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RESTORED DRILL CUTTINGS FOR WETLAND CREATION: RESULTS OF A MESOCOSM APPROACH TO EMULATE FIELD CONDITIONS UNDER VARYING SALINITY AND HYDROLOGIC CONDITIONS

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Executive Summary

Wetland loss, conservation, and restoration have become important environmental issues as the benefits of wetlands and their alarming rate of loss have become known. Louisiana, which contains 40% of the coastal wetlands in the United States, accounts for 80% of the nation's 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 substrate deprivation from alteration of the hydrology of the Mississippi River, 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). Previous research has indicated that processed drill cuttings exhibit a low degree of contaminant migration from the processed drill cuttings to interstitial water and a low toxicity, as tested by seven-day mysid shrimp chronic toxicity trials. Two different types of processed drill cuttings were utilized in these experiments. The first type of processed drill cuttings (hereafter referred to as DC-A) results from physical separation of the drilling fluids from the cuttings. The second type of processed drill cuttings examined (hereafter referred to as DC-B) results from further processing of DC-A by isolation and encapsulation of the cuttings in a silica matrix. Performance of plants grown on the DC-A

substrate was comparable to both dredge spoil, a common wetland restoration substrate, and topsoil. Performance of plants grown on the DC-B substrate was greatly reduced compared to all other substrate types. This lack of plant productivity in DC-B was likely due to the high pH (>10.0) of these drill cuttings under freshwater conditions. Although results of these initial studies were promising, concern was raised over the behavior of the processed drill cuttings under saline conditions, which was not investigated in the these earlier studies. Specifically, under saline conditions it was suggested that metals might be leached from the cuttings through cation exchange. Therefore, this mesocosm study was designed to examine the behavior of the processed drill cuttings and the productivity of common Louisiana brackish and salt marsh plants grown on them as salinities were increased from fresh water to open-ocean salinity conditions.

In this study, as was observed in the previous study, both interstitial water and plant tissue associated with the DC-A substrate exhibited low metal concentrations. Also in agreement with the previous study, plant performance in the DC-A substrate was found to be comparable to plant performance in the dredge spoil and topsoil substrates. This is extremely important because it indicates that the drill cuttings themselves may serve as an excellent substrate for wetland plant growth, but that the processing and stabilization techniques and drilling fluid formulations may require further refinement.

This study has also revealed unexpected results concerning the performance of the DC-B substrate under saline conditions. The pH of the DC-B substrate, although high (alkaline) under fresh conditions as in the previous studies, became substantially less alkaline when exposed to saline conditions. This decrease in pH corresponded with an increase in the performance of plants grown on the DC-B substrate, such that productivity became more comparable to the other substrates. The results of this study indicate that a major impediment of plant performance in the

DC-B substrate, high pH, may be ameliorated in saline environments. Furthermore, metal concentrations in substrate interstitial water and plant tissue were found to be low, even though the drill cuttings were subjected to levels of salinity, pH, and Eh that may allow for maximal release of many contaminants (i.e. maximum solubility) at various times during the study. Our results did indicate that substantial amounts of barite (barium sulfate used in drilling fluids) associated with both drill cuttings substrates, and theoretically encapsulated in the DC-B substrate, did dissociate and become available for plant uptake. Plant tissue concentrations of barium were elevated in the plants grown on both the DC-A and DC-B substrates, but fell within ranges reported for agricultural plants such as wheat. Soluble (available) barium levels in the interstitial water always remained at very low levels. Therefore, it appears that as barite dissociated, the available barium was taken up by the plants, which likely incorporated the barium as an insoluble (unavailable) form. The information generated by this study should resolve concerns of direct metal leaching through cation exchange into substrate interstitial water, and has further elucidated the potential use of processed drill cuttings in wetland rehabilitation. We suggest that future research focus on refinements in drilling fluid formulations and the behavior of barite (or alternative drilling fluid additives, such as hematite) under wetland conditions.

INTRODUCTION

The coastal wetlands of Louisiana are experiencing a high rate of wetland loss. Estimates of current loss rates are between 65 and 91 km^2/year (Barras et al. 1994; Dunbar et al. 1990). Major reasons for this loss include alteration of the Mississippi River, saltwater intrusion, and canal dredging for oil and gas extraction (Turner 1990). Modification of the Mississippi River, particularly flood protection levees, combined with natural subsidence of this area, has resulted in the loss of the allocthonous sediments needed to nourish and maintain the deltaic marshes. With no substrate input, the marshes subside. As the elevation of a marsh decreases relative to sea level, plants are placed under greater degrees of flooding stress. Eventually, the plants succumb to the stress, the marsh vegetation dies, and the stabilizing effect of the vegetation on the marsh substrate is lost. Erosion then increases and the area converts to open water.

Drill cuttings are oil field waste products that result from drilling excavations. Unprocessed drill cuttings are composed of the parent substrate (earthen materials), and have associated with them the drilling fluids or muds (which may contain additives such as organic thinners, hydroxides, barite, etc.) that are used in the drilling process (Gray and Darley 1980). The type of drilling mud used, and therefore the additives, can vary depending on where the drilling has taken place (Gray and Darley 1980). The drill cuttings investigated in this report were obtained with water-based drilling fluids, which are generally more environmentally benign than oil-based drilling fluids.

Several companies have developed methodologies that allow the drilling additives to be recycled as a cost-reduction technique. Another benefit to this recycling is that once the drilling fluids are removed, a substrate byproduct is created that may be low in toxicity and have potential as a medium for plant growth. Several studies have examined the potential of processed drill cuttings for plant propagation. These studies range in application from

agricultural usage (Chaineau et al. 1996) to wetland restoration (Kelley and Mendelssohn 1995). Kelley and Mendelssohn (1995) examined the potential for utilizing processed drill cuttings in the restoration of Louisiana wetlands, which are degrading due to a lack of sediment input.

Large amounts of drill cuttings are produced by the oil industry (Kelley and Mendelssohn 1995). In fact, nearly two million tons (dry weight) of this material are currently produced each year (Louisiana Coastal Wetlands Restoration and Conservation Task Force 1993). Kelley and Mendelssohn (1995) suggested that, following amendment with organic material, processed drill cuttings could serve as a medium for plant growth in wetland environments. Studies have also been performed at Southeastern Louisiana University concerning the applicability of processed drill cuttings for wetland creation (DesRoches 1998; Shaffer et al. 1998; Shaffer et al. 1996). In these studies two processed drill cutting types were assessed in terms of vegetation performance and contaminant release in a mesocosm setting. The first processed drill cutting type (hereafter referred to in this study as 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 (DesRoches 1998; Shaffer et al. 1998; Shaffer et al. 1996). Results from these previous studies indicated that the two types of processed drill cuttings exhibited low toxicity in saline (23-ppt) conditions as tested by mysid shrimp seven-day chronic toxicity trials (DesRoches 1998; Shaffer et al. 1998; Shaffer et al. 1996). These studies also indicated a low probability of heavy metal release to substrate interstitial water (pore water) under freshwater 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 control substrates (i.e. topsoil). The drill cuttings that underwent further processing to encapsulate the cuttings and remaining drilling fluids (i.e. DC-B) showed low levels of toxicity, but only supported very limited growth of wetland vegetation. The poor response of vegetation grown on DC-B has been attributed to both the high pH of the interstitial waters of this substrate (mean pH=11.1) and the coarse texture of the soil (DesRoches 1998). Nonetheless, the successful colonization of DC-A and the high productivity of vegetation in this substrate indicated that drill cuttings have the capacity to support the growth of wetland vegetation, at least under freshwater conditions.

The studies discussed above demonstrated the potential of processed drill cuttings as a substrate for the restoration of wetlands. However, we decided that investigations on the behavior of processed drill cuttings under various salinity regimes needed to be conducted before truly educated decisions could be made concerning their use in wetland restoration. Most of the wetlands that would potentially benefit from this technique are located in coastal environments and would be exposed to some degree of salinity. Therefore, we conducted the mesocosm study presented in this report.

In this most recent study we examined plant performance and potential metal and ion release from processed drill cuttings under fresh, brackish, saline, and open-ocean salinity regimes. This study addressed concerns that under saline conditions ion exchange may occur that would potentially release metals and other ions from the processed drill cuttings into the interstitial water. This study also reports the response of dominant salt marsh plant species, not previously investigated, when grown on processed drill cuttings.

Specifically, this study addressed the following questions:

1. Will increased salinity cause heavy metals to migrate from the processed drill cuttings into interstitial water and/or plant biomass?
2. What are the effects of substrate type, hydrologic regime, and salinity on the growth responses of *Spartina patens* (Ait.) Muhl. (a brackish marsh grass), *Spartina alterniflora* Loisel. and *Spartina cynosuroides* (L.) Roth. (dominant salt marsh grasses), and *Avicennia germinans* (L.) L. (a salt marsh tree)?
3. Do *S. alterniflora* and *S. patens* exhibit significant intraspecific variation in growth responses to substrate type, hydrologic regime, and salinity regime?

Materials and Methods

Experimental Design

Overview

The experimental design for this study consisted of four sequential increases in salinity across all treatments of a 4x3x6 factorial design of four substrates, three hydrologies, and six plant types with two true replicates. The four salinity levels were 0 ppt, 9 ppt, 18 ppt, and 36 ppt. Each of these salinity levels was maintained for at least a one-month time interval prior to measurements. The four substrates were topsoil (control), products of two different drill cuttings processing techniques referred to hereafter as DC-A and DC-B, and a dredge spoil treatment, which consisted of a 40-cm cap of dredge spoil placed on top of DC-A. The three hydrologic treatments were mesic (continually moist), tidal (daily flooding and draining of water relative to sediment surface), and permanently flooded (always flooded to a depth of 20 cm). The tidal treatment was designed to emulate the diurnal tidal range of the northern Gulf Coast of Mexico by ranging from 20 cm above the soil surface to drainage 10 cm below the soil surface. Plant species used include *Spartina alterniflora* (oyster grass), *Spartina patens* (wiregrass), *Spartina cynosuroides* (big hogcane), and *Avicennia germinans* (black mangrove).

Study Facility

Two years prior to this study, a wetland simulation mesocosm facility was constructed to conduct preliminary investigations of processed drill cuttings as a potential substrate source for wetland restoration under freshwater conditions. This wetland simulation mesocosm facility and its substrates, located at the Southeastern Louisiana University horticultural complex, were used and modified as necessary to perform this experiment. A schematic of the mesocosm design is provided in appendix A-5 for better visualization.

Substrate Type

Each treatment mesocosm (200-liter, polyethylene vessel) was filled to approximately 3/4 volume with one of the four test substrates by hand in a cross-classified manner at the time of the original (1996-1998) experiment. As previously mentioned, the same substrates were used for this experiment. This was justified because during the freshwater experiment all substrates were treated in the same fashion and were assigned to the same hydrologic regime used in the present study. The drill cutting materials used in the experiment were generated in Grand Bay, Louisiana (a tidally influenced, intermediate marsh area). All drill cuttings were separated from the drilling fluids, which resulted in the DC-A substrate (processing accomplished by Cameron Corp.). Some of this material was subjected to further processing, resulting in the DC-B substrate (processing accomplished by SWACO Corp.). The further processing of DC-B is proprietary information that is stated to isolate and stabilize the cuttings within a silica matrix. Drill cuttings substrates (DC-A and DC-B) and dredge spoil were transported to the SLU mesocosm facility courtesy of Greenhill Petroleum Company. Topsoil was acquired from a local supplier. All substrates were exposed to the same hydrologic regimes employed in this study during the previous two years of this research. Therefore, substantial time had elapsed to allow these substrates to respond to long-term exposure in simulated wetland environments.

Hydrology Design

The 200-liter mesocosm treatment vessels (144 total) were networked to four, 3,000-liter reservoirs with each substrate type assigned to a different reservoir. The substrate-specific plumbing design prevents any cross contamination of water from different substrate sources. An overflow drainpipe (3/4-inch diameter PVC) was installed 20 cm above the soil surface. A controlled-flow drainpipe was also installed, which extended 20 cm above the soil surface and

was perforated to a depth of 10 cm below soil surface. These drainpipes were used to maintain hydrologies as stated below. All overflow drainpipes were left open for the duration of the experiment. Controlled-flow drainpipes were closed for all permanently flooded barrels, except for a two-day period at the inception of each salinity level when they were opened to allow salinities to equilibrate. Controlled-flow pipes were left open for the duration of the experiment for the tidal and mesic hydrologies. Tidal drainlines were connected to substrate-specific electronic valves that could be set by timer to drain for certain intervals, thus creating a tidal effect. All drainpipes were connected to substrate-specific drainlines (1-inch PVC) that emptied into sumps containing Little Giant Big John Submersible sump pumps (Model #8CMB) that returned water to the substrate-specific reservoirs.

Plant Treatments

Transplants of *S. cynosuroides* (big hogcane) were collected from the Bayou Lacombe area, a local brackish marsh. *Avicennia germinans* (black mangrove) plants were ordered from a horticultural supplier. Three genotypes each of *S. patens* (wiregrass) and *S. alterniflora* (oystergrass) were selected from a collection of genotypes that are maintained by Dr. Mark Hester's laboratory at the SLU horticultural complex. These genotypes represented a range of salt tolerance based on his previous research (Hester et al. 1996, Hester et al. 1998). *Juncus roemerianus* Scheele. (black needle rush) was also collected from the Bayou Lacombe area and was initially included in the study, but the plants persistently showed low survival in all substrates despite several attempts to establish them in the mesocosm. Discussion with other researchers confirmed that this species is highly sensitive to having its roots and rhizomes separated into smaller transplant units. *Juncus romerianus* can be established in larger sods, but since the soil brought in with a sod would confound direct measurement of substrate-specific

plant performance, this treatment was omitted and these barrels were treated as a second mudflat (unvegetated). Test plants were transferred into treatment vessels during the period of April 23 to May 5, 1998. Individual plants were rinsed to remove parent soil and then randomly allocated to the appropriate treatment vessels. Plants were maintained under mesic conditions until June 17, 1998, at which time hydrologic treatments were initiated.

Salinity Levels

Salinity was sequentially increased from 0 ppt, to 9 ppt, to 18 ppt, and then to 36 ppt for all treatment vessels over a one-year period using a commercial aquarium salt (Coralife scientific grade sea salt). The durations of the salinity treatments were four months for the 0 ppt and 9 ppt salinity levels, and two months for the 18 ppt and 36 ppt salinity levels. The increases from 9 ppt to 18 ppt and 18 ppt to 36 ppt were accomplished over a two-week period to allow plants time for acclimation. All treatment vessels were subjected to a 48-hour period with mesic soil conditions and continual water circulation after addition of salt. This ensured that all treatment vessels experienced the same salinity regardless of hydrology. Hydrologies were restored the following day. Salinity levels of each treatment were monitored monthly with both a refractometer and a salinity meter (YSI Model # 30 salinity/conductivity meter) and adjusted accordingly. Salinity ranges were maintained at +/- 2 ppt for the 9-ppt treatment, +/- 2 ppt for the 18-ppt treatment, and +/- 1 ppt for the 36-ppt treatment.

Data Collection and Analysis

Overview

Data collections were performed at the end of each salinity treatment (Oct. 2-18, 1998 for 0 ppt, Feb. 24-March 3, 1999 for 9 ppt, April 14-28, 1999 for 18 ppt, and July 9-22, 1999 for 36 ppt). Measurements taken consisted of plant photosynthetic response, interstitial water samples (pH and elemental analysis), and Eh at 2 cm and 15 cm below the soil surface. Interstitial water samples were filtered and preserved for elemental analysis. Heights and diameters of *A. germinans* were recorded at the conclusion of the experiment. Biomass harvest was performed at the end of the 36 ppt treatment data collection. A subsample of this biomass was used for analysis of metals in plant tissue. A more detailed description of the data collection and analysis methods follows.

Plant Photosynthetic Response

Plant photosynthetic response was determined using a LICOR® 6400 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 selected 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 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 μmol per mol CO_2 per m^2.

Soil Reduction Potential

Soil reduction potential (Eh) was monitored in all experimental vessels using a Hanna model HI 9025 pH/mV meter and brightened platinum redox electrodes. Probes were constructed and calibrated as per Faulkner et al. (1989) and were calibrated and brightened prior

to each measurement period. A redox probe was placed two centimeters below the soil surface in each vessel, allowed to equilibrate overnight, and redox measurements were taken the following day. A redox probe was then placed 15 cm below the soil surface and allowed to equilibrate overnight. Readings were recorded the following day.

Interstitial Water Analysis

Interstitial water pH was monitored with a Hanna model Hi 9025 pH/mV meter which was calibrated prior to each measurement period with pH 7 buffer. Measurements were taken by placing the pH probe into the interstitial well (slotted 3/4 inch PVC pipe extending 10 cm below soil surface) of each treatment vessel, thus getting a pH of interstitial water at 10 cm below the soil surface.

Interstitial water samples for elemental analysis were collected by drawing approximately 20 ml of fluid with a syringe from interstitial water wells. Each sample was then passed through a 2 μm syringe filter into an acid-washed scintillation vial. Samples were preserved by adding two drops of concentrated, reagent-grade nitric acid to each sample and then freezing. Prior to analysis the samples were thawed and replicates of higher order interactions were pooled. Samples were then transported to the Louisiana State University Wetland Biogeochemistry Laboratory for analyses. 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 and Hg, which were subjected to Cold Vapor Atomic Absorption (CVAA) with a Perkin-Elmer Model 5000 Atomic Absorption Spectrophotometer. Samples were analyzed for the following elements: macrocations (P, K, Na, Ca, Mg), microcations (Fe, Mn, Cu, Zn, Al), and heavy metals (Cd, Cr, Pb, As, Ba, Hg). It should be noted that the DC-A treatment experienced a large leak during the 18-ppt treatment; therefore,

interstitial samples were not collected for this treatment. Furthermore, many of the 9-ppt samples were lost due to vial breakage during the thawing period for refiltration. Therefore, these data are presented for qualitative purposes and were not included in the repeated measures analysis of interstitial elements. Because analysis of Hg requires large volumes of sample (i.e. ~100 ml), the remainder of the samples after analysis for other elements were pooled by substrate to achieve this volume. Therefore, no replication was possible and these data are presented for qualitative assessment only.

Biomass Measurements

Plant biomass was harvested at the end of the 36-ppt experimental period. Biomass was separated into aboveground alive, aboveground dead, and belowground partitions and placed into paper bags at harvest. All biomass was dried in a plant-drying oven at 60° C for a minimum of three days or until constant weight was achieved. Ten empty bags were taken from the same batch of paper bags used for plant biomass collection and were subjected to the same drying procedure as the biomass bags. The average dry bag weight was then subtracted from the biomass plus bag weight to determine the biomass weight.

Stem Heights and Diameters

At the conclusion of the experiment heights of *A. germinans* plants were measured with a meter stick and diameters were measured with digital calipers. All *A. germinans* appeared similar in height and weight at the inception of the experiment; therefore, the final heights and diameters were deemed appropriate for analysis as dependent variables.

Genotype Analysis

All treatment vessels that were designated as either *S. patens* or *S. alterniflora* were planted with three clones (genotypes) of the appropriate species. Clones were placed equidistant from each other and from vessel design factors that might affect performance (e.g. water inlet, drain valve, etc.). These plants were then subjected to the same measurements as the other plant species. Because of the profuse growth and production of runners by *S. alterniflora*, discerning genotypes became unreliable towards the conclusion of the experiment. The 36-ppt net CO_2 assimilation rates and final biomass partitioning were therefore excluded from the *S. alterniflora* genotypic analyses.

Plant Tissue Analysis

Subsequent to being weighed, a subsample of dried biomass (approximately 1 g of stem and leaf) was obtained from all vegetated test vessels in the mesic and flooded hydrology treatments. Aboveground tissue was ground in a stainless steel Wiley Mill and acid digested as described below. One g 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 (Al, As, Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, and Zn).

