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RESTORED DRILL CUTTINGS FOR WETLANDS CREATION: RESULTS OF A TWO YEAR MESOCOSM APPROACH TO EMULATE FIELD CONDITIONS UNDER VARYING HYDROLOGIC REGIMES

Topical Report
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Executive Summary

It is well documented that Louisiana has the highest rate of wetland loss in the United States. Deep-water channel dredging and leveeing of the Mississippi River since the 1930s have interrupted the natural delta cycle that builds new marshes through sediment deposition. Several sediment diversion and hydrologic restoration projects are currently in various stages of implementation under the Coastal Wetlands Planning, Protection, and Restoration Act (CWPPRA 1993). Many of the areas that are subsiding and deteriorating are isolated from riverine sediment sources; therefore alternative methods to deposit sediment and build marshes must be implemented.

This project demonstrates that the earthen materials produced when drilling oil and gas wells can be used as a suitable substrate for growing wetland plants. Drilling fluids (muds) are used to lubricate drill bits and stabilize the earth around drill holes and become commingled with the earthen cuttings. Two processes (referred to in this report as A and B) have been reported to restore drill cuttings to acceptable levels by removal of any toxic components found in drilling muds. The main objective of this project was to assess the potential of drill cuttings processed by these two methods in terms of their ability to support wetland vegetation and potential toxicity. It was our belief that if processed drill cuttings can be shown to pose no environmental hazard, support wetland vegetation, and compare favorably in cost to their current disposal, then their use in wetland creation and restoration projects should be considered.

With support from the U. S. Department of Energy and Southeastern Louisiana University's College of Arts and Sciences, a state-of-the-art mesocosm facility (an experimental arena scaled between the laboratory and nature) was constructed to facilitate the two-year investigation concerning wetland creation using restored drill cuttings. Two methods of processing (A and B) were included in the trials, and were compared to a topsoil control as well as dredge spoil, a substrate currently used for building levees and restoring wetlands. Three different hydrologic regimes were imposed across all four substrate types to determine the response of different wetland plant species to these substrates under flooded conditions (as occurs in a subsiding marsh), under tidal conditions where water levels fluctuate daily, and in moist-but -not-flooded conditions. Five different plant species were used in monoculture treatments during the first year along with an unvegetated control. Each treatment was applied in duplicate and thus required one hundred forty four experimental vessels. During the second year of the project, several other wetland emergent plant species were tested for suitability in the different substrates. In addition, two submerged aquatic plant species and benthic algae were tested on all substrates under flooded conditions.

In a separate study, toxicity trials were conducted using mysid shrimp. Both experimental substrates manifested low toxicity when tested by EPA guidelines. Elemental analysis, electrical conductivity, exchangeable sodium percentage, cation exchange capacity, and pH were conducted on samples of the different substrates using acid extraction. A water extraction procedure also was used at that time for comparison. Elemental analysis of the water in all vessels was repeated twice to detect potential leaching. Elemental analyses indicated that the substrates were within Louisiana Department of Natural Resources 29-B standards for reusable material.

Substrate B, however, contained high levels of aluminum (not regulated in LaDNR 29-B) and had a high pH throughout the study. Although two of the plant species grown on Substrate B sequestered heavy metals, results were well within Louisiana Department of Natural Resources No. 29-B standards for reusable materials. Nutrient analysis indicated that Substrate B contained the highest levels of nitrate/nitrite-nitrogen and ammonium-nitrogen while having the lowest levels of phosphate-phosphorus.

Substrate B also had the most reduced soil (lowest Eh), while Substrate A was similar in redox potential to dredge spoil. Similarly, Substrate B supported the least plant growth throughout the study, a response especially evident under the flooded hydrologic regime. Substrate A performed comparably to dredge spoil, a proven substrate for wetlands restoration.

Plant species exhibited some definite preferences for different substrate types and different hydrologic conditions. Bulltongue and elephant's-ear (fresh to intermediate marsh plants) grew best on topsoil while wiregrass (Brackish marsh dominant) produced the greatest biomass when grown on Substrate A or dredge spoil. Maidencane (fresh marsh dominant) produced little biomass on substrates other than topsoil. Bulltongue was most productive and wiregrass was least productive under permanently flooded conditions and wiregrass was most productive when conditions were moist-but-not-flooded. Cattail performed well over all hydrologic regimes and was one of the few species that accumulated substantial aboveground biomass on Substrate B.

Two of the six different wiregrass genotypes demonstrated the ability to establish and grow well on Substrate A, and all genotypes accumulated the greatest biomass under the mesic hydrology. Several of the nine alternative plant species established and grew on Substrate A, while Substrate B proved to be the least favorable for supporting their growth. Submerged aquatic vegetation mimicked the results of emergent species, regarding substrate. In contrast to vascular plant species, benthic algal productivity was greatest on Substrate B. Benthic algal productivity was moderate on Substrate A and was not statistically different from that observed on dredge spoil and topsoil.

Toxicity trials, elemental analysis, soil testing, and plant responses are all encouraging indicators of the potential suitability of restored drill cuttings (particularly Substrate A) as a substrate that may be utilized to produce healthy and stable wetlands. Even under the extreme conditions of a closed, permanently flooded system, Substrate A performed similar to dredge spoil, a substrate already widely used in wetland creation and restoration projects.

Future research needs on restored drill cuttings include assessing their performance under increased salinity regimes, as may be encountered in brackish or salt marshes (preliminary studies are underway), assessing alternative drill cuttings restoration processes, and eventually conducting a field demonstration of wetlands creation utilizing restored drill cuttings.

Introduction

In many areas of Louisiana, wetlands have subsided to open water habitat characterized by depths greater than 0.5 m. Due to waterlogging stress, most of these areas no longer support emergent vegetation (Penfound and Hathaway 1938, DeLaune et al. 1978, Gornitz et al. 1981, Buamann et al. 1984, Mitsch and Gosselink 1993). Furthermore, these open-water areas continue to increase in coastal Louisiana at a rate of three acres each hour, or roughly 35 square miles per year, Boesch et al. 1984. In attempt to redress this loss, several sediment diversion and hydrologic restoration projects have been implemented or are planned under the Coastal Wetlands Planning, Protection, and Restoration Act (CWPPRA 1993). Nevertheless, many areas of Louisiana are either isolated from potential sediment sources or are not suited for such projects because of the need for maintaining navigable waterways. These areas will continue to degrade unless a mechanism for site-specific sediment addition is devised.

One such method involves using restored drill cuttings, a by-product of the oil and gas industry, to build elevations suitable for colonization and establishment of wetland vegetation. Through physical isolation of metals and organics in a silica matrix and removal of toxic constituents with stabilizing agents, the cuttings (referred to herein as Substrates A and B) may be restored to acceptable levels. Although cuttings from a single drilling project would yield perhaps less than one-acre of emergent wetland, cumulatively such projects could add significantly to current restoration activities. Certainly, if restored sediments can be shown to cause no environmental hazards, support wetland vegetation, and have a restoration cost comparable to current disposal costs, then this method warrants serious consideration.

A study of Kelley and Mendelssohn (1995) has demonstrated that restored sediments can support emergent wetland vegetation, albeit at lower rates of productivity than organic or natural wetland soils. Although the pilot study of Kelley and Mendelssohn was quite informative, it was limited in scope; several governmental agencies (U. S. Fish and Wildlife Service, National Marine Fisheries Service, Louisiana Department of Wildlife and Fisheries, U. S. Army Corps of Engineers) voiced concerns that extrapolating from this pilot study to a field study would be quite risky. Consequently, we designed an intensive mesocosm (i.e., a controlled system scaled between lab and field) project that would emulate field conditions.

During the first year of a proposed field study, the site is to be fully contained. If elemental analysis from the field site indicates that the restored cuttings are indeed safe, it is proposed that the containment be breached to enhance wetlands productivity. To accommodate this scenario, we emulated both hydrologic regimes in the mesocosm facility. Two drill cuttings processing methods (processes A and B) were assessed along with a dredge spoil substrate and a topsoil (control).

Materials and Methods

Experimental Approach

Design. One hundred forty four 200-liter vessels, fully networked to four 3000-liter supply reservoirs, were subjected to a 3 x 4 x 6 factorial treatment arrangement with two true replicates per treatment combination (detailed in Appendix A). Specifically, three hydrologic regimes, four substrates, and six vegetative conditions were applied in a factorial arrangement as described below.

Hydrologic Regimes. Three hydrologic regimes were established. The three hydrologic regimes consisted of moist-but-not flooded, permanently flooded, and daily tidal cycle conditions. Water was provided to 200-liter experimental vessels (mesocosms) from the appropriate 3000-liter reservoirs by using on-off switches controlled by timers. The moist-but-not-flooded treatment was maintained by leaving the vessel's low tide drains in the open position and trickling supplemental moisture into the vessel during the high tide cycle. Permanently flooded conditions were maintained at a depth of 20 cm above the sediment surface by setting the vessels' low tide drains in the closed position and the vessels' high tide drains in the open position. The daily tidal cycle regime was achieved in four distinct phases. First, water from the 3000-liter reservoirs was distributed to mesocosm units with the vessels' high tide drains set in the open position to yield a flood-tide depth of 20 cm above the sediment surface. The vessels' low tide drains were only slightly open on tidal vessels and once the flooding stage was completed, four (one for each reservoir) timer-controlled electric drain valves held water in the vessels until a period of no water movement (slack tide) had occurred. At the end of the high tide phase, the reservoir drain valves were opened to allow water to slowly flow from the 200-liter vessels back into the large reservoirs. After several hours of low tide, the 24-hour cycle began again.

Substrate Types. The drill cuttings used in this project were generated in Grand Bay, Louisiana. The raw cuttings were then treated via two processes. First, they were treated with a process designed to separate and recycle drilling mud (lubricants) from drill cuttings (this process yields what we refer to as Substrate A). This process decreases the weight of material to be transported to a hazardous waste facility, thereby decreasing transportation and waste costs. A second process further remediates the drill cuttings via physical isolation of metals and organics in a silica matrix. Any remaining toxic agents are then diluted with stabilizing agents yielding the end product referred to as Substrate B. Processing of the cuttings occurs until constituents reach stabilized, acceptable (Louisiana Department of Natural Resources (LaDNR) No. 29-B) levels.

Six cubic yards each of cuttings that had undergone treatment with the Substrate A and B processes, plus six cubic yards of dredge spoil were transported to the mesocosm facility at Southeastern Louisiana University, courtesy of Greenhill Petroleum Corporation. Topsoil was ordered from a local supplier. In a completely cross-classified manner, one hundred and forty four 200-liter vessels were filled by hand with the following substrates: (1) topsoil, (2) Substrate A, (3) Substrate B, and (4) Substrate A capped with 40 cm of dredge spoil. The dredge spoil cap treatment was included in case the vegetation failed to establish directly on the cuttings material, and to enable direct comparison of the restored cuttings material with the substrate currently being used in wetlands creation projects in coastal Louisiana (i.e., dedicated dredging projects, CWPPRA 1993). Rarely do the roots of herbaceous wetland vegetation penetrate below a 30-cm depth (Mitsch and Gosselink 1993), and our belowground harvest during fall 1997 verified that no roots penetrated through the dredge spoil.

Vegetation. Six vegetation conditions were established across the hydrologic regime and substrate type combinations. Individual plants of each species were collected in the field and rinsed of all marsh soil, and planted during June1996. One of the species, arrowhead (*Sagittaria latifolia*) had zero survival and was subsequently replaced with elephant's-ear (*Colocasia esculenta*). The other five vegetative conditions were bulltongue (*Sagittaria lancifolia*), maidencane (*Panicum hemitomon*), two treatments of wiregrass (*Spartina patens*, six genotypes isolated by Dr. Hester (Hester et al. 1996) which have been shown to demonstrate low, intermediate, and high stress tolerance to elevated salinity levels, planted as separate treatments as three genotypes of varying salt tolerance per treatment), and the unvegetated control (mudflat). *Sagittaria* species tend to be fresh marsh pioneer species (Godfrey and Wooten 1979). Chabreck (1972) reported that *Panicum hemitomon* and *Sagittaria lancifolia* are the dominant fresh-marsh emergent macrophytes in Louisiana. *Spartina patens* is a dominant brackish marsh species and is the most frequently encountered coastal grass species in Louisiana (Chabreck 1972, Godfrey and Wooten 1979). *Colocasia esculenta* is a widespread fresh to intermediate marsh species (Godfrey and Wooten 1979).

In attempt to explore the establishment and growth potential of several other wetland plant species, six additional species were transplanted into the maidencane treatment vessels (maidencane performed poorly in all vessels). These six species included alligatorweed (*Alternanthera philoxeroides*), duck-potato (*Sagittaria platyphylla*), flat sedge (*Cyperus* sp.), seaside goldenrod (*Solidago sempervirus*), soft rush (*Juncus effusus*), and spike-rush (*Eleocharis* sp.). Three additional species were transplanted into the mudflat treatment. These species were baldcypress (*Taxodium distichum*), cattail (*Typha latifolia*), and swamp primrose (*Ludwigia alternifolia*). To study the establishment and growth potential of submerged aquatic vegetation, two species of aquatic vascular plants, elodea (*Egeria densa*) and fanwort (*Cabomba caroliniana*), were planted in the flooded mudflat treatments. Finally, net benthic algal productivity was monitored on five clear days between 10 am and 2 pm on all substrate types using a YSI Model 59 dissolved oxygen meter. To minimize oxygen diffusion during the incubations, the treatment vessels were sealed with transparent polyethylene covers.

Variables Measured

Plant photosynthetic response, biomass partitioning, and elemental analysis of plant tissue, sediment and sediment interstitial water were determined as described below.

Plant Photosynthetic Response. Instantaneous measurements of plant photosynthetic response (net CO_2 assimilation and stomatal conductance) were conducted during August and October 1996, and October 1997, using a LICOR 6400 portable photosystem. Two measurements were obtained from each of three individual stems per mesocosm vessel under uniform, light saturated conditions and net CO_2 assimilation expressed as µmol of CO_2 fixed per m^2 per second, with conductance expressed as mol H_2O per m^2 per second. Net primary productivity of benthic algae was measured via changes in dissolved oxygen and expressed as mg CO_2 fixed per m^2 per hour (Shaffer 1988).

Biomass Partitioning. In early November 1996, aboveground biomass was harvested at the sediment surface and partitioned into live and dead components. Harvested tissue was then oven-dried until constant weight was achieved and weighed. During November 1997, above and belowground biomass was harvested. Belowground biomass was obtained by extracting all root material from each vessel for all species except elephant's-ear and bulltongue. The belowground tissue of these two species was extremely dense and was estimated by extracting two 7.7 x 40 cm cores from each vessel. All belowground tissue was isolated by carefully washing the sediment from the root material, and weighing the oven-dried material.

Elemental Analysis. For all treatment combinations, sediment, sediment interstitial water, and water from the 3000-liter reservoirs were collected for elemental analysis. Plant aboveground tissue was collected at the 1996 harvest for elemental analysis.

At the beginning of the experiment (April 1996) acid digestions of dried homogenized (mortar and pestle) samples of each substrate were conducted using concentrated HNO_3 at 130° C. The digested samples were then subjected to elemental analysis. Water extractions were also conducted at that time (April 1996) by placing 10 g of dried substrate samples in centrifuge tubes and extracting with 30 ml of distilled water while shaking for one hour. The extract was then filtered through a 0.45 μ filter and subjected to elemental analysis. Interstitial water samples from all vessels and reservoirs were subjected to elemental analysis during peak season (June) and end of season (November) 1996. Additionally, elemental analysis and nutrient analysis for each of three parameters (nitrate/nitrite-nitrogen, ammonium-nitrogen, and phosphate-phosphorus) were conducted on interstitial water samples taken from all vessels except those containing topsoil (control) during peak season (July) 1997. Plant tissue elemental analysis was performed on dried (live) aboveground tissue (November 1996 harvest) digested in concentrated nitric acid at 130° C.

Elemental analyses were conducted on acid (HNO_3) preserved samples analyzed with a Jarrel-Ash inductively coupled argon plasma - optical emission (ICP-OES Atom Comp Series 800, later referred to as ICP) spectrophotometer for concentrations of Al, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, and Zn. For the analyses of NO_3^- / NO_2^- -N, NH_4^+ -N, and PO_4^{-2} -P in interstitial water, samples were immediately frozen upon collection and concentrations determined with a Technicon Auto Analyzer.

Redox Potential (Eh) and pH. Substrate redox potential was measured at 1 cm and 10 cm depths using bright platinum electrodes. Electrodes were allowed to equilibrate with the substrate for twenty minutes prior to taking each measurement using a calomel reference electrode. For all readings the potential of the calomel reference electrode (+244 mV) was added to each value (Faulkner et al. 1989). Substrate pH was measured on a sample of interstitial water taken 10 cm below the substrate surface in each of the 144 vessels using a (Hanna Model HI 9023C) pH-millivolt meter.

Toxicity Trials. Drill cuttings treated by the two restoration methods were used in 96-hour Static Definitive Toxicity trials (U.S. EPA, Federal register, Vol. 58, No. 41, 1993 (40 CFR Part 435/12507)) by Environmental Enterprises, Slidell, Louisiana. Testing was conducted using mysid shrimp (*Mysidopsis bahia*) and the suspended particulate phase (SPP) of the drill cuttings. The mysid shrimp were 3 to 6 days old, cultured and maintained in 23 ppt ($\pm$ 2) and 23° C ($\pm$ 2) and fed a daily ration of fairy shrimp (*Artemia* sp.) nauplii. The shrimp were acclimatized to a salinity of 20 ppt ($\pm$ 1) and a temperature of 20° C ($\pm$ 2) prior to initiation of the toxicity trials. Standard reference toxicant (95 % pure sodium dodecyl sulfate, Sigma Chemical) tests yielded a 96-hour LC_{50} of 7.5 ppm, with a 95 % confidence interval of $\pm$ 0.60. The drill cuttings-seawater slurry, mixed at a 1:9 ratio, was shaken for two hours and allowed to settle (EPA 1993). After one hour, the suspended particulate phase (SPP) was decanted and monitored for pH, temperature, dissolved O_2, and salinity. When necessary, pH and dissolved O_2 were adjusted (EPA 1993). Sixty mysids (three replicates of twenty) were exposed to five SPP concentrations and controls. Surviving mysids were counted and recorded at 24-hour intervals, and measurements made of temperature, dissolved O_2, pH, and salinity.

Data Analysis

The data were analyzed using the general linear models procedure of SYSTAT 6.1 statistical software (SYSTAT Inc. 1996). With the exception of year-one photosynthetic response, benthic algal productivity, and exploratory test plantings (nine other emergent plant species and two species of submerged aquatic vegetation) all dependent variables (above- and belowground biomass, plant tissue elemental composition, interstitial water elemental composition, and year-two photosynthetic response) were analyzed using a completely randomized design with a 3 x 4 x 6 factorial treatment arrangement. Year-one photosynthetic response was analyzed in two ways: initially as a split-plot design and then as separate ANOVAs. In the split-plot design the time effect (peak or end of growing season) was placed in the subplot, along with all interactions pertaining to time. The time effect was highly significant ($F = 104.7$, $P < 0.0001$), indicating that photosynthetic response behaved differently during the two time periods, thereby warranting analysis of the two periods separately. Algal productivity was measured only in the flooded hydrology (1-way ANOVA on substrate). The test plantings of alternative plant species and the submerged aquatic vegetation were qualitatively scored on performance (survivorship and biomass).

Results

Elemental Analysis

Results of acid digestion elemental analysis conducted on Substrates A and B, dredge spoil, sediment from the proposed wetland creation site, and a composite sample of unprocessed raw cuttings (i.e., a homogenized sample of the raw cuttings taken at various depths from the well) prior to the initiation of the experiment are presented in Table 1. It is important to note that acid digestion is a measure of the total potentially extractable elements, not what would be extracted under normal environmental conditions. The last column (Table 1) displays the 29-B standards of LaDNR; none of the substrates exceeded these 29-B limits. However, Substrate B is quite alkaline (pH = 10, Table 2). A subsequent water extraction elemental analysis of Substrates A and B (Table 2) indicates that elements in high concentrations in the cuttings material tend to be tightly bound to the substrates. Corresponding concentrations of those elements in the water extract are generally several orders of magnitude lower than their acid digestion extract counterparts. The cuttings, regardless of whether they were processed into Substrates A or B, tended to be high in iron, calcium, and magnesium. The two processes differ in that Substrate B contains high levels of aluminum. The corresponding high pH is almost certainly associated with an abundance of aluminum hydroxides (Mengel and Kirby 1987).

Two months following initiation of the study, the interstitial water of dredge spoil was more similar to that of the processed drill cuttings (Substrates A and B) than to interstitial water samples from topsoil, particularly with regard to cation concentrations (Table 3). For example, potassium in topsoil was 14.6 μg/g compared to 34.4, 41.9, and 48.1 μg/g in Substrates A, B, and dredge spoil, respectively. Furthermore, topsoil sodium concentrations were 94.5 μg/g compared to 912.9 (Substrate A), 702.6 (Substrate B), and 1276.1 μg/g (dredge spoil). Calcium concentrations were similar between dredge spoil and topsoil (31 to 33 μg/g), whereas calcium concentration was slightly higher in Substrate A (50.8 μg/g) and lowest in Substrate B (2.6 μg/g). Aluminum concentrations were low to non-detectable in Substrate A, dredge spoil, and topsoil, but remained elevated in Substrate B (Table 3). These patterns in elemental differences between substrates in interstitial water two months into the study are similar to the initial

Table 1. Elemental analysis (μg/g of substrate) for the acid extractions of Substrates A and B, dredge spoil, the proposed field site, and a composite of raw cores ranging in depth from 1000 to 9000 feet below the earth's surface. Also displayed are Louisiana Department of Natural Resources 29-B standards for reusable material.

	Substrate B	Substrate A	Dredge spoil	Field Site	Composite	Standards Not to Exceed
[Zn]	63.800	60.600	76.300	64.300	56.900	5000
[Cd]	2.000	0.700	1.300	1.100	1.100	100
[Pb]	25.500	14.700	17.200	21.400	13.900	500
[Cr]	41.100	17.500	18.500	15.800	18.500	500
[As]	2.100	ND*	ND	0.900	3.000	500
[Ba]	14500.000	12500.000	ND	ND	4500.000	10000
[Ag]	ND	ND	ND	ND	ND	500
[Hg]	ND	ND	ND	ND	ND	20
[Se]	ND	ND	ND	ND	ND	100

*ND -- Non Detectable Level

	Substrate B	Substrate A	Dredge spoil	Field Site	Composite	Standards Not to Exceed
Electrical Conductivity (mmhos/cm)	6.94	26.10	7.31	Not Available	1.44	8
Exchangeable Sodium (%)	43.0	115.90	52.30	Not Available	10.80	15
Cation Exchange Capacity (meq/100g)	16.0	22.00	23.00	Not Available	17.00	NA
Soil pH (-log[H$^+$])	10.11	8.83	7.94	Not Available	8.20	6.5-9.0

Table 2. Elemental analysis (µ g/g) of Substrates A and B and water extraction taken from drill cuttings prior to initiation of the mesocosm study (April 1996).

