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Microbial Community Structure of Salt Marsh Macrophyte Rhizosphere as an Indicator of Contamination

Project Report
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This report describes the results of our comparison of sediment microbial communities associated with *Spartina alterniflora* and *Phragmites australis*. The study was based on the hypothesis that large differences in macrophyte root morphology, biomass, and depth would result in rhizosphere microbial community differences under different species of vegetation. We further hypothesized that microbial community composition and functional capabilities would be different under contaminated versus non-contaminated conditions. The overall goal of the project was to identify microbial molecular indicators that could be linked to contamination and possibly used as an index of pollution. There were three specific objectives of this project: 1) to use molecular methods for characterization of microbial communities associated with *Phragmites australis, Spartina alterniflora*, and *Typha latifolia*, 2) to characterize differences in microbial community populations and functions under contaminated and non-contaminated conditions, and 3) through use of a degradation assay, determine microbial community functional ability to biotransform a contaminant.

The proposed experimental design called for a comparison of brackish *Phragmites australis* (hereafter *Phragmites*) and *Spartina alterniflora* (hereafter *Spartina*) populations from the Hackensack Meadowlands and the Mullica River. The Meadowlands have been hydrologically altered and impacted by multiple contaminants, while the Mullica site has had low anthropogenic impacts. The experimental design also called for a comparison of the brackish vegetation with freshwater *Phragmites* and *Typha latifolia* from Kearny Marsh. However, *Typha latifolia* was growing on a very watery substrate in mixed species stands, so it was not possible to obtain single-species sediment cores. Therefore only *Phragmites* sediments were sampled in Kearny Marsh (communicated to Dr. Kirk Barrett at the time of sampling).

The original proposed measurement of microbial functional ability was a phenol bioassay to determine a rate of biodegradation. Due to the ubiquitous presence and recalcitrant nature of halogenated contaminants in estuarine systems, we determined that the use of tetrabromol-bisphenol-A (TBBPA) as a model contaminant was of greater interest than the simpler phenol bioassay. We also added the analysis of extracellular enzyme activity as a second microbial functional comparison. This was done because we thought it important to compare a functional capacity not associate with contamination,

although one which could potentially be impacted by the presence of contaminants.

Due to the volume of samples processed, the additional time needed for anaerobic microcosm incubation (120 days) and TBBPA analysis, and the addition of the extracellular enzyme analysis, this study is somewhat longer than originally anticipated. We are currently in the process of analyzing selected DNA samples, which we expect to complete by December 2002. Based on the results obtained from the TBBPA microcosms, we will work on isolating dehalogenase genes. A separate report detailing the findings of the genetic work will be submitted to MERI upon completion of that analysis.

Methods

Field sites were located on Saw Mill Creek and Kearny Marsh in the Hackensack Meadowlands and on an unnamed tidal creek of the Mullica River, west of the Garden State Parkway about 7 km from the river mouth (Fig.1). Brackish salinities of 12-18 ppt were similar at both riverbank sites. Kearny Marsh salinity was <1 ppt. The Hackensack Meadowlands sites have experienced a lengthy history of disturbance caused by a variety of local industrial and regional watershed contaminants such as heavy metals, chlorinated hydrocarbons, polycyclic hydrocarbons, and other toxic compounds, as well as numerous physical disturbances such as ditching and tidal obstruction (Kennish 1992, 1998, Quinn 1997). In contrast, the Mullica River site is bounded on the inland side by the largest protected watershed in the northeast (the Pinelands National Reserve), and is part of the Jacques Cousteau National Estuarine Research Reserve on the seaward side; there is little industrial activity within the watershed. At each site *Phragmites* and *Spartina* stands were growing adjacent to each other.

Transects 12 m in length were established at each site parallel to the main surface water channel, starting at a randomly chosen point within monospecific populations of both plant species. Three plots, each 2 m^2, were marked at 2 m intervals along the transect. Three replicate sediment cores extending to a depth of 70 cm were randomly extracted from each plot using a Russian peat corer. All cores were extracted within 2 hours of low tide and immediately wrapped in gas-impermeable Saran^R wrap for transport to the laboratory, where they were subdivided into 10 cm segments. Each

section was subsampled for moisture content, organic matter content, and bulk density. Two additional subsets of samples were taken from the 0-10, 30-40, and 60-70 cm sections. These samples were: 1) processed immediately for extracellular enzyme activity or 2) frozen at –20° C for later PLFA and DNA analysis. Sediment from each 10 cm section was also immediately added to anaerobic microcosms to determine potential microbial dehalogenation activity. The Kearny Marsh site does not experience tidal flushing, and the vegetation within this marsh was found to be growing as a root mat on top of the fresh water. Therefore in this location, surface (0-10 cm) grab samples were obtained in lieu of sediment cores.

Root and rhizome biomass was determined from samples obtained by excavating soil blocks within each plot. Aboveground vegetation was clipped to the marsh surface and a metal frame was inserted into the sediments in 10 cm increments to a depth of 30 cm. Intact sediment blocks were removed and transported to the laboratory where sediments were washed from the roots and rhizomes. Because of the large mass of material and the interwoven nature of different root sizes and status, no attempt was made to separate live and dead roots or rhizomes. Washed biomass was dried to constant weight. Due to the hydrology and sediment character in Kearny Marsh, only the top 20 cm *Phragmites* sediment blocks were excavated.

Phospholipid fatty acids (PLFAs) were extracted using a slight modification of the method described by White et al. (1979). Samples were thawed and dewatered by centrifugation. A single-phase chloroform:methanol:0.05M phosphate buffer (pH 7.4) solvent was used for the extraction. The concentrated chloroform extract was separated into lipid fractions on a silicic acid column using the method of King et al. (1977). The phospholipid fraction was eluted, saponified, and methylated according to MIDI (1995) protocols. Individual fatty acid methyl esters were identified based on their GC retention times, and then quantified as a percentage of the total fatty acids recovered from the sample.

Analysis of the activity of 5 extracellular enzymes related to carbon, nitrogen, phosphorus, and soil organic matter cycling were measured using the methods of Sinsabaugh et al. (1993). β-glucosidase (E.C. 3.2.1.21), an enzyme important in the degradation of carbohydrates, was used as an indicator of carbon cycling potential. β-*N*-

acetylglucosaminidase (E.C. 3.2.1.30), commonly known as chitobiase, is involved in degradation of the nitrogen-containing fungal cell wall compound acetylglucosamine, and was used as the nitrogen-related enzyme. Acid phosphatase (E.C. 3.1.3.2), an enzyme necessary for the degradation of phosphorus containing organic molecules, was used as an indicator of phosphorus cycling potential. Two enzymes active in the degradation of complex organic substrates, phenol oxidase (E.C. 1.10.3.2 and E.C. 1.14.18.1) and peroxidase (E.C. 1.11.1.7) were used as indicators of SOM cycling potential.

Substrates used for β-glucosidase, chitobiase, and acid phosphatase were p-nitrophenol derivatives that release pNP when hydrolyzed. The resulting supernatant is then analyzed spectrophotometrically and enzyme activity expressed as μM substrate/g sediment/hr. The substrate was dissolved in an acetate buffer (pH 5), then added to a fresh sediment slurry, which was incubated at 20° C for the time specified for each enzyme. The reaction was stopped by centrifugation followed by the addition of 1N NaOH to the supernatant. The same procedure was used to measure phenol oxidase and peroxidase activity using L-DOPA as the substrate, but the supernatant was directly used to measure absorbance. The peroxidase assay includes the addition of H_2O_2, and measures the sum of phenol oxidase and peroxidase activity. Peroxidase activity alone was obtained by subtracting phenol oxidase results from the peroxidase total results.