Sediment Analysis

One g of substrate was collected from 10 randomly chosen DC-B barrels and dried for 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 8.0 (SPSS Inc. 1998). Factor analyses employed a Varimax rotation and minimum eigenvalue of one. All ANOVAs were performed using the general linear model procedures of SYSTAT version 8.0 (SPSS Inc. 1998). Repeated measure ANOVAs, with salinity treated as the repeat factor (Girden 1992), were used to analyze all dependent variables that were measured at each salinity level (soil redox potential, interstitial pH, interstitial elemental concentration, and photosynthetic response). Univariate ANOVAs were employed to test for differences within a time period when a significant interaction with time was detected (Day et al. 1999). As previously mentioned, plants that died were not replaced at the start of each salinity level. In order to prevent confounding of the repeated measures analysis of photosynthetic rates, dead plant photosynthetic rates were entered as 0 for the first salinity level

in which they were dead and thereafter as missing data for all subsequent salinity levels. This maintained independence among the repeat factors in the repeated measures analysis. Genotypic photosynthetic rates were analyzed as strip-plot (i.e. split-split plot) ANOVAs (Neter et al. 1990). Genotypic final biomass weights were analyzed as split-plot ANOVAs (Neter et al. 1990). Final biomass weights and plant tissue metal concentrations were analyzed using univariate ANOVAs. Final DC-B substrate elemental concentrations were compared with an expected value obtained from previous studies (under non-saline conditions) on this substrate using one sample t-tests. All data were tested for meeting the criteria of parametric analysis. Interstitial element concentration, pH, and redox values for the 0-ppt treatment were subjected to factor analysis to determine if any variables were behaving in similar fashion. Factor analysis was also performed on the 36-ppt data set for interstitial element concentration, pH, and redox values. Plant tissue elemental concentrations were subjected to a factor analysis to determine similar behavior in elemental concentration. Repeated measures ANOVAs were adjusted for departures from sphericity using the Hunyh-Feldt technique, which compensates for these deviations (Girden 1992). Although total, aboveground, and belowground biomass for species data exhibited mild departures from normality, it was determined acceptable because the ANOVA technique is considered robust with regard to these departures (Neter et al. 1990).

Results

Factor Analysis

Factor analysis indicated that Pb, Ni, As, and Cr behaved in a similar fashion in 0-ppt interstitial water, 36-ppt interstitial water, final DC-B substrate, and final plant tissue concentrations. Factor scores were not employed for ANOVA calculations because we felt that the identity of all metals should be maintained. However, in all but one case Pb, Ni, As, and Cr behaved in a similar fashion. The only exception was in regard to salinity, where Cr exhibited no significant increase, and Pb, Ni, and As all increased, although only slightly, as salinity increased. Factor scores are presented in appendices A-1, A-2, A-3, and A-4.

Elemental Analysis (Substrate)

Acid-digested substrate concentrations of Ba, Zn, Cr, Fe, Mn, Mg, Al, P, and K were significantly lower in the DC-B substrate at the end of the study compared with substrate metal concentrations at the end of year one (Table 1). This indicates migration of metals occurred, to some extent, during the three-year time period between samples. No significant difference was detected with regard to DC-B substrate Ni, Cu, Cd, As, and Pb concentrations, whereas the concentrations of Ca and Mg actually increased over time (Table 1).

Elemental Analysis (Interstitial)

Mercury concentration at each salinity level is presented for qualitative assessment in Table 2. The results of substrate interstitial water analysis on all four test substrates (topsoil, dredge spoil, DC-A, and DC-B) at the end of the 0-ppt and 36-ppt salinity level exposures are presented in Tables 3 and 4 respectively. Because of lost sample data from the 9-ppt salinity level (vial breakage upon freezing) these values were not included in the analysis, but the data is presented in Table 5 for qualitative evaluation. There was a significant trend toward increases in the concentration of Ba, Na, Pb, Ni, As, Fe, Ca, Mg, and P in the 36-ppt salinity level compared

Table 1. Total potentially extractable (acid-digestion) elemental concentrations in the DC-B substrate prior to being subjected to any treatments in 1996 (initial) and at the end of the current study (final). Values are means (µg/g soil) plus or minus standard errors in parentheses for final concentration values (n=10).

Element	DC-B (initial) Mean	DC-B (final) Mean	t value	P value
Ba	1310.47	779.7 (26.999)	-66944.8	< 0.001
Cu	58.070	57.567 (3.531)	-0.143	0.890
Zn	61.860	54.082 (1.386)	-5.480	< 0.001
Cd	2.490	1.939 (0.251)	-2.199	0.055
Pb	0.000	1.063 (1.063)	1.000	0.346
Cr	32.690	15.442 (0.836)	-20.622	< 0.001
Ni	26.060	26.061 (0.606)	0.002	0.998
As	32.180	36.756 (7.555)	0.606	0.560
Fe	244418.4	23241.96 (29.984)	-388.069	< 0.001
Mn	177.660	127.58 (2.797)	-17.904	< 0.001
Ca	78576.98	89382.6 (2606.5)	4.146	0.003
Mg	17507.53	19569.8 (586.3)	3.517	0.007
P	692.44	838.63 (32.449)	-1446.572	< 0.001
Al	68546.59	34730.5 (766.239)	-44.133	< 0.001
K	1462.36	1078.33 (34.16)	-11.242	< 0.001

Table 2. Mercury concentration in interstitial water (µg/g) pooled by substrate (across hydrology and species; n=12) at the 0 ppt, 9 ppt, and 36 ppt salinity levels.

Salinity	Substrate			
	Topsoil	Dredge	DC-A	DC-B
0 ppt	0.140	1.140	1.210	0.030
9 ppt	1.390	0.570	0.620	5.610
36 ppt	0.650	1.940	1.720	0.460

Table 3. Results of elemental analysis of substrate interstitial water at the end of the 0-ppt salinity level. Values are mean and maximum concentrations (µg/g) plus or minus standard error in parentheses (n=36).

Element	Substrate							
	Topsoil		Dredge		DC-A		DC-B	
	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.
Ba	0.041	0.202	0.069	0.166	0.115	0.251	0.100	0.238
	(0.013)		(0.009)		(0.016)		(0.012)	
Cu	0.016	0.062	0.030	0.101	0.019	0.074	0.011	0.062
	(0.005)		(0.008)		(0.006)		(0.005)	
Zn	0.171	0.949	0.104	0.351	0.160	0.026	0.123	0.635
	(0.059)		(0.021)		(0.381)		(0.032)	
Cd	0.000	0.000	0.006	0.062	0.006	0.003	0.004	0.033
	(0.000)		(0.062)		(0.048)		(0.002)	
Pb	0.015	0.050	0.037	0.148	0.010	0.056	0.006	0.066
	(0.005)		(0.011)		(0.004)		(0.004)	
Cr	0.000	0.000	0.003	0.030	0.001	0.012	0.000	0.000
	(0.000)		(0.002)		(0.001)		(0.000)	
Ni	0.000	0.000	0.006	0.060	0.000	0.000	0.000	0.000
	(0.000)		(0.004)		(0.000)		(0.000)	
As	0.004	0.076	0.023	0.130	0.000	0.000	0.000	0.000
	(0.004)		(0.010)		(0.000)		(0.000)	
Fe	1.453	6.090	0.873	3.807	1.900	0.657	0.304	2.374
	(0.429)		(0.204)		(10.35)		(0.135)	
Mn	0.271	1.489	0.064	0.333	0.130	0.881	0.014	0.147
	(0.092)		(0.023)		(0.054)		(0.009)	
Ca	15.912	33.49	52.72	448.9	22.30	62.22	16.60	79.63
	(2.217)		(25.21)		(3.478)		(4.792)	
Mg	5.164	9.190	31.48	193.7	12.79	84.76	4.008	50.17
	(0.627)		(13.46)		(4.325)		(2.860)	
P	4.645	29.37	1.675	3.910	1.773	4.000	0.311	1.270
	(1.628)		(0.214)		(0.268)		(0.085)	
Al	1.004	3.680	1.005	3.040	1.153	4.480	8.034	20.99
	(0.236)		(0.165)		(0.304)		(1.228)	
K	5.127	15.31	16.274	48.33	13.92	41.47	22.79	50.65
	(1.197)		(2.698)		(1.744)		(2.777)	
Na	138.2	269.8	274.1	796.9	276.0	699.3	274.0	508.7
	(10.82)		(42.72)		(30.33)		(27.08)	

Table 4. Results of elemental analysis of substrate interstitial water at the end of the 9-ppt salinity level. Values are mean and maximum concentrations (µg/g) plus or minus standard error in parenthesis (n=36).

Element	Substrate							
	Topsoil		Dredge		DC-A		DC-B	
	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.
Ba	0.020	0.024	0.046	0.134	0.074	0.195	0.055	0.131
	(0.004)		(0.004)		(0.014)		(0.009)	
Cu	0.020	0.059	0.035	0.135	0.000	0.000	0.035	0.082
	(0.020)		(0.010)		(0.000)		(0.007)	
Zn	0.092	0.134	0.815	12.78	0.220	2.186	0.062	0.146
	(0.022)		(0.748)		(0.179)		(0.010)	
Cd	0.007	0.020	0.626	10.54	0.002	0.023	0.000	0.000
	(0.007)		(0.626)		(0.002)		(0.000)	
Pb	0.040	0.070	18.10	307.0	0.000	0.000	0.016	0.059
	(0.021)		(18.05)		0.000		(0.006)	
Cr	0.004	0.011	13.49	0.809	0.002	0.018	0.005	0.016
	(0.004)		(0.793)		(0.002)		(0.002)	
Ni	0.000	0.000	0.022	0.080	0.003	0.037	0.003	0.042
	(0.000)		(0.007)		(0.003)		(0.003)	
As	0.000	0.000	0.089	0.706	0.005	0.063	0.000	0.000
	(0.000)		(0.040)		(0.005)		(0.000)	
Fe	8.392	22.781	1.776	4.898	1.205	3.744	0.712	1.354
	(7.192)		(0.385)		(0.257)		(0.119)	
Mn	6.999	13.52	3.002	36.67	0.577	1.625	0.003	0.027
	(3.712)		(2.144)		(0.177)		(0.002)	
Ca	171.3	281.26	254.2	748.2	118.9	196.5	126.0	294.2
	(55.25)		(45.52)		(13.70)		(22.51)	
Mg	92.21	107.30	138.4	229.6	76.46	138.2	34.93	105.3
	(9.079)		(14.38)		(8.148)		(10.12)	
P	0.610	1.200	0.548	1.450	0.406	0.820	0.254	0.570
	(0.295)		(0.074)		(0.061)		(0.037)	
Al	1.543	0.459	1.899	8.290	0.908	1.450	1.475	2.740
	(1.543)		(0.425)		(0.096)		(0.212)	
K	72.583	136.80	75.66	160.3	54.98	86.99	74.97	117.8
	(32.394)		(9.292)		(4.472)		(5.346)	
Na	800.27	1075.0	883.2	1041	746.1	1010.0	709.6	943.5
	(139.8)		(34.38)		(39.18)		(34.84)	

Table 5. Results of elemental analysis of substrate interstitial water at the end of the 36-ppt salinity level. Values are mean and maximum concentrations (µg/g) plus or minus standard error in parenthesis (n=36).

Element	Substrate							
	Topsoil		Dredge		DC-A		DC-B	
	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.
Ba	1.003	1.730	1.132	4.860	0.931	2.500	1.346	3.290
	(0.081)		(0.276)		(0.126)		(3.290)	
Cu	0.183	0.070	0.827	13.745	0.281	1.784	0.108	0.595
	(1.137)		(0.760)		(0.123)		(0.040)	
Zn	0.062	0.246	0.474	7.780	0.110	0.576	0.020	0.198
	(0.019)		(0.430)		(0.034)		(0.012)	
Cd	0.025	0.301	0.017	0.268	0.015	0.084	0.000	0.002
	(0.017)		(0.015)		(0.005)		(0.000)	
Pb	0.115	0.855	0.090	0.249	0.264	2.307	0.140	0.510
	(0.054)		(0.022)		(0.135)		(0.038)	
Cr	0.006	0.071	0.001	0.018	0.013	0.134	0.001	0.025
	(0.004)		(0.001)		(0.008)		(0.001)	
Ni	0.000	0.000	0.010	0.046	0.056	0.360	0.030	0.043
	(0.000)		(0.003)		(0.021)		(0.020)	
As	0.062	0.302	0.050	0.190	0.172	1.500	0.023	0.236
	(0.021)		(0.016)		(0.083)		(0.015)	
Fe	19.87	77.82	7.838	30.89	16.89	66.80	0.297	0.850
	(6.040)		(2.508)		(4.888)		(0.053)	
Mn	0.064	0.330	6.228	18.97	3.647	12.54	0.064	0.330
	(0.027)		(1.478)		(0.888)		(0.027)	
Ca	331.9	829.7	405.9	1678	690.4	1837	668.2	1466
	(64.20)		(103.6)		(112.7)		(117.5)	
Mg	190.2	1102	169.9	848.0	295.1	1136	0.000	0.000
	(74.38)		(74.31)		(75.81)		(0.000)	
P	1.610	8.960	0.201	1.660	0.348	1.990	0.033	0.190
	(0.680)		(0.126)		(0.157)		(0.013)	
Al	22.21	347.0	0.236	2.450	1.484	7.510	0.078	0.660
	(22.21)		(0.156)		(0.452)		(0.046)	
K	53.40	546.1	79.46	695.1	116.5	1408	53.40	546.1
	(34.93)		(50.35)		(78.67)		(34.93)	
Na	9658	18536	274.1	796.9	9816	20316	8496	13566
	(979.3)		(42.72)		(969.0)		(712.6)	

with the 0-ppt salinity level (Tables 3 and 4; all $P < 0.05$). This trend was the most pronounced in DC-A and topsoil, and the least pronounced in DC-B. In fact, a significant interaction of salinity by substrate was present for both Mg and Ni, because although all other substrates showed an increase in these elements, their concentrations in DC-B were below detection throughout the study.

A significant interaction between salinity and substrate existed for Ca concentrations (Fig. 1; $F = 7.504$, $P < 0.001$). This can be explained by the large increase of Ca concentration in the DC-A and DC-B substrates in the 36-ppt salinity treatment. A significant interaction was also detected between salinity and hydrology at the 0-ppt salinity level, in which Ca concentrations were highest under mesic conditions, whereas at the 36-ppt salinity level, Ca concentrations were highest under tidal conditions (Fig. 2; $F = 14.336$, $P < 0.001$). A significant species and salinity interaction was detected because *S. cynosuroides* had the highest interstitial Ca levels at the 0-ppt salinity level, whereas other species (especially *S. alterniflora*) displayed increased interstitial water Ca concentrations at the 36-ppt salinity level (Fig. 3 $F = 6.325$, $P < 0.001$). In general, the main effect of salinity level on interstitial water Ca concentrations was consistent across all interactions, with Ca concentration being greater at the 36-ppt salinity level than at the 0-ppt salinity level.

A significant three-way interaction between salinity, substrate, and hydrology was detected for Fe concentrations (Fig. 4; $F = 2.679$, $P = 0.025$). This was likely due to the increase in the Fe concentrations of the topsoil substrate under all hydrologies, whereas dredge spoil and DC-A Fe concentrations increased only under flooded and tidal conditions, and DC-B Fe concentrations did not change. This interaction limits the interpretation of the main effect of

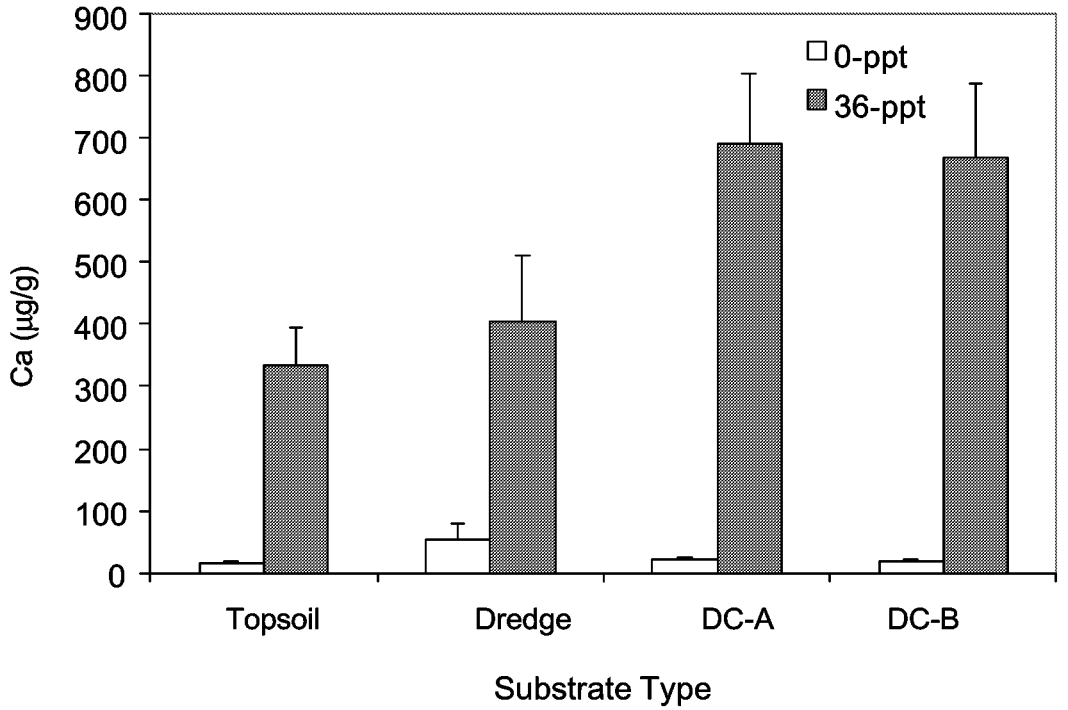

Fig. 1. The effect of salinity level and substrate type on interstitial Ca concentration (mean +/- standard error) averaged across hydrologic regime and plant species.

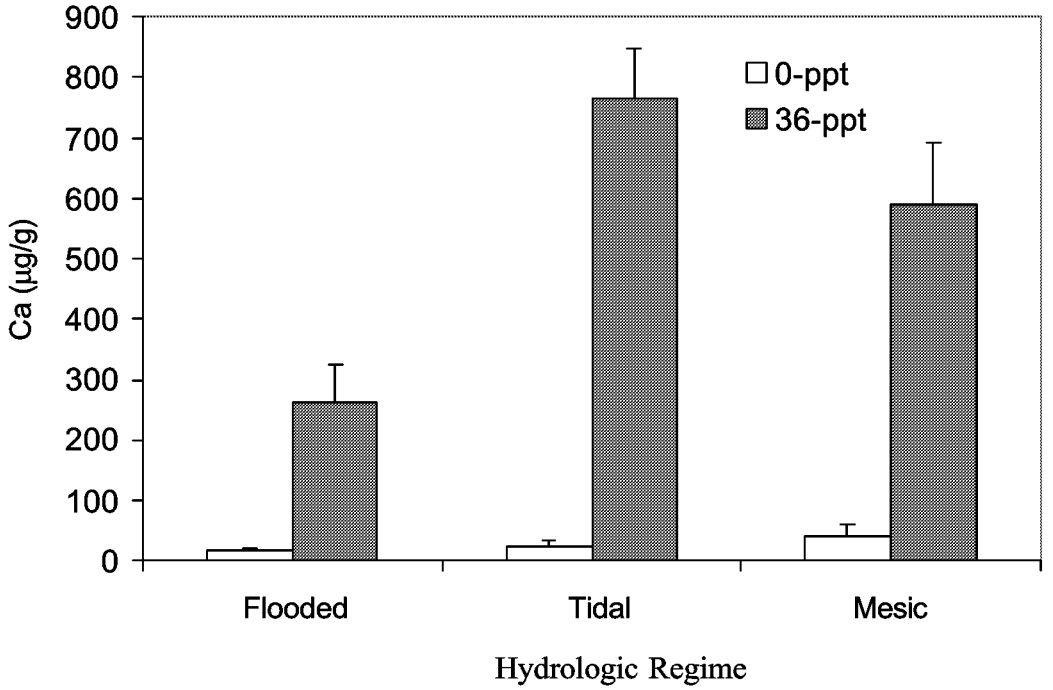

Fig. 2. The effect of salinity level and hydrologic regime on substrate interstitial water Ca concentration (mean +/- standard error) averaged across substrate type and plant species.