Element	Substrate B	B Water	Substrate A	A Water
[P]	692.44	0.40	318.01	2.34
[K]	1462.36	71.04	3734.09	76.49
[Ca]	78576.98	1099.70	8457.61	136.40
[Mg]	17507.53	3.61	5674.88	30.83
[Fe]	244418.39	9.32	17651.39	2.34
[Mn]	177.66	0.03	262.19	0.00
[Cu]	58.07	0.34	24.90	0.55
[Zn]	61.68	0.07	51.06	0.03
[Al]	68546.59	145.04	2129.81	1.80
[Cd]	2.49	0.00	1.35	0.00
[Cr]	32.69	0.85	24.49	0.00
[Ni]	26.06	0.00	16.93	0.06
[Pb]	0.00	0.00	0.00	0.00
[As]	32.18	0.32	33.42	0.00
[Ba]	1310.47	0.71	226.48	0.20
[Ag]	124.54	1.12	41.55	0.69

Table 3. Elemental analysis (µ g/g) of interstitial water from mesocosms and reservoir supply vessels two months after initiation of the mesocosm project (June 1996).

Element	Substrate B	Substrate A	Dredge Spoil	Topsoil
[P]	0.16	2.58	0.00	0.02
[K]	41.94	34.12	48.13	14.64
[Na]	702.65	912.91	1276.14	94.50
[Ca]	2.56	50.84	32.74	30.99
[Mg]	0.00	23.15	97.40	5.80
[Fe]	0.00	0.02	0.00	0.00
[Mn]	0.00	0.00	0.00	0.00
[Cu]	0.03	0.02	0.00	0.01
[Zn]	0.00	0.00	0.00	0.00
[Al]	16.49	0.11	0.00	0.00
[Cd]	0.00	0.00	0.00	0.00
[Cr]	0.06	0.00	0.00	0.00
[Ni]	0.00	0.02	0.01	0.00
[Pb]	0.00	0.01	0.00	0.00
[As]	0.00	0.00	0.00	0.00
[Mo]	0.33	0.05	0.01	0.00
[S]	203.33	291.97	309.52	25.05
[Si]	0.00	13.30	0.00	0.55
[Co]	0.00	0.00	0.00	0.00

analyses (Table 2). As in the initial analysis, none of the analyzed elements exceeded the limits set by LaDNR 29-B standards.

At the end of the 1996 growing season, elemental analyses were conducted of interstitial water extracted 10 cm below the substrate surface in each of the one hundred and forty four experimental vessels (Table 4). The patterns in elemental differences between substrates in interstitial water at the end of the first growing season were similar to those of the initial analysis and two months into the study (Tables 2 and 3). Again, the most striking difference between substrates was the elevated aluminum concentration of Substrate B. As in the other analyses, none of the analyzed elements exceeded the limits set by LaDNR 29-B standards. Furthermore, an additional one hundred eight subsurface samples of interstitial water extracted during the 1997 peak-growing season (Table 5), also demonstrate a stable pattern of low levels across elements. In summary, the elemental makeup of Substrate A is more similar to dredge spoil than Substrate B. The primary concern with Substrate B is the elevated concentrations of aluminum.

An elemental analysis was also conducted on the aboveground tissue of the four dominant wetlands plant species (bulltongue, elephant's-ear, maidencane, and wiregrass) grown on the four substrate types. For the data averaged across species for each substrate (Table 6), the tissue grown on Substrates A and B fell well below LaDNR 29-B standards (testing criteria for reusable material) for all constituents. Relative to the other three substrates, Substrate B was high in aluminum, chromium, and arsenic. LaDNR 29-B standards does not set a criterion for aluminum, but the sequestering of high levels of aluminum in the plant tissue may potentially be a cause for concern, especially if these primary producers are preferred herbivore forage. The arsenic concentrations in all four substrates may actually reflect organic compounds that have similar signatures as arsenic (Robert Gambrell, Wetlands Biogeochemistry Institute, Louisiana State University, pers. com.). For the data averaged across each substrate for each species (Table 7), it appears that bulltongue sequesters heavy metals (e.g., aluminum, chromium, and arsenic) to a greater extent than the other wetland species. Maidencane also had relatively high concentrations of aluminum.

For the analysis of NO_3^- / NO_2^- -N, significant differences were isolated for substrate type ($F = 17.07$, $P < 0.0001$) and hydrologic regime ($F = 11.43$, $P < 0.0001$) effects. Substrate B contained the highest levels of NO_3^- / NO_2^- -N (Figure 1) and NO_3^- / NO_2^- -N decreased from the permanently flooded to the tidal hydrologic regime (Figure 2).

Table 4. Elemental analysis of interstitial water (µg/g) extracted 10 cm below the substrate surface in mesocosm vessels (n=144) averaged for each substrate (Substrates A, B, dredge spoil, and topsoil) at the end of the 1996 growing season.

Element	Substrate B	Substrate A	Dredge spoil	Topsoil
[P]	0.06	1.21	0.80	1.91
[K]	32.54	29.88	35.56	14.76
[Na]	496.18	548.73	543.86	107.06
[Ca]	10.44	46.43	68.86	37.21
[Mg]	0.02	22.93	48.31	10.66
[Fe]	0.02	0.13	0.10	4.50
[Mn]	0.00	0.10	0.38	1.82
[Cu]	0.08	0.05	0.10	0.01
[Zn]	0.02	0.02	0.02	0.06
[Al]	25.04	0.25	0.00	1.19
[Cd]	0.00	0.00	0.01	0.00
[Cr]	0.03	0.01	0.02	0.01
[Ni]	0.00	0.00	0.01	0.00
[Pb]	0.00	0.03	0.06	0.06
[As]	0.14	0.16	0.15	0.12

Table 5. Elemental analysis (µg/g) from the interstitial water of composite samples taken from three substrate types (Substrate A, Substrate B, and dredge spoil) during the peak growing season 1997.

Elements	Substrate A	Substrate B	Dredge Spoil
[P]	0.726	0.195	1.138
[K]	20.591	24.45	16.956
[Na]	48.203	47.493	38.964
[Ca]	25.846	5.054	25.047
[Mg]	17.567	0.142	20.744
[Fe]	0.441	0.016	0.713
[Mn]	0.045	0	0.008
[Cu]	0.011	0.013	0
[Zn]	0.002	0.003	0.002
[Al]	0.92	11.157	1.339
[Cd]	0	0	0
[Cr]	0	0.003	0.002
[Ni]	0	0	0
[Pb]	0.002	0	0.003
[As]	0	0	0

Table 6. End of 1996 growing season elemental analysis (μg/g) of aboveground biomass averaged across species (mean ± SE, n = 4).

Element	Substrate B	Substrate A	Dredge spoil	Topsoil
[P]	1744.04 ± 201.76	1653.02 ± 484.60	1537.22 ± 285.16	2096.81 ± 316.36
[K]	9410.30 ± 4387.37	7540.19 ± 3473.69	5291.968 ± 2336.29	10109.10 ± 1849.64
[Na]	13155.01 ± 4573.12	11344.31 ± 4335.95	10554.95 ± 3799.37	8087.38 ± 4345.42
[Ca]	6055.81 ± 469.02	3521.69 ± 958.71	4597.03 ± 1170.77	4264.33 ± 1437.75
[Mg]	1531.66 ± 210.24	1347.14 ± 290.62	2338.80 ± 504.82	1349.88 ± 346.43
[Fe]	783.42 ± 208.10	653.63 ± 283.31	951.92 ± 463.40	145.26 ± 18.08
[Mn}	142.33 ± 44.74	312.22 ± 92.72	239.35 ± 50.65	307.83 ± 85.39
[Cu]	0.34 ± 0.34	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
[Zn]	46.68 ± 11.30	25.98 ± 5.28	24.41 ± 5.27	13.40 ± 2.03
[Al]	3052.32 ± 1085.55	683.14 ± 347.24	1169.19 ± 598.21	191.41 ± 55.13
[Cd]	0.37 ± 0.37	0.87 ± 0.52	0.53 ± 0.53	0.00 ± 0.00
[Cr]	17.85 ± 9.01	2.78 ± 0.50	2.43 ± 0.65	0.87 ± 0.18
[Ni]	2.72 ± 1.93	7.46 ± 1.56	4.85 ± 2.11	0.70 ± 0.70
[Pb]	0.69 ± 0.69	1.05 ± 0.83	0.35 ± 0.35	0.00 ± 0.00
[As]	16.05 ± 4.98	9.02 ± 1.77	8.54 ± 1.98	4.36 ± 0.91

Table 7. End of 1996 growing season elemental analysis (µg/g) of aboveground biomass averaged across substrates (mean ± SE, n = 4).

Element	Elephant's-ear	Panicum	Bulltongue	Spartina
[P]	2165.25 ± 176.70	1232.96 ± 222.28	2379.50 ± 161.2	1253.39 ± 218.57
[K]	9163.97 ± 5472.67	4165.63 ± 1101.08	11700.73 ± 1711.83	7321.22 ± 831.65
[Na]	3781.76 ± 823.81	6650.30 ± 1473.20	22495.26 ± 702.45	10214.32 ± 2498.73
[Ca]	6263.41 ± 234.54	3016.72 ± 665.59	6102.73 ± 560.77	3056.00 ± 1261.27
[Mg]	2182.98 ± 283.34	1269.23 ± 279.63	2170.98 ± 372.31	944.29 ± 174.19
[Fe]	216.80 ± 35.33	1085.49 ± 415.80	867.50 ± 250.71	364.45 ± 232.73
[Mn]	309.87 ± 87.99	240.39 ±17.60	337.05 ± 93.41	114.42 ± 10.32
[Cu]	0.00 ± 0.00	0.00 ± 0.00	0.34 ± 0.34	0.00 ± 0.00
[Zn]	42.335 ± 12.55	24.10 ± 3.50	23.90 ± 4.93	20.13 ± 9.30
[Al]	274.09 ± 71.33	1644.53 ± 603.72	2096.99 ± 1157.70	1080.17 ± 947.21
[Cd]	0.87 ± 0.52	0.00 ± 0.00	0.53 ± 0.53	0.38 ± 0.37
[Cr]	1.56 ± 0.44	3.81 ± 1.28	12.18 ± 9.87	6.39 ± 5.00
[Ni]	3.30 ± 1.25	5.00 ± 2.89	3.68 ± 2.48	3.75 ± 1.69
[Pb]	1.21 ± 0.59	0.00 ± 0.00	0.88 ± 0.88	0.00 ± 0.00
[As]	7.47 ± 1.34	8.49 ± 1.71	13.54 ± 5.01	8.46 ± 4.34

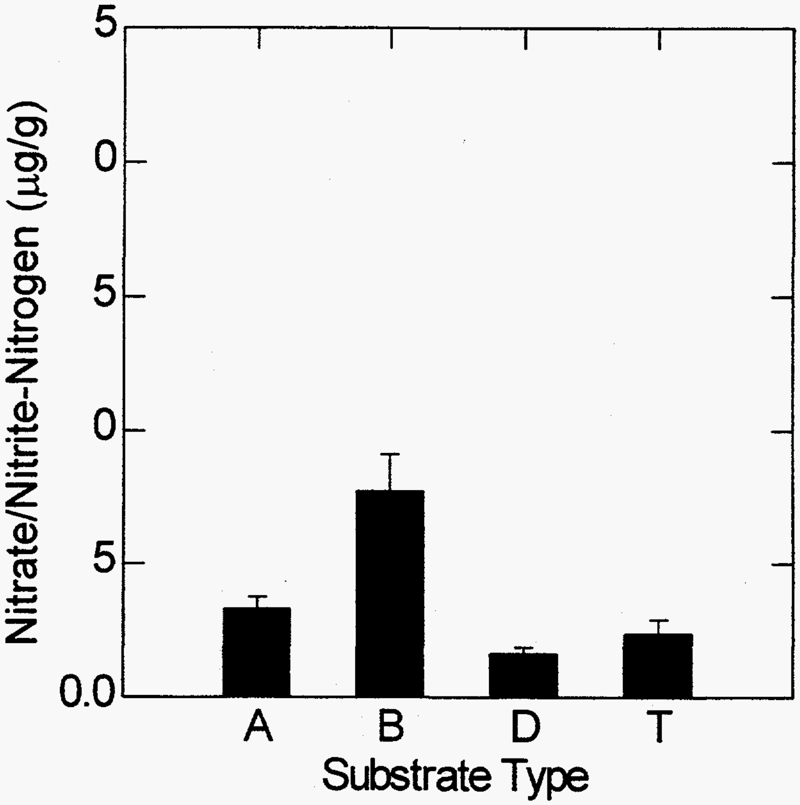

Figure 1. Interstitial water nitrate/nitrite-nitrogen in the four different substrate types during peak growing season 1997 (A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

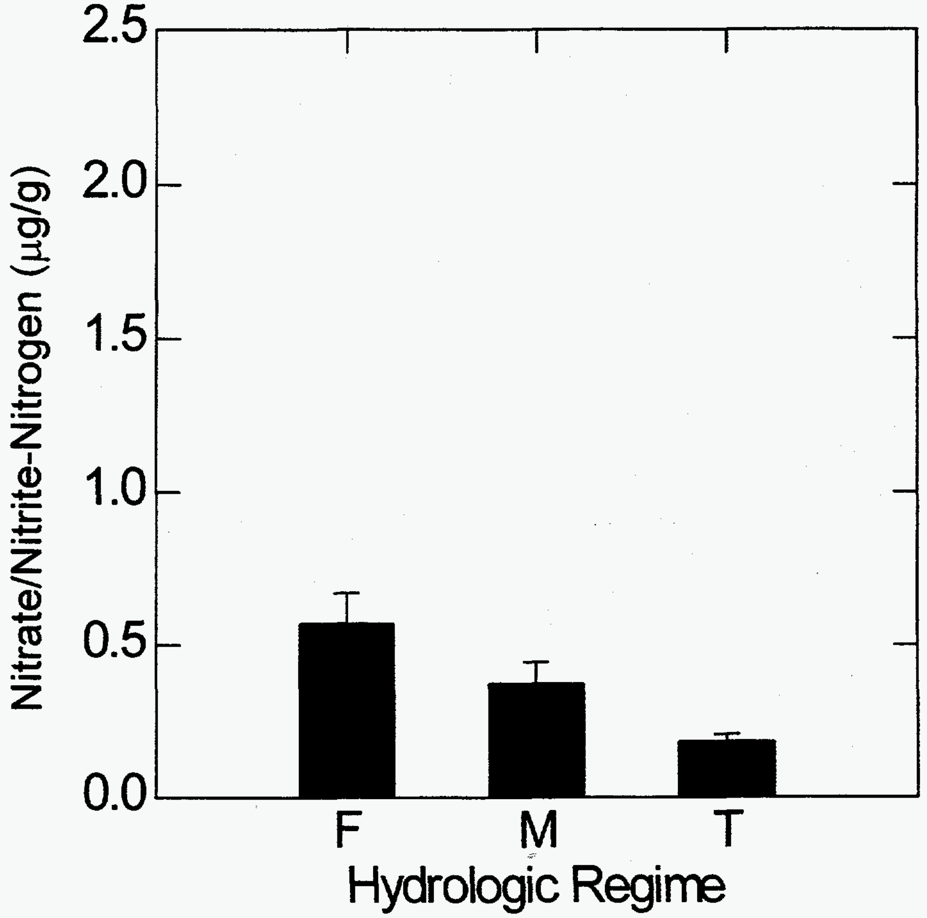

Figure 2. The effect of hydrologic regime on interstitial water nitrate/nitrite-nitrogen during peak growing season 1997 (F = flooded, M = moist-but-not flooded, T = tidal).

NH_4 -N concentrations were highest in Substrate B and topsoil (Figure 3; contrast F = 25.15, P = 0.0001). In contrast, PO_4^{-2} -P was lowest in Substrate B (Figure 4; contrast F = 15.73, P < 0.0001) and PO_4^{-2} -P increased from permanently flooded to the tidal hydrologic regime (Figure 5; contrast F = 6.95, P = 0.001).

Redox Potential (Eh) and pH

Redox potentials at both the 1 cm and 10 cm sediment depths were highest in dredge spoil and Substrate A (Figures 6 and 7, respectively; F = 5.42, P = 0.002, F = 18.51, P <0.0001), and at the 10 cm depth Substrate B had significantly lower redox potentials than the other substrates (Figure 7; contrast F = 25.18, P < 0.0001). At the 10 cm depth the permanently flooded treatments had lower (contrast F = 28.52, P < 0.0001) redox potentials than the moist and tidal treatments (Figure 8). Species differences were significant (Figure 9; F = 2.99, P = 0.014) only at the 1 cm depth with unvegetated mudflat tending to have the lowest redox potentials.

The pH levels of interstitial water samples extracted 10 cm below the substrate surface were highly elevated in Substrate B (Figure 10; contrast F = 2054.92, P < 0.0001). These alkaline levels were almost certainly attributable to aluminum hydroxides generated by the restoration process.

Toxicity Trials

Baseline toxicity limits are established at 30,000 ppm (EPA 1993), indicating that the suspended particulate phase (SPP) concentrations causing toxicity below 30,000 ppm are deemed toxic, whereas those above that threshold are considered safe. The 96-hour exposure to 6 %, 13 %, 25 %, and 100 % suspended particulate phase concentrations resulted in an LC_{50} of 639,700 ($\pm$ 71,000, 95 % confidence limit) for Substrate B and greater than 1,000,000 (the upper limit of detection) for the Substrate A treated cuttings. Survival in the 100 % SPP was 90 % in both substrates, compared to 100 % survival in the Control. The very high SPP concentrations of restored drill cuttings required to cause mysid shrimp death is an indicator of their low level of toxicity.

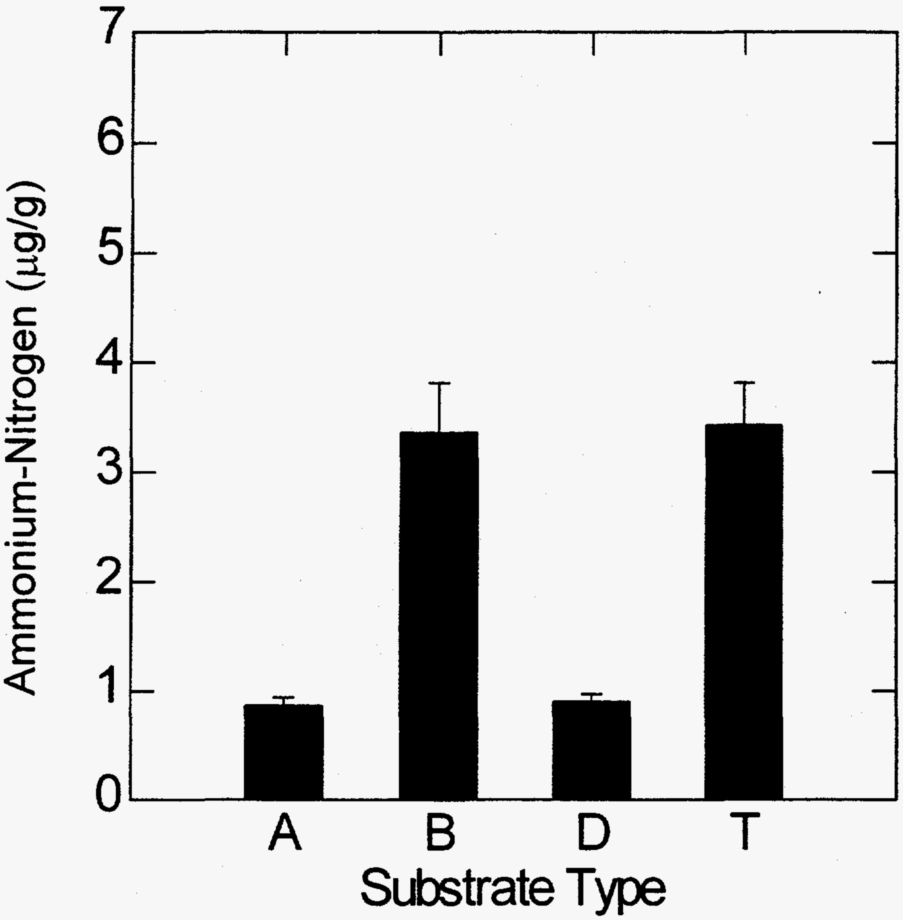

Figure 3. Interstitial water ammonium-nitrogen in the four different substrate types during peak growing season 1997 (A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

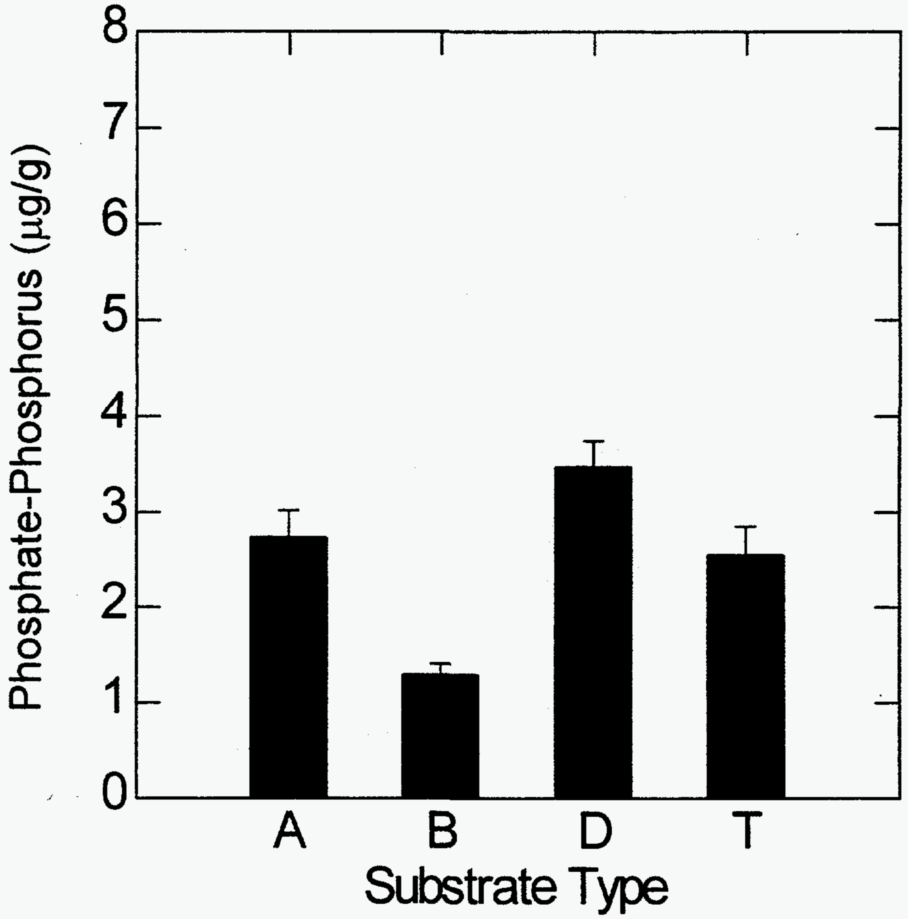

Figure 4. Interstitial water phosphate-phosphorus in the four different substrate types during peak growing season 1997 (A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