To determine the functional ability of the sediment microbial community to biotransform halogenated compounds, TBBPA was chosen as a model contaminant. This brominated flame retardant, which is used in the manufacture of plastics and personal computers, has been documented as a problem contaminant in estuarine systems (Sellstrom & Jansson 1995). Sterilized sediment previously collected from the Hackensack Meadowlands was used as the contaminant delivery medium. TBBPA concentrations of 100 μM were dissolved in acetone and added to a gram of dried sterilized sediment. After allowing the acetone to evaporate, fresh sediment from each plant species at each site was added to the contaminated sediment under anaerobic conditions maintained by working in a Coy chamber. Anaerobic methanogenic medium was then added, the jars were sealed and incubated in the dark at 28° C for 120 days. Jars were periodically sampled during the incubation period, and changes in TBBPA concentrations were monitored using HPLC analysis.

Statistical Analysis

Sediment characteristics, including root biomass, were analyzed using two-factor analyses of variance (ANOVA SITE factor = Saw Mill Creek (SMC) and Mullica (MUL), SPECIES factor = *Phragmites* (P) and *Spartina* (S), n=3 for each site-species combination). Two-way ANOVAs were also applied to the data from each enzyme and each fatty acid. Contrast analysis was used to compare vegetation effects within each site. Principal components analyses were used to examine patterns among the four site-species combinations based on the multivariate sets of enzyme activities and fatty acids. Because conditions within Kearny Marsh were not found to be comparable to the salt marsh locations, the data are presented separately and were not included in the multivariate analyses. Significant differences were not seen in the pattern of species responses as depth increased (Table 1), so results discussed are for the surface 0-10 cm depth where sediment activity and root biomass was greatest.

Results

Sediment characteristics were different between the two brackish sites (Fig. 2a-c.), although the quantitative differences for any given variable were relatively small. Contrast analysis revealed significant differences between sediments of the two plant species at the Mullica site, where *Spartina* organic matter was higher and bulk density was lower than that of *Phragmites*. Significant sediment differences between vegetation species were not found at Saw Mill Creek. Total *Spartina* root and rhizome biomass (Fig. 3) was significantly higher than *Phragmites* in the surface 10 cm at both sites due to the presence of a high density of fine roots. Overall belowground biomass for both species was significantly higher at the Saw Mill Creek site than at the Mullica site (Fig. 3). No significant differences in root biomass were found at either site at the 10-30 cm depths. Kearny Marsh (Table 3) *Phragmites* belowground biomass was similar to that of Saw Mill Creek *Phragmites*. Kearny Marsh organic matter, determined by loss-on-ignition, was approximately double that of the brackish system (> 40% vs. 12-20%), as was moisture content (>300% vs. 150%). Conversely, Kearny Marsh sediment bulk density was less than half that of the brackish samples (0.18 g/c^3 vs. 0.35-0.50 g/c^3).

Patterns of extracellular enzyme activity varied with both the species and the site (Figs. 4a,b). Contrast Analysis (Table 1) showed all Mullica surface layer enzymes, except β-glucosidase, exhibited significant differences between plant species, but only phenol oxidase exhibited significant species differences in the Saw Mill Creek sediment. The pattern of more significant differences between species at Mullica was repeated in the depth profile. All sediment samples from Mullica exhibited higher activity than the corresponding sediments from Saw Mill Creek (Fig. 4a). Principal components analysis using the five enzymes at the four site-species combinations (Fig. 5a) indicates that although the two species are separable at each site, there is a greater difference between species clusters at the Mullica site than at Saw Mill Creek where both species cluster closely together. There was no tendency for the two populations of each species to overlap in the ordination space.

Kearny Marsh extracellular enzyme activity (Fig. 4b) was different from that observed in the salt marsh systems. Acid phosphatase activity of > 70 μM/g sediment/hr was the highest observed at any site, as was β-glucosidase activity of > 30 μM/g sediment/hr. Conversely, activity of chitobiase, peroxidase, and phenol oxidase was lower in the Kearny Marsh sediments than in brackish sediments from either location.

PLFA distribution for each site-species combination suggests differences between both species and sites (Figs. 6a,c, 7-9). The abundance of ubiquitous fatty acids contributes a much higher percentage to the total fatty acids extracted from SMC sediments (Fig. 8), indicating a less diverse microbial population than at Mullica or Kearny Marsh. Total PLFAs recovered from Saw Mill Creek (38) were lower than the number recovered from Mullica (92) or Kearny Marsh (44). Contrast analysis indicates PLFA differences between species are significant at Mullica (Fig. 9), but not at Saw Mill Creek. PLFA principal components analysis (Fig. 5b) suggests a pattern somewhat similar to that observed with enzyme activities, where ordination separation is much greater between species at Mullica than at Saw Mill Creek. The first PCA axis, which accounts for nearly half the variation in the dataset, significantly separates the two sites.

The functional ability to biotransformation TBBPA was found in sediments from all three sites. However, disappearance of TBBPA varied depending on the location and vegetation species. TBBPA concentrations in the Kearny Marsh (Fig. 10) sediments

decreased from 100 μM to <20 μM after 20 days of incubation; after approximately 65 days of incubation in *Spartina* sediments from both sites (Fig. 11), and in approximately 120 days of incubation in *Phragmites* sediments from both sites (Fig. 12).

Discussion

Differences in microbial communities were found to be markedly different between the two sites. The data in this study suggests that differentiation of microbial communities associated with *Spartina* and *Phragmites* is contingent on qualities associated with the individual sites. Although differences in microbial community structure and function occurred beneath the two plants, the pattern of difference for individual descriptors of the microbiota was site dependent. Most frequently, patterns occurring at the Mullica site were opposite to those occurring at the heavily impacted Saw Mill Creek site, while patterns at the contaminated Kearny Marsh freshwater site differed from those found in the brackish systems.

We found significant differences in enzyme activity and fatty acid profiles at the Mullica site, but not at Saw Mill Creek. Our results indicate that root-mediated effects on microbial communities appear to be impacted in wetland sediments of disturbed systems. The Saw Mill Creek and Kearny Marsh results may reflect the disturbed and contaminated nature of the sediments. Enzyme activities are sensitive to metal contamination (Dick & Tabatabai 1993), and heavy metal contamination has been found to correlate with a 10- to 50-fold reduction in enzyme activity (Kuperman & Carreiro 1997). The lower enzyme activities and lower number of fatty acids recovered from the Saw Mill Creek sediments suggests that the history of contamination and physical disturbance in the Meadowlands has had a direct effect on microbially-mediated processes through diminution of microbial abundance, activity, and diversity. The environmental history of Saw Mill Creek may have overwhelmed the effects of the two plant species, contributing to the conclusion that brackish marsh microbial communities are primarily affected by the nature of the site, which in highly disturbed systems may be of greater importance in determining microbial community composition than species of macrophyte forming the plant cover. Without more specific data on contaminant loads and comparative studies within the Meadowlands district, this remains speculation.