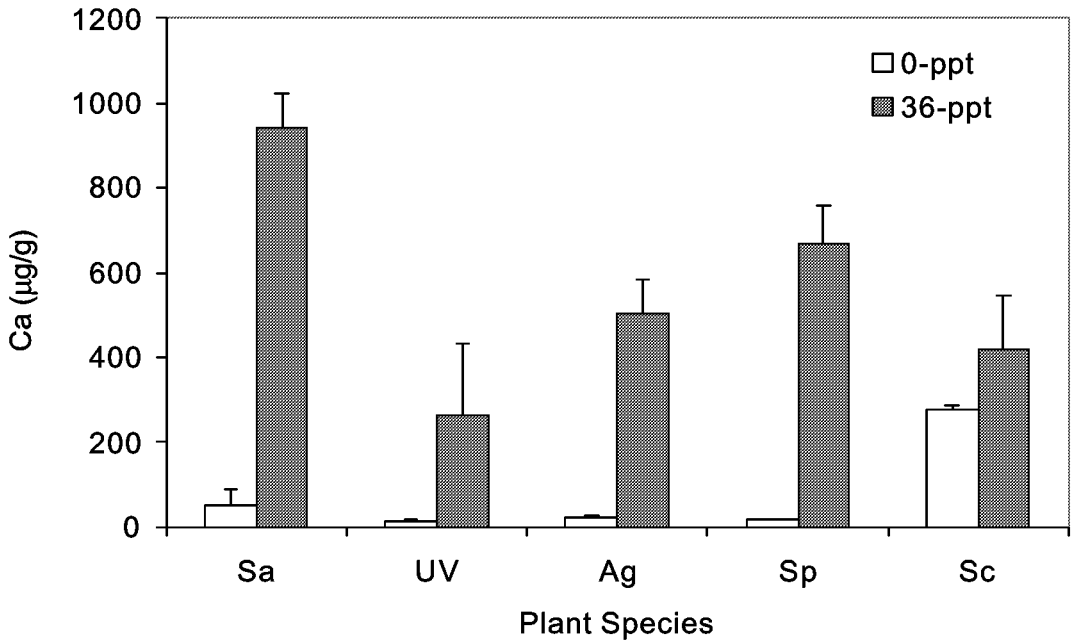

Fig. 3. The effect of salinity level and plant species on interstitial Ca concentration (mean +/- standard error) averaged across substrate type and hydrologic regime. Species abbreviations are as follows: Sa= *S. alterniflora*, Ag= *A. germinans*, Sp= *S. patens*, Sc= *S. cynosuroides*.

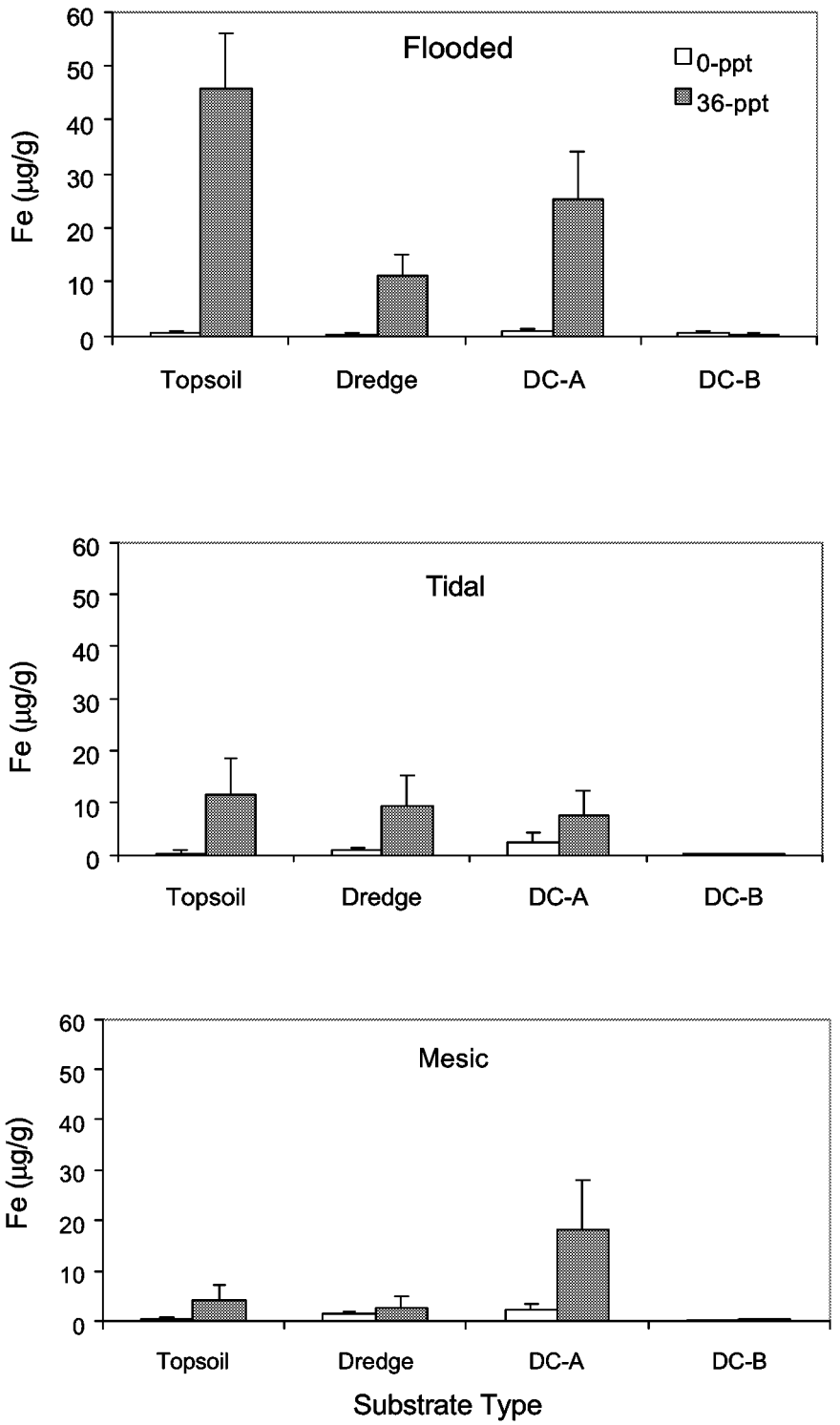

Fig. 4. The effect of salinity level, substrate type, and hydrologic regime on interstitial Fe concentration (mean +/- standard error) averaged across plant species.

salinity, but the interactions between salinity and substrate (F= 5.425, P= 0.003), and salinity and hydrology (F= 7.407, P= 0.002), are consistent with the explanation of the three-way interaction.

A significant three-way interaction between salinity, substrate, and hydrology was also detected for Mg concentrations (Fig. 5; F= 2.330, P= 0.046). This can best be explained by the high concentration of Mg in dredge spoil under the flooded, 36-ppt salinity level. This explanation is consistent with a significant salinity by hydrology interaction (F= 11.406, P< 0.001). A significant interaction also occurred between salinity and substrate (Fig. 6; F= 7.338, P< 0.001). This interaction appears to be due to the large increase in Mg concentration in all substrates except DC-B under increasing salinity levels.

Elemental Analysis (Vegetation)

Results of elemental analysis of acid-digested plant material are presented in Table 6. No significant effects of substrate, hydrology, species, or the interactions thereof were detected in plant tissue concentrations of Cu, Zn, Cd, Pb, Cr, Ni, As, Ca, Al, K, or Na. Plants grown in the DC-A and DC-B substrates had higher concentrations of Ba than those grown in topsoil or dredge spoil (Fig. 7; Contrast F= 6.318, P= 0.020). Plants grown in the DC-B substrate had significantly lower Mn levels than plants grown on any other substrates (Fig. 8; Contrast F= 7.351, P= 0.014). *Avicennia germinans* had significantly higher Mg concentrations than all other species regardless of substrate (Fig. 9; Contrast F=8.774, P= 0.008). Plants grown on topsoil had a greater concentration of P than those grown on other substrates (Fig. 10; Contrast F= 12.601, P= 0.002).

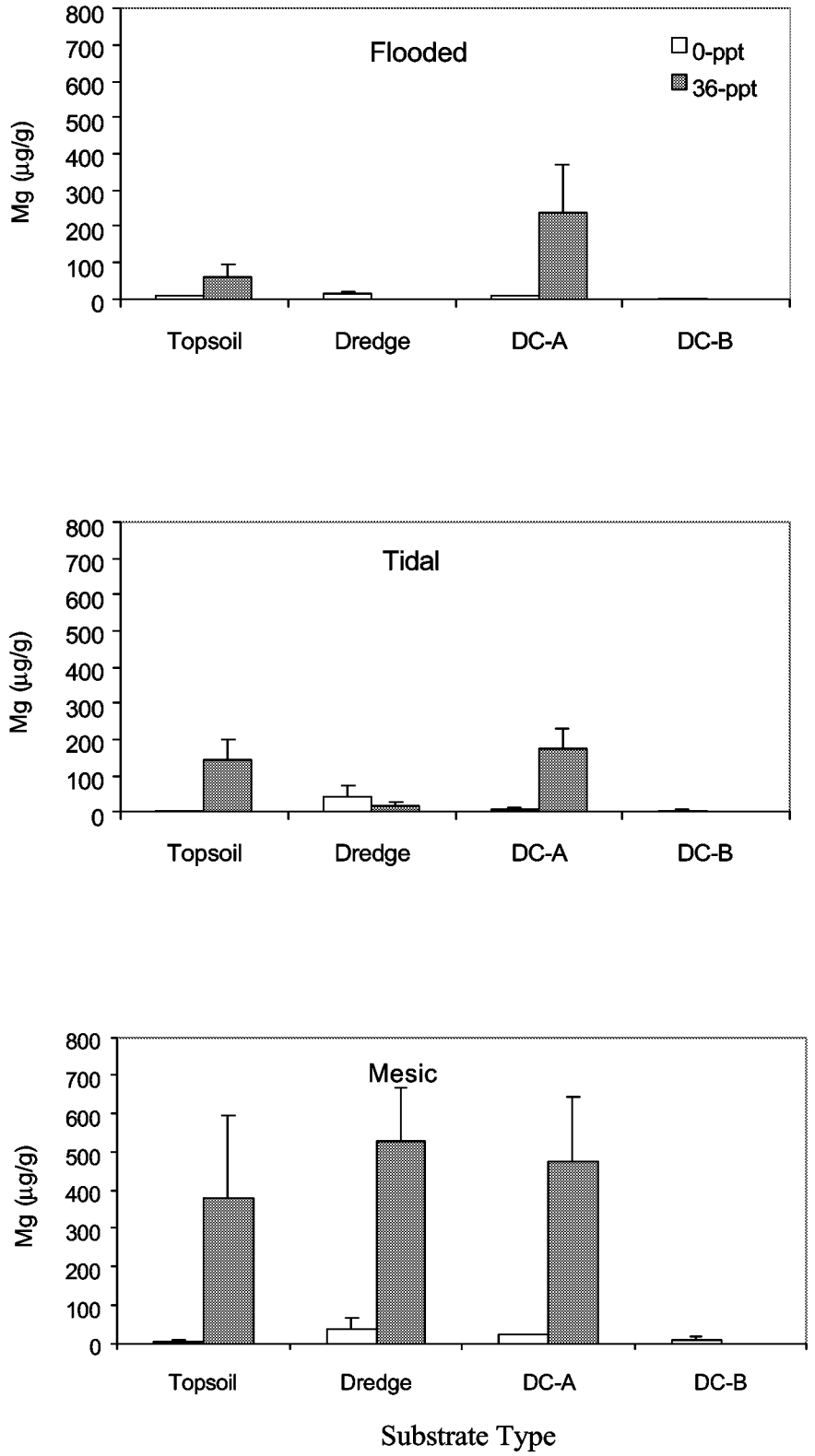

Fig. 5. The effect of salinity level, substrate type, and hydrologic regime on interstitial Mg concentration (mean +/- standard error) averaged across plant species.

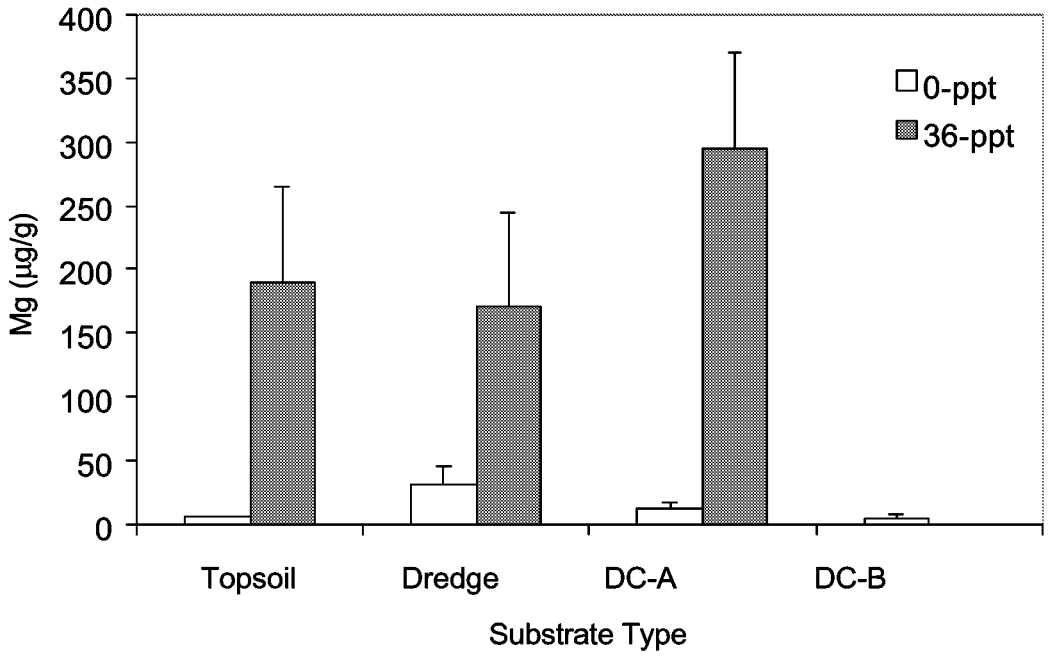

Fig. 6. The effect of salinity level and substrate type on interstitial Mg concentration (mean +/- standard error) averaged across hydrologic regime and plant species.

Table 6. Results of elemental analysis of aboveground plant tissue at the end of the 0-ppt salinity level. Values are mean and maximum concentrations (µg/g), plus or minus standard error in parentheses (n=36).

Element	Substrate							
	Topsoil		Dredge		DC-A		DC-B	
	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.
Ba	6.030	15.60	6.728	25.66	22.15	45.08	20.25	66.99
	(1.656)		(2.724)		(6.917)		(7.803)	
Cu	1.780	12.46	2.380	8.820	2.310	6.370	5.504	27.30
	(1.780)		(1.267)		(1.142)		(3.485)	
Zn	32.98	111.8	33.54	97.790	20.24	39.48	29.628	63.70
	(13.41)		(10.60)		(4.169)		(6.039)	
Cd	0.280	1.960	0.219	1.750	0.220	1.540	0.411	1.750
	(0.280)		(0.219)		(0.220)		(0.270)	
Pb	0.630	4.410	0.814	3.570	0.000	0.000	0.971	4.830
	(0.630)		(0.536)		(0.000)		(0.660)	
Cr	0.440	1.960	0.429	1.470	0.250	0.910	0.560	1.890
	(0.298)		(0.216)		(0.162)		(0.281)	
Ni	1.470	10.29	1.689	6.020	1.250	4.830	1.146	4.690
	(1.470)		(0.862)		(0.813)		(0.751)	
As	1.300	9.100	3.089	15.260	3.280	10.57	1.645	7.770
	(1.300)		(1.896)		(1.645)		(1.100)	
Fe	205.5	395.0	202.5	513.3	186.0	377.4	124.8	259.1
	(38.57)		(53.19)		(39.04)		(26.53)	
Mn	420.5	845.5	289.5	620.7	223.0	635.9	62.97	199.6
	(115.8)		(62.58)		(78.25)		(21.55)	
Ca	5919.0	13529	4406.8	8798.3	5026.4	10170.	6361.7	13508
	(1156.0)		(806.7)		(1027.8)		(1327.6)	
Mg	2339.0	5063.1	2327.2	5159.0	1957.6	4137.0	1457.3	2398.2
	(624.6)		(624.7)		(427.6)		(284.6)	
P	1541.0	2907.1	1049.7	1985.2	925.7	1416.8	613.6	1086.4
	(277.7)		(184.8)		(128.9)		(100.0)	
Al	49.90	212.1	73.237	194.6	86.4	156.8	131.6	435.4
	(28.69)		(21.20)		(20.76)		(60.91)	
K	5554.8	11158.	4948.6	6913.2	5611.4	7994.0	4665.1	9758.0
	(996.2)		(548.2)		(821.0)		(1140.9)	
Na	29017.	48013.	22913.	36029	20094.	41692	22850	41692.
	(4613.5)		(2804.4)		(3197.0)		(4099.2)	

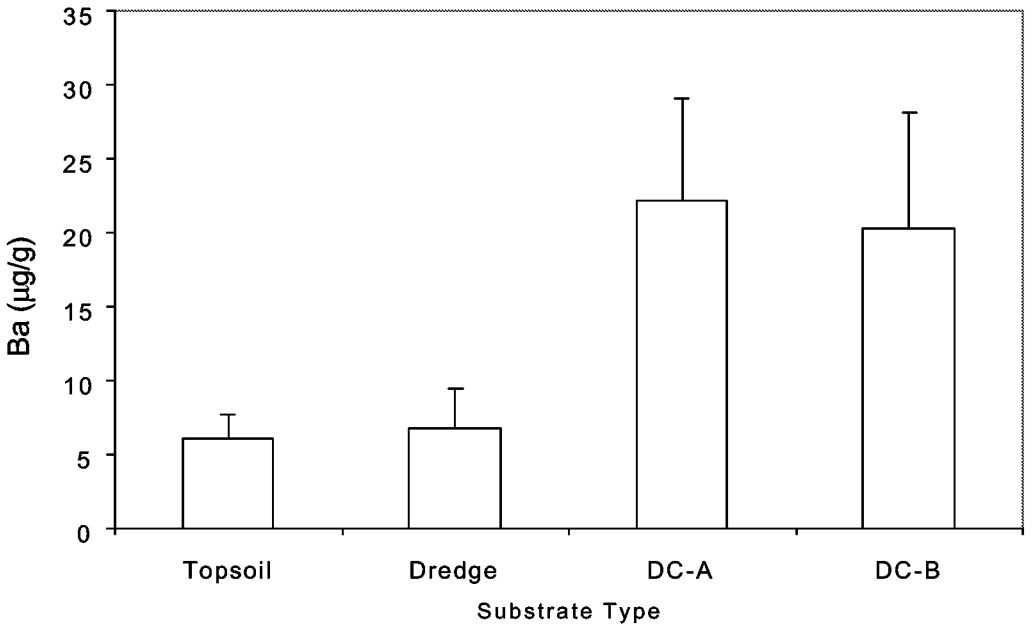

Fig. 7. The effect of substrate type on Ba concentration (mean +/- standard error) in plant tissue averaged across hydrologic regime and plant species.

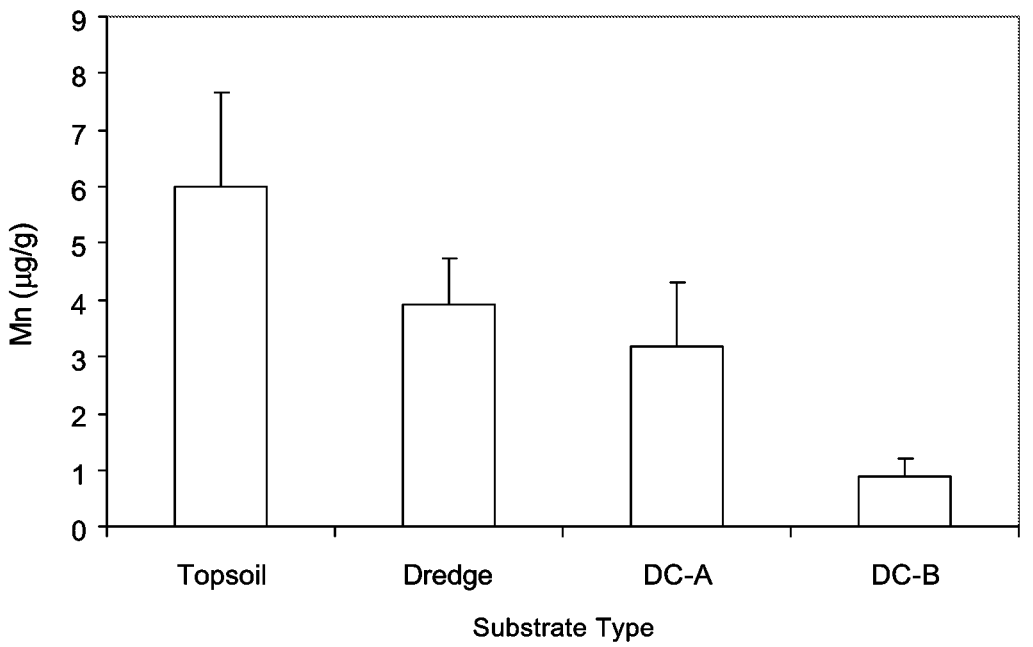

Fig. 8. The effect of substrate type on Mn concentration (mean +/- standard error) in plant tissue averaged across hydrologic regime and plant species.

