evaluations should be made in closed systems prior to field implementation.

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