It is interesting to note that in the Kearny Marsh freshwater system the number of PLFAs recovered from *Phragmites* sediments was 37.5% greater than from Saw Mill Creek (44 vs. 32). It is also interesting to note that *Phragmites* sediments from both Saw Mill Creek and Kearny Marsh exhibit similar oxidase (SOM cycling) and chitobiase activity (N cycling). However, Kearny Marsh β-glucosidase (carbon cycling) and acid phosphatase (phosphorus cycling) activity was 5-fold greater than that observed in Saw Mill Creek *Phragmites* sediments. These differences may be related to 1) the high level of organic matter in Kearny Marsh relative to the brackish system (41% versus <15%), 2) naturally occurring differences between freshwater and saltwater systems, or 3) a response due to exposure to the contents of effluents or leachates released into Kearny Marsh.

The results of the biodegradation analysis were quite striking. Concentrations of TBBPA were observed to decrease from 100 μM to less than 20μM within 20 days in the Kearny Marsh samples. This transformation required approximately 60 days in *Spartina* sediments from both locations, and 120 days in *Phragmites* sediments. It is possible that the microbial population which effects this transformation in less active under the sulfide reducing conditions found in brackish sediments versus freshwater sediments. It is interesting to note that *Spartina* sediments from both the contaminated and non-impacted brackish systems biotransform TBBPA in approximately half the time as *Phragmites* sediments from both systems, which might indicate the microbial population responsible for this transformation is more numerous under rhizosphere conditions associated with *Spartina*. We are hoping that genetic analysis will elucidate DNA/RNA patterns that might be helpful in explaining these results. There is a notable lack of studies in other wetland soils using a combination of these microbial analytical techniques, and so there is a clear need for additional work to test the hypotheses generated by this study.

Conclusions

The results of this study suggest that in relation to biogeochemical functions 1) macrophyte species differences may be of greater importance in uncontaminated sites than in contaminated sites and 2) that contaminated freshwater sediments are not the equivalent of contaminated saltwater sediments, but more study is clearly needed.

Acknowledgements

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White, D.C., W.M. Davis, J.S. Nickels, J.D. King and R.J. Bobbie. 1979. Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia 40:51-62.

Fig. 1. Map of New Jersey showing the location of the Hackensack Meadowlands and the Mullica River tributary where sediment samples were obtained.

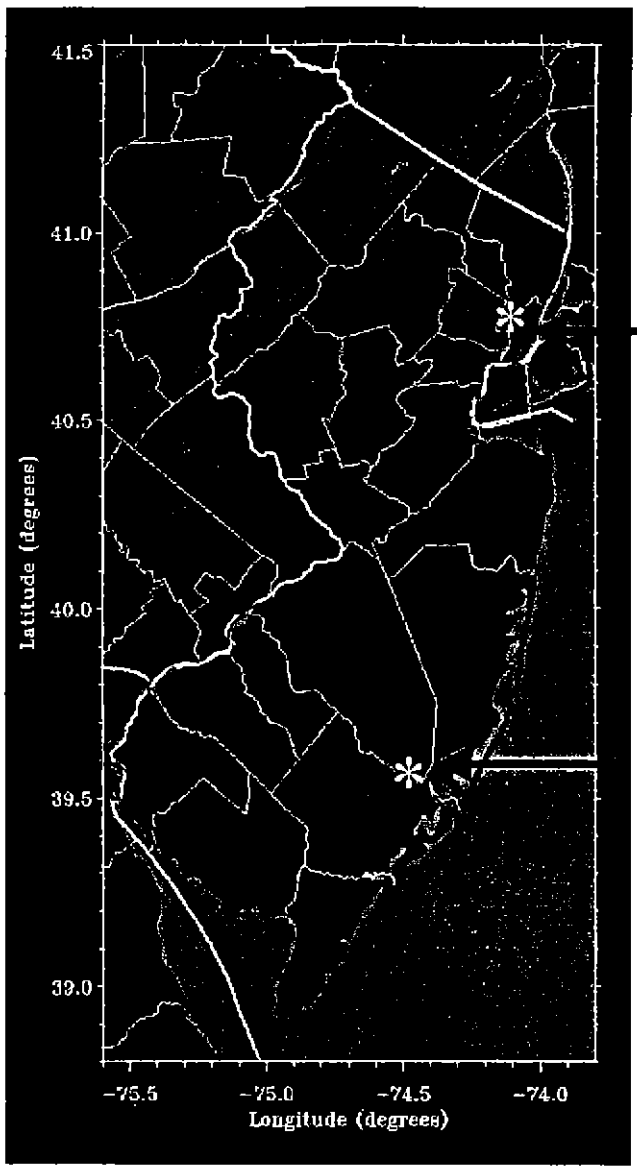
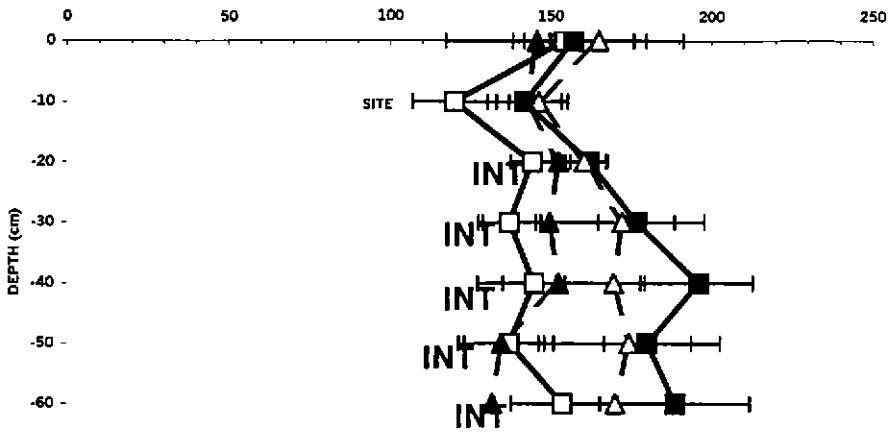
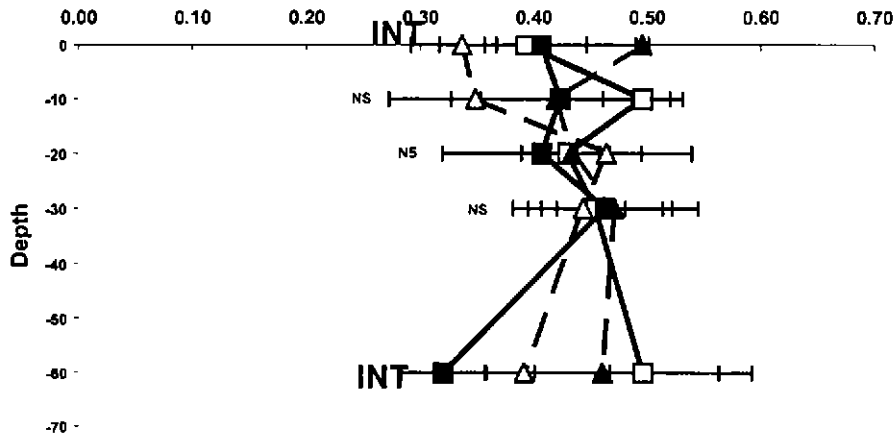


Figure 2a-c. Saw Mill Creek and Mullica sediment characteristics.

a. Soil Moisture (%)



b. Bulk Density (g/cc)



c. Soil Organic Matter (%)