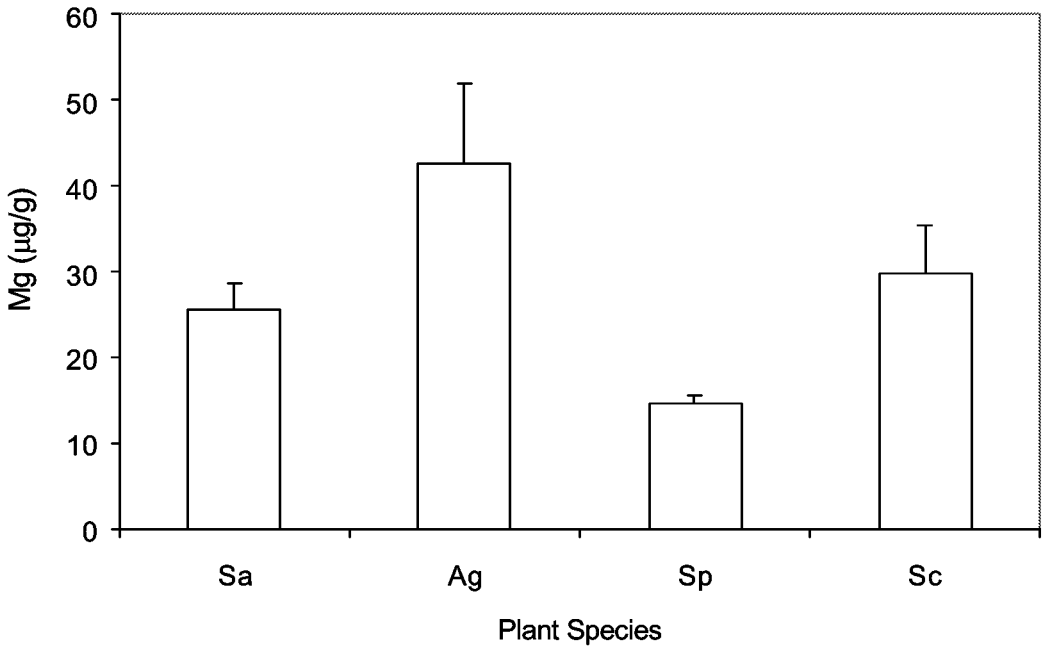

Fig. 9. The effect of plant species on Mg concentration (mean +/- standard error) in plant tissue averaged across substrate type and hydrologic regime. Species abbreviations are as follows: Sa= *S. alterniflora*, Ag= *A. germinans*, Sp= *S. patens*, Sc= *S. cynosuroides*.

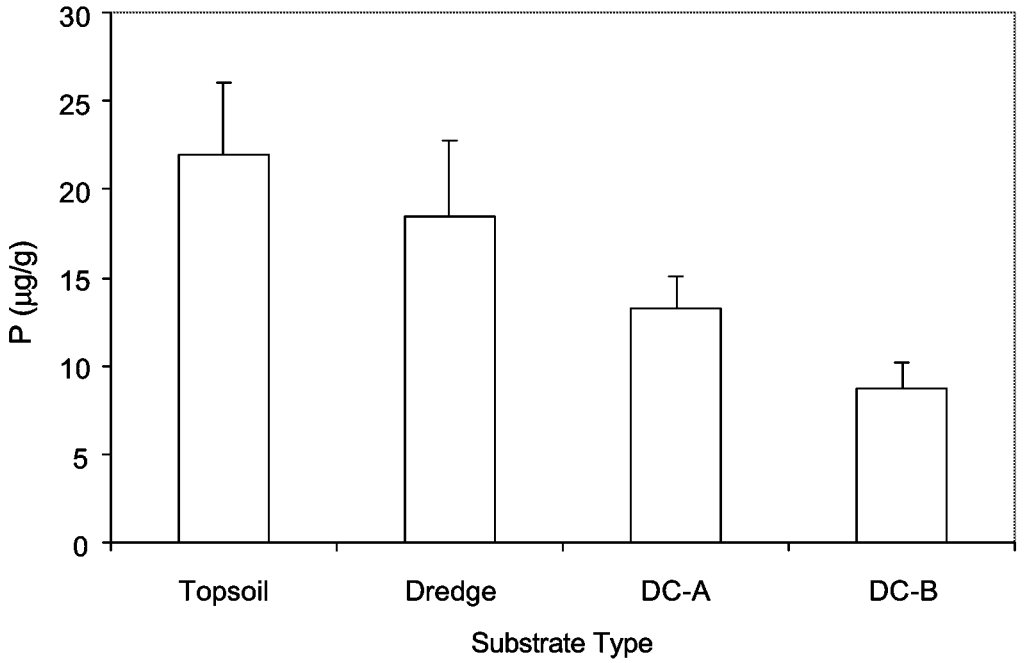

Fig. 10. The effect of substrate type on P concentration (mean +/- standard error) in plant tissue averaged across hydrologic regime and plant species.

Soil Redox Potential

Soil redox potential at 1 cm depth displayed a highly significant decrease with increasing salinity levels (Fig. 11; F= 108.741, P< 0.001). Soil redox potential at the 15 cm depth also became more reduced as salinity level increased, although this trend was not as pronounced (Fig. 11; F= 177.368, P< 0.001). A marginally significant salinity level by hydrologic regime interaction was detected, probably because at the 18-ppt salinity level the mesic hydrology did not display the usual increase in redox potential relative to the flooded hydrology. (Fig. 12; F= 2.276, P= 0.038). Although the mean soil redox for each treatment was greater than -100 mV, values in the range of sulfide formation and methanogenisis (less than -100 mV) were detected for each substrate type for all soil depths and salinity levels measured. This is important to note because most metals are maximally soluble and have the greatest potential for bioaccumulation at redox potentials less than -100 mV (see discussion).

pH

There was a significant effect of salinity level and substrate type on pH (Fig. 13; F= P< 0.001). As the prior studies indicated, the pH of DC-B was highly elevated compared to the other substrates under freshwater conditions. However, the pH of DC-B was significantly lower under all saline treatments than under freshwater conditions (Fig. 13; Contrast F= 59.882, P< 0.001). Under non-saline conditions, the flooded hydrologic regime displayed the highest pH, but this effect disappeared as salinity increased (Fig. 14; F= 3.936, P= 0.001).

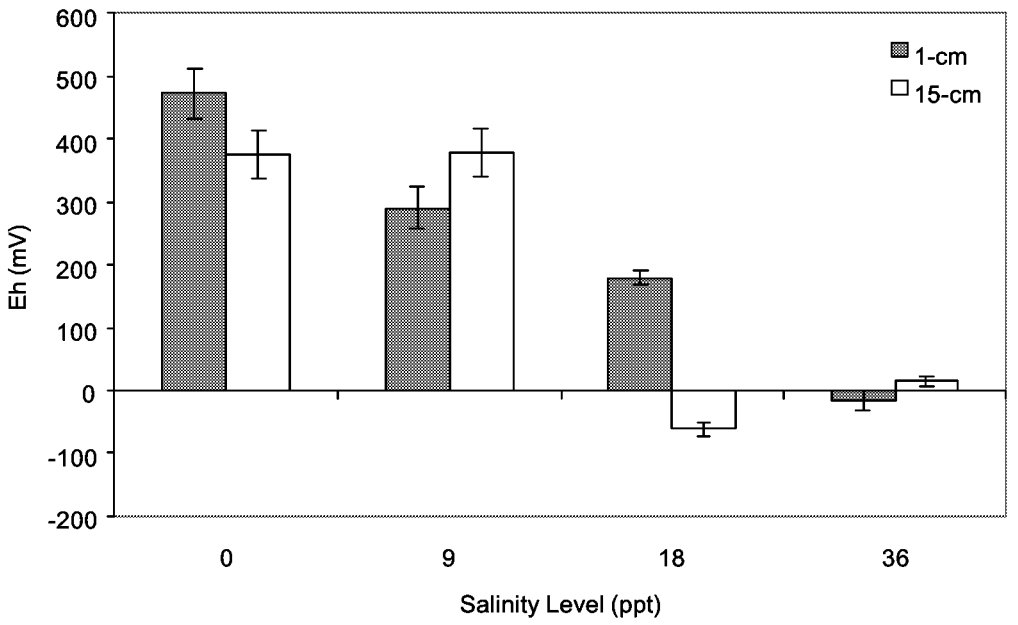

Fig. 11. The effect of salinity level on soil redox potential (mean +/- standard error) at two soil depths (1-m and 15-cm) averaged across substrate type, hydrologic regime, and plant species.

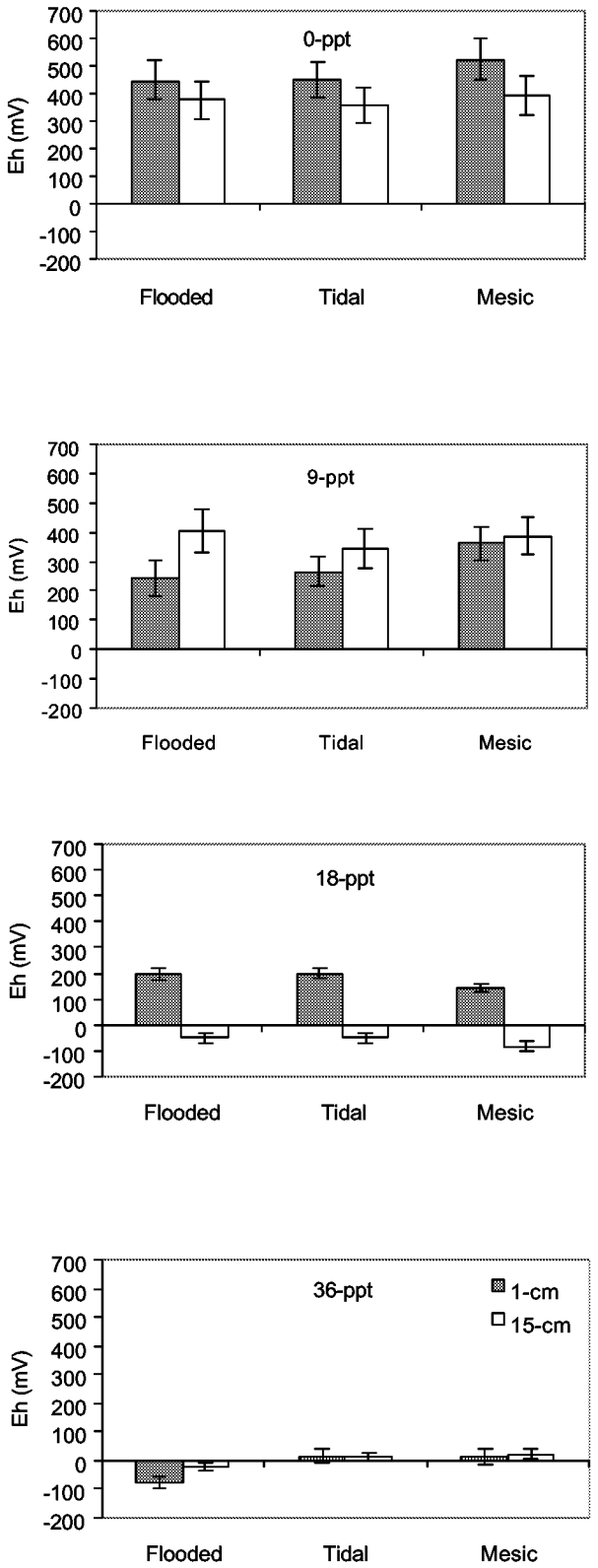

Fig. 12. The effect of hydrologic regime and salinity level on soil redox (mean +/- standard error) averaged across substrate type and plant species.

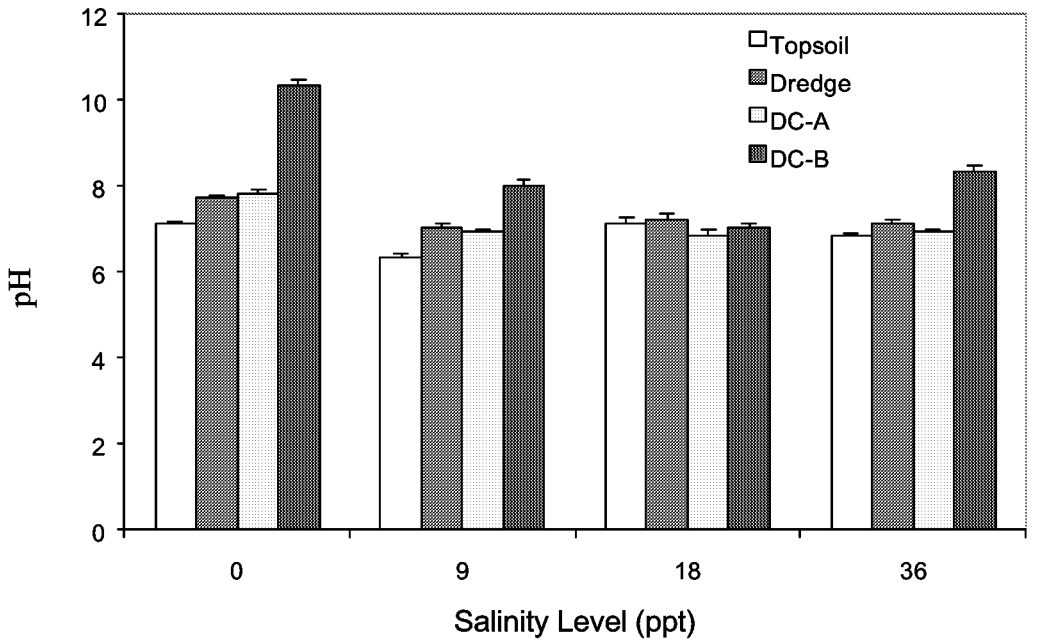

Fig. 13. The effect of salinity level and substrate type on interstitial pH (mean +/- standard error) averaged across hydrologic regime and plant species.

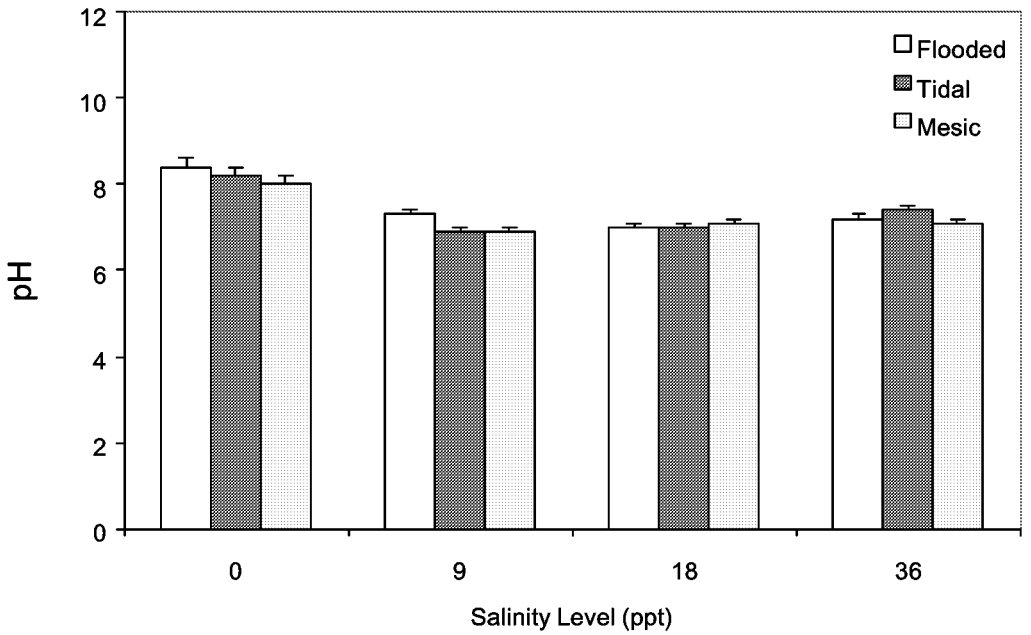

Fig. 14. The effect of salinity level and hydrologic regime on interstitial pH (mean +/- standard error) averaged across hydrologic regime and plant species.

Photosynthetic Response

There was a significant effect of salinity level on net CO_2 assimilation rate (Fig. 15; F= 14.434, P< 0.001). Analysis within salinity levels showed that at the 0-ppt salinity level, plants had significantly reduced net CO_2 assimilation rates when grown in DC-B compared to the other substrates (Fig. 16; Contrast F= 9.206, P= 0.003). This was probably due to the elevated pH that DC-B exhibits under freshwater conditions. No significant differences were detected among substrate types at any other salinity level. It is important to note that no significant differences were detected in net CO_2 assimilation rates due to substrate type, hydrologic regime, or their interaction under saline conditions. This indicates that plants were performing comparably over time regardless of substrate type and hydrologic regime as long as the pH was less alkaline than under non-saline conditions. There was no significant effect of hydrologic regime on plant photosynthesis. Overall, net CO_2 assimilation rates were greatest in the 9-ppt salinity level.

Total Biomass

Substrate type, hydrologic regime, and plant species all had marginally significant effects on total plant biomass (Fig. 17; F= 1.768, P= 0.05). This was likely driven by the apparent high performance of *S. cynosuroides* under all hydrologies in DC-B, and the high performance of *S. patens* in DC-B under mesic conditions. This limits the interpretation of the main effect of substrate type, but it appears that DC-B total biomass was significantly reduced compared to all other substrates (Fig. 18; Contrast F= 8.397, P= 0.006). No significant differences were detected among hydrologies in total biomass production. A significant effect of species was discerned (Fig. 19; F= 7.860, P= 0.001). This was likely driven by the reduced biomass of *A. germinans* compared to the other plant species and probably simply reflects the difference between herbaceous versus woody growth forms.

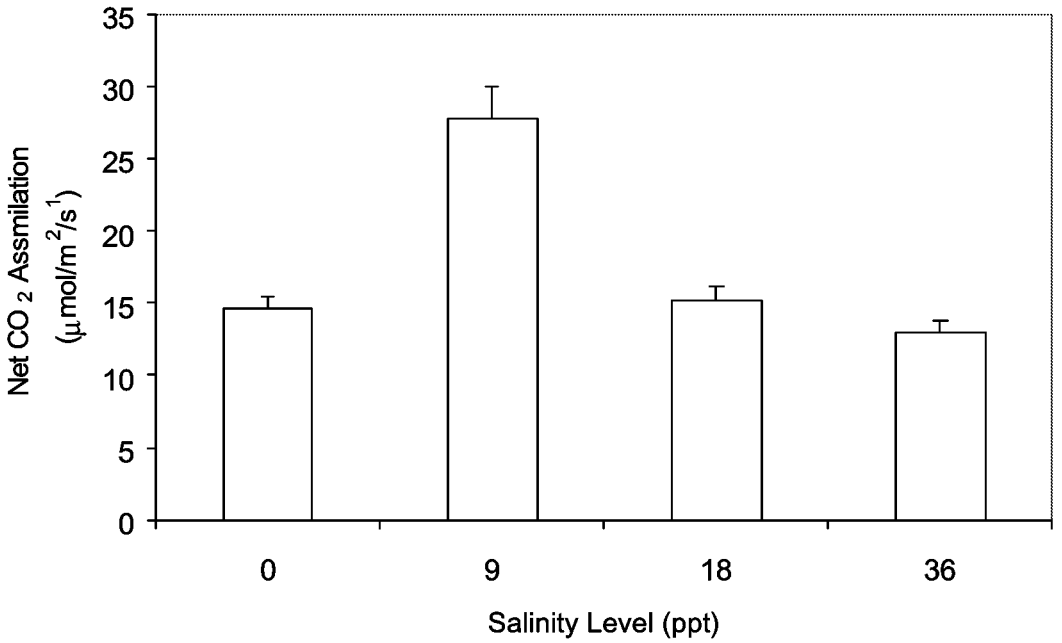

Fig. 15. The effect of salinity level on net CO_2 assimilation rate (mean +/- standard error) averaged across substrate type, hydrologic regime, and plant species.