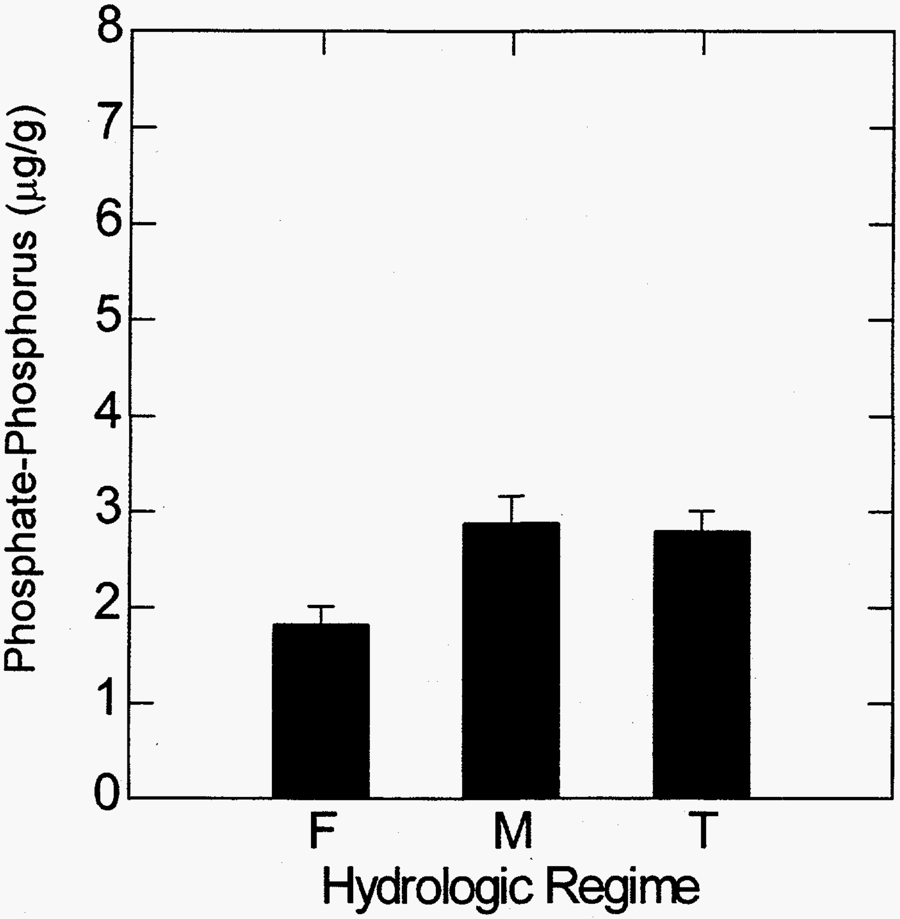

Figure 5. The effect of hydrologic regime on interstitial water phosphate-phosphorus during peak growing season 1997 (F = flooded, M = moist-but-not-flooded, T = tidal).

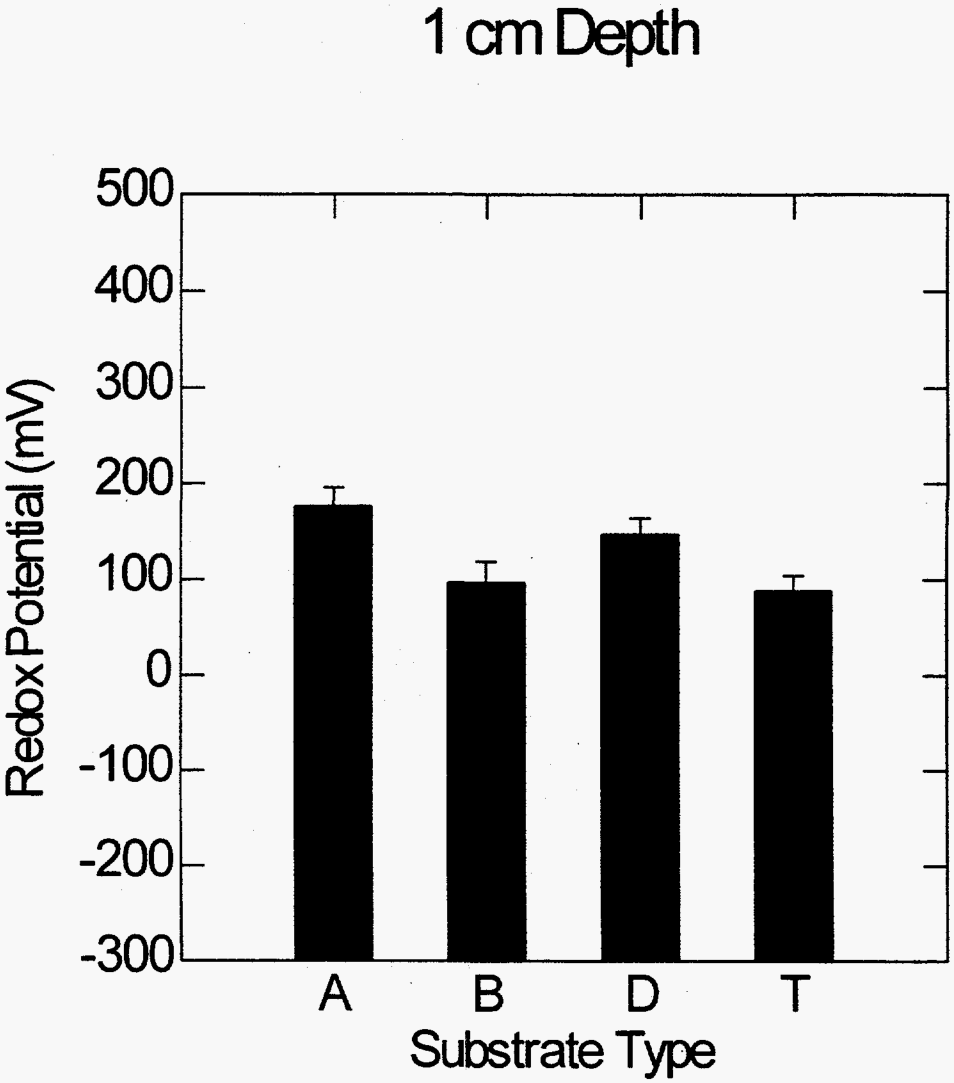

Figure 6. Soil redox potential (Eh) at 1 cm depth in the four different substrate types during peak growing season 1997 (A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

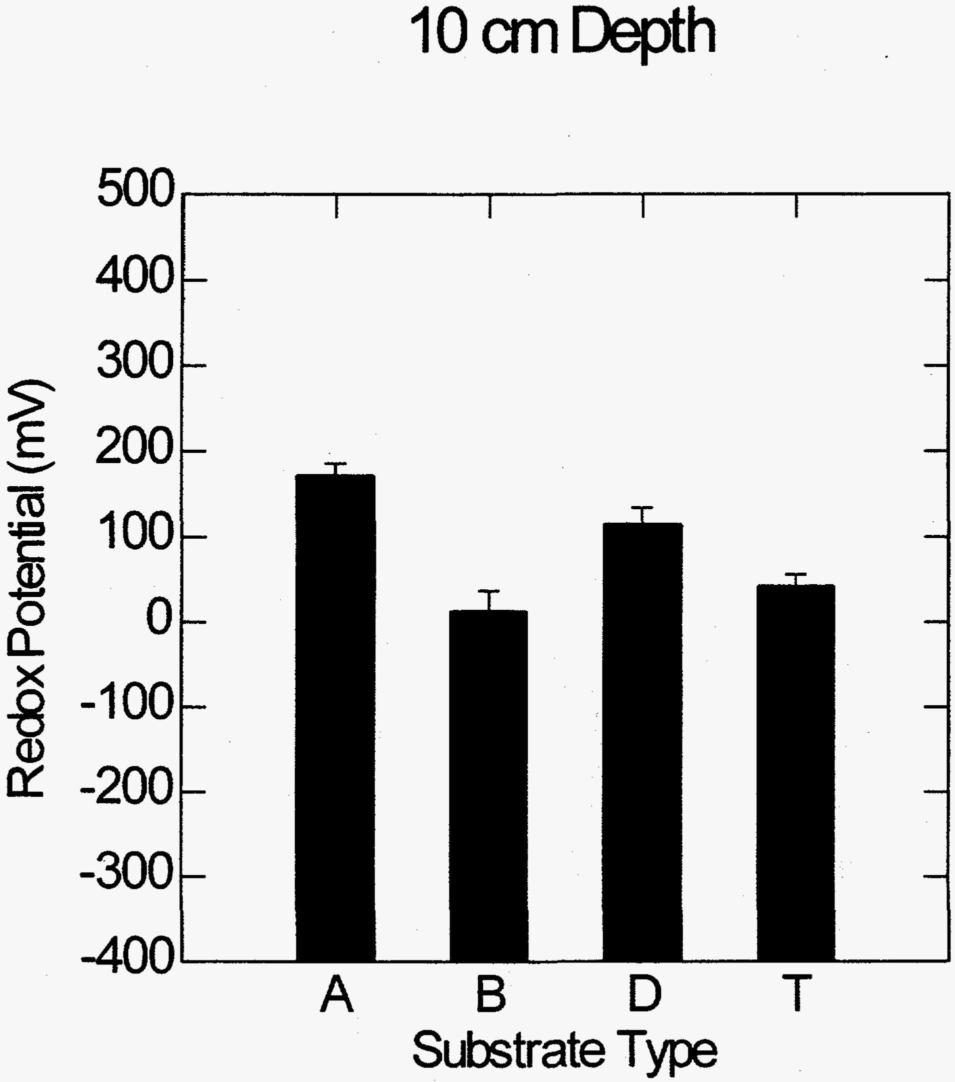

Figure 7. Soil redox potential (Eh) at 10 cm depth in the four different substrate types during peak growing season 1997 (A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

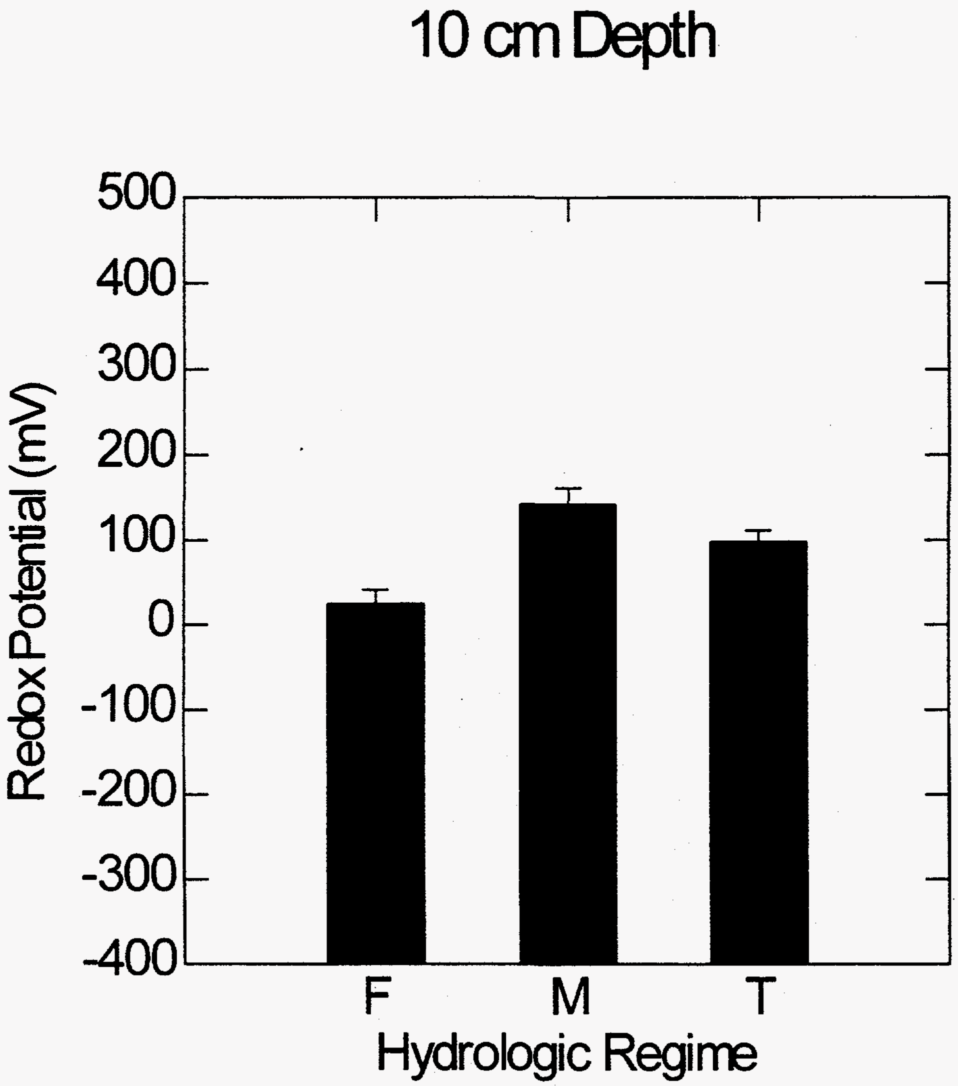

Figure 8. The effect of hydrologic regime on soil redox potential (Eh) at 10 cm sediment depth during peak growing season 1997 (F = flooded, M = moist-but-not flooded, T = tidal).

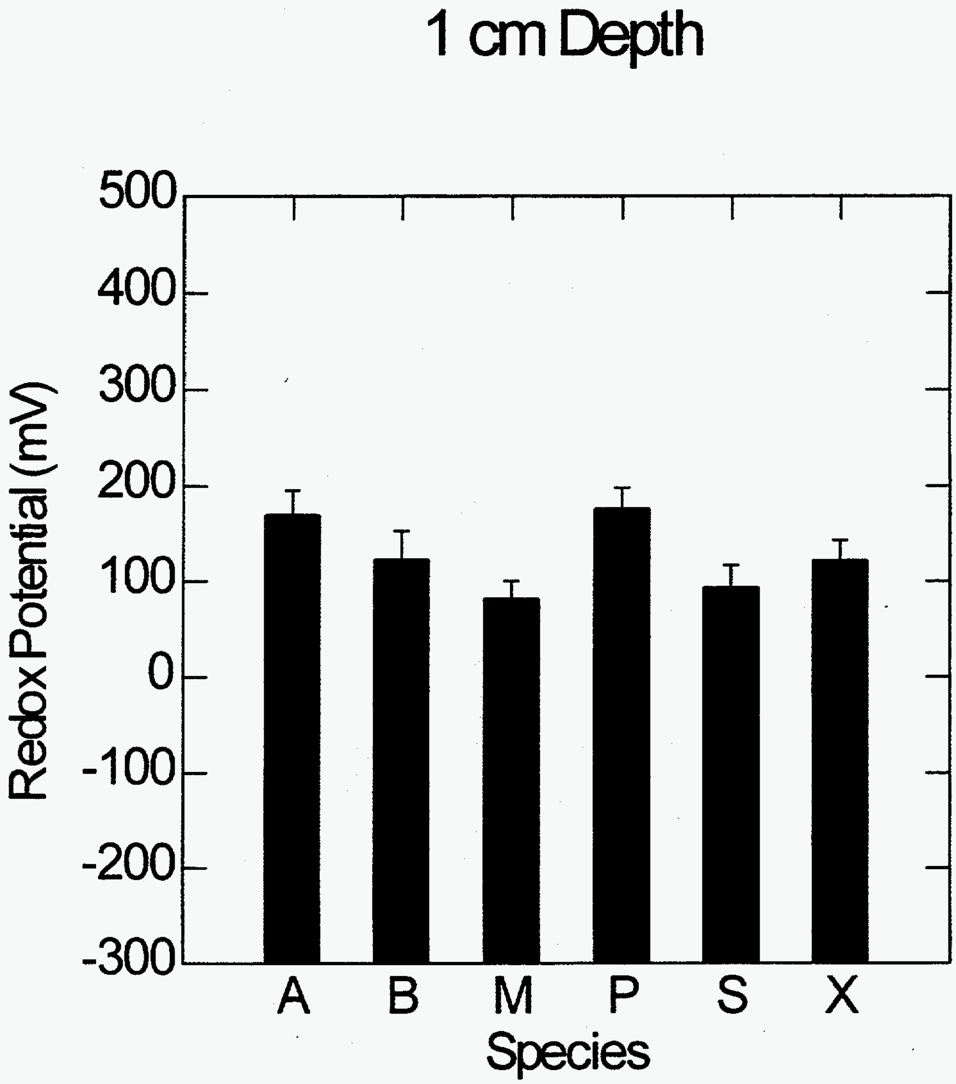

Figure 9. The effect of plant species on soil redox potential (Eh) at 1 cm depth during peak growing season 1997 (A = elephant's ear, B = bulltongue, M = cattail, P = alligator weed, S = wiregrass group #1, X = wiregrass group #2).

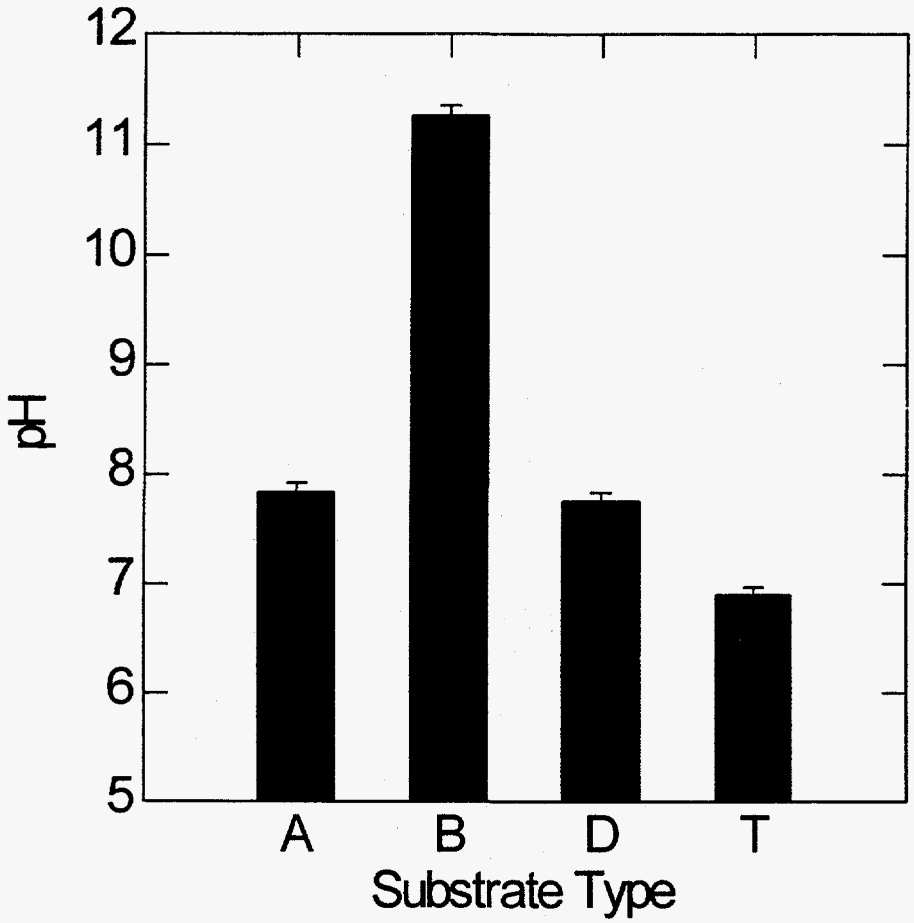

Figure 10. Peak growing season 1997 pH of interstitial water for the four substrate types (A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

Photosynthetic Response

Substrate Effect. During the 1996 peak growing season, perhaps the most striking effect was the main effect of substrate, both for net CO_2 assimilation rate (Figure 11; $F = 14.94$, $P < 0.0001$) and stomatal conductance (Figure 12; $F = 17.84$, $P < 0.0001$). Net CO_2 assimilation of the topsoil was significantly greater (contrast $F = 9.83$, $P < 0.002$) than all other substrates. Dredge spoil resulted in intermediate rates of CO_2 assimilation that were greater than those of Substrate A (contrast $F = 5.75$, $P = 0.018$), which, in turn, were greater than Substrate B (contrast $F = 34.60$, $P < 0.0001$). When comparing Figure 11 and 12, it is interesting to note that Substrate A has the highest water use efficiency (ratio of amount of CO_2 assimilated per amount of water transpired through stomatal conductance).

In October of 1996, the main effect of substrate type on photosynthetic rate (net CO_2 assimilation rate) remained highly significant ($F = 7.08$, $P <0.0001$, Figure 13). Substrate B had photosynthetic rates that were significantly lower than all other substrates (contrast $F = 13.85$, $P < 0.0001$). Importantly, Substrate A supported higher rates of photosynthesis than Substrate B ($F = 4.27$, $P = 0.042$). Although the photosynthetic rates of plants grown on Substrate A appeared similar to those grown on dredge spoil, a contrast revealed that dredge spoil slightly outperformed Substrate A ($F = 4.33$, 0.040). As was the case with photosynthetic rates, the effect of substrate on stomatal conductance remained highly significant ($F = 10.44$, $P < 0.0001$) and tightly paralleled the peak growing season response (Figure 14).

At the end of the 1997 growing season, the effect of substrate on the rate of photosynthesis remained significant (Figure 15, $F = 10.59$, $P < 0.0001$). As was the case with the 1996 measurements, Substrate B resulted in the lowest photosynthetic rates (contrast $F = 22.94$, $P < 0.0001$). With respect to stomatal conductance, a significant substrate effect ($F = 6.74$, $P < 0.0001$) was evident and tracked the pattern observed in photosynthetic response (Figure 16). In addition, an interaction occurred between the species effect and hydrologic regime on stomatal conductance (Figure 17; $F = 2.26$, $P = 0.029$), but revealed no clear pattern.