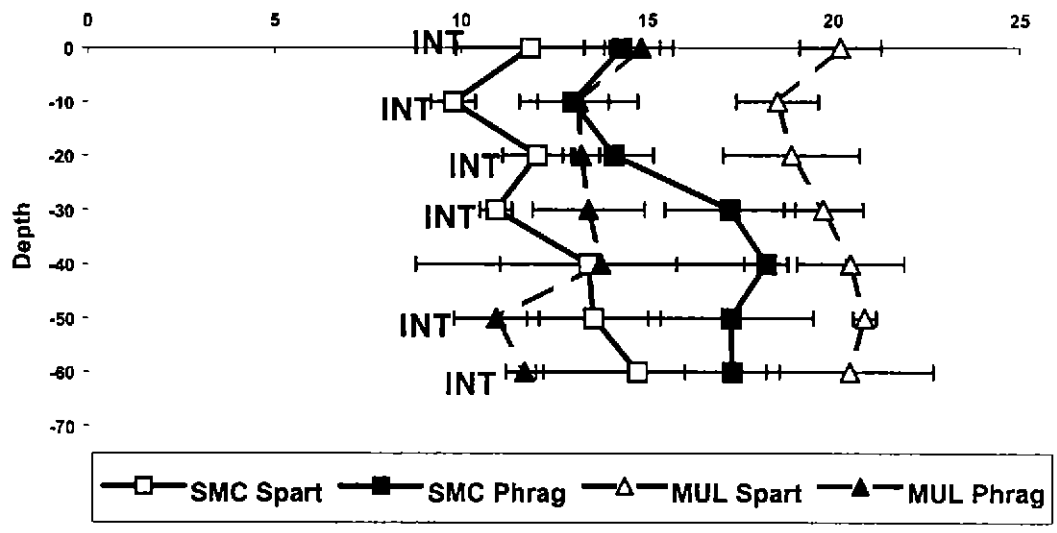


Figure 3. *Spartina alterniflora* and *Phragmites australis* belowground biomass at the Saw Mill Creek and Mullica sites. Depth measurements are in 10 cm increments.

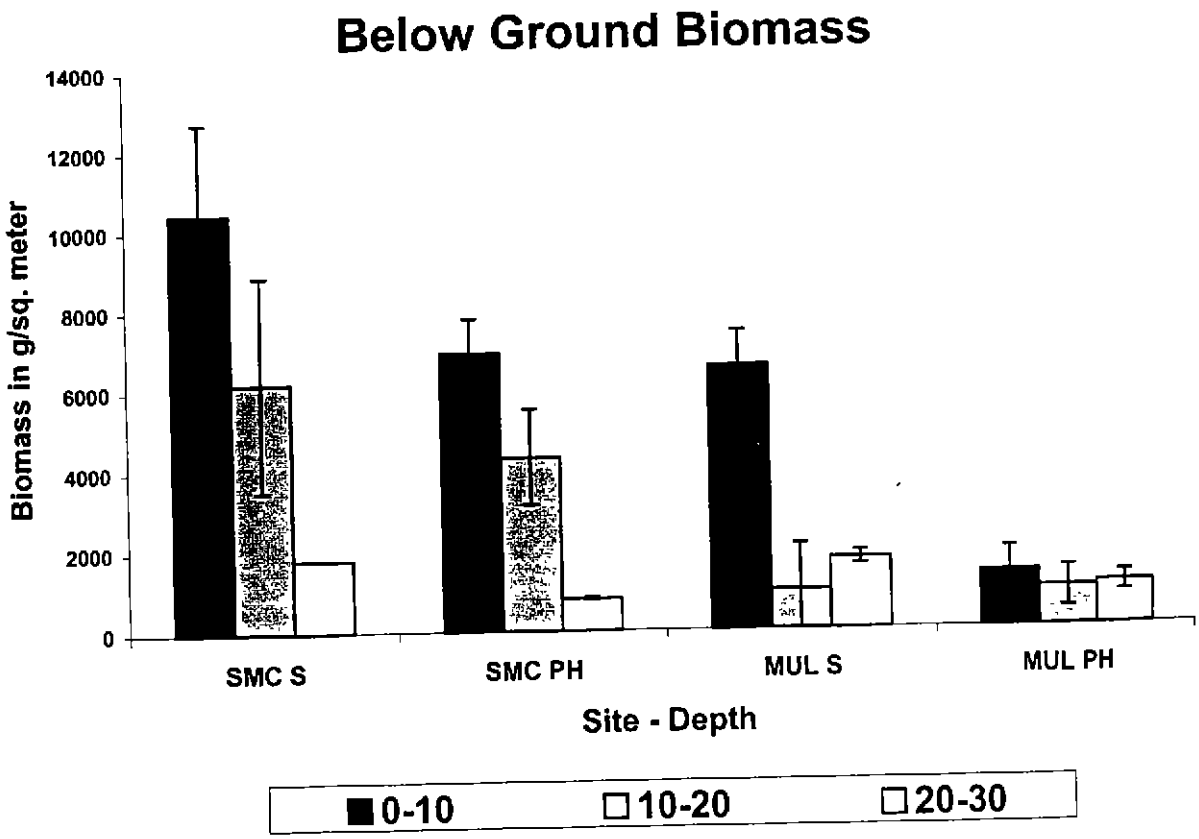
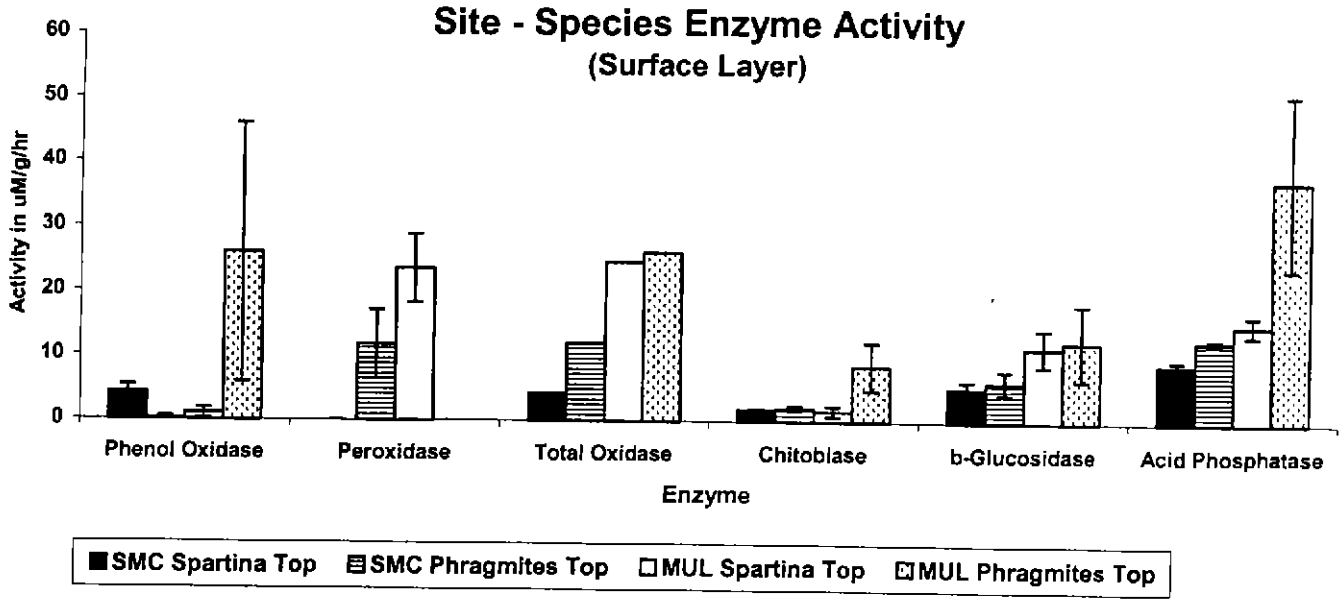


Figure 4a-b. Extracellular enzyme activity of surface sediments (0-10 cm depth) from Saw Mill Creek, Mullica and Kearny Marsh *Spartina alterniflora* and *Phragmites australis*.

a. Saw Mill Creek and Mullica



b. Kearny Marsh *Phragmites australis*. Note value of Y-axis is greater than Y-axis value of figure 4a.