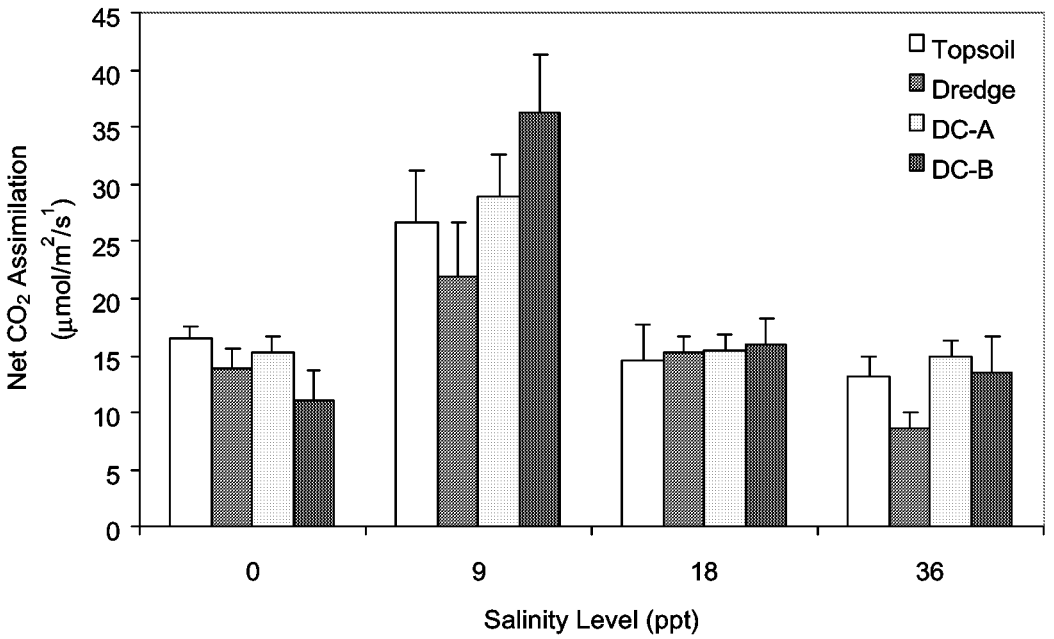

Fig. 16. The effect of salinity level and substrate type on net CO_2 assimilation rate (mean +/- standard error) averaged across hydrologic regime, and plant species.

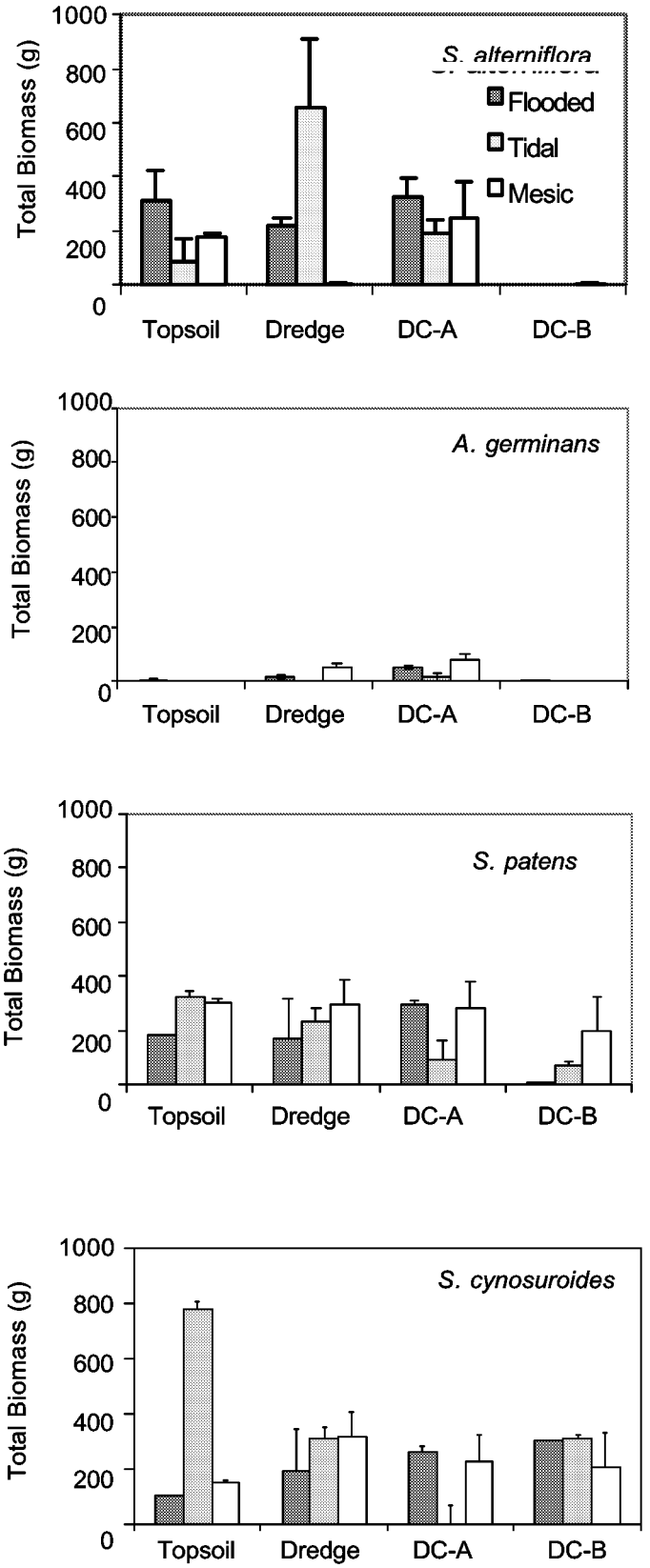

Fig. 17. The effect of substrate type, hydrologic regime, and plant species on total biomass (mean +/- standard error).

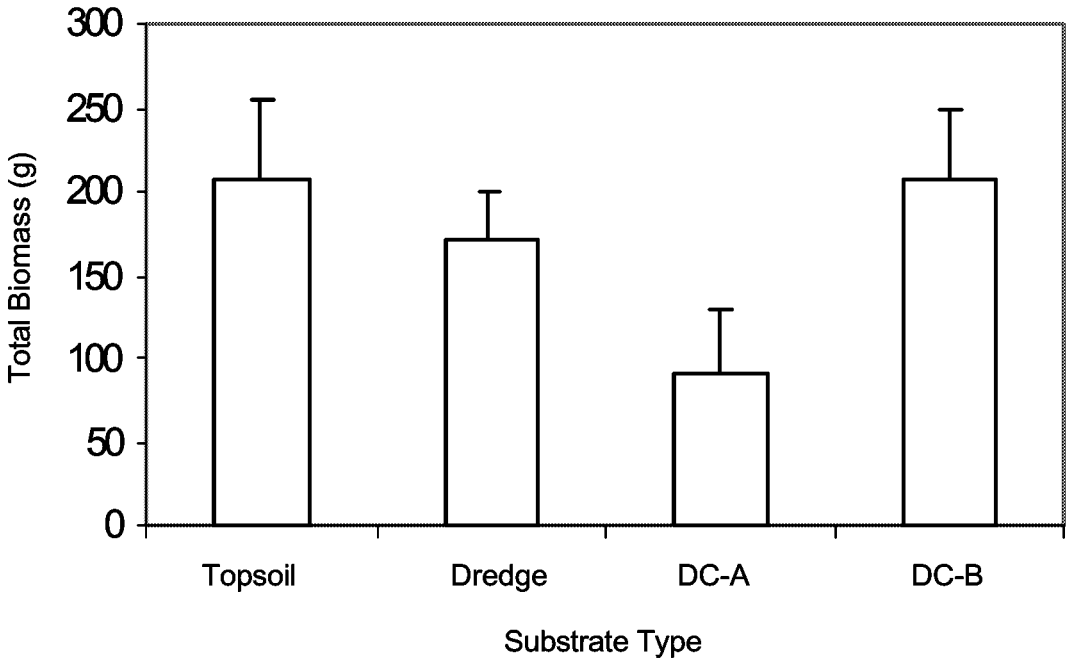

Fig. 18. The effect of substrate type on total biomass (mean +/- standard error) averaged across hydrologic regime and plant species.

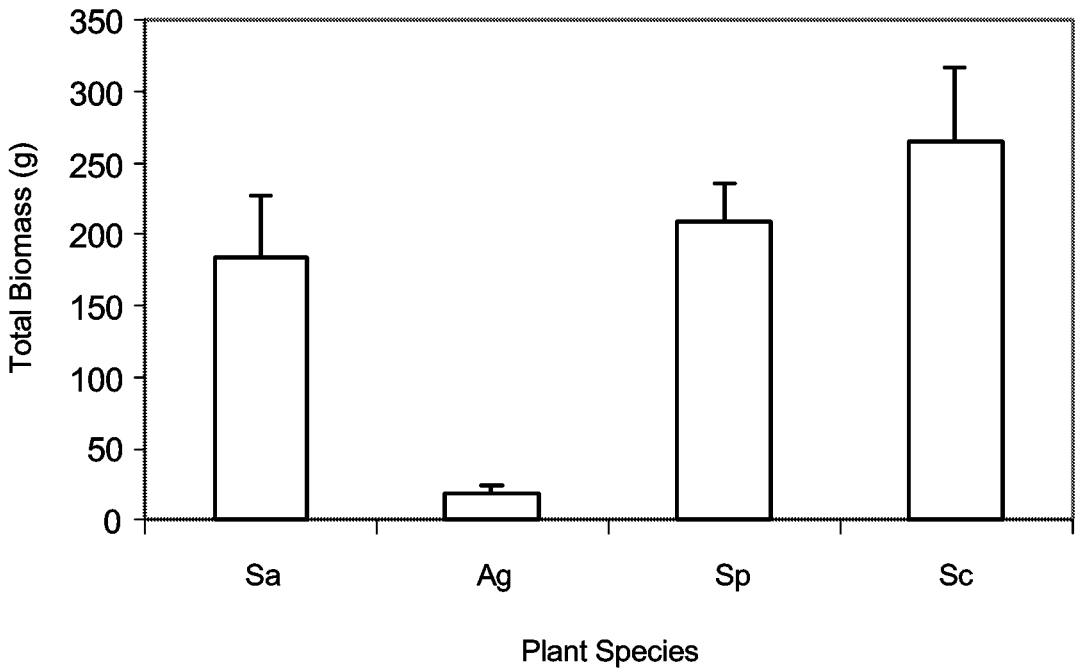

Fig. 19. The effect of plant species on total biomass (mean +/- standard error) averaged across substrate type and hydrologic regime. Species abbreviations are as follows: Sa= *S. alterniflora*, Ag= *A. germinans*, Sp= *S. patens*, Sc= *S. cynosuroides*.

Aboveground Biomass

A significant three-way interaction was noted between substrate, hydrology, and species on aboveground biomass (Fig. 20; F= 2.901, P= 0.002). This may have been due to the reduced biomass of *S. patens* in DC-B under flooded conditions, the reduced biomass of *S. alterniflora* in all DC-B treatments, and also the poor performance of *S. alterniflora* when grown in the dredge spoil substrate under mesic conditions. Plants in DC-B had significantly reduced aboveground biomass compared to plants in other substrates (Fig. 21; Contrast F= 54.945, P< 0.001). A significant species effect was also detected (F= 41.549, P< 0.001). As stated above, this is likely due to the performance of *A. germinans* (woody species) compared to the herbaceous species and the fact that *S. cynosuroides* had significantly lower aboveground biomass than other *Spartina* species (Fig. 22; Contrast F= 6.816, P< 0.012).

Belowground Biomass

A significant difference in belowground biomass was detected among species (F= 4.995, P= 0.005). Again, this is believed to be due to the reduced biomass of *A. germinans* compared to the other species (Fig. 23; Contrast F= 8.887, P=0.005). No other main effects or interactions were found to be significant.

Stem Heights and Diameters

Substrate type was found to significantly affect both *A. germinans* stem diameter and height (Fig. 24; F=9.573, P< 0.002: Fig. 25; F=5.172, P< 0.012 respectively). Bonferroni adjusted pair-wise comparisons indicated that DC-A and dredge spoil resulted in greater stem height and diameter than DC-B and topsoil. No significant effect of hydrologic regime was detected.

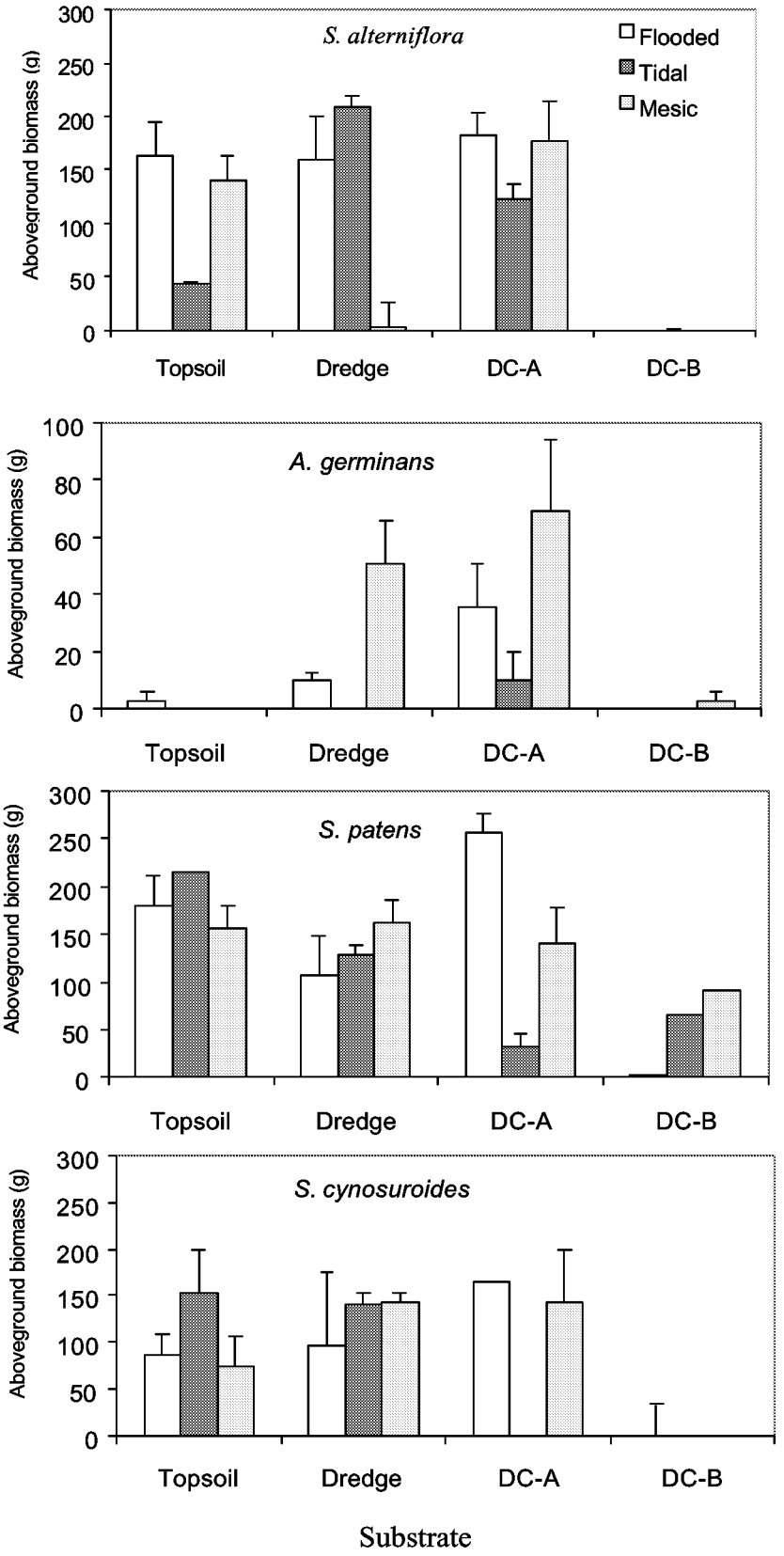

Fig. 20. The effect of substrate type, hydrologic regime, and plant species on aboveground biomass (mean +/- standard error).

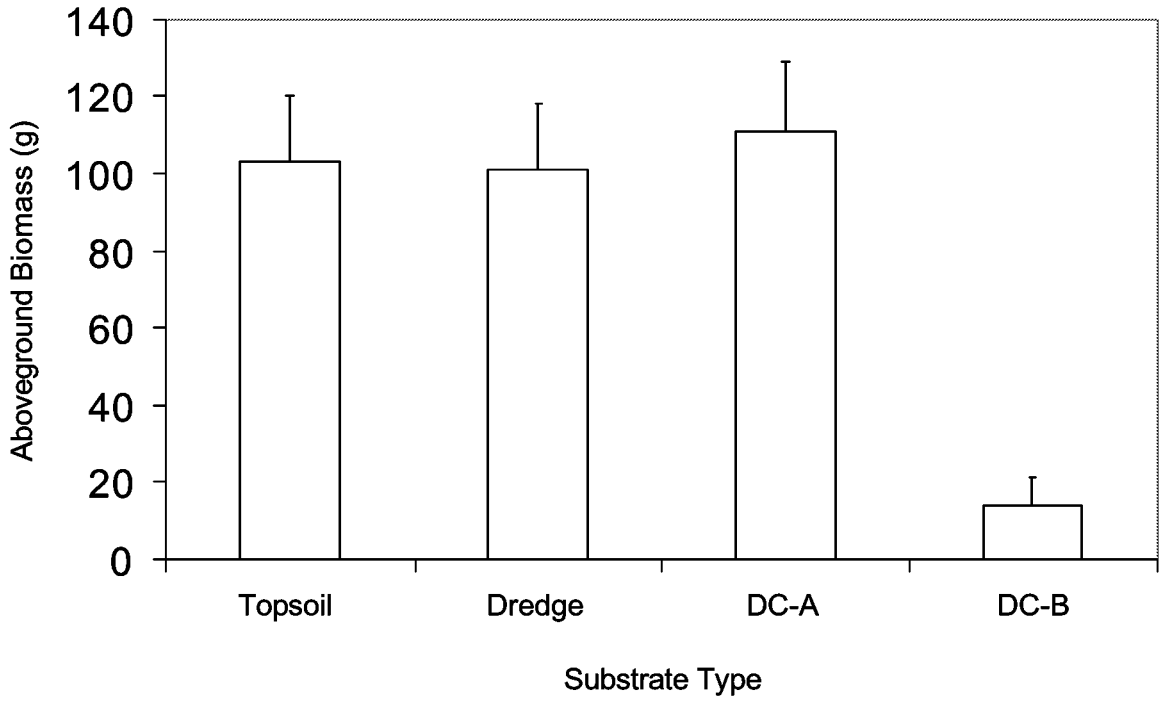

Fig. 21. The effect of substrate type on aboveground biomass (mean +/- standard error) averaged across hydrologic regime and plant species.