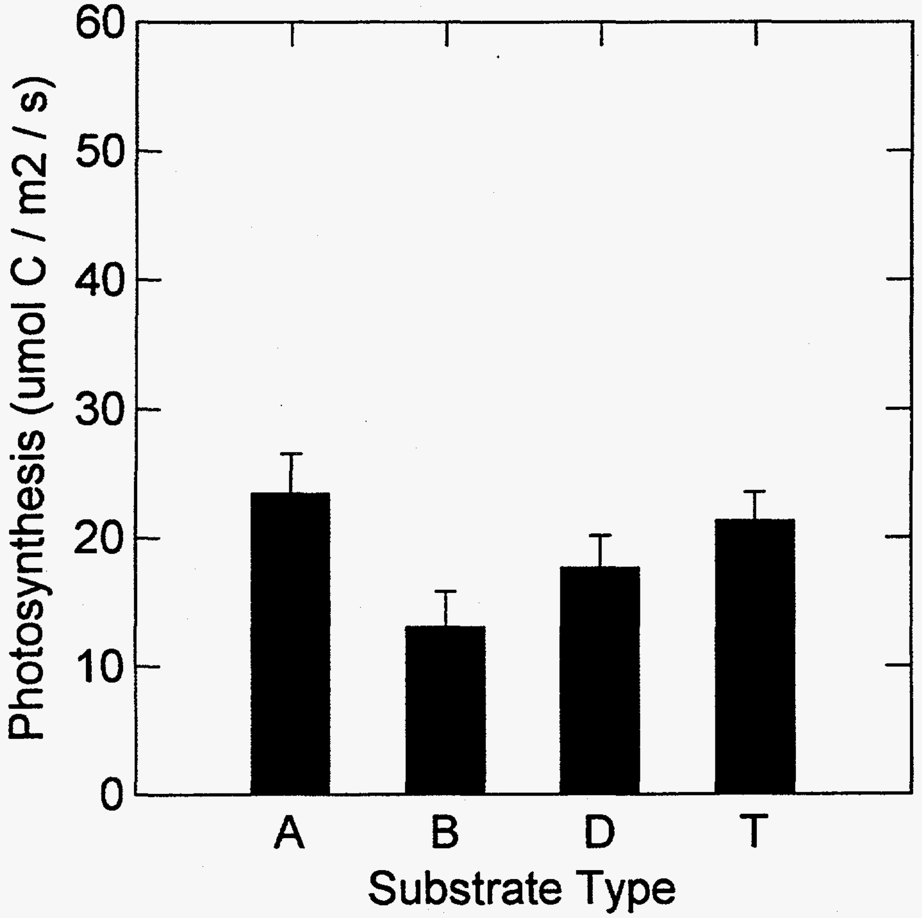

Figure 11. The effect of substrate type on the peak growing season 1996 photosynthetic response averaged across species (A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

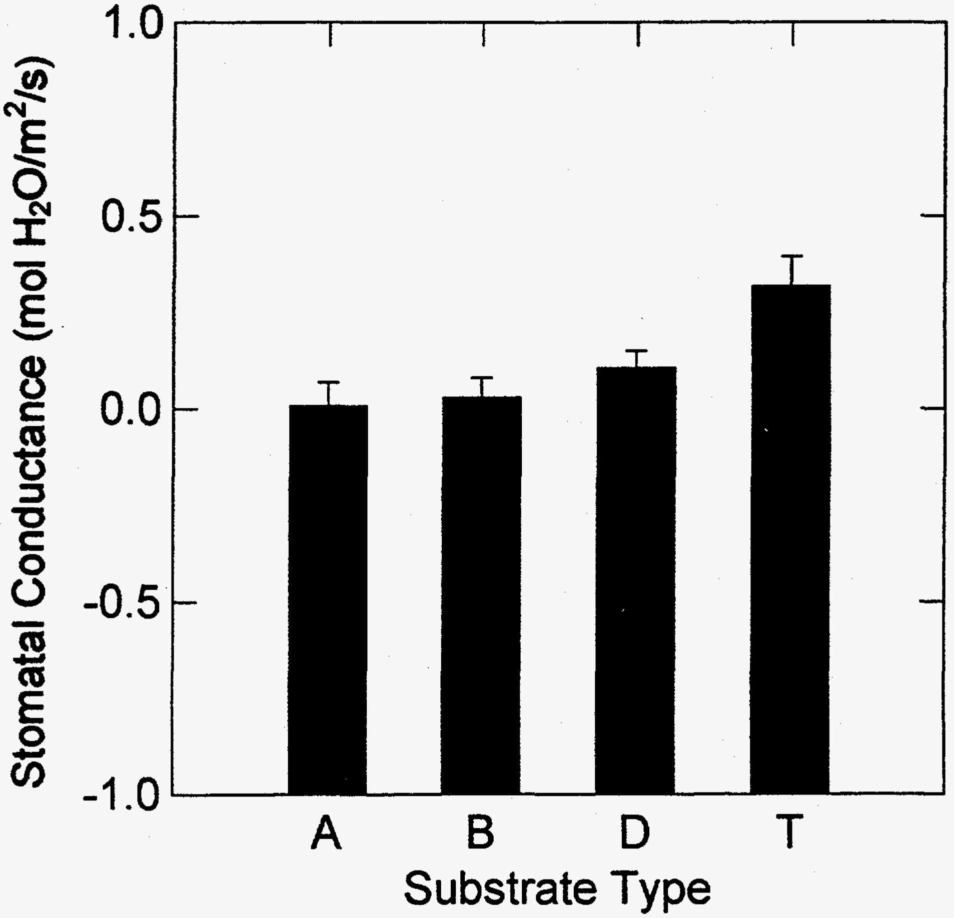

Figure 12. The effect of substrate type on stomatal conductance averaged across species during peak growing season 1996 (A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

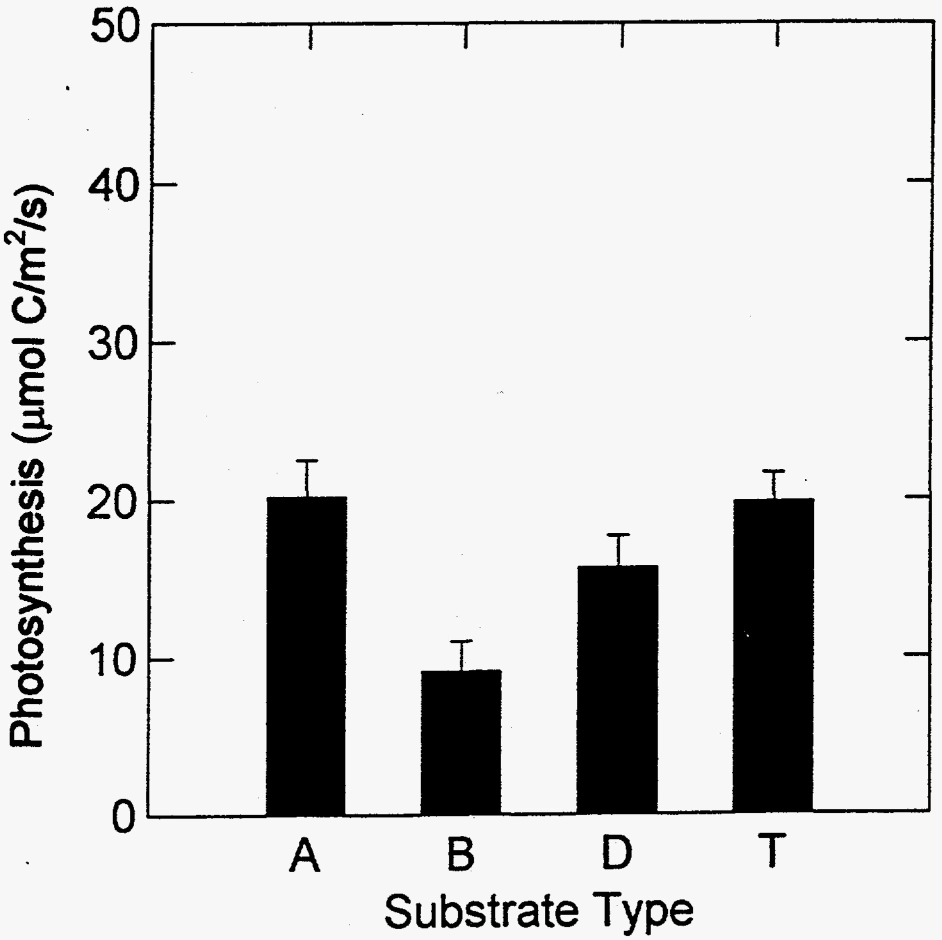

Figure 13. The effect of substrate type on photosynthetic response at the end of the 1996 growing season averaged across species (A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

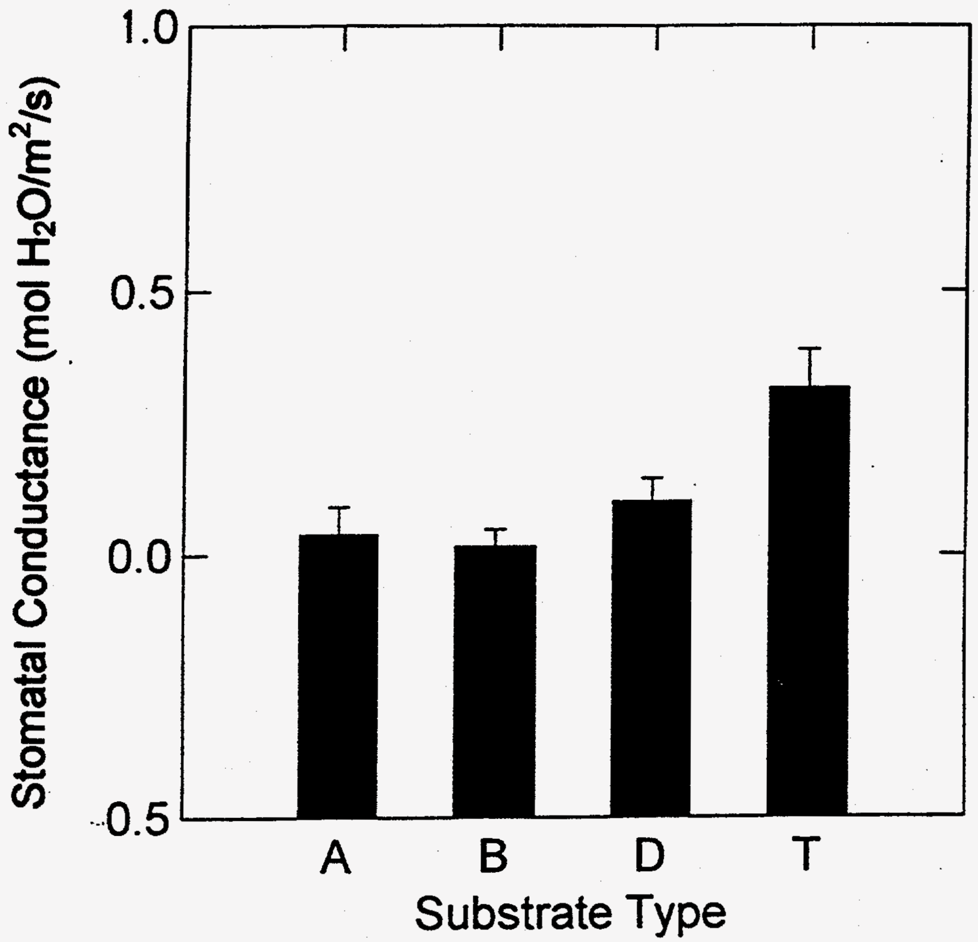

Figure 14. The effect of substrate type on end of 1996 growing season stomatal conductance averaged across species (A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

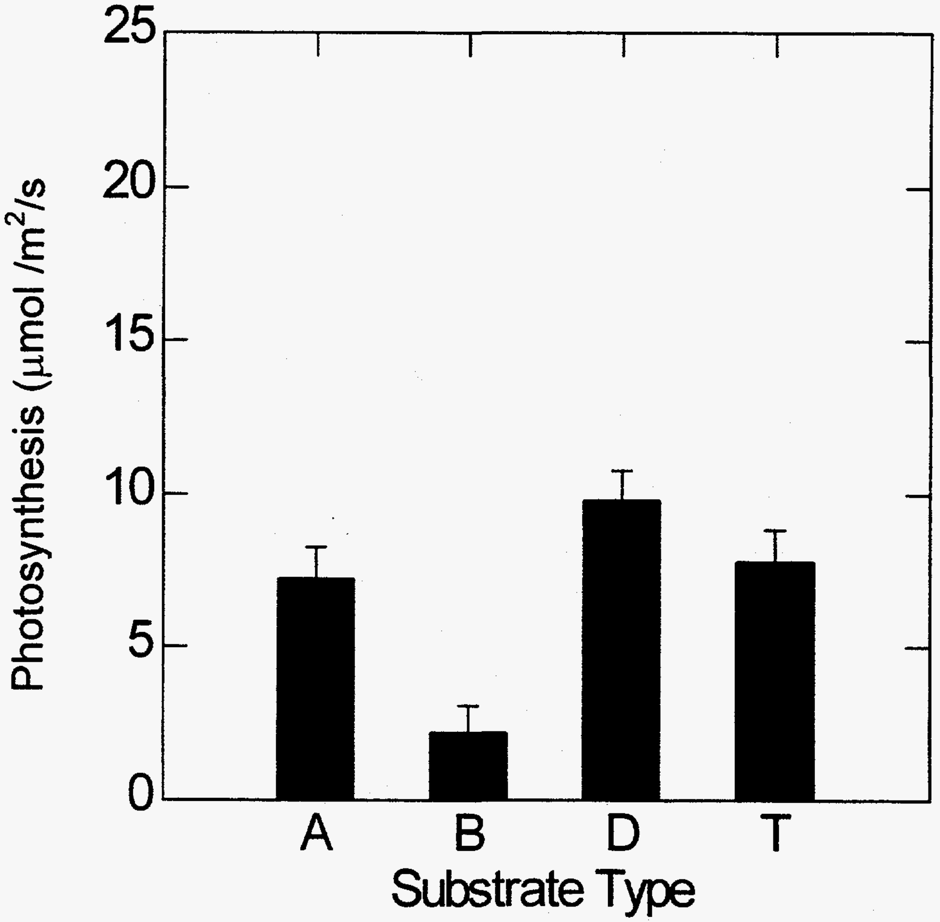

Figure 15. The main effect of substrate type on photosynthetic response at the end of the 1997 growing season averaged across species (A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

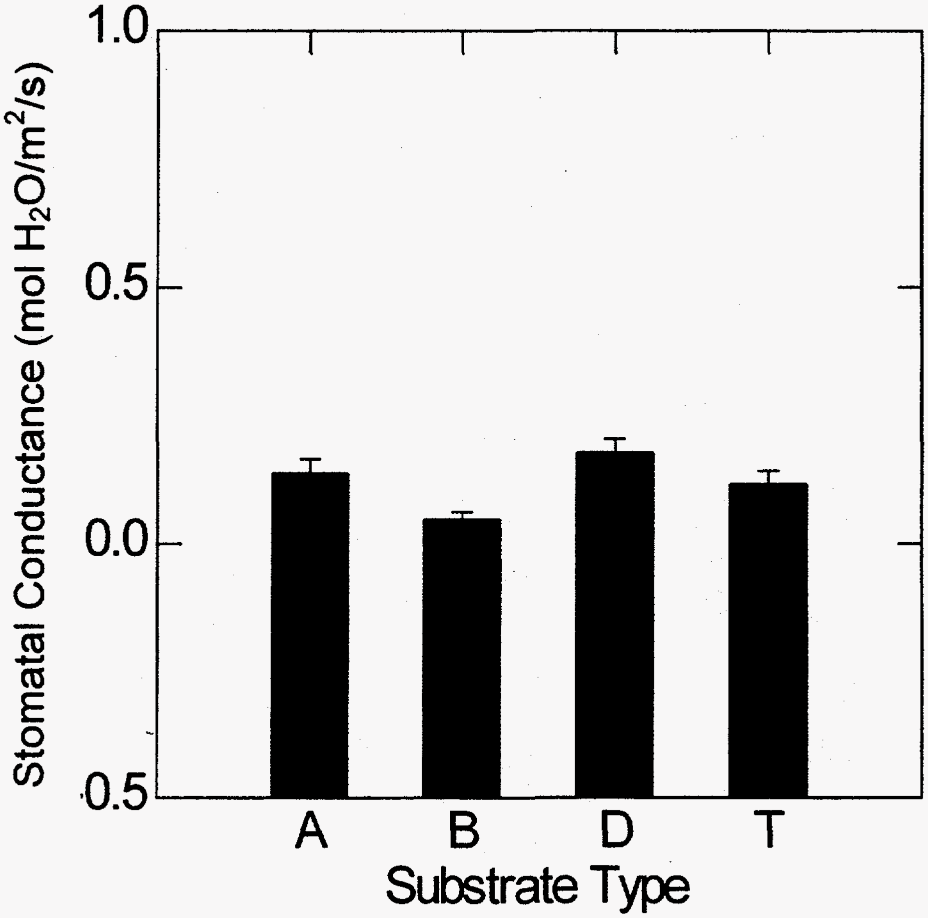

Figure 16. The effect of substrate type on stomatal conductance at the end of the 1997 growing season averaged across species (A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

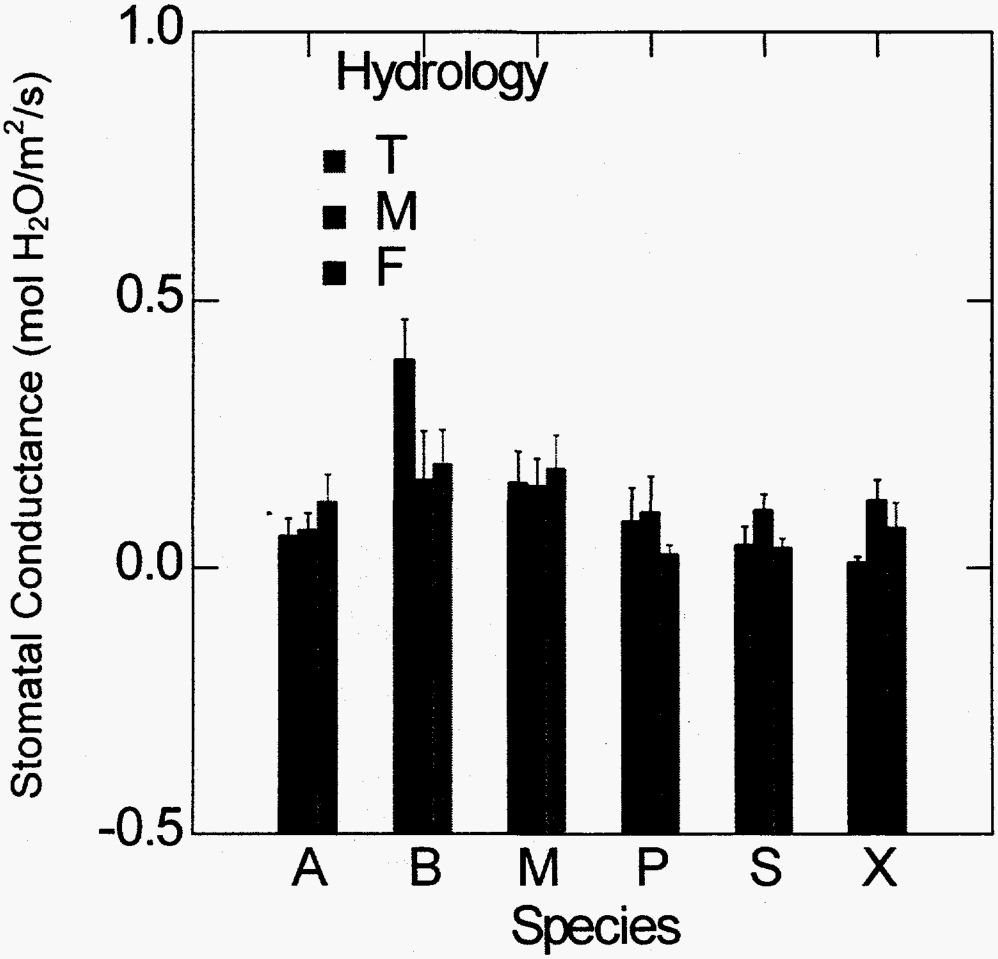

Figure 17. The interaction of plant species and hydrologic regime on stomatal conductance at the end of the 1997 growing season (A = elephant's-ear, B = bulltongue, M = cattail, P = alligator weed, S = wiregrass group #1, X = wiregrass group #2; F = flooded, M = moist-but-not flooded, T = tidal).

Hydrologic Regime. With respect to photosynthetic rate (net CO_2 assimilation) the interaction between hydrologic regime and vegetative species was significant during the 1996 peak growing season (Figure 18; $F = 2.55$, $P = 0.014$), as well as at the end of the 1996 growing season ($F = 2.92$, $P = 0.006$) indicating that the species responses were not consistent across hydrologic regimes. For example, elephant's-ear and maidencane displayed a the highest net CO_2 assimilation in the tidal hydrologic regime not displayed by the other species (Figure 18). The main effect of hydrologic regime, as well as the interaction of hydrologic regime and substrate type, was not significant in 1996 (neither peak nor end of the growing season).

Plant Species. There was a highly significant plant species effect on photosynthetic response during 1996 (both peak and end of the growing season). At the end of the growing season these differences were most evident (Figure 19; $F = 55.21$, $P < 0.0001$). All of the species included in the project, across substrates, fixed CO_2 at moderate to high rates (Figure 19). However, the clones of wiregrass (*Spartina patens*), a plant that utilizes the C_4 photosynthetic pathway, displayed rates of photosynthesis that were much higher than the other species (contrast $F = 218.91$, $P < 0.0001$).

There was a significant species effect on stomatal conductance (Figure 20; $F = 18.12$, $P < 0.0001$). The stomatal conductance rates of *Spartina patens* were the among the lowest of the species assessed, thereby resulting in much greater water use efficiencies in this species (compare Figures 19 and 20).