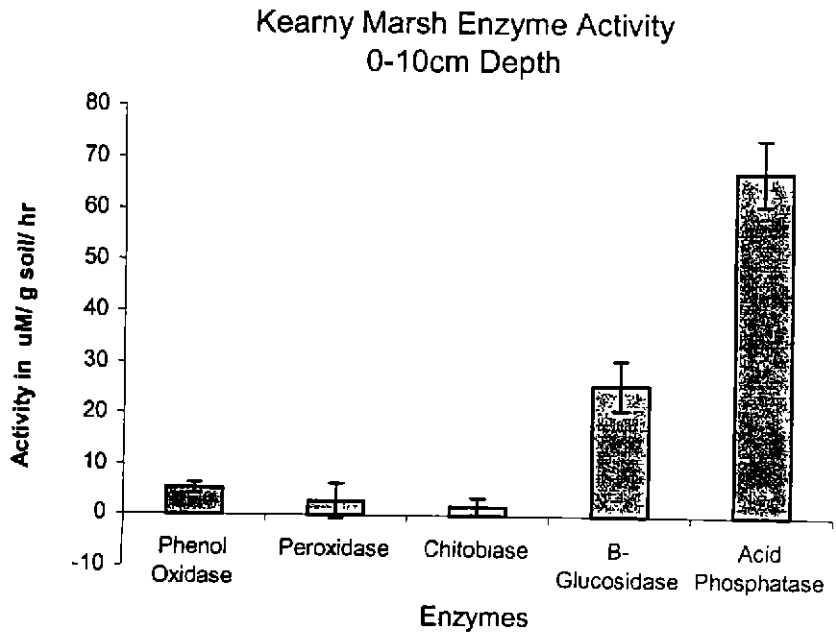
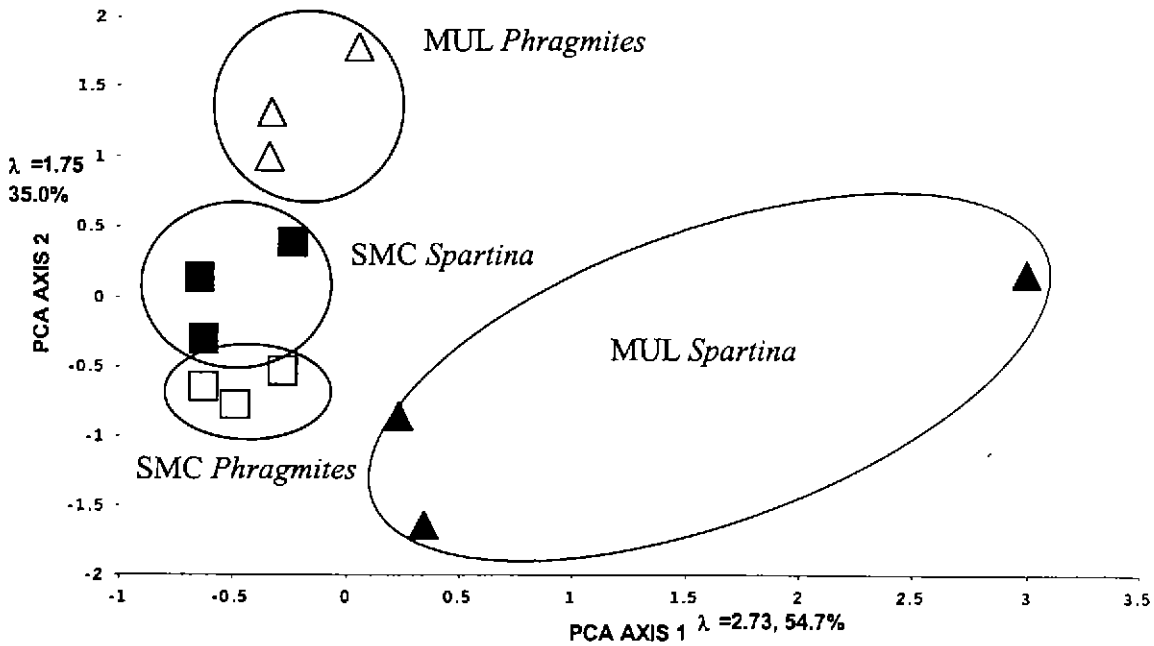


Figure 5a-b. Saw Mill Creek and Mullica Enzyme and PLFA Principal Components Analysis.

a. Enzyme PCA



b. PLFA PCA

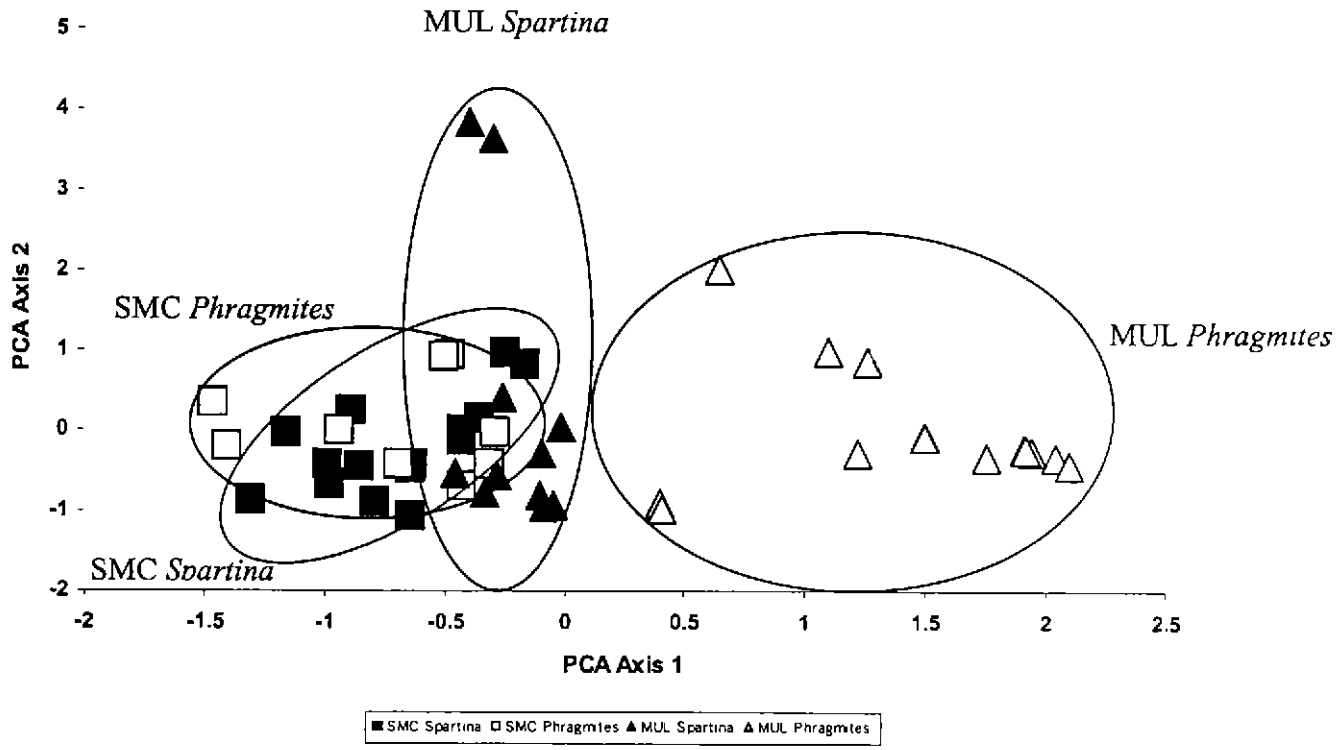
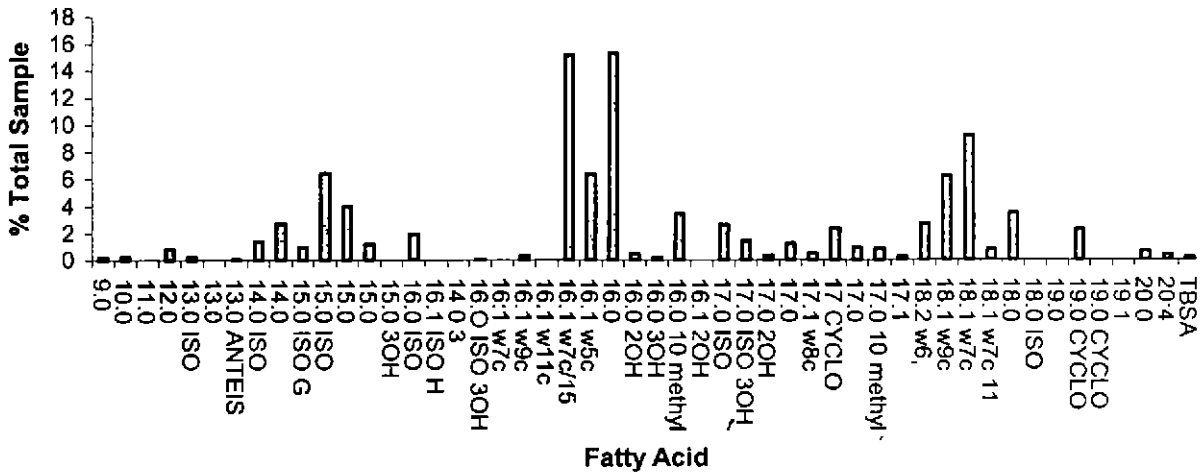