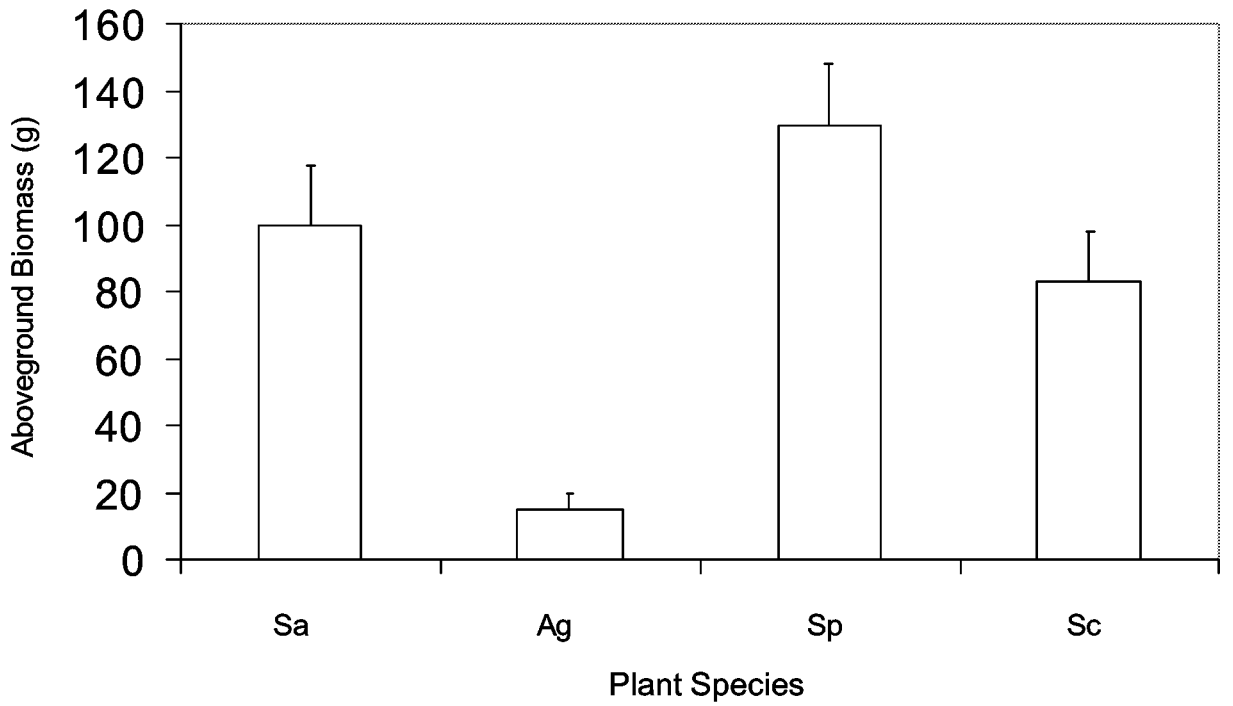

Fig. 22. The effect of plant species on aboveground biomass (mean +/- standard error) averaged across substrate type and hydrologic regime. Species abbreviations are as follows: Sa= *S. alterniflora*, Ag= *A. germinans*, Sp= *S. patens*, Sc= *S. cynosuroides.*

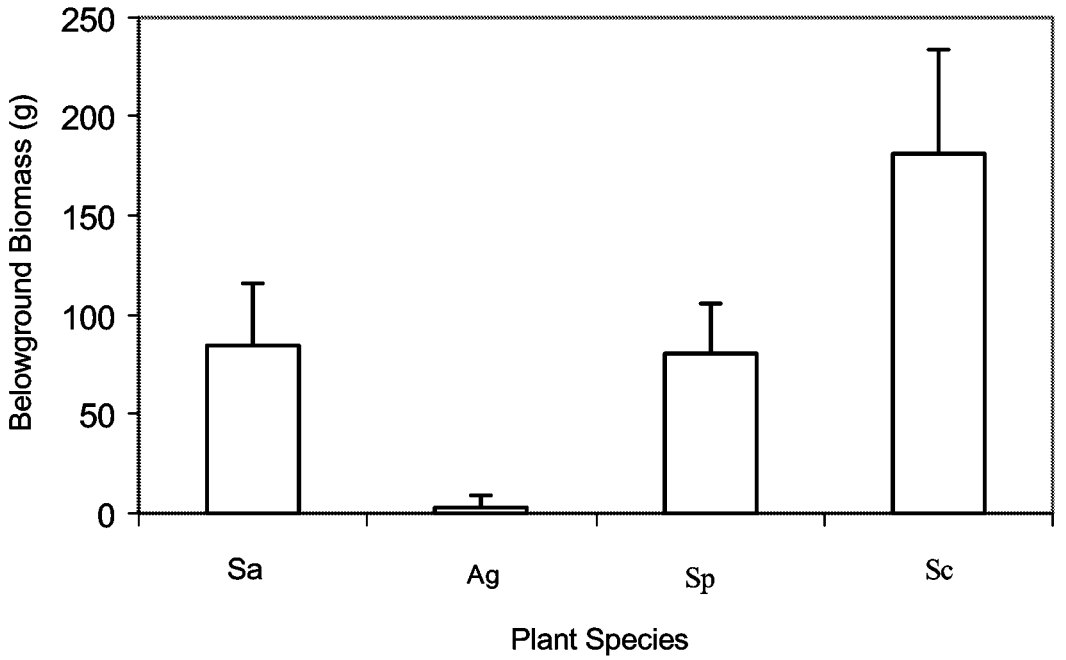

Fig. 23. The effect of plant species on belowground biomass (mean +/- standard error) averaged across substrate type and hydrologic regime. Species abbreviations are as follows: Sa= *S. alterniflora*, Ag= *A. germinans*, Sp= *S. patens*, Sc= *S. cynosuroides.*

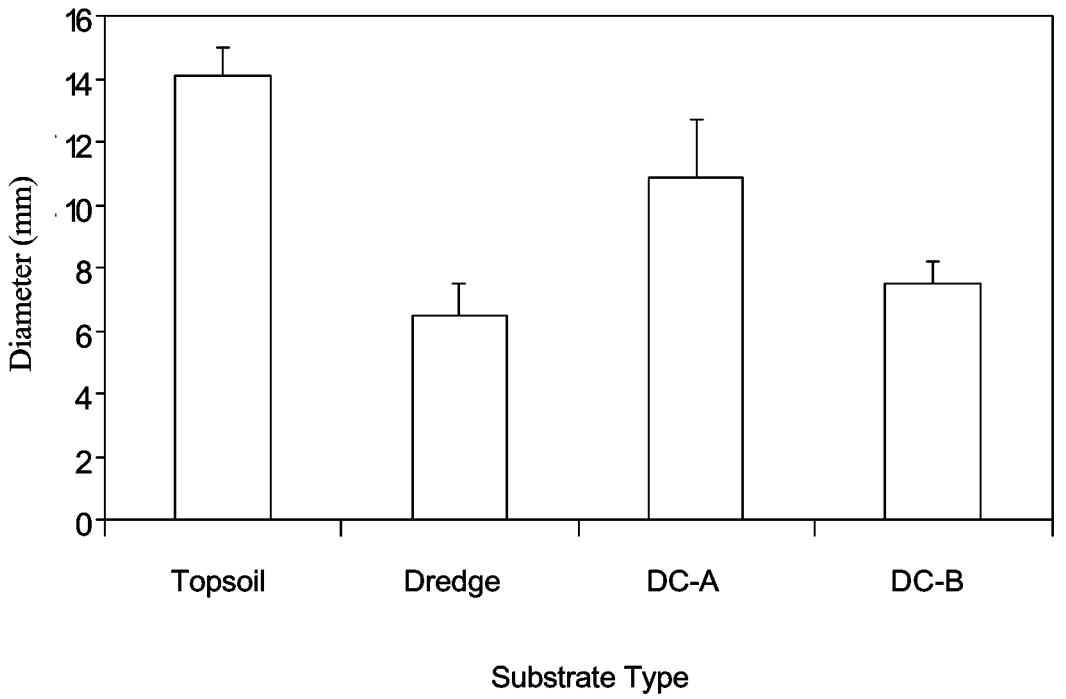

Fig. 24. The effect of substrate type on *Avicennia germinans* final diameter (mean +/- standard error) averaged across hydrologic regime.

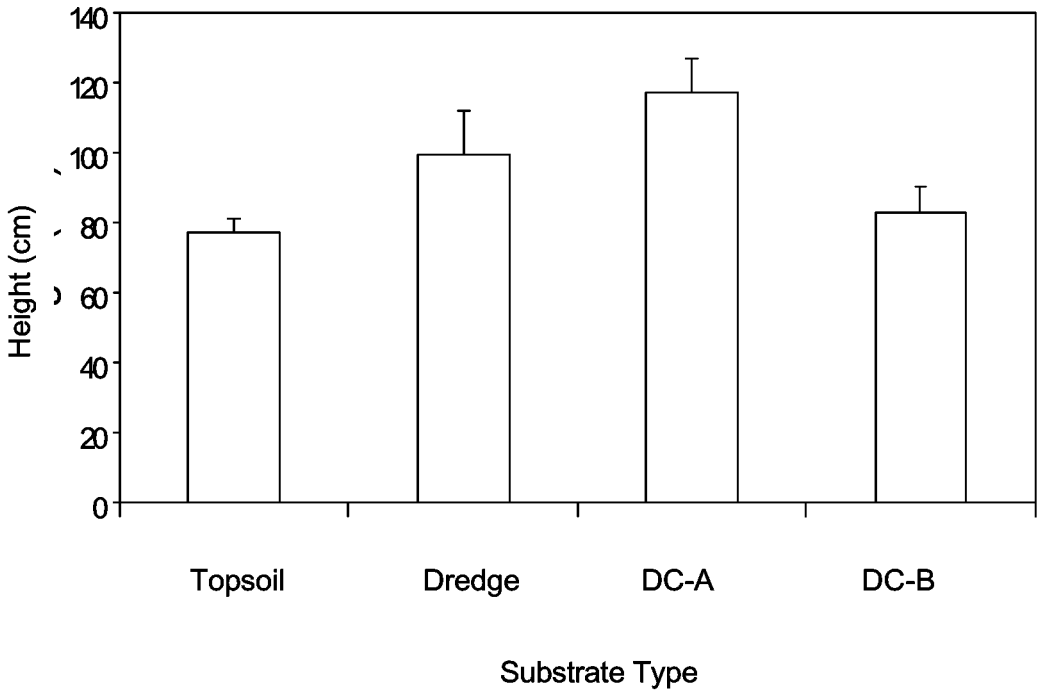

Fig. 25. The effect of substrate on *Avicennia germinans* final height (mean +/- standard error) averaged across hydrologic regime.

Genotypic Response

Spartina patens genotypes in the 9-ppt treatments had higher net CO_2 assimilation rates than in the 0-ppt or 18-ppt treatments (Fig. 26; F= 9.040, P< 0.01). Net CO_2 assimilation rates of *S. patens* under the 0-ppt salinity level were reduced in the DC-B substrate, however under all saline treatments *S. patens* net CO_2 assimilation rates were comparable across substrates (Fig. 27; F= 2.937, P< 0.028). No other significant differences in photosynthetic response were discerned among *S. patens* genotypes.

A significant interactive effect of substrate, genotype, and hydrology on *S. patens* aboveground biomass was detected (Fig. 28; F= 2.181, P= 0.029). This is best explained by the low biomass of genotype 26, compared with genotypes 8 and 9 under flooded conditions in DC-B. Although the presence of this three-way interaction somewhat limits the interpretation of the main effect, it should be noted that biomass production by genotype 9 appears to be significantly reduced from genotypes 8 and 26 (Fig. 29; Contrast F= 7.309, P= 0.01).

Genotype 26 had significantly greater production of belowground biomass than genotypes 8 and 9 (Fig. 30; Contrast F= 10.508, P= 0.002). Overall *S. patens* produced less belowground biomass under flooded conditions than under mesic or tidal conditions (Fig. 31; Contrast F=11.066, P= 0.001). As was observed with photosynthesis *S. patens* belowground biomass was lowest in the DC-B substrate (Fig. 32; Contrast F= 6.942, P= 0.011). No significant interactions on belowground biomass were discerned.

Genotype 26 had significantly greater total biomass production than genotype 8, which in turn had significantly higher biomass production than genotype 9 (Fig. 33; Contrast F= 5.568, P= 0.006). Mesic conditions resulted in significantly higher total biomass than observed under flooded conditions (Fig. 34; 11.123, P= 0.001). As in the belowground biomass analysis, *S.*

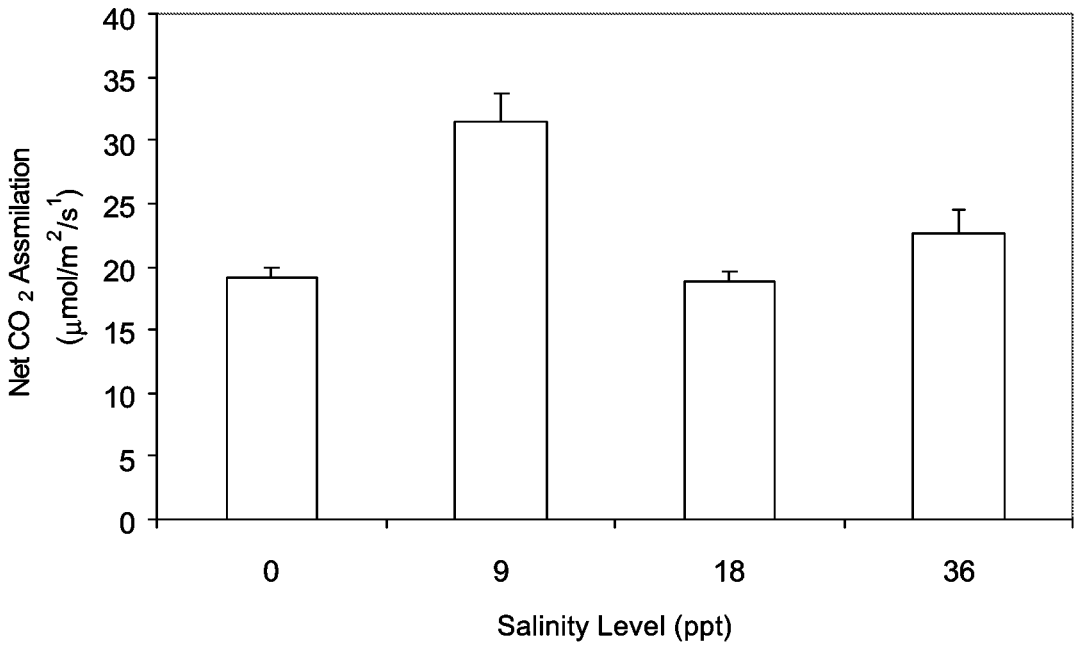

Fig. 26. The effect of salinity on *Spartina patens* net CO_2 assimilation rates (mean +/- standard error) averaged across substrate type, hydrologic regime, *Spartina patens* genotype.

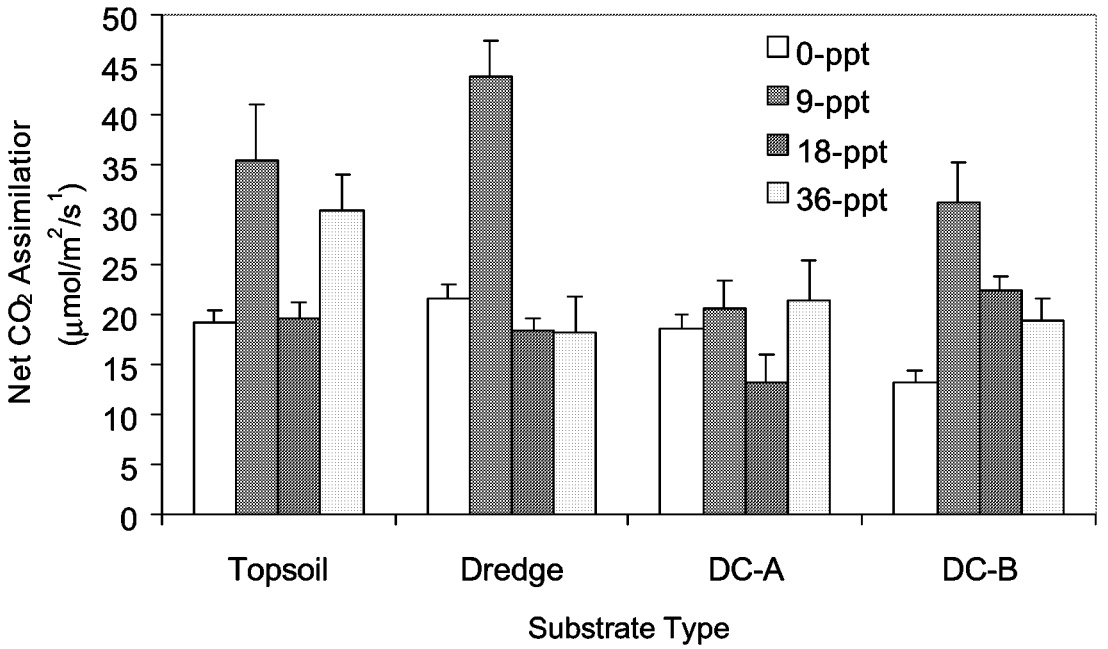

Fig. 27. The effect of salinity level and substrate type on *Spartina patens* net $C0_2$ assimilation (mean +/- standard error) averaged across hydrologic regime and *Spartina patens* genotype.

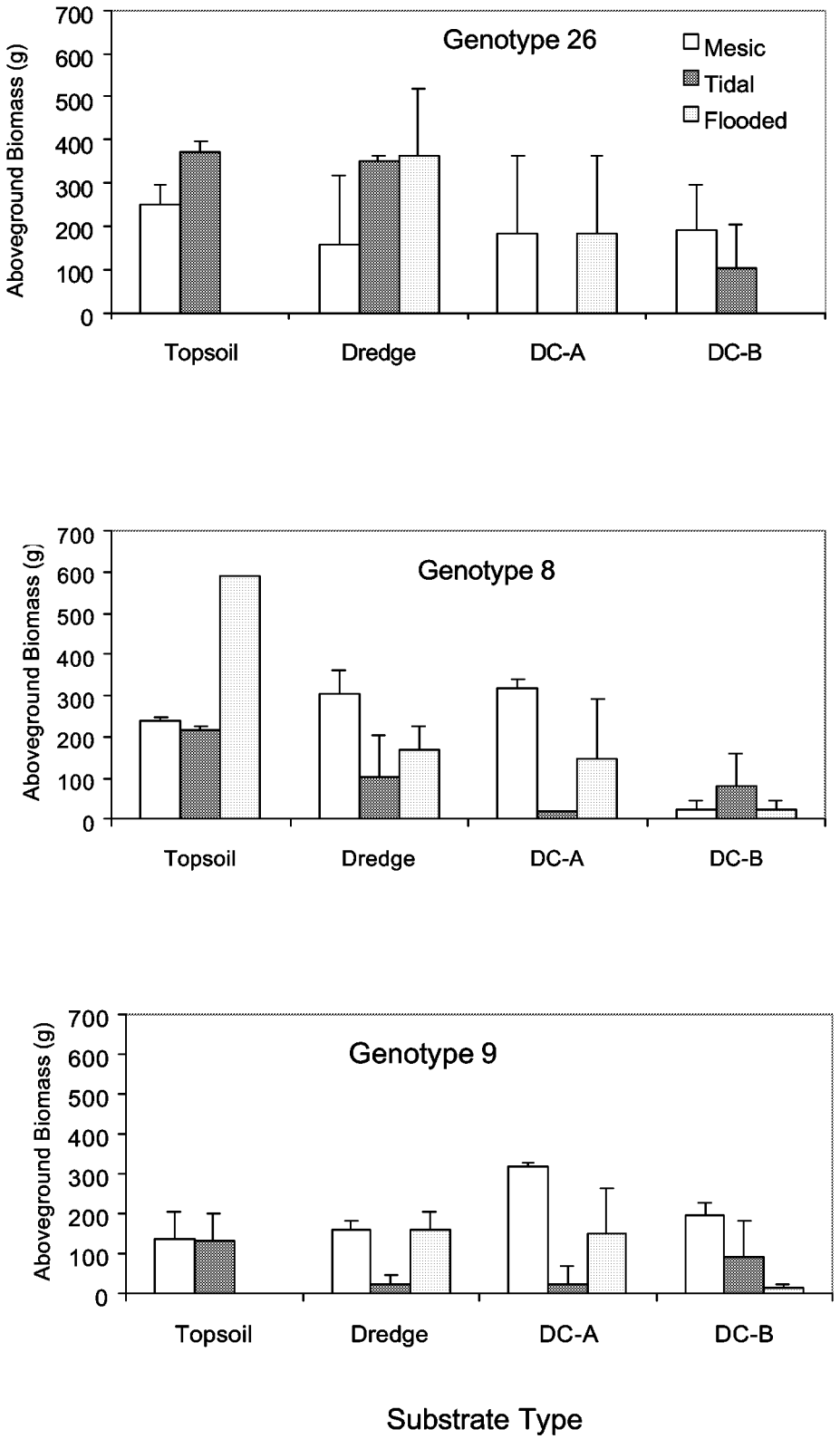

Fig. 28. The effect of substrate type, hydrologic regime, and *Spartina patens* genotype on *Spartina patens* aboveground biomass (mean +/- standard error).