Aboveground Biomass

At the end of the 1996 growing season the main effects of substrate and species were significant ($F = 97.20$, $P < 0.0001$; $F = 54.92$, $P < 0.0001$, respectively). However, a highly significant interaction of species with substrate type ($F = 37.07$, $P < 0.0001$, Figure 21) precluded direct interpretation of these main effects. In all species, Substrate B yielded the poorest biomass production (contrast $F = 141.94$, $P < 0.0001$). The forbs (bulltongue and elephant's-ear) produced the greatest biomass when grown on the topsoil substrate. Interestingly, the wiregrass (*Spartina patens*) clones had the greatest biomass when grown on either Substrate A or dredge spoil and actually displayed a slight decrease when grown

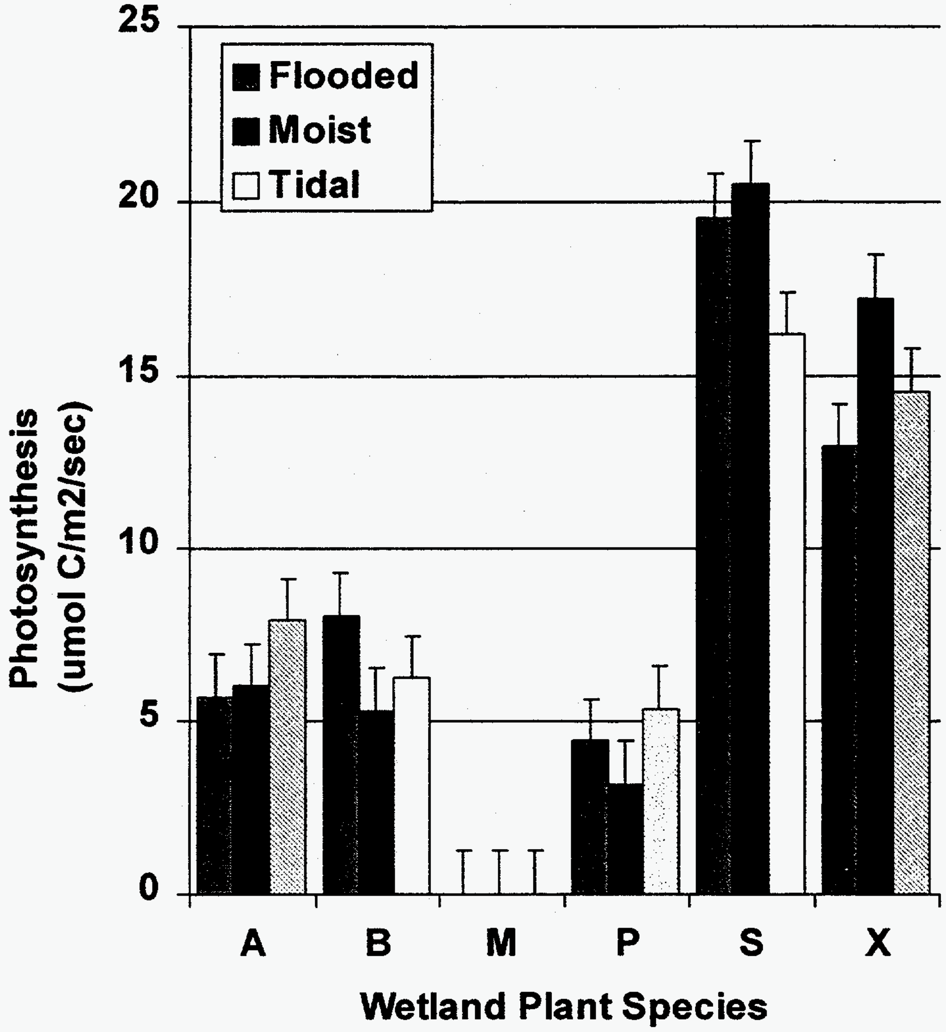

Figure 18. Peak season 1996 photosynthetic rate for the interaction between plant species and hydrologic regime (A = elephant's-ear, B = bulltongue, M = mudflat, P = maidencane, S = wiregrass group #1, X = wiregrass group #2, F = flooded, M = moist-but-not flooded, T = tidal).

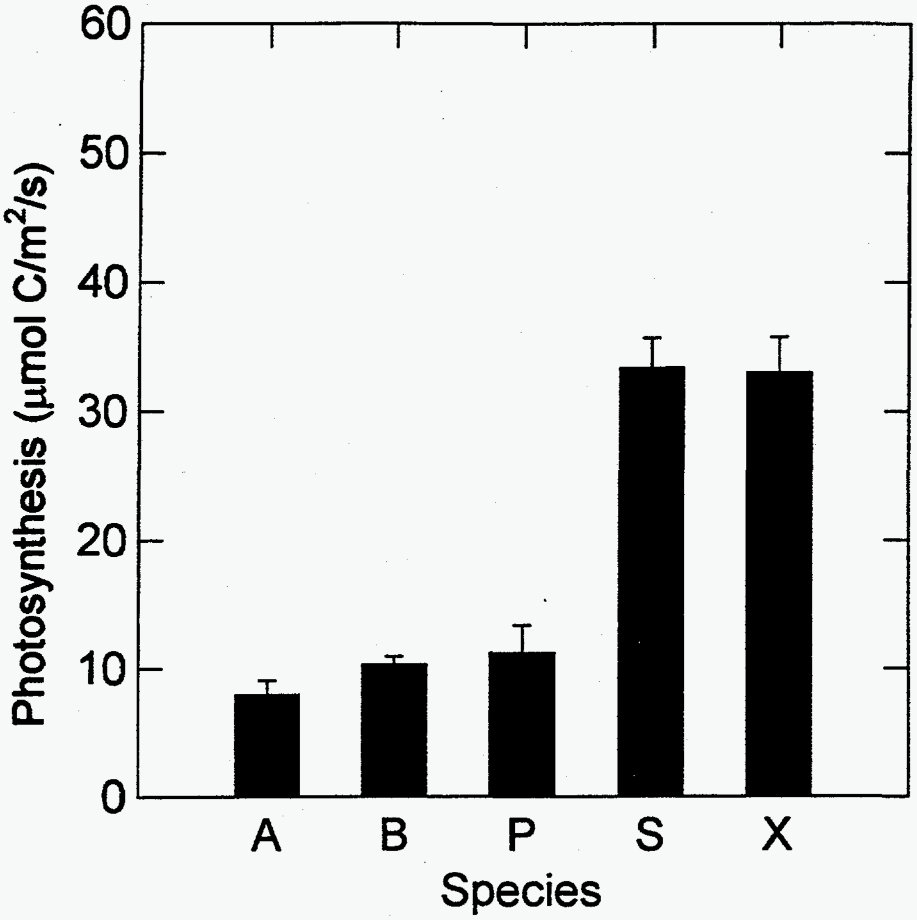

Figure 19. The main effect of plant species on photosynthetic rate at the end of 1996 growing season (A = elephant's-ear, B = bulltongue, M = mudflat, P = maidencane, S = wiregrass group #1, X = wiregrass group #2).

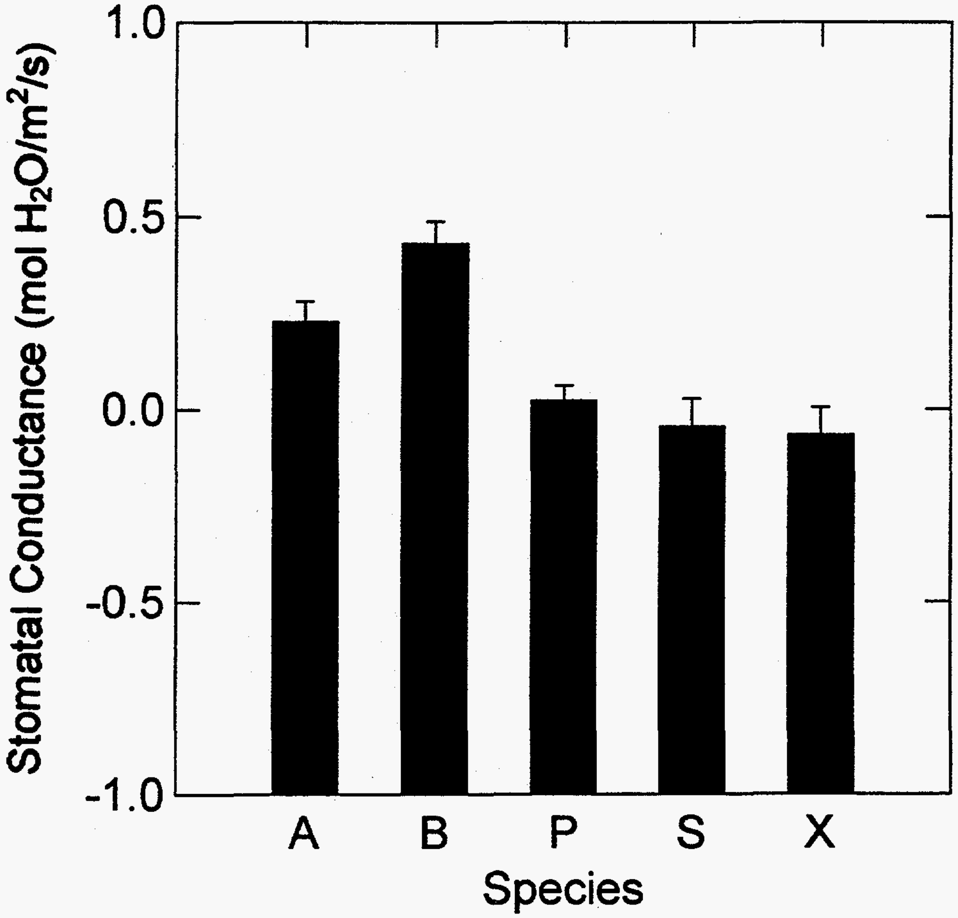

Figure 20. The main effect of plant species on stomatal conductance at the end of 1996 growing season (A = elephant's-ear, B = bulltongue, M = mudflat, P = maidencane, S = wiregrass group #1, X = wiregrass group #2).

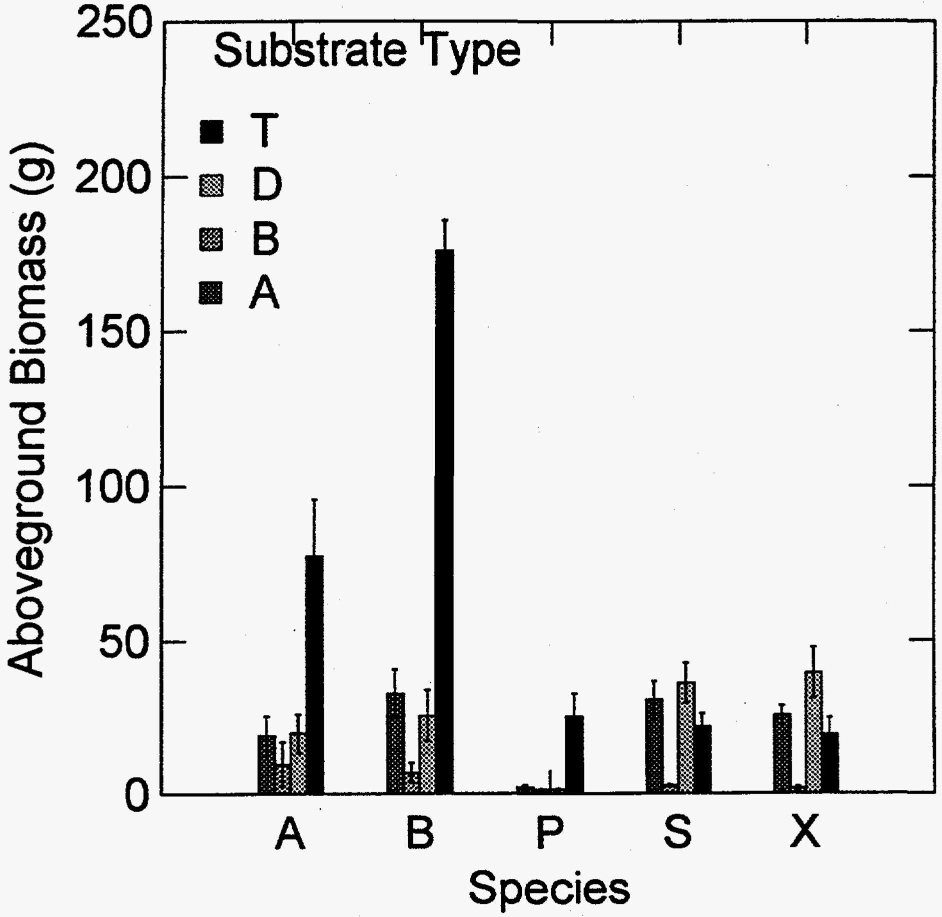

Figure 21. The interaction of plant species and substrate type on aboveground biomass for the 1996 growing season (A = elephant's-ear, B = bulltongue, M = mudflat, P = maidencane, S = wiregrass group #1, X = wiregrass group #2; A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

on topsoil (Figure 21). However, maidencane had depressed biomass production on all substrates except topsoil.

At the end of the 1997 growing season a significant three-way interaction on aboveground biomass was detected between substrate, hydrology, and species effects (Figure 22; F = 2.02, P = 0.008). This three-way interaction was primarily due to decreased aboveground growth on Substrate B, especially under flooded conditions (Figure 22). Cattail was one of the few species that accumulated any aboveground biomass in Substrate B. Although wiregrass generally produced the most aboveground biomass, it produced little to no aboveground biomass under permanently flooded conditions (Figure 22). Furthermore, when grown on dredge spoil or top soil substrates wiregrass tended to produce the greatest aboveground biomass under the mesic condition, but when grown on Substrate A, this preference was not apparent (Figure 22).

Belowground Biomass

At the end of the 1997 growing season belowground biomass response displayed significant interactions of substrate by species (F = 3.57, P < 0.0001) and species by hydrologic regime (F = 5.59, P < 0.0001). As was observed in aboveground biomass, the primary interaction between substrate and species was due to the poor plant growth observed in Substrate B and also the exceptional belowground biomass produced by elephant's-ear when grown in the dredge spoil and topsoil substrates (Figure 23). The significant interaction between species and hydrology was driven largely by the increased belowground biomass production of wiregrass and the other grass species (cattail) when grown under mesic conditions, whereas the forbs tended to have higher belowground biomass under tidal or permanently flooded conditions (Figure 24).

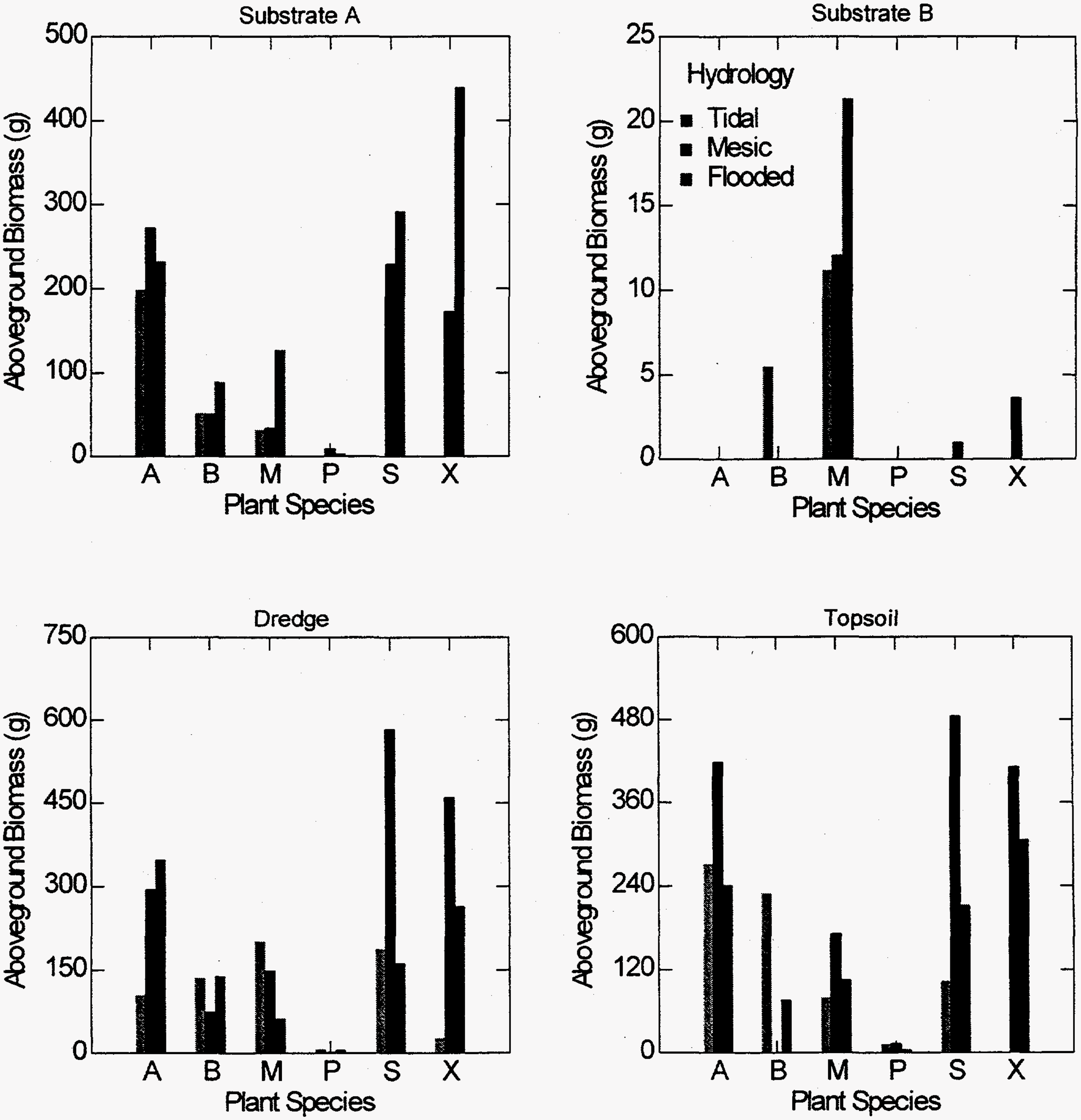

Figure 22. The effect of plant species (A = elephant's-ear, B = bulltongue, M = cattail, P = alligator weed, S = wiregrass group #1, X = wiregrass group #2) and hydrologic regime on aboveground biomass produced during the 1997 growing season for each of the four substrate types. Note: y-axis scales are not uniform across substrate types.

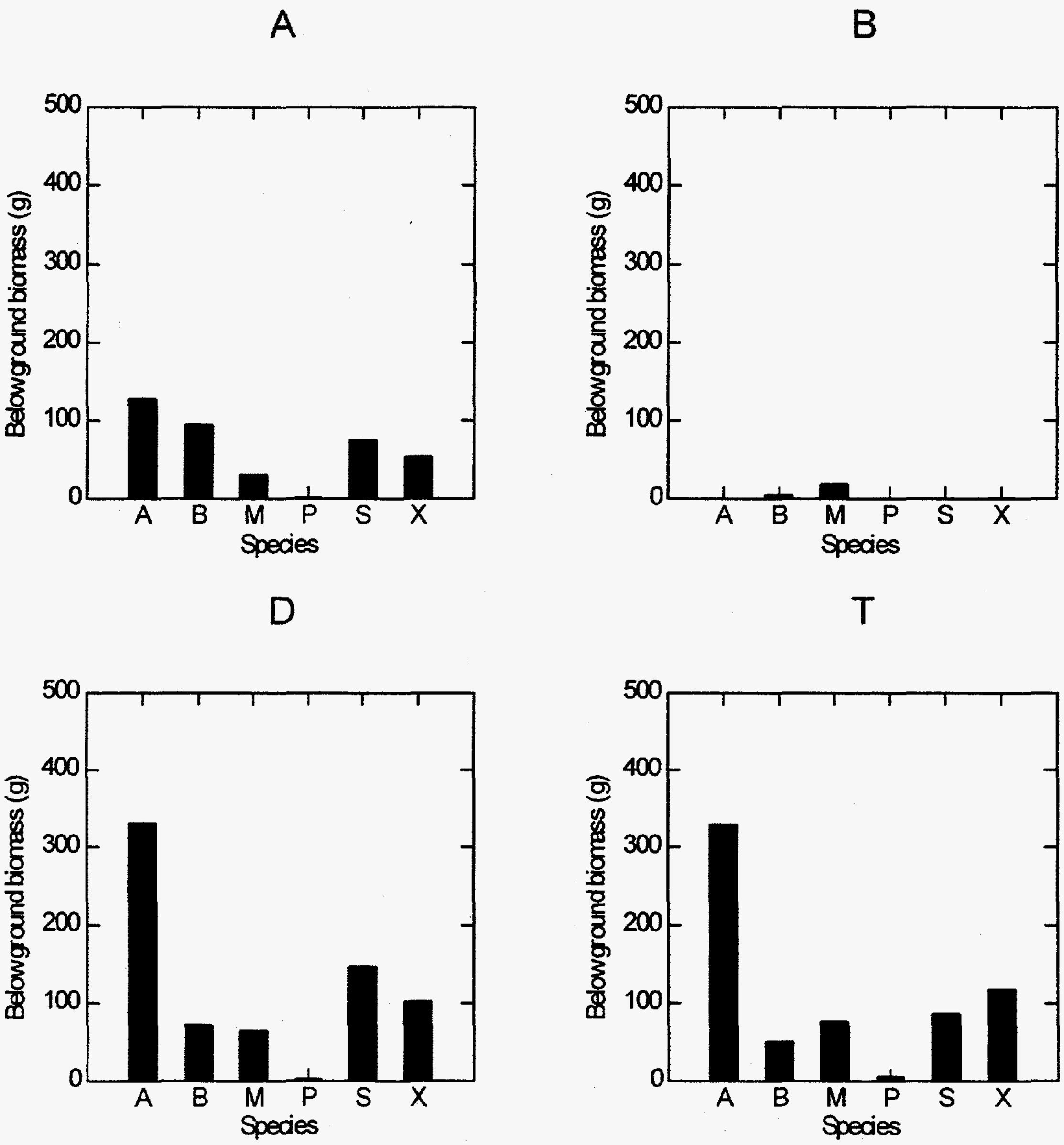

Figure 23. The effect of plant species (A = elephant's-ear, B = bulltongue, M = cattail, P = alligator weed, S = wiregrass group #1, X = wiregrass group #2), on belowground biomass production by substrate type (A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

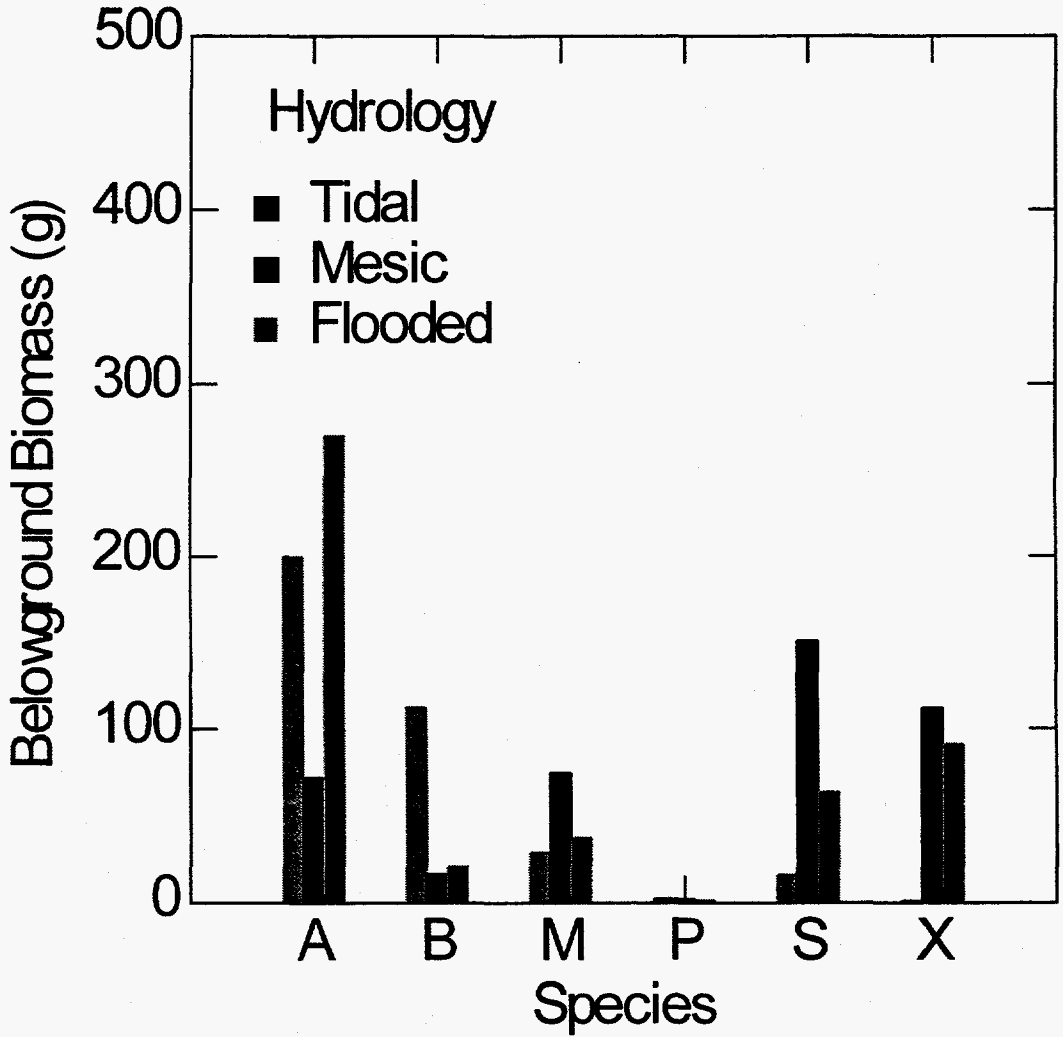

Figure 24. The effect of plant species (A = elephant's-ear, B = bulltongue, M = cattail, P = alligator weed, S = wiregrass group #1, X = wiregrass group #2) and hydrologic regime on belowground biomass production.

