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Effects of vegetation on root-associated microbial communities: A comparison of disturbed versus undisturbed estuarine sediments

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Abstract

While it has been demonstrated that microbial communities are influenced by plant species composition in upland systems, less is known about how estuarine vegetation may influence the structure and functional capabilities of sediment microbial communities. In this study, we adapted terrestrial catabolic response profile (CRP) methods for use under brackish anaerobic conditions. CRPs were employed to determine whether salt marsh vegetation had an effect on sediment microbial community function. Phospholipid fatty acids (PLFAs) were utilized as a descriptor of microbial community structure. Samples were obtained from monospecific *Spartina alterniflora* and *Phragmites australis* plots located in disturbed and undisturbed estuarine salt marsh systems. In response to the addition of a range of 30 organic substrates we observed significant CO_2 production in vegetated versus non-vegetated sediments from the undisturbed site. Vegetated sediments from the undisturbed system produced greater amounts of both CO_2 and CH_4, while sediments from the disturbed site consumed CO_2 except in response to carboxylic acid substrates. In response to nitrogen-containing (amino acid) substrates undisturbed *Phragmites* sediments produced the highest amount of CO_2 seen in any sample. Few differences in CRPs or PLFAs were seen in sediments from the disturbed site. The production of CO_2 was significantly correlated with microbial community structure as described by PLFA profiles. This study is the first to use CRPs to describe estuarine sediment microbial community functional ability, and to link this ability with community PLFA composition. Our results suggest that individual plant species have a less pronounced effect than has been observed in upland soils in structuring salt marsh sediment microbial communities. Under anthropogenically disturbed conditions the effects of the macrophyte root zone on the sediment microbial communities may be overwhelmed, resulting in different ecosystem level functional abilities.

Keywords: Catabolic response profile; CRP; Phospholipid fatty acid; PLFA; Microbial community structure; Microbial community function; *Spartina alterniflora*; *Phragmites australis*; Estuarine; Wetland sediments

1. Introduction

In terrestrial soils, the composition and functional abilities of microbial communities are influenced by their association with specific plant species (Ehrenfeld et al., 2005; Grayston et al., 2001, 1998; Marschner et al., 2001; Boschker et al., 1999). Studies in diverse ecosystems (peatlands—Borga et al., 1994; forests and grasslands—Kourtev et al., 2003; Degens and Harris, 1997; agricultural—Schutter and Dick, 2001) have shown plant species composition to be an important factor, both in selecting for specific microbial populations and in influencing the diversity and functional capacity of these communities. These soil microbial communities provide ecosystem level functions such as the decomposition of soil organic matter (SOM), and variations in microbial decomposition rates have been associated with different plant species (Kourtev et al., 2003; Boschker et al., 1999; Liljeroth et al., 1994).

Differences in microbial numbers, community composition, and variations in nutrient cycling activities and contaminant transformations have also been observed in salt marsh microbial communities associated with vegetation (Ravit et al., 2003, 2005; Burke et al., 2002; Nielsen et al., 2001; Bagwell et al., 2001; Daane et al., 2001; Hines et al., 1999). However, few studies of either estuarine

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vegetation or microbiota have compared the effect of different macrophyte species on biogeochemical functions that occur in brackish sediments. In the highly organic sediments often encountered in wetlands (Richert et al., 2000) a large pool of belowground dead biomass and labile organic compounds excreted by live plant roots support microbial activity. Root transfer of oxygen (O_2) from the atmosphere to the rhizosphere is well documented (Armstrong et al., 1996; Grosse et al., 1996; Mendelssohn et al., 1981; Teal and Kanwisher, 1966). While root-derived carbon (C) may be a less important factor in highly organic estuarine sediments than in upland mineral soils, the root-derived O_2 inputs may be an important influence in determining microbial community composition (Ludemann et al., 2000). Because estuarine plant species differ in their root biomass, turnover, exudation, and O_2 leakage, it can be expected that macrophyte vegetation contributes to the structuring of these sediment microbial communities.

The increasing dominance of *Phragmites australis* (Cav.) Trin. ex. Steud. (hereafter *Phragmites*) in brackish estuarine marshes of the northeastern US has sparked extensive efforts to restore *Spartina* species to tidal marshes (Meyerson et al., 2000; Rooth and Stevenson, 2000; Rice et al., 2000). Restoration efforts are often justified in terms of lost or altered ecosystem functions associated with the *Phragmites* invasions. While these restoration efforts have stimulated many studies of the comparative ability of *Phragmites* and *Spartina* to support vertebrate and invertebrate fauna (Weis and Weis, 2000; Wainwright et al., 2000; Weinstein and Balletto, 1999), relatively little attention has been paid to a comparison of the microbial communities associated with the root zones of these two plant species. The dissimilar root morphologies and processes of root oxidation in *Phragmites* and *Spartina alterniflora* (hereafter *Spartina*) can result in differences in characteristics of the sediments associated with these two plants (Armstrong et al., 2000; Windham and Lathrop, 1999; Chambers, 1997; Hwang and Morris, 1991), and these differences may be a factor in structuring sediment microbial communities.

Catabolic response profiles (CRPs) measure CO_2 production after the addition of a range of simple substrates, and this technique has been used to differentiate microbial ability to mineralize various organic compounds and to describe microbial functional diversity in upland soils (Kourtev et al., 2003; Degens et al., 2001; Degens, 1999; Degens and Harris, 1997). However, this approach has not yet been used in the highly organic anaerobic sediments typical of salt marshes. CRP substrates include a range of organic compounds (carboxylic and amino acids, simple sugars, complex polymers) and a short incubation time (2–3 h) that more closely reflects in situ microbial activity, thus minimizing the selective biases associated with microbial culturing techniques (Øvreås, 2000).