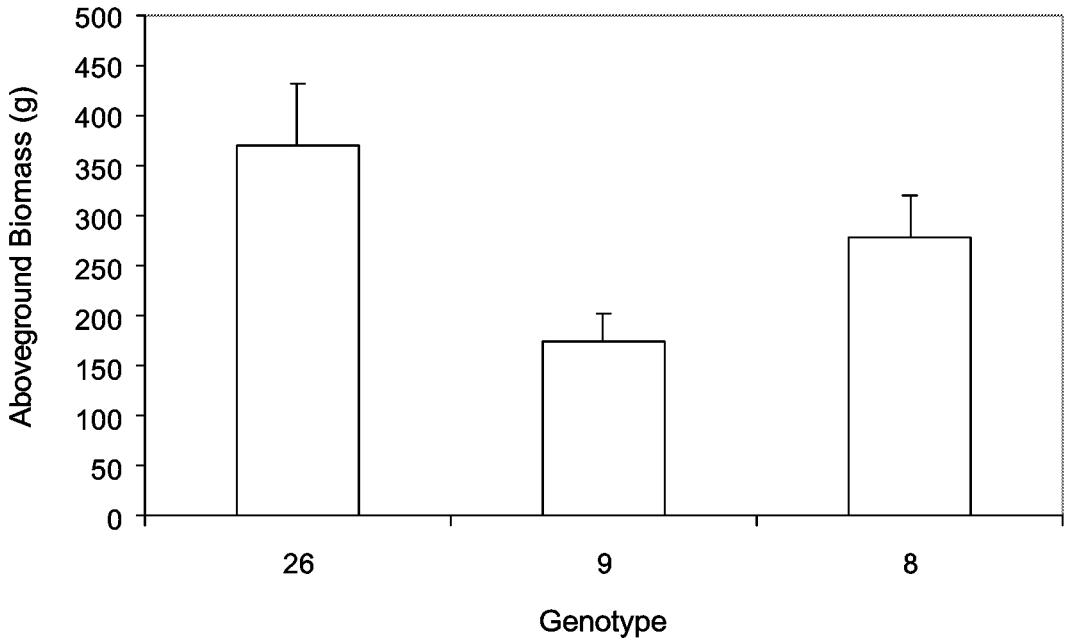

Fig. 29. The effect of *Spartina patens* genotype on *Spartina patens* aboveground biomass (mean +/- standard error) averaged across substrate type and hydrologic regime.

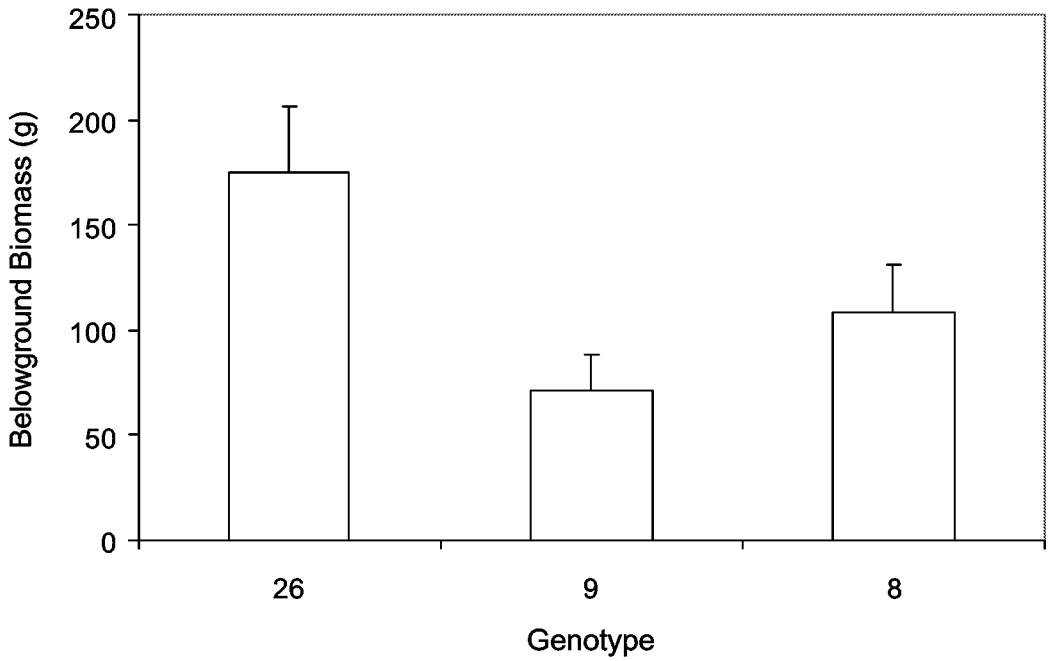

Fig. 30. The effect of *Spartina patens* genotype on *Spartina patens* belowground biomass (mean +/- standard error) averaged across substrate type and hydrologic regime.

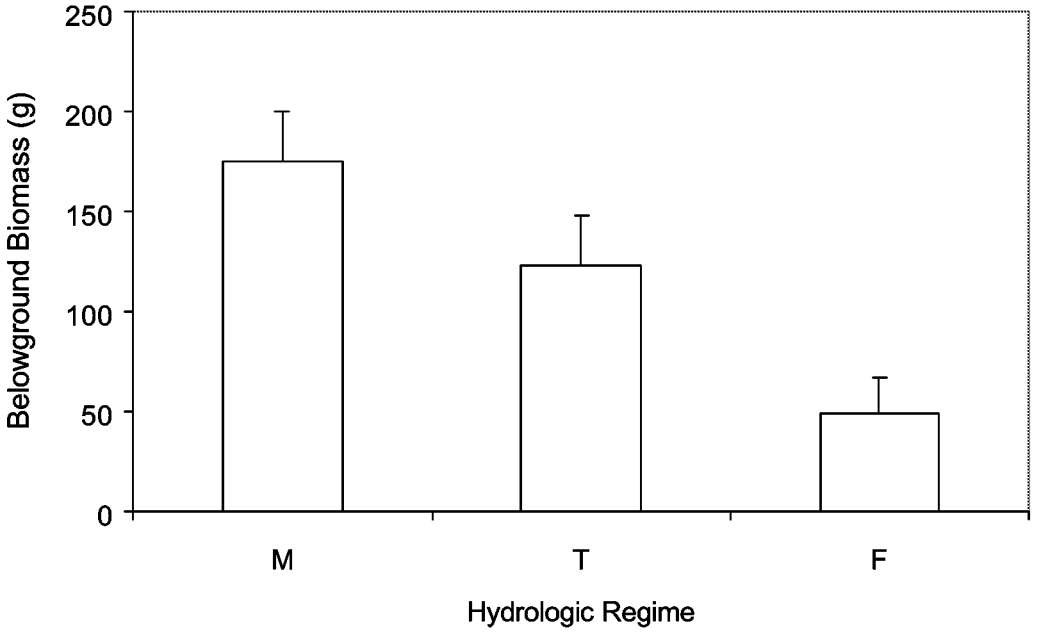

Fig. 31. The effect of hydrologic regime on *Spartina patens* belowground biomass (mean +/- standard error) averaged across substrate type and *Spartina patens* genotype.

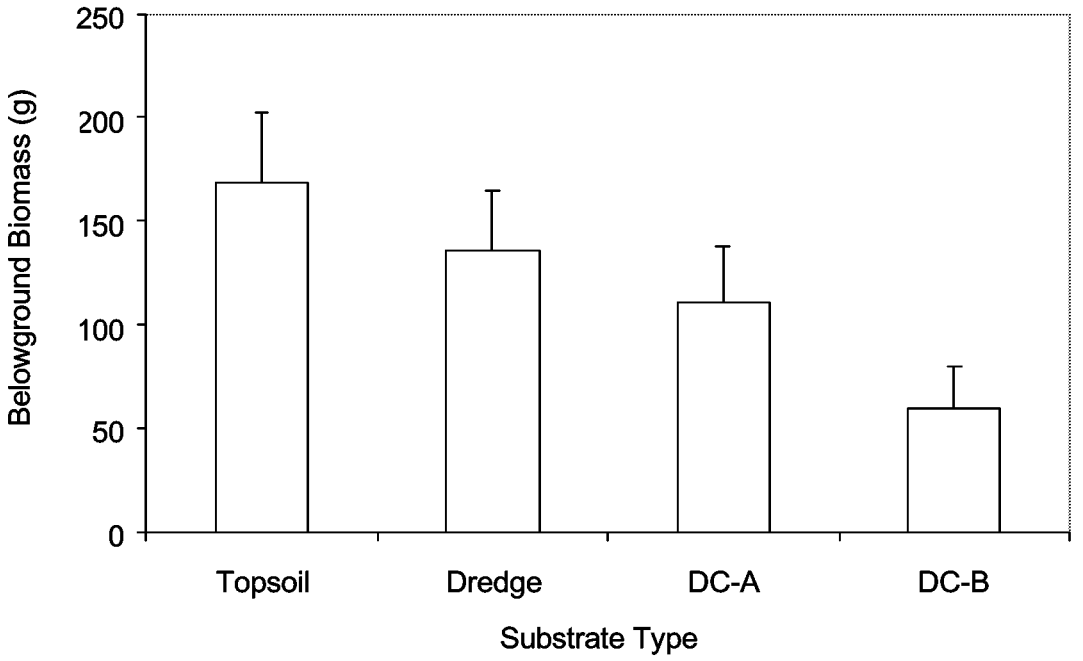

Fig. 32. The effect of substrate type on *Spartina patens* belowground biomass (mean +/- standard error) averaged across hydrologic regime and *Spartina patens* genotype.

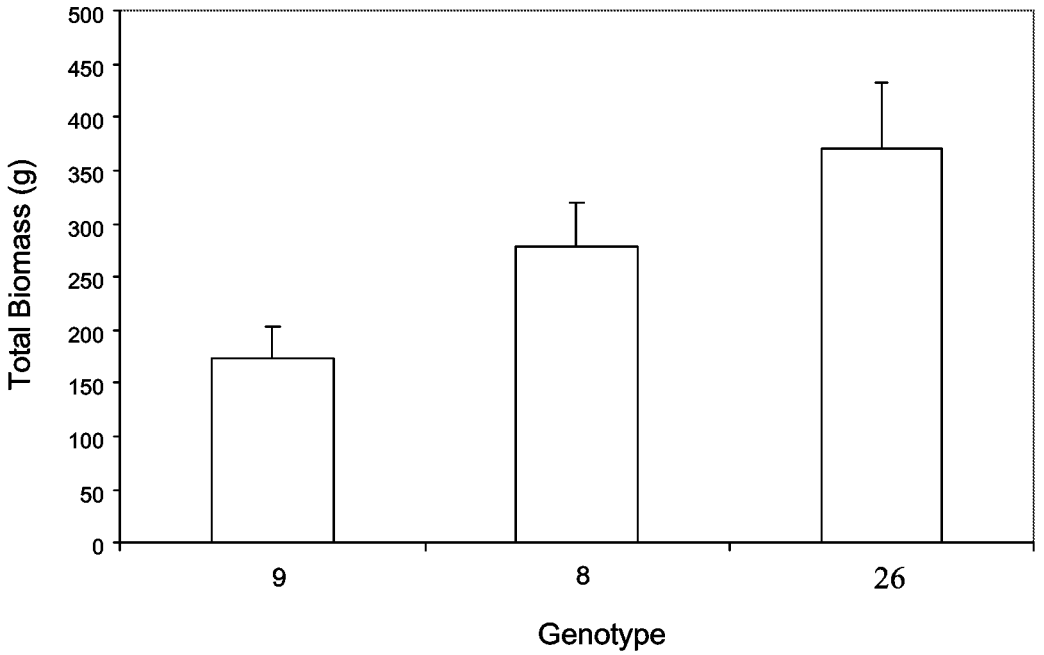

Fig. 33. The effect of *Spartina patens* genotype on total *Spartina patens* biomass (mean +/- standard error) averaged across substrate type and hydrologic regime.

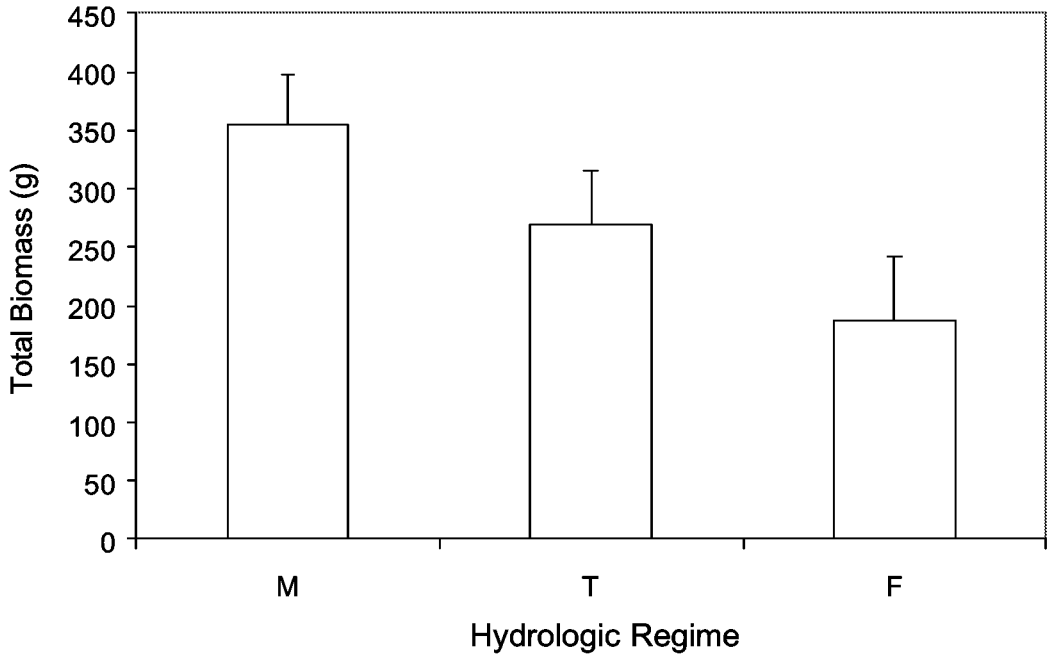

Fig. 34. The effect of hydrologic regime on *Spartina patens* total biomass (mean +/- standard error) averaged across substrate type and *Spartina patens* genotype.

patens total biomass was lowest in the DC-B substrate compared to all other substrates (Fig. 35; Contrast F= 10.185, P= 0.002). No significant interactions on total biomass were detected.

Similar to the responses observed in *S. patens*, the 9-ppt salinity level resulted in significantly higher net CO_2 assimilation rates in *S. alterniflora* than in the other salinity levels (Fig. 36; F= 4.828, P= 0.025). All genotypes of *S. alterniflora* showed depressed net CO_2 assimilation under fresh water conditions in the DC-B substrate, but exhibited net CO_2 assimilation rates comparable with, or superior to, other substrates under the 9-ppt and 36-ppt salinity level (Fig. 37; F= 2.826, P= 0.025).

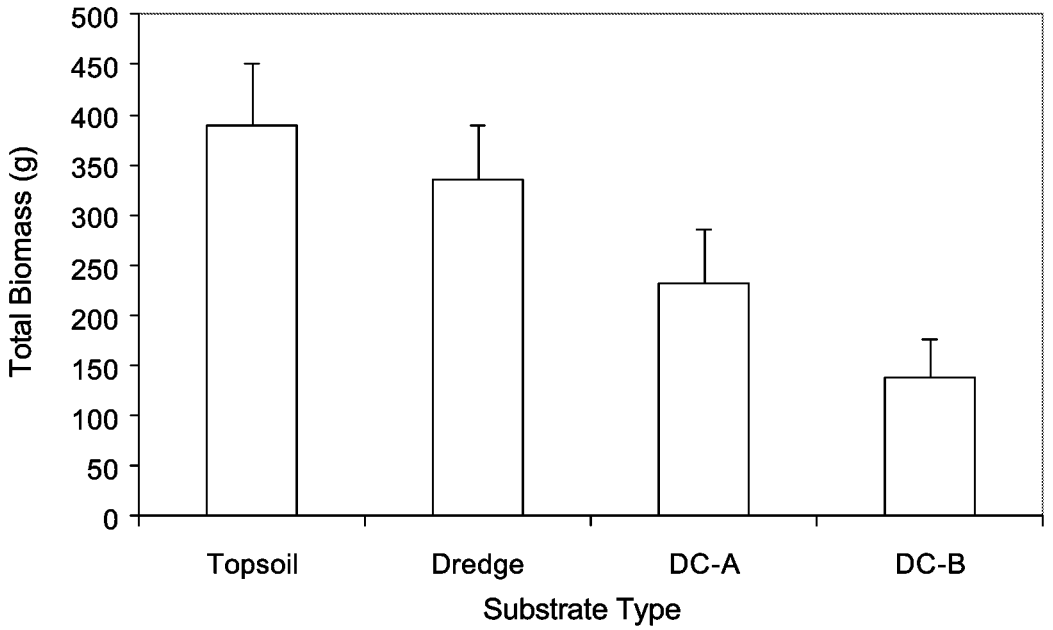

Fig. 35. The effect of substrate type on total *Spartina patens* total biomass (mean +/- standard error) averaged across hydrologic regime and *Spartina patens* genotype.

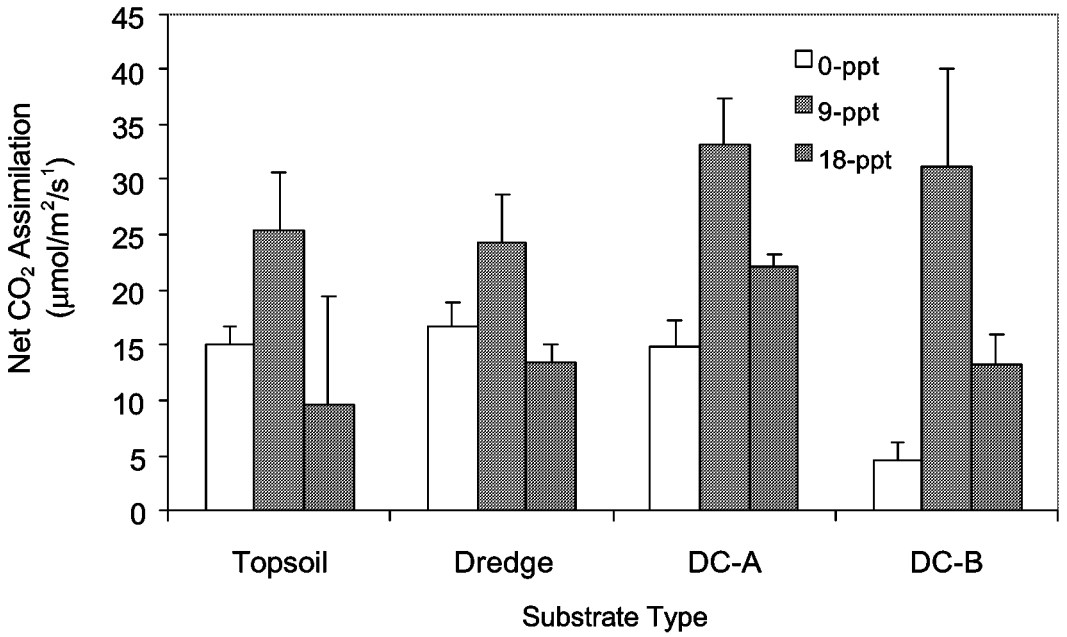

Fig. 36. The effect of salinity level and substrate type on *Spartina alterniflora* net CO_2 assimilation rates (mean +/- standard error) averaged across hydrologic regime and *Spartina alterniflora* genotype.

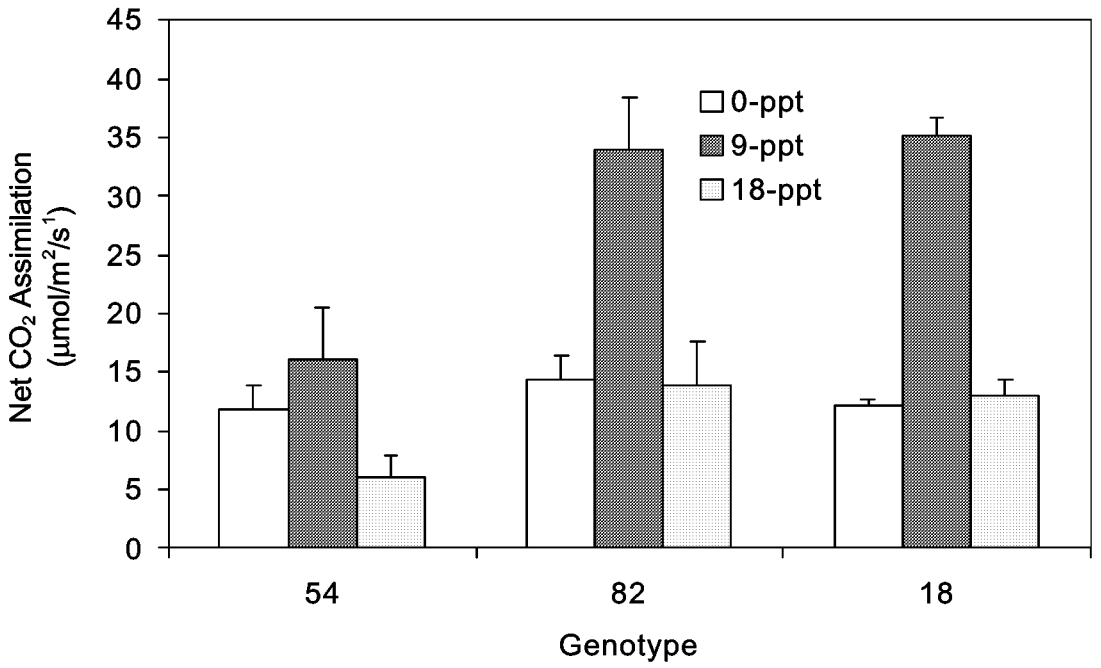

Fig. 37. The effect of salinity type and *Spartina alterniflora* genotype on *Spartina alterniflora* net CO_2 assimilation rates (mean +/- standard error) averaged across substrate type and hydrologic regime.