Figure 6a-b. *Phragmites australis* PLFAs from Kearny Marsh, Saw Mill Creek, and Mullica sites.

a. Kearny Marsh PLFAs from surface (0-10 cm) sediments.



b. Saw Mill Creek, Mullica, and Kearny Marsh *Phragmites australis* PLFA comparison for surface 0-10 cm depth.

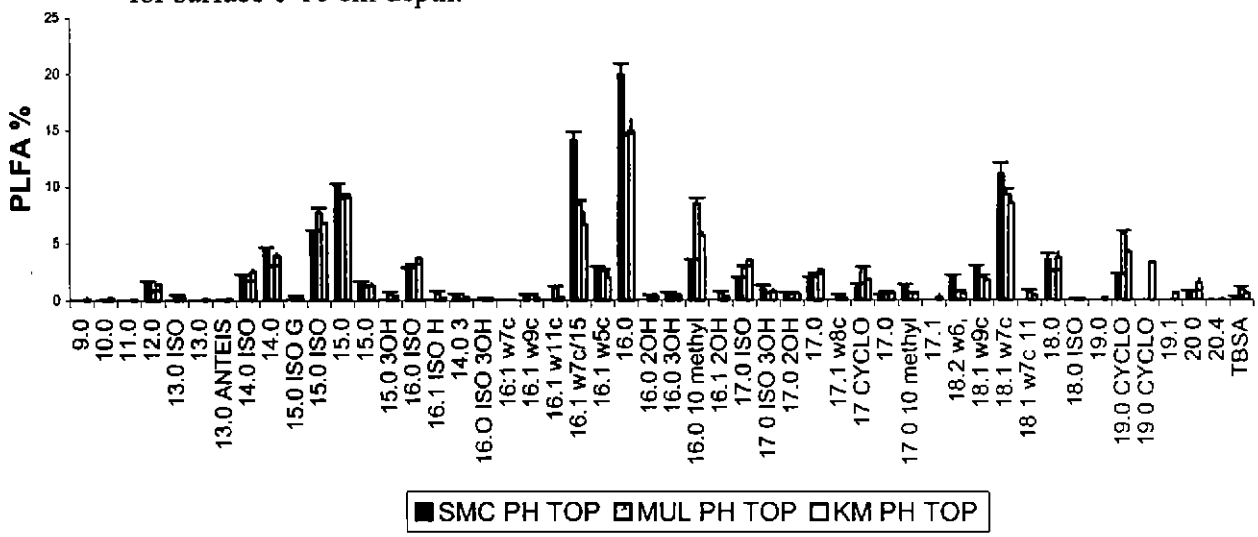


Figure 7. Saw Mill Creek and Mullica *Spartina alterniflora* PLFAs from surface sediments (0-10 cm).

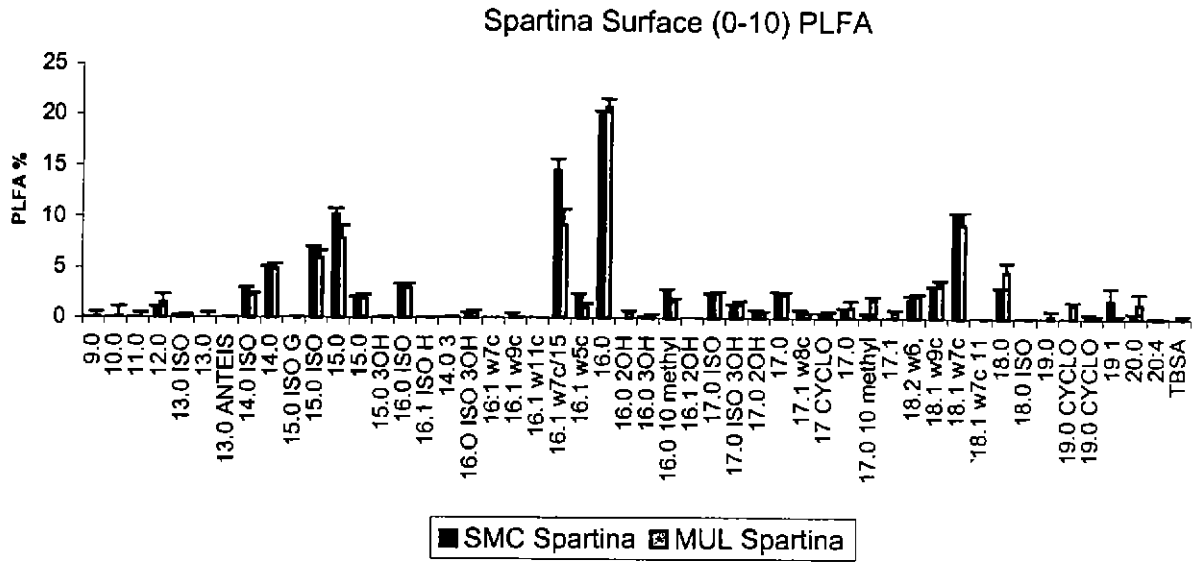


Figure 8. Saw Mill Creek *Spartina alterniflora* and *Phragmites australis* PLFAs from surface sediments (0-10 cm). There is not a significant difference between vegetation species.