Assessment of Interclonal Variability in *Spartina patens*

Analysis of photosynthetic response during the peak 1996 growing season revealed highly significant genotype ($F = 4.66$, $P = 0.001$) and substrate ($F = 11.59$, $P < 0.0001$) effects. During this peak 1996 growing season, two genotypes (S69 and X3) had greater rates of photosynthesis than the other genotypes (Figure 25; contrast $F = 20.72$, $P < 0.0001$). Substrate B resulted in significantly lower rates of photosynthesis across genotypes than all other substrates (Figure 26; contrast $F = 29.05$, $P < 0.0001$). Interestingly, Substrate A resulted in rates of photosynthesis that were significantly higher than those of dredge spoil and top soil (Figure 26, contrast $F = 4.35$, $P = 0.039$).

At the end of the1996 growing season, the effect of genotype on photosynthesis was no longer significant. However, the main effect of substrate on rates of photosynthesis (across genotypes) remained highly significant (Figure 27; $F = 4.89$, $P = 0.003$). As was observed during the peak 1996 growing season, Substrate B resulted in significantly lower rates of photosynthesis than all other substrates ($F = 12.16$, $P = 0.001$). However, by the end of 1996 growing season photosynthetic response on Substrate A was no longer different than those on dredge spoil or top soil (Figure 27; contrast $F = 2.42$, $P = 0.12$).

Aboveground biomass harvested at the end of the 1996 growing season revealed significant interactions of substrate by genotype ($F = 3.49$, $P < 0.0001$) and substrate by hydrology ($F = 2.57$, $P = 0.023$). The interaction of substrate by genotype (Figure 28) was largely driven by a preference of most genotypes for dredge spoil, whereas genotype S26 (and X8 to some extent) showed greater aboveground biomass on Substrate A. It is important to note that all genotypes produced the least amount of biomass on Substrate B (Figure 28). The interaction of substrate by hydrology is interpretable and was caused by the low aboveground biomass produced on Substrate B, regardless of hydrologic regime (Figure 29). In all other substrates, aboveground biomass was greatest under the mesic hydrologic regime (Figure 29 and 30), which had greater aboveground biomass than the tidal hydrologic regime (contrast $F = 8.61$, $P = 0.004$), which in turn had greater aboveground biomass than the flooded hydrologic regime (contrast $F = 3.92$, $P = 0.050$).

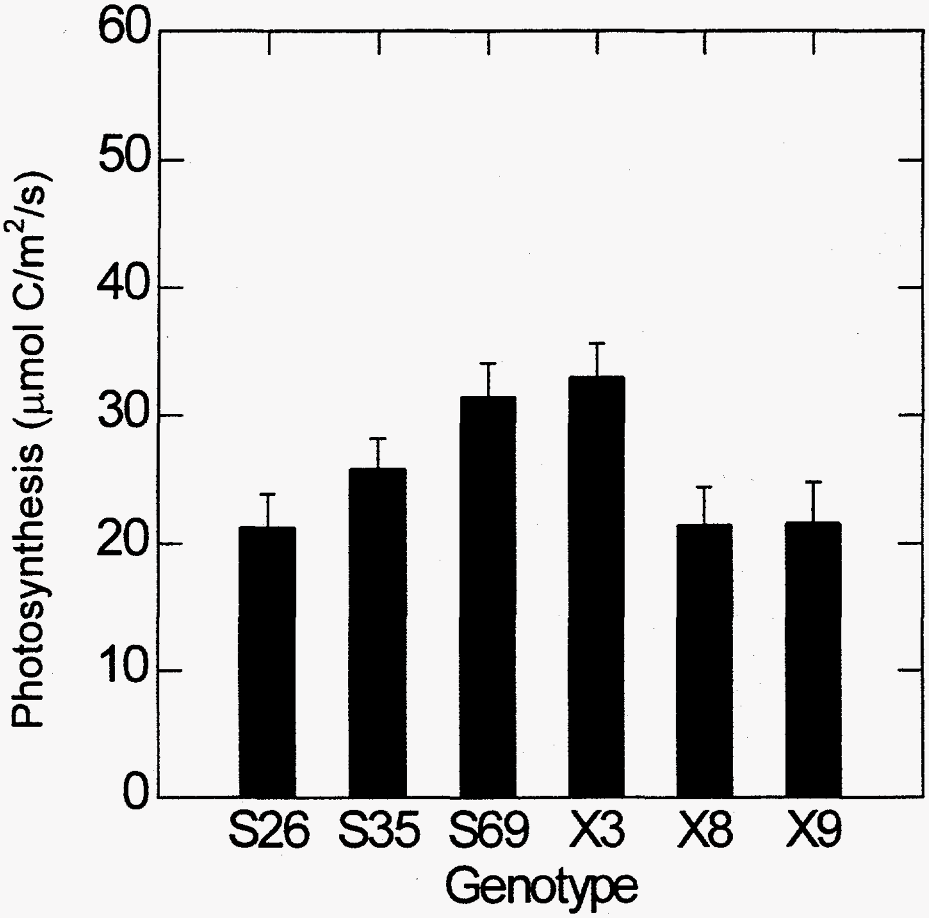

Figure 25. The main effect of genotype on photosynthetic rate of wiregrass during the peak 1996 growing season.

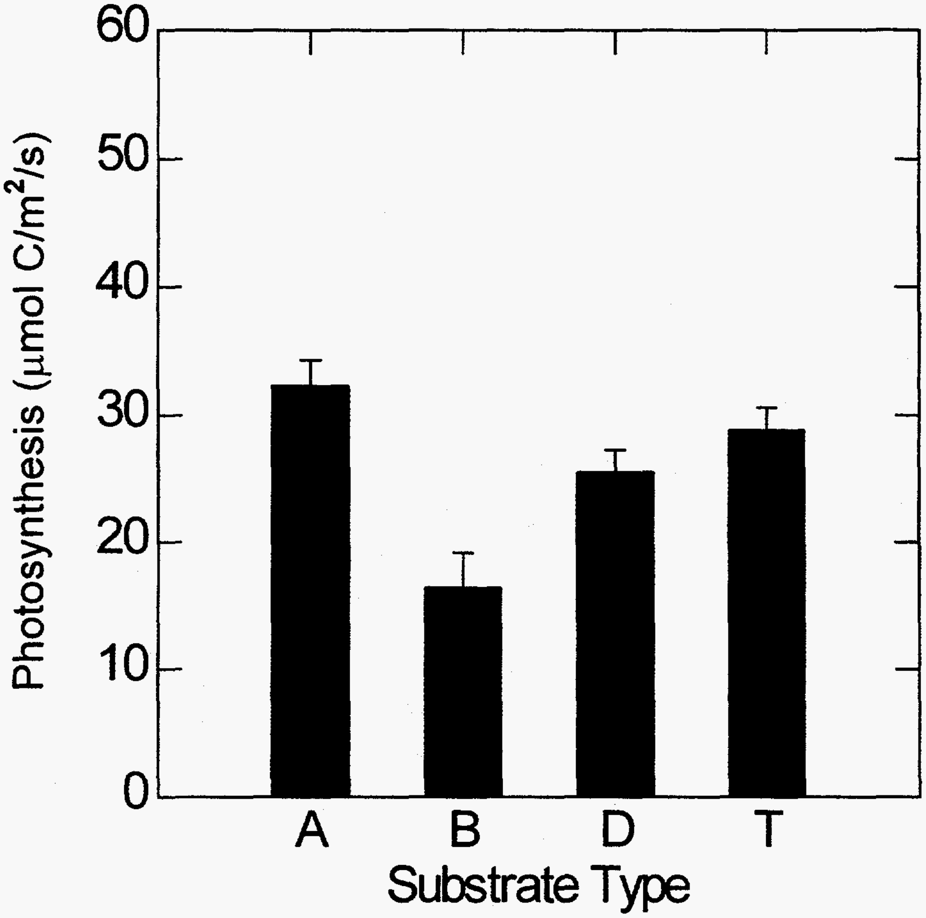

Figure 26. The effect of substrate type on photosynthetic rate of wiregrass during the peak 1996 growing season (A = Substrate A, B = Substrate B, D = dredge spoil, and T =topsoil).

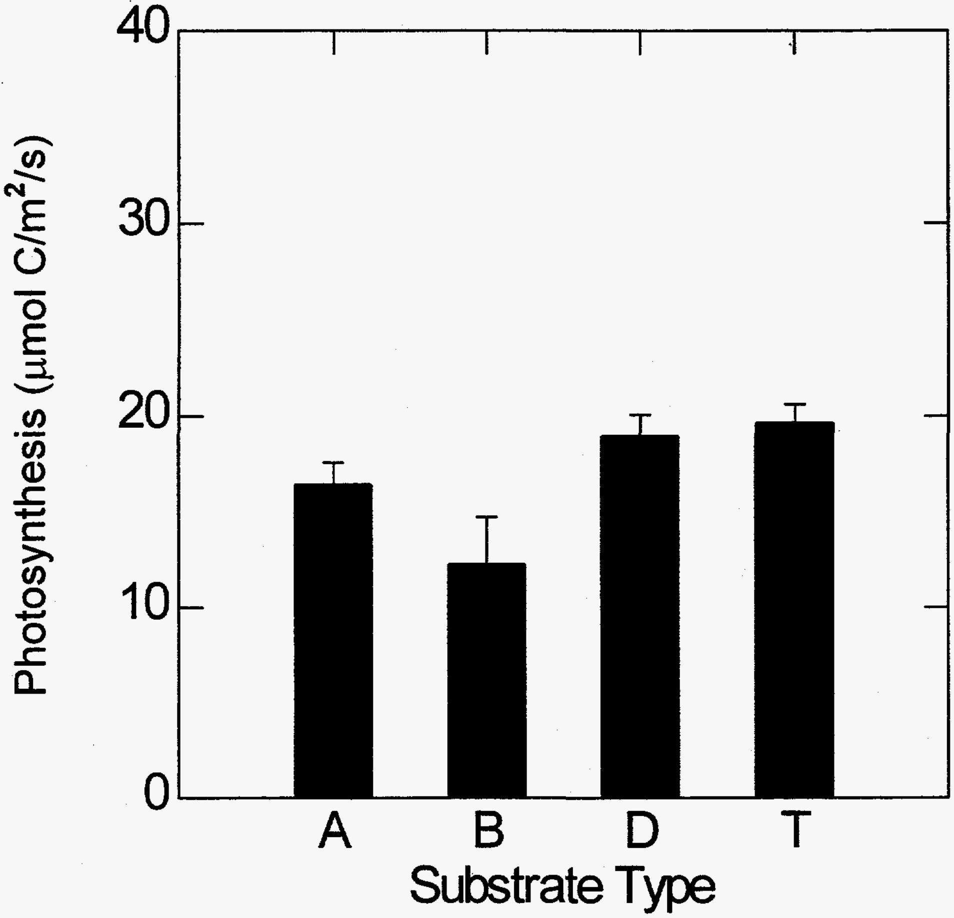

Figure 27. The effect of substrate type on photosynthetic rate of wiregrass averaged across genotypes at the end of the 1996 growing season (A = Substrate A, B = Substrate B, D = dredge spoil, and T = topsoil).

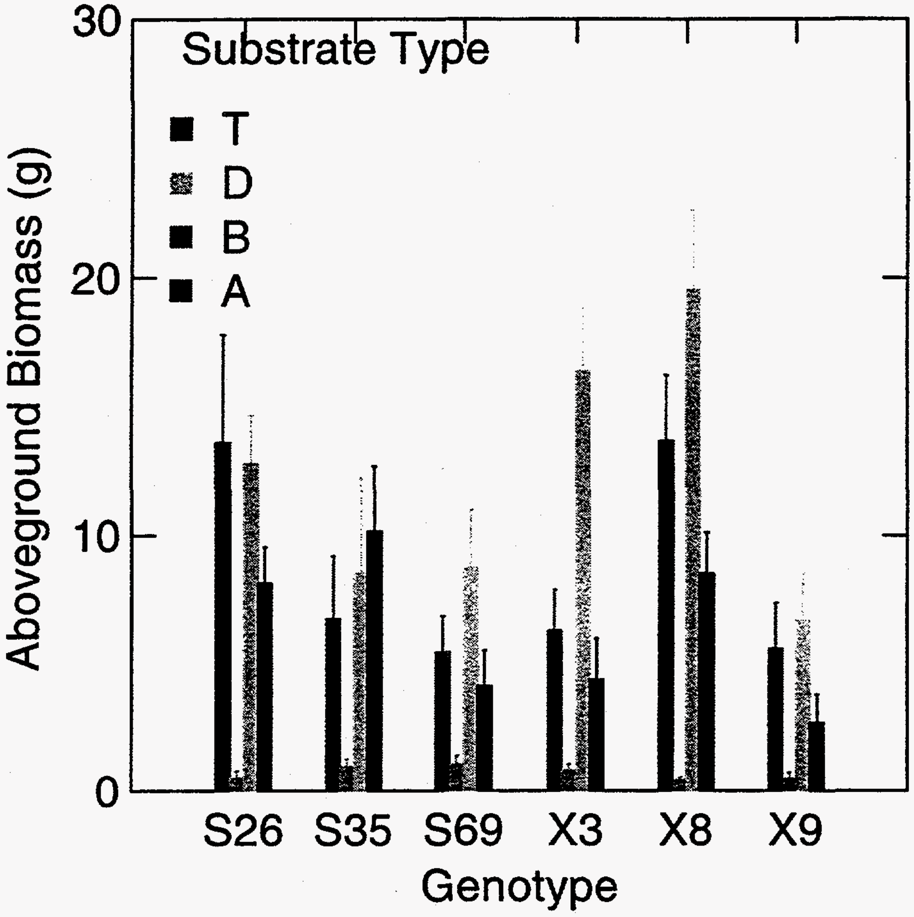

Figure 28. The effect of genotype and substrate type on aboveground biomass production during the 1996 growing season (A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

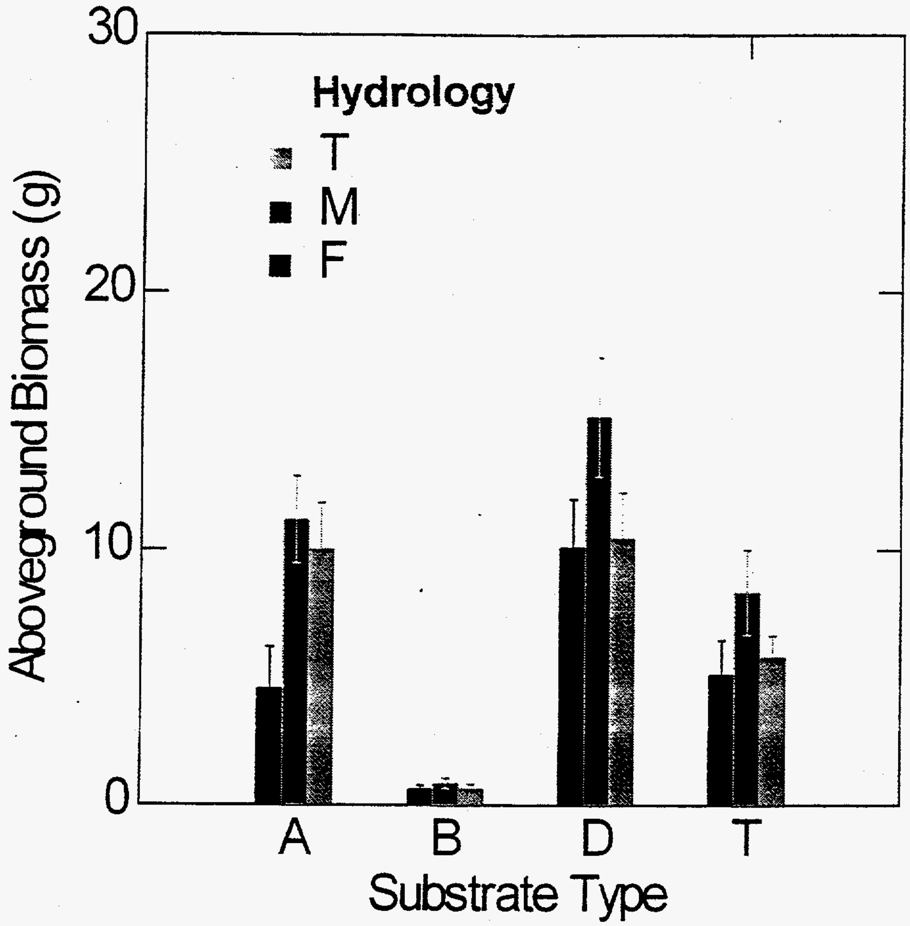

Figure 29. The interaction of substrate and hydrology on aboveground biomass production of wiregrass for the 1996 growing season (A = Substrate A, B = Substrate B, D = dredge spoil, and T = topsoil; F = flooded, M = moist-but-not flooded, T = tidal).

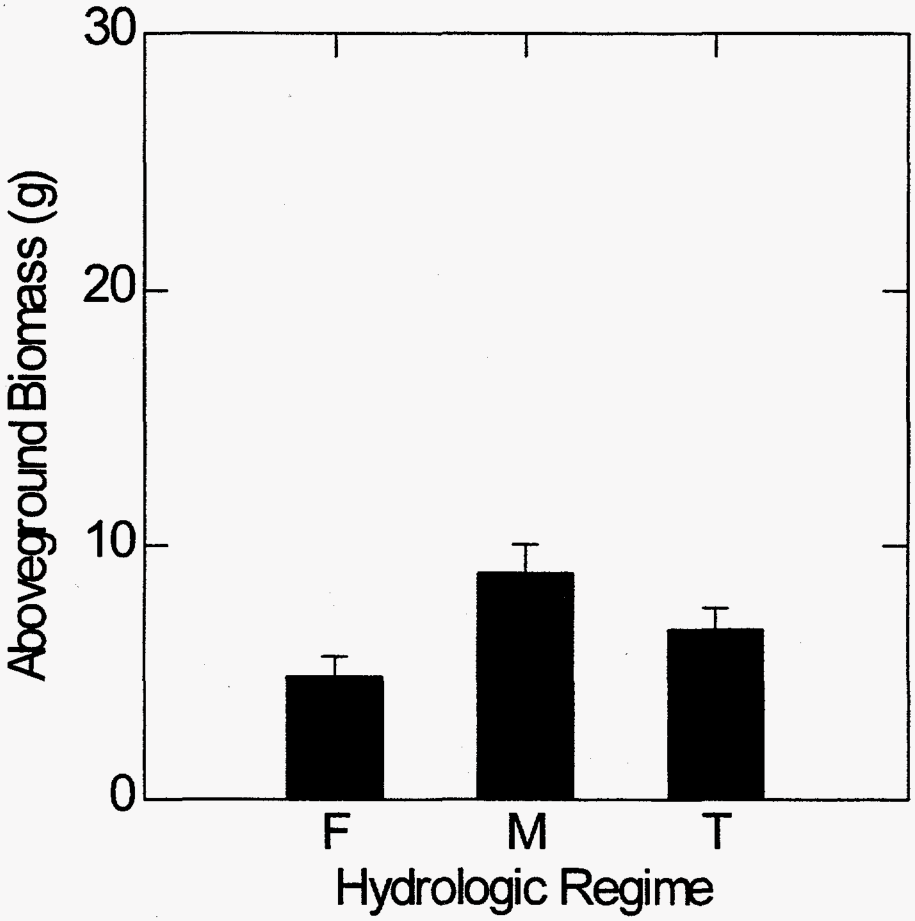

Figure 30. The effect of hydrologic regime on aboveground biomass production of wiregrass for the 1996 growing season (F = flooded, M = moist-but-not flooded, T = tidal).