Discussion

The previous mesocosm (freshwater) studies that were conducted under non-saline conditions indicated that the DC-A substrate had greater potential as an alternative wetland substrate than DC-B, which supported much more limited plant growth. The low growth of plants in the DC-B substrate compared to the other substrates was attributed to the high pH and very coarse texture of the substrate. This study has further elucidated the potential of both DC-A and DC-B as alternative wetland substrates in terms of edaphic variables and vegetation response under scenarios of increasing salinity.

Edaphic Variables

As one would expect, soil redox potentials became significantly more reduced as salinity levels were increased (DeLaune et al. 1976). Because salinity levels were increased over time, this reduction in soil redox potential likely reflects both an increase in the duration of flooding and the presence of sulfate in the sea-salt mix. Sulfate is reduced to sulfide by microbiological activity at a redox potential of approximately -100 mV (Mitsch and Gosselink 1993). Although soil redox potentials as low as -500 mV were reported, the mean values were above –100 mV at each salinity level. This indicates that oxidized Fe and Mn were probably present, at least part of the time, in the interstitial water. Both of these metals can serve as electron acceptors for metabolic activity that are energetically more efficient than sulfate, and the usage of these alternative acceptors is reflected in higher redox potentials (100-150 mV) (Mitsch and Gosselink 1993).

Substrate interstitial pH in the DC-B substrate was dramatically lowered from extreme alkaline values (~ 10.1) to moderate pH values (~8.5) by the addition of seasalt. Seawater has a strong buffering capacity because of the carbon dioxide-calcium carbonate equilibrium system, which allows it to buffer both basic and acidic systems (Dietrich et al. 1980). Therefore, it would seem likely that some interaction of this carbon dioxide-calcium carbonate system with aluminum hydroxide (thought to be a major constituent of DC-B) was involved in the lowering of alkalinity in DC-B as salinity levels increased above 0 ppt. This is of great significance because the high alkalinity was considered to be a major obstacle to the successful establishment

of vegetation in the DC-B substrate. This pH-salinity phenomenon was not noted in the Kelly and Mendelssohn (1995) study. This is likely due to the difference in size of the experiments, specifically the size of the water reservoirs. Because of the large volume of the mesocosm water reserves in this study, a larger buffering capacity was likely available to ameliorate the high pH of the DC-B substrate. It is documented that the interstitial pH of submerged soils will tend to approach neutrality over time (Ponnamperuma 1972). However, it is unlikely that this is the main factor behind the amelioration of the DC-B substrate pH because no reduction in pH was detected after the submergence of the DC-B substrate in the previous studies. Therefore, the reduction of the DC-B substrate pH is likely a direct result of the increase in salinity level, especially since this pH reduction corresponds exactly with increase in the salinity level.

Previous drill cuttings research indicated that the use of processed drill cuttings to create wetlands may require anthropogenic amelioration of the high pH in order to be successful, which would reduce cost-effectiveness of the method (Kelley and Mendelssohn 1995; Shaffer et al. 1996; DesRoches 1997). However, an implication of this study is that if these processed drill cuttings are used for coastal wetland restoration where natural saline waters are present, then there may be no need to amend the alkalinity of the substrate with organic materials as was previously thought necessary (Kelley and Mendelsson 1995).

Substrate interstitial pH and soil redox potential are considered primary variables that control the release and solubility (and hence bioavailability) of heavy metals in substrates (Fergusson 1990). Bourg and Loch (1995) also include organic matter interactions with interstitial pH and soil redox potential as master variables that control metal release from sediments. For the majority of heavy metals in aquatic systems, maximum potential release occurs at moderately low and extremely high pHs, and under slightly reduced or oxidized conditions (Fergusson 1990; Bourg and Loch 1995). The low solubility of metals under low soil redox (i.e., very reduced conditions) is linked to the amount of sulfate present (Bourg and Loch 1995). If sulfate is not available to precipitate metals under low Eh, then contaminants will increase in solubility (Bourg and Loch 1995). Soil redox potential also plays a major role in the

availability of metals by controlling interactions with Mn, Fe, and redox-sensitive, precipitate-inducing anions (Bourg and Loch 1995). Gambrell et al. (1991) examined the effect of salinity, pH, and Eh on the solubility of certain metals (Cd, Cr, Cu, Zn, Ni, and Pb) in a contaminated estuarine substrate and found that reduced, basic conditions promoted inavailibility, whereas oxidized, acidic conditions increased solubility. The conditions reported by Gambrell et al. (1991) for minimal solubility (pH= 8.0 and soil redox potential of -150 to –100 mV) are similar to the environmental conditions in the mesocosm at the 18-ppt and 36-ppt salinity levels.

As previously discussed, this mesocosm design is a closed system. Any metals that might be released from the substrates can not escape from the system or become diluted by outside water sources; therefore, all should be accounted for by the elemental analyses of sites of potential sorption/solution (i.e., substrate matrix, interstitial water, algae, macrophytes, and fauna). Essentially, all values for pH and Eh that could be expected in natural systems were reached during the experiment under fresh and saline conditions, with no significant differences from the control (i.e., topsoil) in any of the metals of concern.

One concern from our previous studies was potential aluminum phytotoxicity. Aluminum toxicity can be ameliorated through two major mechanisms: the addition of ligand forming agents and the addition of certain cations (Kinraide and Parker 1987). Amelioration can occur by binding of the Al^{+} cations by chelators such as hydroxides (i.e, $Al^{+} + 3OH^{-} = Al[OH]_3$; Kinraide and Parker 1987). The presence of certain cations, markedly Ca and Mg, relieve Al toxicity through competitive binding to extracellular surfaces, thereby limiting Al uptake (Kinraide and Parker 1987). Whatever mechanism was responsible for the reduction in Al concentration in the interstitial waters of the DC-B substrate, the increased concentrations of Ca and Mg that naturally occur in seawater should limit the uptake of Al by vegetation. Indeed, plant tissue concentrations in this study were much less than observed in the previous studies.

Examination of aboveground plant tissue indicated significantly higher levels of Ba in the DC-A substrate (22.15 μg/g) and DC-B substrate (20.25 μg/g) compared to topsoil (6.03 μg/g) and dredge spoil (6.73 μg/g). However, normal levels of Ba for plant foliar levels are considered

to be between 10-50 μg/g (Chaudhry et al. 1977; also see references in Cipollini and Pickering 1985). For example, Smith (1971) examined uptake of total barium by *Triticum vulgare* (wheat) in three agricultural soils in Western Australia and found concentrations ranging from 19.7 μg/g to 76.9 μg/g in stem and leaf material. It should also be noted that studies of Ba toxicity in laboratory mice have indicated no discernable effect when given drinking water containing up to 250 μg/g of Ba (USEPA 1985). Therefore, the levels of Ba in plant tissue grown on the two processed drill cutting substrates, although elevated compared to topsoil and dredge spoil, are not exceptionally high. Barite ($BaSO_4$) is often employed in drilling fluids and this is presumed to be the major source of Ba in the processed drill cuttings substrates. Solubility (i.e., potential for accumulation) of Ba compounds varies from extremely low ($BaSO_4$) to extremely high ($BaCl_2$) (USEPA 1985). Carbonell et al (1999) determined that barite can be responsible for significant levels of soluble barium under acidic, reduced conditions. Although no pH levels were ever detected below 7.0 during the experiment, it should be noted that pH was measured on interstitial water. Because plants generate proton gradients to absorb nutrients in the root zone, it is possible that the pH immediately surrounding the roots was low enough to increase Ba solubility for enhanced plant uptake (Larcher 1995). Once Ba is assimilated by the plant, it is likely converted to some nonionic, low solubility form, which greatly reduces its toxicity (availability).

Substrate interstitial Hg concentration appeared to increase slightly in all substrates with increasing salinity levels. Although the interstitial Hg concentration in the DC-B substrate reached 5.61 μg/g at the 9 ppt salinity level, it was reduced to 0.46 μg/g at the end of the 36 ppt salinity level, indicating that the substrate had become stable by the end of the study. No significant differences among substrates were detected for any other metals of concern (Cd, Cr, Pb, and Ni). Accumulation is known to vary among metals and plants tend to accumulate metals in belowground tissue at higher rates than in aboveground tissue (Fergusson 1990). However, belowground tissue can experience a high rate of leaching of metals when the soil is rinsed of sediment prior to analysis. Furthermore, accumulation of metals up the food chain often begins with consumption of plant material that is available above the sediment surface. Therefore, only

aboveground material was analyzed in this study to maintain consistency during analysis. Acid digestions of the DC-B substrate at the end of this study did indicate that some metals had been removed from the substrate by the third year of the study. The lack of these contaminants in aboveground tissue, especially given the high levels of metals in both in the DC-B substrate and plant tissue in the first year study, suggests that uptake of certain elements by the plants and their subsequent harvest in year two of the study, were involved in these processed drill cuttings becoming more stable and usable by the third year. This is especially true of Ba in the DC-B substrate, which had an initial acid-digestion sediment Ba concentration of 14,500 μg/g that was reduced to 779.7 μg/g by the end of the third year. If the vegetation is indeed responsible for the reduction in soil contaminants, then phytoremediation (via plant uptake and harvest) of processed drill cuttings is an aspect of drill cuttings remediation that should be further investigated.

Vegetation Performance

The two prime indicators of plant growth used in this experiment were net CO_2 assimilation rate (photosynthesis) and biomass partitioning. Net CO_2 assimilation rates were measured at each salinity level and can thus be used to evaluate plant responses to substrate and hydrologic regime at different salinity levels. Biomass was harvested at the conclusion of the experimental period and therefore represents the cumulative effects of substrate and hydrology across all salinity levels through time. Plant biomass is recognized as an integrated measure of plant response to various environmental stresses through time (Larcher 1995).

Net CO_2 assimilation rates under freshwater conditions indicated significantly lower plant performance when plants were grown on the DC-B substrate, corroborating the previous studies. However, a significant interactive effect of salinity and substrate was detected, in which plants grown on the DC-B substrate had rates of net CO_2 assimilation comparable to other substrates once salinity levels were increased. This may be explained by the observed shift to a more

moderate pH in the DC-B substrate under saline conditions. Although analysis of plant biomass would indicate that plants were performing significantly worse in the DC-B substrate than in other substrates, one must take into account that biomass values were influenced by the time spent at 0-ppt, and therefore, may not accurately reflect salinity-substrate interactions. All net CO_2 assimilation rates under saline conditions showed no significant differences among either substrate type or hydrology regime for all species. This indicates that, after two years in a simulated wetland environment, drill cuttings processed by this technique are as suitable for wetland restoration projects as topsoil, or a dredge spoil/drill cuttings mix, in terms of supporting appropriate wetland vegetation. Two of the most wide-spread brackish and salt marsh plant species of Louisiana, *S. alterniflora* and *S. patens*, exhibited excellent growth, both in terms of net CO_2 assimilation and total biomass, indicating that they would be good candidates for this type of wetland restoration project. Although no differences were detected in the performance of *S. cynosuroides* when grown on processed drill cuttings or on topsoil or dredge spoil, this species is still not a viable candidate for creating wetlands in this fashion. *Spartina cynosuroides* exhibited high mortality of tissue and relatively low photosynthetic rates throughout the experiment regardless of substrate type. This can probably be attributed to a low tolerance to transplanting stress. Because the grasses utilized in this experiment are widespread throughout Atlantic and Gulf Coast marshes (*S. alterniflora*, *S. patens,* and *S. cynosuroides*) and *Avicennia germinans* is a common tropical and subtropical mangrove, the results of this project may be applied to many coastal areas (Godfrey and Wooten 1979).

No advantage was discerned by any of the genotypes tested in regard to establishing and growing in either of the processed drill cuttings, suggesting that intraspecific variation in tolerance of *S. patens* and *S. alterniflora* to substrate type is quite low. No differences were

detected among genotypes with regard to hydrologies, again indicating that intraspecific variation in flooding tolerance may be low in the genotypes assessed. However, these statements are made cautiously because our earlier studies on more recently processed drill cuttings did reveal several differences among genotypes (Shaffer et al. 1998). Also, it is important to note that although there is a bounty of useful applications for genotype research and plant selection, the dangers of over-reliance on single genotypes (or varieties), whether in agriculture or wetland creation, can be disastrous should the environment change drastically over time, or should a disease or pest outbreak occur. Therefore, we recommend that the use of selected genotypes be limited to extremely stressful environments in need of rehabilitation.

In summary, this study has important implications for the use of processed drill cuttings in wetland rehabilitation in Louisiana and other portions of the globe. Previous studies on processed drill cuttings have indicated that the potential for their utilization in wetland rehabilitation existed, but that plant performance when grown on processed drill cuttings was often reduced from that of other substrates, or there was concern about the safety of the processed material under various environmental scenarios. This study has illustrated that processed drill cuttings have the potential to serve as suitable and nontoxic substrates capable of supporting high rates wetland plant growth under a range of environmental conditions. However, if processed drill cuttings are to become a viable option as a substrate source in wetland rehabilitation, we recommend further refinement in the formulation of environmentally safe, water-based drilling fluids. Rather than attempt to further develop encapsulation techniques that are both truly safe and conducive to plant growth in wetland environments, it may prove more expedient to formulate water-based drilling fluids with wetland rehabilitation in mind.

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Appendix

Table. A-1 Factor Analysis of the 0-ppt salinity level substrate interstitial water elemental concentrations

Principal Component:	PC 1	PC 2	PC 3	PC 4
Eigenvalue:	6.555	2.526	1.870	2.203
Percent explained variation:	32.77	12.63	9.352	11.02

Rotated Loading Matrix (VARIMAX)

Variable	PC 1	PC 2	PC 3	PC 4
Mg	0.978	0.026	0.032	-0.009
Cr	0.949	-0.031	0.000	0.940
Ni	0.939	0.005	0.010	-0.008
Ca	0.906	0.023	-0.003	-0.035
Cd	0.840	-0.104	0.106	-0.106
As	0.773	0.124	-0.094	-0.320
Na	0.759	-0.303	-0.003	-0.200
Pb	0.698	0.113	0.137	0.358
pH	-0.133	-0.810	-0.059	-0.264
Al	-0.144	-0.734	0.009	0.018
P	-0.082	0.576	0.059	0.071
Redox 1 cm	0.059	0.152	0.918	0.047
Redox 15 cm	-0.107	-0.030	0.910	0.036
Fe	0.006	0.065	-0.062	0.922

Table. A-2 Factor Analysis of the 36-ppt salinity level substrate interstitial water elemental concentrations.

Principal Component:	PC 1	PC 2	PC 3	PC 4
Eigenvalue:	4.818	2.213	2.153	2.147
Percent explained variation:	25.36	11.65	11.33	11.30

Rotated Loading Matrix (VARIMAX)

Mg	0.151	0.008	0.116	0.794
Cr	0.952	0.077	-0.097	0.009
Ni	0.975	0.045	0.033	0.116
Ca	-0.041	-0.147	-0.331	0.665
As	0.925	0.087	0.001	-0.001
Na	0.549	-0.376	0.060	0.338
Pb	0.961	0.078	-0.079	-0.125
pH	-0.009	-0.099	-0.651	-0.369
Al	0.713	-0.009	0.129	0.481
Fe	0.111	-0.016	0.807	-0.014
Cu	0.098	0.945	0.005	-0.120
Mn	-0.114	0.035	0.780	-0.201
Zn	-0.024	0.948	0.050	-0.088

Table. A-3 Factor Analysis of totally potentially extractable DC-B sediment elemental concentrations (resulting from acid digestion).

Principal Component:	PC 1	PC 2	PC 3	PC 4
Eigenvalue:	5.665	4.947	2.099	1.836
Percent explained variation:	35.41	31.92	13.12	11.47

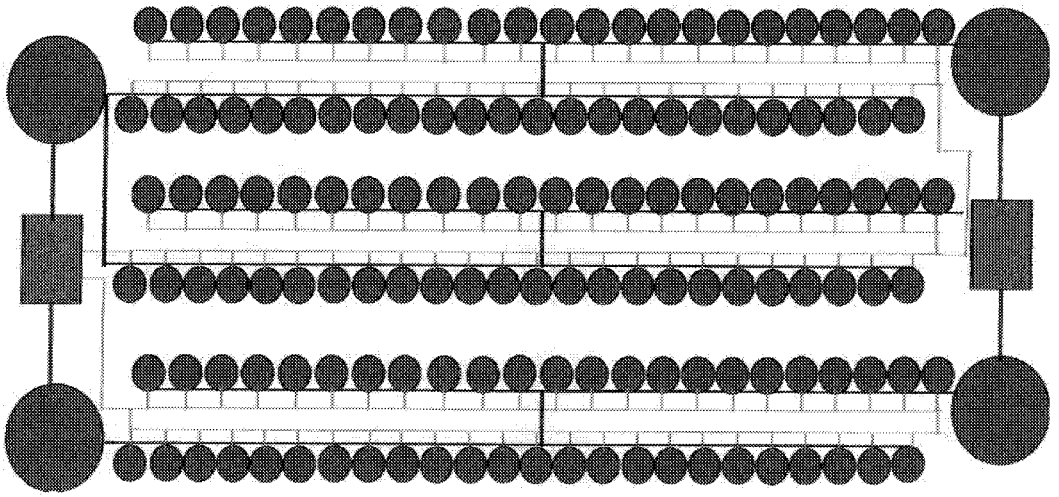
Rotated Loading Matrix (VARIMAX)

Variable	PC 1	PC 2	PC 3	PC 4
Fe	0.974	-0.088	0.188	0.010
Mg	0.959	-0.210	0.067	0.127
Al	0.943	-0.226	0.077	0.049
Ca	0.924	0.118	0.110	-0.031
P	0.817	0.464	-0.085	0.060
Ni	0.789	0.534	0.117	-0.172
Pb	-0.090	0.970	-0.037	0.145
As	-0.039	0.924	-0.092	-0.340
Cd	0.225	-0.848	0.469	0.027
Cr	0.089	0.833	0.110	-0.379
Na	-0.024	-0.167	0.911	0.209
Ba	0.008	-0.158	0.087	0.921
K	0.272	-0.053	0.445	0.583

Table. A-4 Factor Analysis of acid digested plant tissue elemental concentrations.

Principal Component:	PC 1	PC 2	PC 3	PC 4
Eigenvalue:	5.189	2.688	2.374	1.885
Percent explained variation:	32.43	16.80	14.84	11.78

Rotated Loading Matrix (VARIMAX)

Variable	PC 1	PC 2	PC 3	PC 4
Cu	0.884	0.017	-0.058	-0.322
Zn	0.316	0.658	0.383	-0.224
Cd	0.871	0.097	0.169	-0.121
Pb	0.878	-0.007	0.180	0.129
Cr	0.850	0.347	0.014	0.008
Ni	0.714	0.101	0.563	-0.098
As	0.849	-0.014	0.060	0.217
Fe	0.286	0.297	0.664	0.033
Mn	-0.078	-0.013	0.941	0.030
Ca	0.184	0.646	0.410	-0.119
Mg	0.122	0.815	0.053	0.197
P	0.158	0.264	0.417	0.637
K	0.081	0.078	-0.277	0.745
Na	-0.397	0.783	-0.169	0.243

A-5 Schematic of Mesocosm Facility