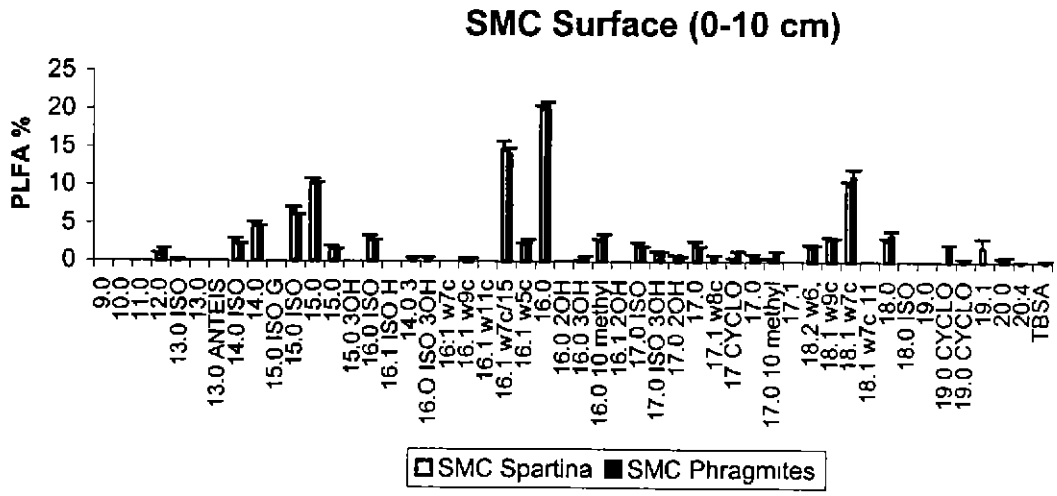


Figure 9. Mullica *Spartina alterniflora* and *Phragmites australis* PLFAs from surface sediments (0-10 cm). There are significant differences between vegetation species.

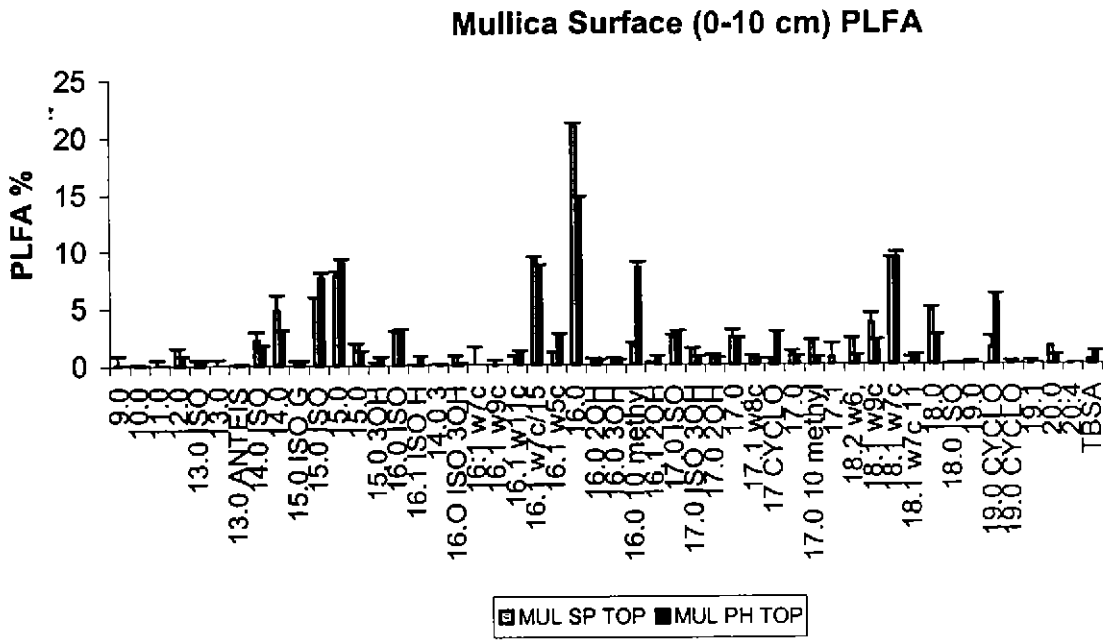


Figure 10. Kearny Marsh TBBPA microcosm incubation. Presence of TBBPA was measured for a period of 90 days.

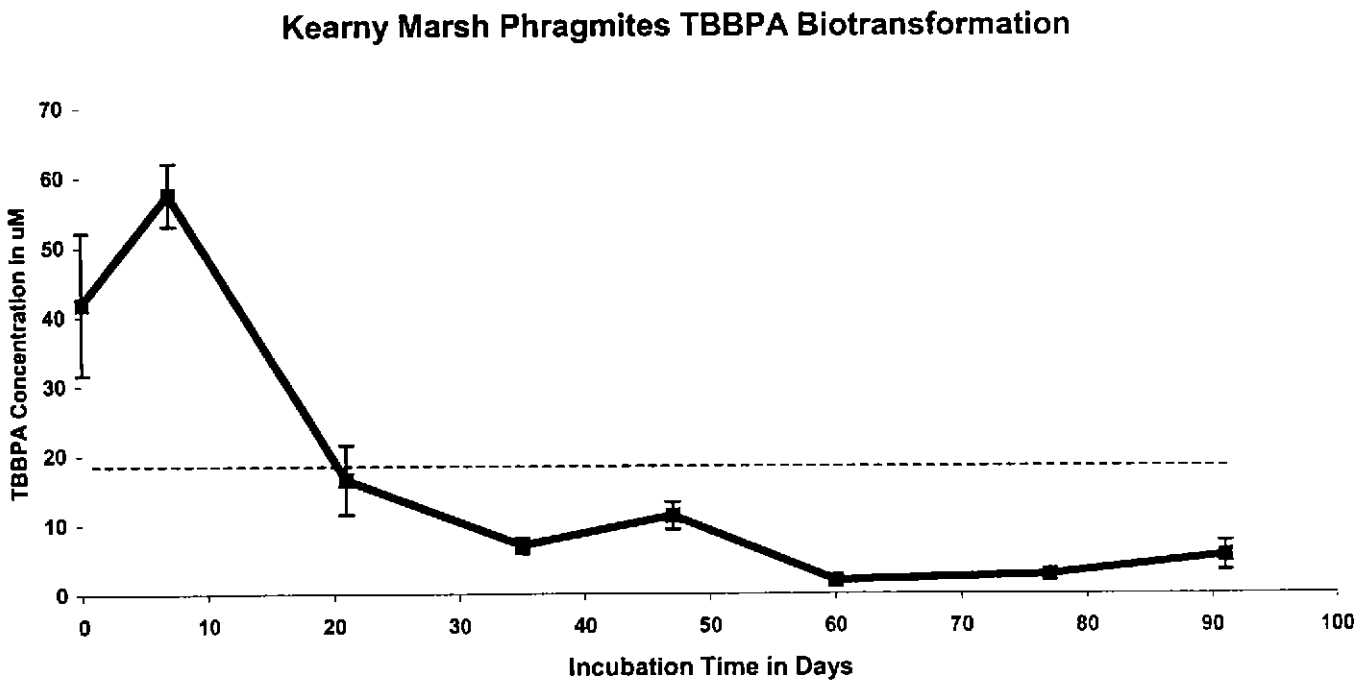


Figure 11. *Spartina alterniflora* TBBPA microcosm incubation. Presence of TBBPA was measured for a period of 120 days.