At the end of the 1997 growing season, there remained a significant amount of variation between clones of wiregrass in their ability to become established and grow in the various substrate types. In some cases, differences were as large or greater than those observed between different species. In general, all clones grew poorly, or not at all, in Substrate B. However, there was a significant substrate by genotype interaction (Figure 31; $F = 4.97$, $P < 0.0001$) on total plant biomass. This is of particular interest because it demonstrates the superior ability of genotypes S26 and to a lesser extent S35 to grow and establish on Substrate A (Figure 31). A significant substrate by hydrology interaction ($F = 6.06$, $P < 0.0001$) showed a general lack of growth on Substrate B regardless of hydrology, whereas the other substrates generally produced the poorest growth under permanently flooded conditions (Figure 32). Furthermore, a significant interaction of genotype by hydrology (Figure 33, $F = 3.10$, $P = 0.002$) is explained by the relatively poor growth of genotypes S69 and X3 under all hydrologic regimes and the extremely limited production of S69, X3, and X9 under the flooded hydrologic regime. As was observed during the1996 growing season, most genotypes accumulated the greatest biomass under the mesic hydrology followed by the tidal hydrology (Figure 33).

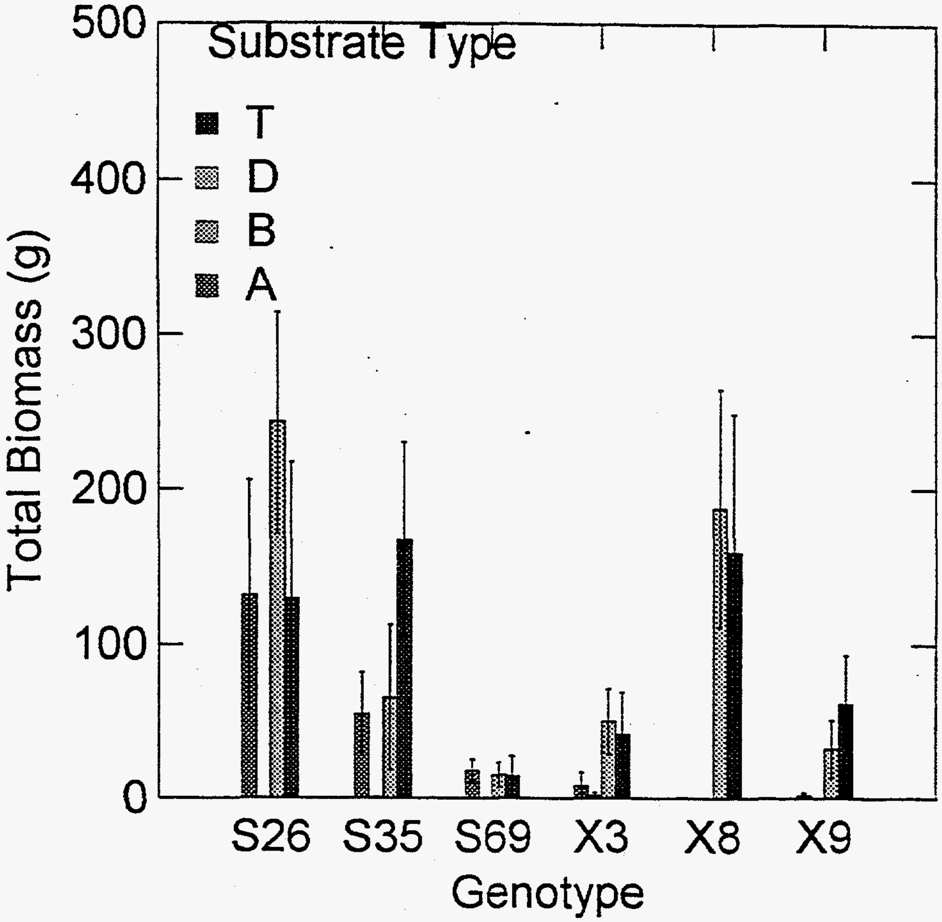

Figure 31. The interaction of genotype and substrate type on total biomass production of wiregrass for the 1997 growing season (A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

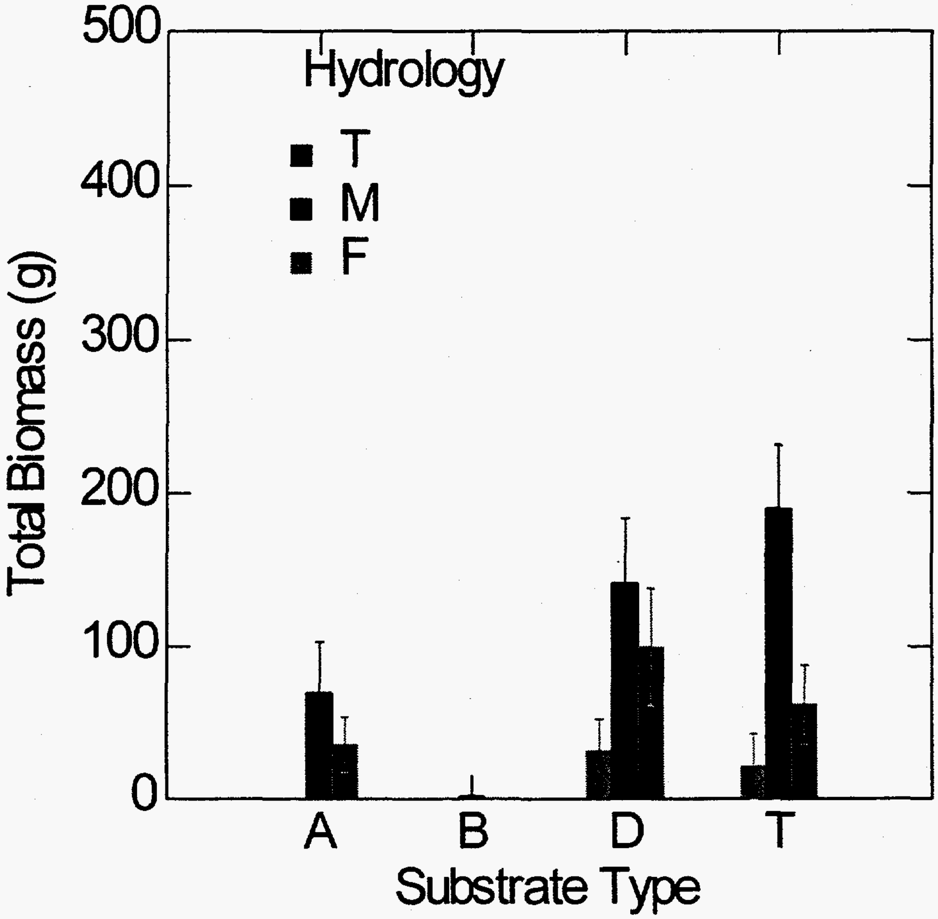

Figure 32. The effect of substrate type and hydrologic regime on the total biomass production of wiregrass for the 1997 growing season (F = flooded, M = moist-but-not flooded, T = tidal; A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

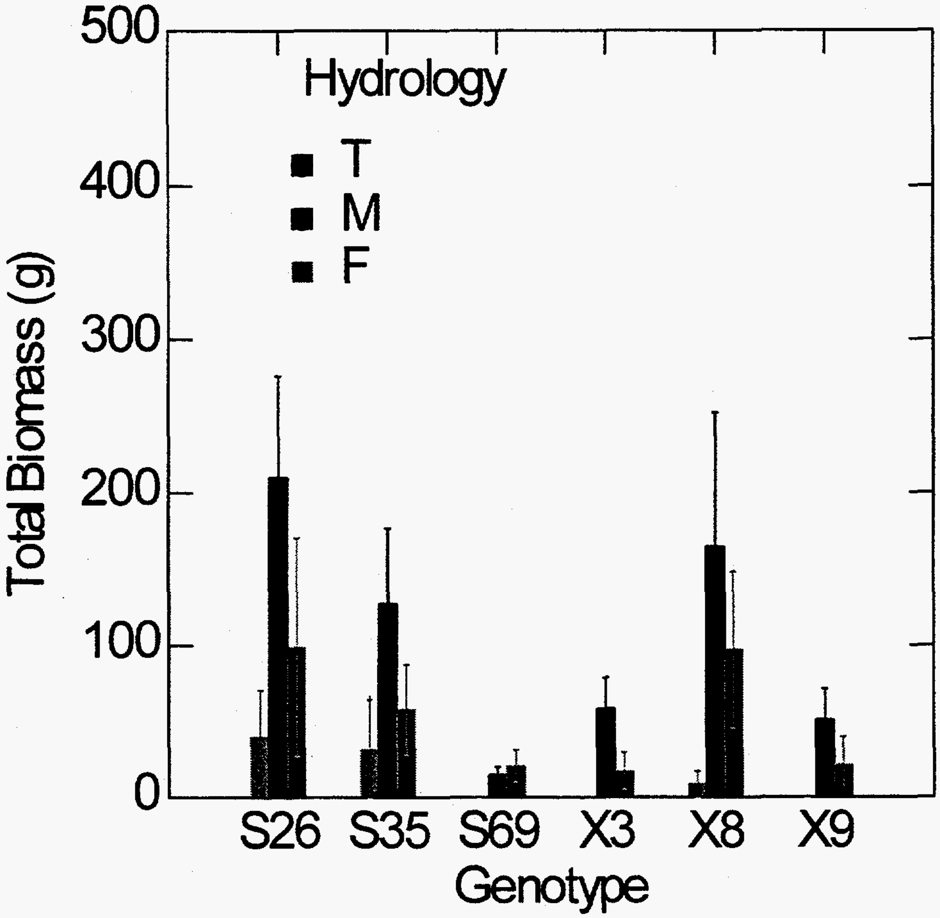

Figure 33. The interaction of genotype and hydrologic regime on total biomass production of wiregrass for the 1997 growing season (F = flooded, M = moist-but-not flooded, T = tidal).

Assessment of Submerged Aquatic Vegetation

A qualitative assessment of the ability of two species of submerged aquatic vegetation to grow on the four substrates supported our other findings (Table 8). Top soil resulted in the highest growth and establishment, followed by dredge spoil and Substrate A, which performed similarly (Table 8). In agreement with our previous findings on emergent vegetation, submergent vegetation performed poorly or failed to survive on Substrate B. Out of six mesocosm vessels of Substrate B utilized in this assessment, *Cabomba caroliniana* failed to survive in any of the vessels and *Egeria densa* survived in only one vessel with correspondingly weak growth (Table 8).

Assessment of Alternative Emergent Plant Species

Of the nine species assessed during 1997, eight survived and established across substrates. However, as was observed with all the plant species that were evaluated (emergent and submergent), Substrate B proved to be the least favorable for supporting the establishment and growth of wetland vegetation (Figure 34). Substrate A supported considerable growth of these alternative species (Figure 35), especially cattail, baldcypress, duck-potato, flat sedge, and seaside goldenrod. Of these alternative species, cattail tended to produce the greatest biomass regardless of substrate type (Figure 34).

Benthic Algal Productivity

All substrate types displayed colonization by benthic algae during the course of this study. In stark contrast to the responses of the vascular plant species (submergent and emergent), benthic algal productivity was greatest on Substrate B (Figure 36, contrast $F = 37.04$, $P < 0.0001$). Net benthic algal productivity was moderate on the other three substrate types (Figure 36) with Substrate A resulting in productivity not different from dredge spoil or top soil (contrast $F = 0.620$, $P = 0.44$).

Table 8. Qualitative performance rankings for the submerged aquatic vegetation species *Egeria densa* (elodea) and *Cabomba caroliniana* (fanwort) conducted at the end of the 1997 growing season. Results are presented for each mesocosm vessel containing these species. Performance ranking scores are as follows:
-- = mortality; * = survived; ** = survived and grew; *** = survived and grew vigorously.

Substrate Type	Elodea	Cabomba
Substrate A	**	***
Substrate A	**	**
Substrate A	--	*
Substrate A	*	*
Substrate A	*	*
Substrate A	*	*
Substrate B	--	--
Substrate B	--	--
Substrate B	*	--
Substrate B	--	--
Substrate B	--	--
Substrate B	--	--
Dredge Spoil	*	*
Dredge Spoil	***	**
Dredge Spoil	**	**
Dredge Spoil	*	*
Dredge Spoil	**	**
Dredge Spoil	***	--
Top Soil	***	***
Top Soil	***	***
Top Soil	***	***
Top Soil	*	*
Top Soil	**	***
Top Soil	***	--

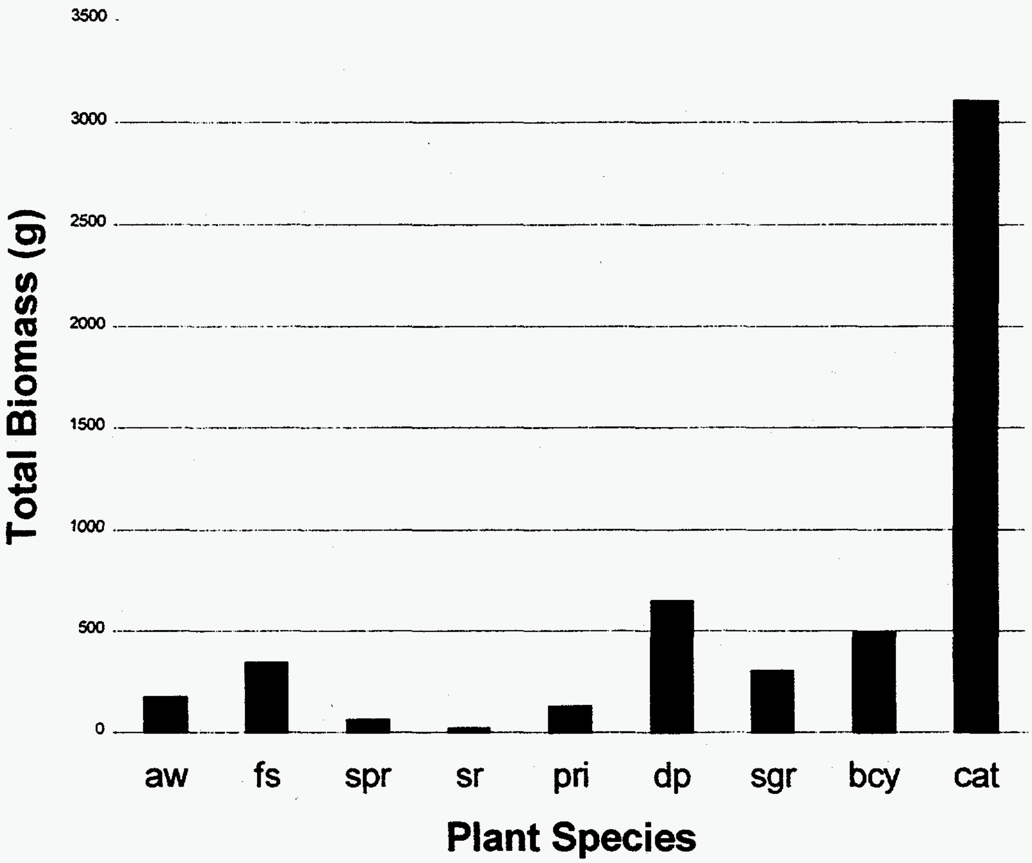

Figure 34. Total biomass production of nine alternative emergent plant species averaged across substrate types and hydrologic regimes (aw = alligator weed, fs = flat sedge, spr = spike-rush, sr = soft rush, pri = swamp primrose, dp = duck-potato, sgr = seaside goldenrod, bcy = baldcypress, cat = cattail).

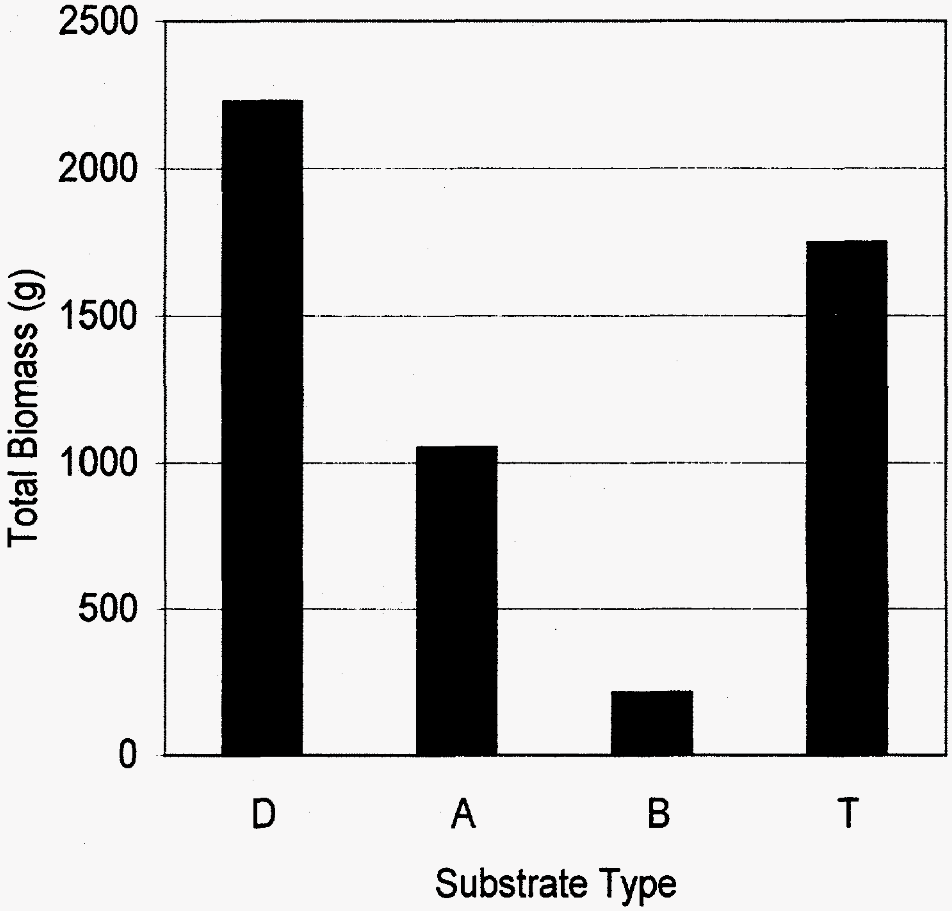

Figure 35. The effect of substrate type on total biomass production of nine alternative emergent plant species averaged across species and hydrologic regime (D = dredge spoil, A = Substrate A, B = Substrate B, T = topsoil).

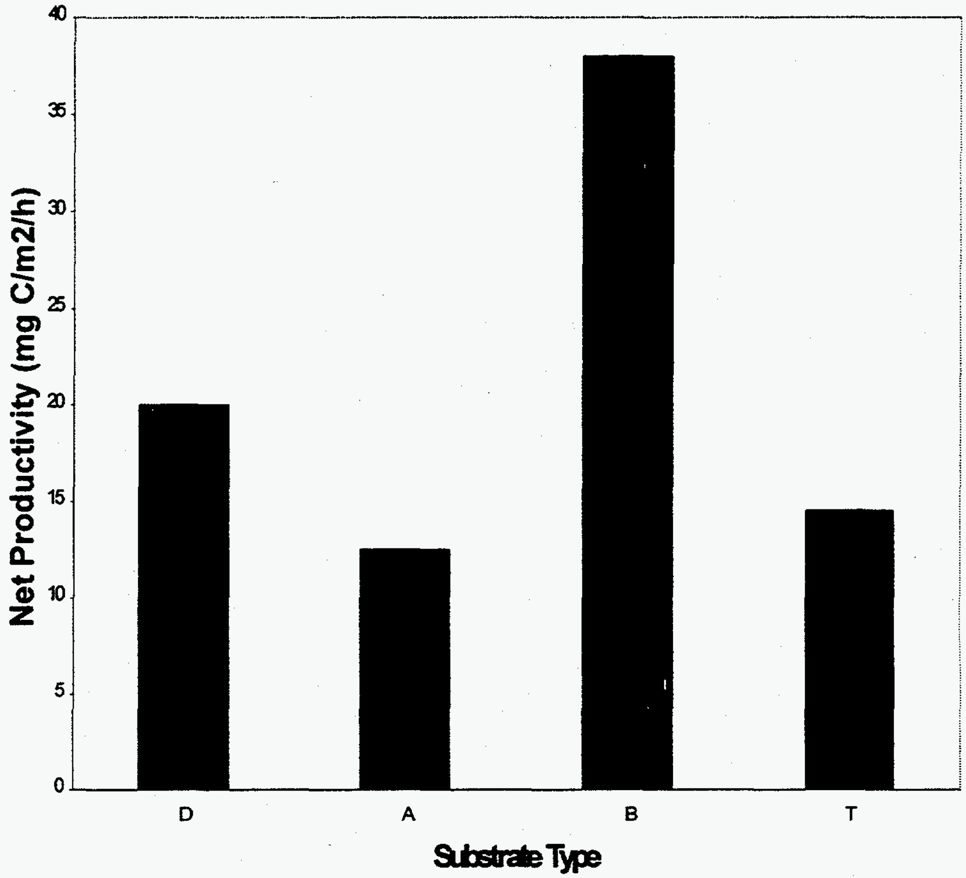

Figure 36. Net benthic algal productivity on the four substrate types at the end of the 1997 growing season (A = Substrate A, B = Substrate B, D = dredge spoil, T = topsoil).

Discussion

The results obtained thus far are promising with regard to the low toxicity of restored drill cuttings and their ability (particularly Substrate A) to support healthy wetland vegetation. Water extraction, acid digestion, and interstitial water samples from the restored drill cuttings all yielded elemental analyses that fell within the LaDNR 29-B guidelines. This is particularly encouraging since acid digestion represents the worst case scenario of total potentially extractable elements being released upon complete digestion of the substrate, a situation highly improbable in nature.

The toxicity trial results based on mysid shrimp showed that both restoration processes had acceptable toxicity levels (LC_{50}) at all percentages of suspended particulate phase (6% to 100%). Survival in the 100% suspended particulate phase was 90% in both substrates, further demonstrating the low toxicity of these restored drill cuttings.

Substrate A is remarkably similar to dredge spoil which is currently being used as a wetlands creation substrate (i.e., dedicated dredging, CWPPRA 1993). The few elements that were extracted into the interstitial water were primarily cations (Ca, K, and Mg) and were not elevated to a level that would pose a threat to wetlands productivity. Substrate B remained high in aluminum with concomitant high pH, which likely resulted in limited plant productivity through hindered nutrient uptake (Larcher 1995).

Varying methods have been successful at ameliorating alkaline soils and providing a suitable substrate for plant growth. Clemens and Singer (1992) found that basalt powder was an inexpensive form of iron additive and soil conditioner that produced superior results to the proven iron chelate ferric-ethylenediaminedihydrochloric acid (Fe-EDDHA). Organic litter, ranging from barnyard manure (Sharma and Yadav 1986) to leaves, twigs, and prunings of trees (Swarup 1988) has been shown to improve alkaline soil by providing an organic complex where macro and micronutrients may be available to plants. A study by Kelley and Mendelssohn (1995) demonstrated that restored drill cuttings support emergent wetland vegetation when combined with organic compost. The mixture of the two substrates resulted in interstitial water concentrations of K and Na that were significantly lower than in drill cuttings alone, possibly due to a higher cation exchange capacity (Mitsch and Gosselink 1993). Once a plant community has been established, root respiration and organic litter will aid in the continued reduction of soil pH (Chhabra and Abrol 1977, Kumar et al. 1994, Swarup 1988).

One potential negative aspect of Substrate A is elevated electrical conductivity (Table 1). However, other than the response of maidencane (restricted to fresh marsh), the other plant species tested did not appear to be severely affected. In particular, wiregrass (widespread intermediate to brackish marsh grass) and elephant's-ear (fresh to intermediate marsh forb) performed nearly as well on Substrate A as dredge spoil over the two-year study.

Plant photosynthetic response showed interesting trends due to hydrology during the peak-growing season, with the tidal regime tending toward the highest rates. However, photosynthetic responses measured at the end of both the 1996 and 1997 growing seasons did not display any particular trend with respect to hydrologic regime. This may be due to temperature buffering in the permanently flooded treatments, thereby resulting in a delayed onset of dormancy. It is important that the permanently flooded hydrologic regime supported healthy growth in most of the species tested because the pending field demonstration project will be carried out under similar hydrologic conditions. The total biomass production of elephant's-ear was similar over all hydrologic regimes, though it is interesting to note the large amount of adventitious root production (included with belowground biomass due to its lack of chlorophyll) by this species in tidal and permanently flooded conditions. This species performed nearly as well on Substrate A as on dredge spoil.

Substrate A supported higher rates of photosynthesis, across species, than Substrate B. Biomass measured at the end of year one showed that average biomass production on Substrate A was essentially identical to that on dredge spoil (Figure 21). This finding is important in documenting the suitability of Substrate A for wetland restoration because plant growth response is an integrated indicator of a species cumulative stress response (Osmond et al. 1987, Ewing et al. 1995, Larcher 1995, Bazzaz 1996). In particular, elephant's-ear, wiregrass, cattail, and bulltongue appear promising for stabilizing wetlands restored with drill cuttings treated by process A due to their successful establishment and biomass production on that experimental substrate (Figures 22 and 23). Although none of the species tested performed well on the process B-restored cuttings, cattail did outperform the other plant species on that substrate. Maidencane only performed well on the topsoil treatment and appears unsuitable for use in wetlands restoration projects that utilize drill cuttings or dredge spoil. This plant is restricted to fresh marshes and prefers organic soils (Chabreck 1972). Arrowhead, a species that did not survive the initial planting, also appears unsuitable for this type of project, at least during summer transplantings since it is very sensitive to handling during the warmer months.

Wiregrass had the highest overall photosynthetic rates across substrates at the end of the 1996 growing season, but for the 1997 growing season this trend did not continue. Although wiregrass grew well under the moist-not-flooded hydrology, it performed poorly in flooded conditions during 1997. Genotypes S69 and X3 had the highest rates of photosynthesis at peak season 1996, S26 and X8 yielded the greatest aboveground biomass by season's end. It is interesting to note that 1996 biomass production for these two genotypes was equal across the four substrate types (Figure 28). Although Hester et al. (1996) reported that genotypes S69 and X9 had the highest salt tolerance (when challenged with weekly increases in salt concentration), S69 and X9 did not perform better than any other genotype on the alkaline drill cuttings (Substrate B). Interestingly, genotypes X8 and S26 (only intermediate in their tolerance of high salinity) performed best under fresh water conditions in this study, regardless of substrate. Similar to their performance in 1996, genotypes S26 and X8 along with genotype S35, accumulated the greatest biomass during the 1997 growing season. All genotypes performed poorly on Substrate B. Genotypes S26 and S35 were fairly productive on experimental Substrate A (Figure 31), emerging as clear favorites for restoration work using drill cuttings of that type.

Of the nine alternative plant species investigated for restoration initiatives on restored drill cuttings, cattail was by far the most successful. It was highly productive on Substrate A and even grew, to some extent, on Substrate B where most other species failed. Baldcypress, duck-potato, and seaside goldenrod all showed promise for wetland restoration plantings on Substrate A; these species established and grew with moderate success (Figure 34).

The submerged aquatic species showed favorable establishment and growth in the flooded experimental vessels of Substrate A, comparable to the vessels containing dredge spoil. Inteestingly, benthic algal productivity was highest on Substrate B. This is an important finding because benthic algae are a crucial food source in estuarine systems as they are easily assimilable and are available year-round unlike most vascular plants.

In summary, results from this project have yielded several interesting findings. Most importantly, Substrate A-restored drill cuttings have a low toxicity and are capable of supporting several wetland plant species at levels of biomass production directly comparable to dredge spoil. It is important to note that the mesocosm facility has enabled emulation of a closed system, as would be the case in a contained field demonstration project, where any subsurface extraction of metals would remain in the system and not be diluted by water flowing out of the system. Even under these extreme conditions the restored drill cuttings appeared to be non-toxic and supported vigorous vegetative biomass production. The forthcoming investigation into plant responses to restored drill cuttings as influenced by varied salinities should provide important information for future restoration in tidally influenced marshes. In short, results from this mesocosm project indicate that a field demonstration project utilizing restored drill cuttings is safe and will likely result in the creation of healthy and stable wetlands.

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Appendix A

The Mesocosm Facility

The mesocosm facility itself consists of one hundred forty-four 200-liter industrial strength polypropylene vessels networked by PVC pipe to 3000-liter fiberglass supply reservoirs (Figure 1). The water level within each mesocosm is externally controlled and different substrates may be included as treatments. Each Mesocosm unit contains approximately 150 liters of soil. There are four soil treatments in this experiment: Process A restored drill cuttings, Process B restored cuttings, Process A restored cuttings capped with 40 cm of dredge spoil, and topsoil. Each one of the four treatments is replicated in 36 mesocosm vessels. Each mesocosm has potential access to each supply reservoir. Acclimation of substrate within the mesocosm system occurred for two months prior to planting.

The Mesocosms:

Each of the 144 mesocosms are individually wrapped with one-half inch industrial grade black neoprene for insulation, then covered with heavy-duty cellophane to attach and seal the neoprene to each vessel. Each mesocosm is plumbed with two bulkhead fittings centered approximately 12.5 cm from the bottom of the mesocosm vessel and oriented approximately 45 degrees around the barrel from one another (Figure 2). One bulkhead fitting is attached to an adjustable, internal high tide plumbing arrangement and the other bulkhead fitting to an adjustable, internal low tide plumbing arrangement. The low tide plumbing arrangement consists of a ¾ inch PVC elbow fitted to the inside surface of the bulkhead fitting, changing the orientation of the pipe from horizontal to vertical. The elbow is then fitted with a series of ¾ inch PVC pipes which are jointed at distinct levels according to desired level for the low tide. The terminal piece of ¾ inch PVC has approximately one hundred 1/8 inch drilled holes to allow water to enter the low tide drain but prevent the soil substrate from entering, since the low tide drains 10 cm below the substrate/air interface. The high tide bulkhead fitting is plumbed similarly, with the terminal piece of PVC pipe reaching approximately 20 cm higher than the sediment surface. The bulkhead fittings are attached externally to a series of pipes that will return the water to one of the four reservoirs in the mesocosm facility (Figure 3). Once each mesocosm is plumbed internally and its specific substrate type added, an interstitial water catchment well is placed into the center of the mesocosm. The interstitial water catchment well consists of a two-foot piece of ¾ inch PVC pipe with a cap on both the upper and lower ends. The lower end of the pipe received a 1 mm slit vertically, and was then capped. This allows subsurface water to drain into the catchment tube. The upper portion of the catchment tube is capped and contains a 1/16 inch hole to prevent a pressure gradient from forming. The interstitial water catchment pipe is then inserted to a depth of 15 cm below the substrate surface.

The Circulatory System:

The circulatory system of the mesocosm facility consists of two subsystems, namely the water delivery and recovery subsystem and the air delivery subsystem.

The Water Delivery and Recovery Subsystem

Four 3000-liter fiberglass reservoirs form the basis of the water system. These reservoirs allow the delivery of four independent, water-soluble treatments to as many as 36 individual mesocosms. In this particular instance, they represent each of the four substrate treatments: Substrate A, Substrate B, dredge spoil, and topsoil. The system is gravity-fed from the supply tanks to stand-pipes located between each series of four mesocosms (Figure 2 and Figure 4). From the standpipes, solutions are fed to each mesocosm (Figure 4).

The Air Delivery Subsystem

The air lift portion of the mesocosm facility circulatory system consists of three Sweetwater™ regenerative blowers connected to 2 inch PVC pipe spanning each row of mesocosm units. At each series of standpipes, airline tubing provides the conduit for air to lift from the standpipe to each mesocosm (Figure 4). Fine adjustment of air via brass valves allows the precise control of the amount of fluid lifted from each standpipe. In this way, adjustments can be made for tidal versus moist-but-not-flooded conditions (Figure 5).

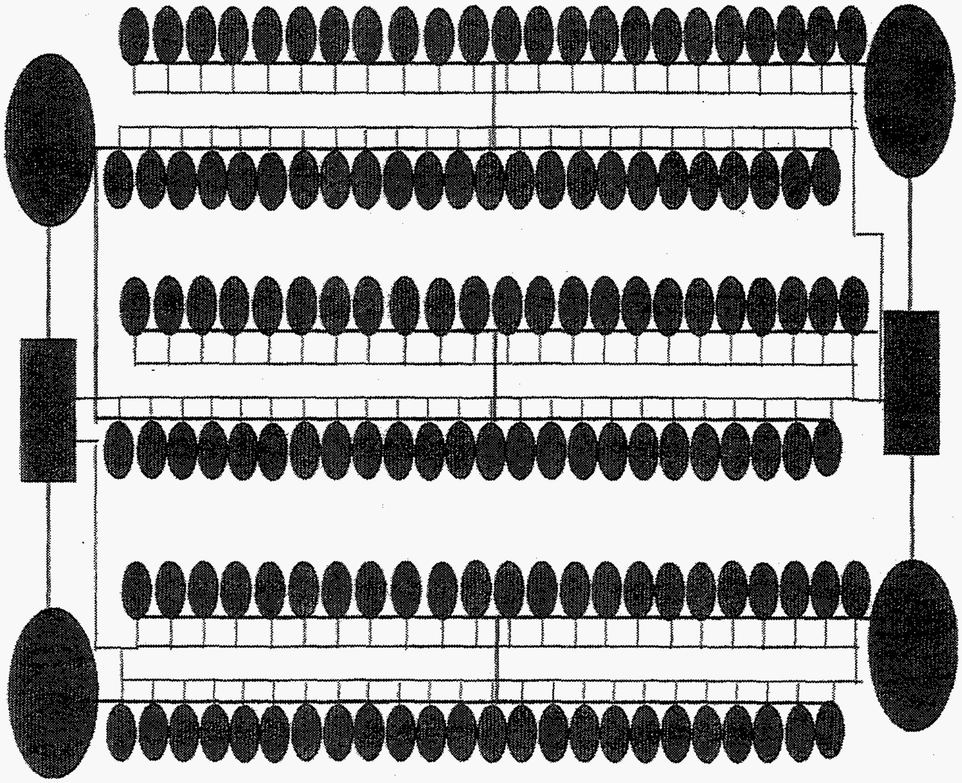

Figure 1
Diagram of mesocosm system containing 4 supply tanks, 144 two-hundred liter mesocosms, and 2 delivery stations with blowers, timers and switches. Mesososms are housed in a 90'x34' greenhouse, which are climate-controlled. NOTE: diagram does not represent actual experimental design or pipe layouts.

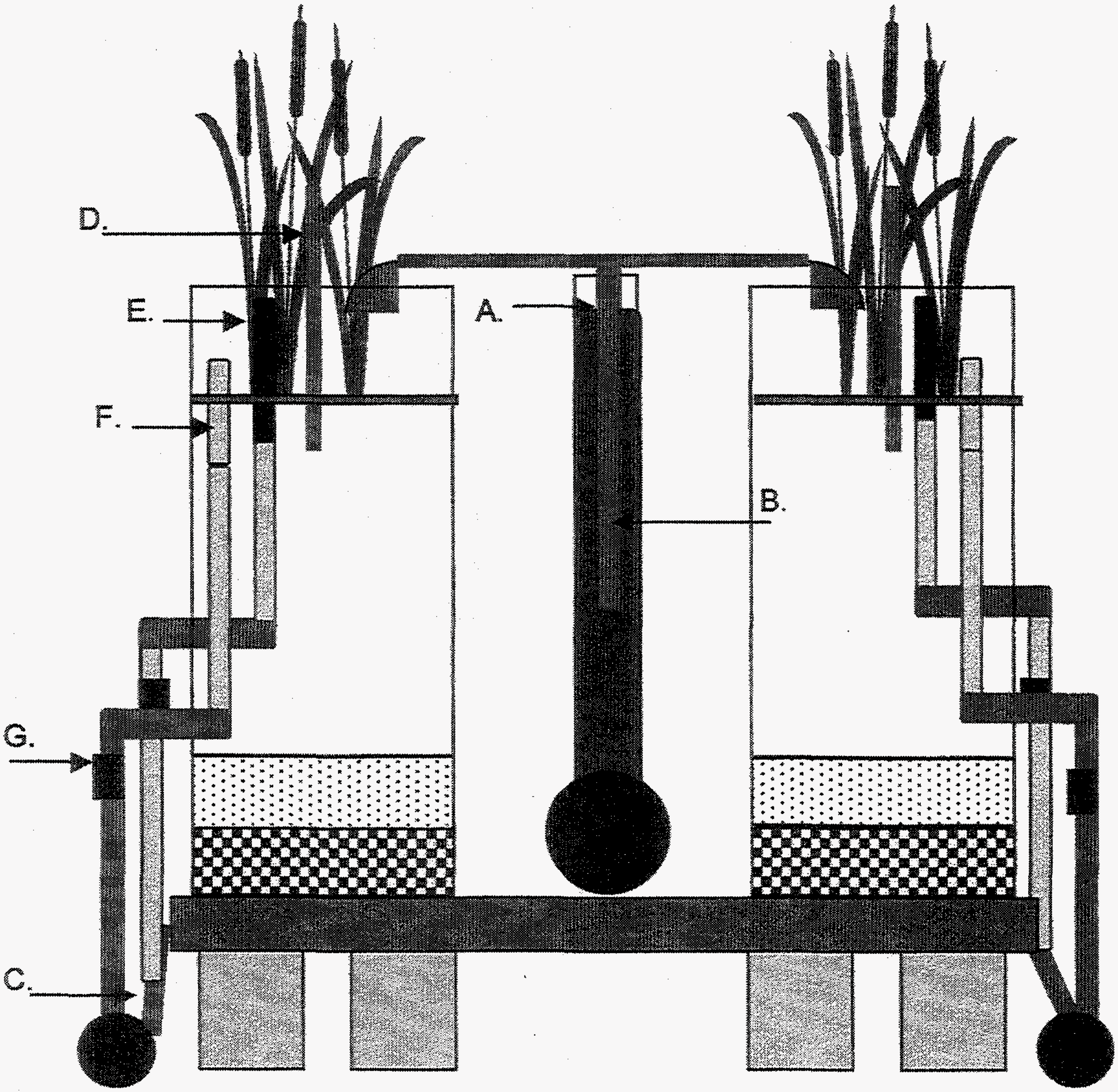

FIGURE 2
ONE PAIR OF MESOCOSMS WITH (A.) SUPPLY WATER STAND PIPE, (B.) WATER LIFTED BY VERTICAL AIR STREAM, AND (C.) GRAVITY RETURN TO SUPPLY RESERVOIRS. TIDE LEVELS ARE REGULATED BY EITHER (E.)HIGH TIDE OVERFLOW OR (F.)LOW TIDE OVERFLOW. SOIL WATER SAMPLING IS FACILITATED BY (D.) INTERSTITIAL WATER CATCHMENT PIPE. RATE OF TIDAL DRAINAGE IS CONTROLLED BY (G.) BALL VALVES

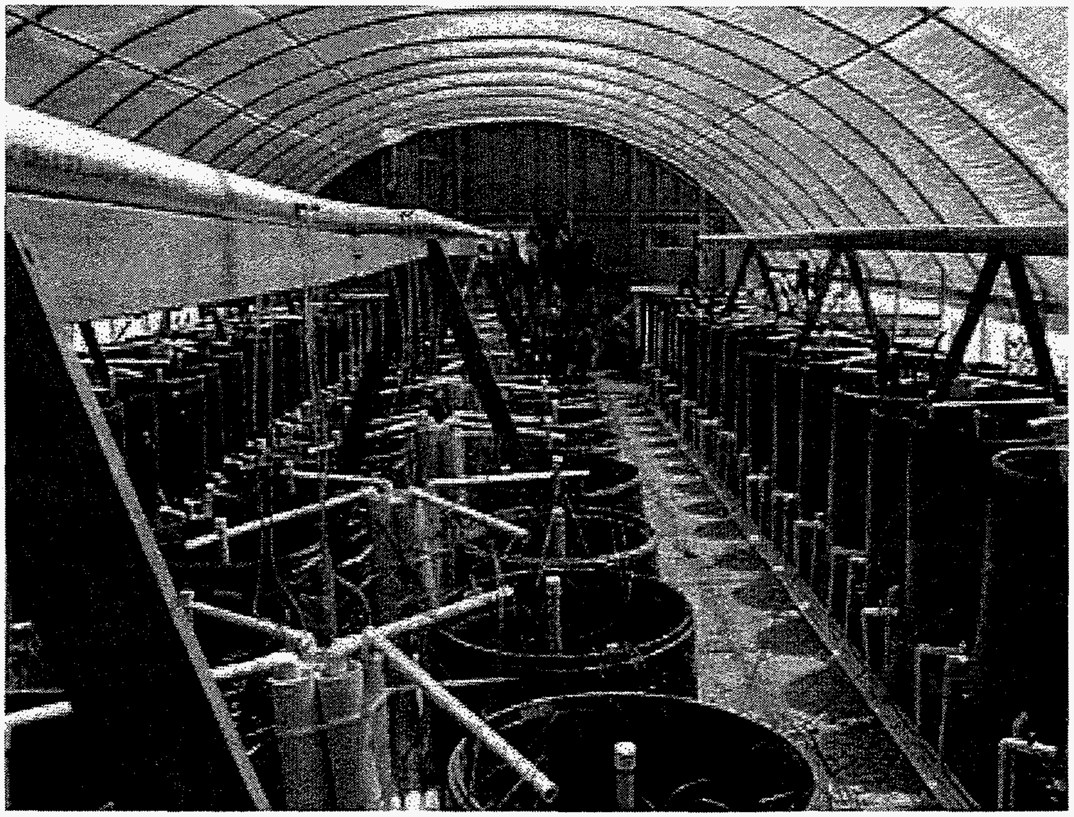

Figure 3
Mesocosm layout showing individual mesocosm units, ball valves for regulating tidal drain rates, and pipes for drain water returning to supply reservoirs.

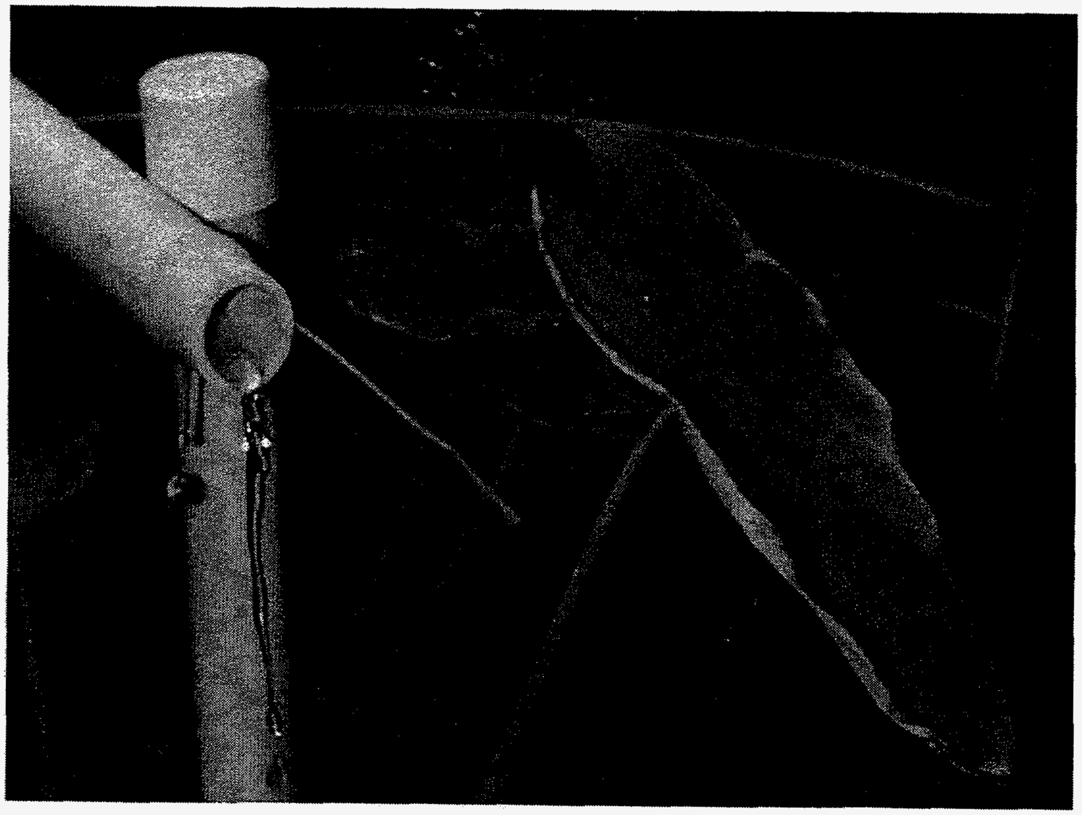

Figure 4
View of individual mesocosm unit showing tidal influx controlled by timers and regenerative blowers. Capped interstitial water catchment pipe is in background.