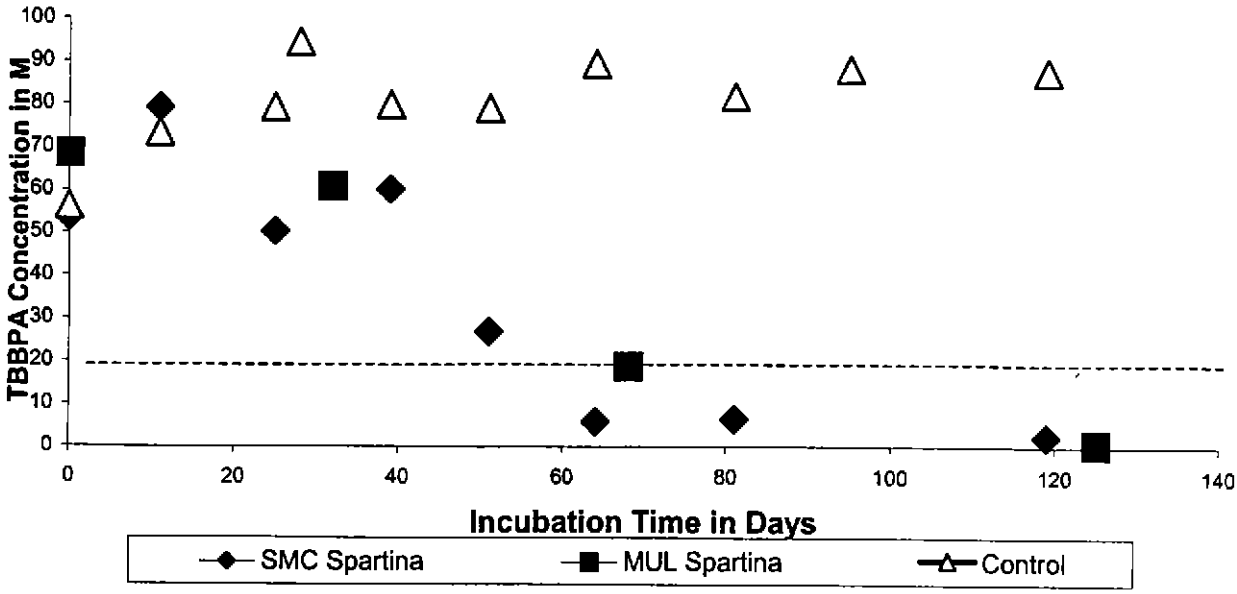


Figure 12. *Phragmites australis* TBBPA microcosm incubation. Presence of TBBPA was measured over a period of 130 days.

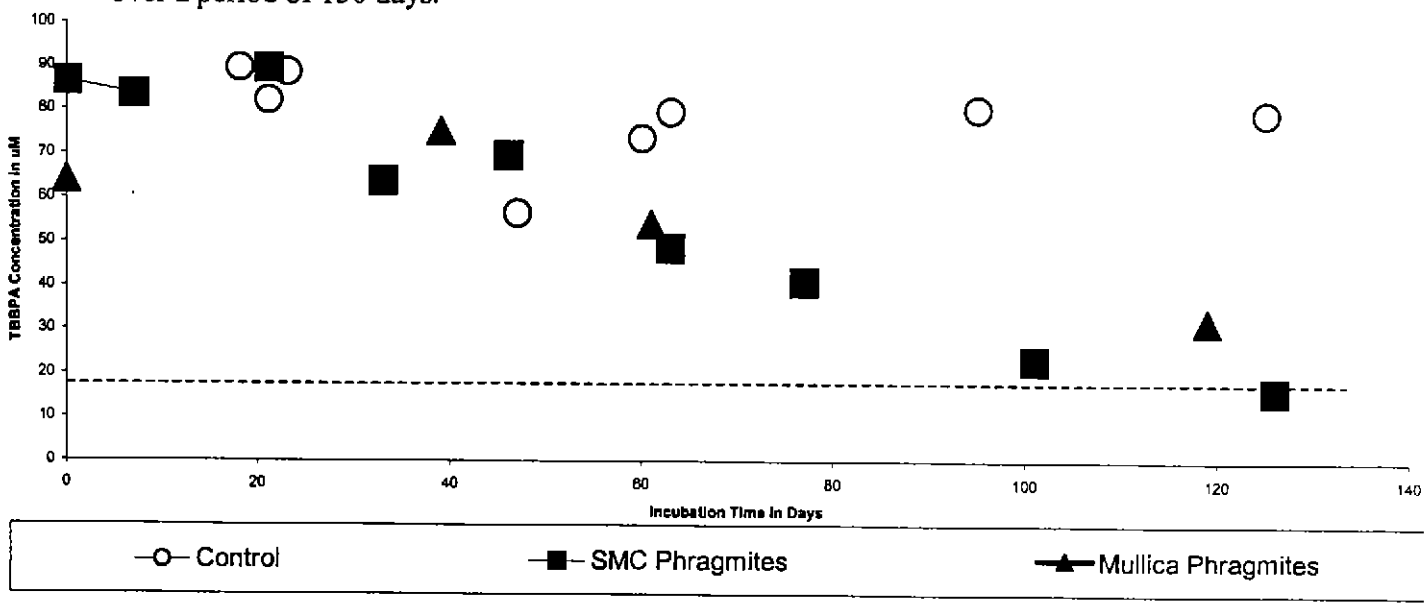


Table 1. Patterns of significance in two-way analyses of variance and 1 *df* contrasts of site and species effects on extracellular enzyme activities, for each of three sediment depths (TOP = 0-10 cm, MIDDLE = 30-40 cm, BOTTOM = 60-70 cm). P = *Phragmites*, S=*Spartina*. Letters (a,b,c) indicate significance level of the overall 2-way ANOVA, and numbers (1,2,3) indicate significance of 1-*df* contrasts comparing species within each site.

ENZYME	SITE	TOP	MIDDLE	BOTTOM
Phenoxidase		Int[c]	Int[c]	Int[c]
	MUL	P>S[3]	P>S[3]	P>S[3]
	SMC	S=P	S>P[3]	S>P[3]
Peroxidase		Int[c]	Site[c]	Int[b]
	MUL	S>P[3]	S=P	S>P[3]
	SMC	P>S[3]	S=P	P>S[3]
Chitobiase		Int[b]	Int[a]	Int[b]
	MUL	P>S[3]	P>S[3]	P>S[3]
	SMC	P=S	P=S	P> S[3]
β-Glucosidase		Site[a] (M>S)	Int[b]	Site[a]
	MUL	P=S	S>P[3]	S>P[3]
	SMC	P=S	S= P	S = P
Acid phosphatase		Site[b] (M>S)	Site[b] (M>S)	Site[b] (M>S)
	MUL	P>S[3]	P=S	P>S[1]
	SMC	P=S	P=S	P>S[1]

INT = Interaction factor

[a]P<0.05, [b]P<0.01, [c] = P<0.001; "P=S" indicates no significant difference in 1-*df* contrasts

Table 2. Number of significant factors of each factor in two-way analyses of variance of site and species effects for the phospholipid fatty acids in the surface sediments.

DEPTH	SITE	SPECIES	INTERACTION	NONE
0-10 cm	6	3	13	18
30-40 cm	7	0	7	23
60-70 cm	8	4	8	19

Table 3. Kearny Marsh Sediment Characteristics.

Characteristic		Std. Err.
Bulk Density	0.18	0.03
Moisture Content	353%	0.56
Organic Matter (LOI)	41%	1.14
Belowground Biomass		
0-10 cm	8835 g/m^2	2537
10-20 cm	6015 g/m^2	1856