Our study modified the procedures of Degens and Harris (1997) for use under anaerobic conditions. Substrate concentrations were chosen for preliminary experiments from a range of 90 organic compounds found in roots growing under anaerobic conditions [based on the studies of Campbell et al. (1997), Gopal and Goel (1993), Bertani and Reggiani (1991), and Crawford (1980)]. Various substrate concentrations were tested at multiple incubation time intervals. From these experiments we determined the substrates that produced measurable responses under anaerobic conditions during 3–4 h benchtop incubation. During these initial studies we observed that the addition of certain substrates resulted in a reduction in CO_2 with respect to the sediment control (addition of 1 ml deionized H_2O only), and so added the analysis of methane (CH_4) to our methods.

Our experimental goal was to determine whether the *Phragmites* and *Spartina* root zones supported different microbial communities, and whether anthropogenic disturbance overrides the effects of these plants on the sediment microbiota. A second goal was to determine whether microbial functionality in anaerobic sediments (as measured by CRPs) could be linked to microbial community structure as described by phospholipid fatty acids (PLFAs), molecular biomarkers that have been used extensively to describe microbial communities in soils and sediments (Ravit et al., 2005; Kourtev et al., 2003; Ibekwe and Kennedy, 1998; Borga et al., 1994; White et al., 1979).

2. Materials and methods

2.1. Sediment samples

Root-associated sediment samples were obtained from adjacent populations of *Phragmites australis* and *Spartina alterniflora* and unvegetated intertidal mudflats in two New Jersey coastal marshes. The first site, Saw Mill Creek (SMC) in the Hackensack Meadowlands, has a lengthy history of disturbance. Anthropogenic inputs of a variety of industrial contaminants including heavy metals, chlorinated hydrocarbons, polycyclic aromatic hydrocarbons, and other toxic compounds (Kennish, 1992), as well as numerous physical disturbances such as ditching and tidal obstruction (Quinn, 1997) have impacted this site for over 200 years. The second site is on the Maurice River (MAUR), which links the southwest portion of the Pinelands National Reserve to the Delaware River estuary. In contrast to the industrialized Hackensack River site, the Maurice River site is bounded on the inland side by the largest protected watershed in the northeastern US and the river forms the western boundary of the Pinelands Preserve. Both sites are brackish, with salinities typically ranging from 10 to 18 $g\,L^{-1}$ and SOM ranging from 10% to 15%, depending on site and plant species. Due to the higher than normal precipitation during the summer of 2003 (5th wettest calendar year since 1895; 82.65 cm of rainfall June–November (Robinson, 2004)), salinity in the river channels at the time of sampling (late August and early September MAUR and SMC, respectively) was 5–7 $g\,L^{-1}$.

Transects were established at both sites in *Spartina*-dominated and *Phragmites*-dominated zones (''treatments'') parallel to the main surface water channel; five plots (4 m^2, 2 m apart) were delineated along each transect. For the purposes of this study, we wanted the sediment to remain anaerobic, and so traditional sediment coring methods that maintain anaerobiosis of the core interior were used. Vegetation within a plot was clipped to the marsh surface and five replicate sediment cores (5 cm diameter, 10 cm depth) were extracted with a coring device from within the root zone of each plant (one from each plot). Unvegetated cores were obtained from exposed mudflats parallel to the vegetation transects. Cores were immediately placed in individual plastic bags and sealed. Two additional sediment subsamples (~50 g) were obtained at ~5 cm depth from the hole left after extracting a core. These samples were placed in separate 50 ml centrifuge tubes, which were immediately sealed. All sediment samples were taken within 2 h of low tide and transported on ice to the laboratory. Field site pH was determined with a portable Orion probe inserted directly into the sediment. The pH measurement was repeated in the laboratory to confirm that sample pH was equivalent to that observed in the field.

In the laboratory, the cores were stored anaerobically in their sealed bags at 20 °C for CRP processing within 72 h. One of the 50 g subsamples was frozen for future PLFA analysis and the other 50 g subsample was processed immediately to determine moisture content and pH. To determine sediment organic matter, all visible roots were removed from the sediment samples, which were then dried at 105 °C to a constant weight and ground to a fine powder prior to ashing in a 500 °C muffle furnace. Total organic matter percent was determined by loss on ignition.

2.2. Catabolic response profiles (CRP)—microbial community function

Based on the results of the preliminary studies, 30 organic substrates were chosen for the CRP experiment. Substrates (Sigma Aldrich) were prepared by adding the required amount of substrate to deionized H_2O to obtain the following final concentrations of substrate solution g dry wt sediment^{-1}: carboxylic acids and simple sugars 200 mM, phenolic acids, amino acids and simple sugars 100 mM, lignin and tannic acid 0.5% by weight, dried root extracts 1.0% by weight. The pH of all substrates was adjusted (using 1 N HCl or 1 N NaOH) to a range of 5.5–6.5, which was the pH that was observed in the field rhizosphere sediments. To obtain the water soluble root extracts, 0.5 g of dried *Spartina* or *Phragmites* roots were ground to a fine powder, added to 50 ml deionized H_2O and placed in a 100 °C water bath for 60 min (Ladd et al., 1995). After centrifugation for 30 min at 2000 rev. min^{-1} the supernatant was recovered.

Substrates were filter sterilized (0.2 μm Millipore) into sterile serum vials that had been capped with Teflon-lined butyl rubber septa and crimp sealed. Headspace gas in the serum vials was evacuated and replaced with argon. Substrates were stored at 4 °C until use; vials containing phenolic substrates and complex polymers were wrapped in aluminum foil to prevent light degradation.

Prior to benchtop incubation, sediment subsamples from each core were processed inside an anaerobic Coy chamber (97% N_2/3% H_2 atmosphere). Approximately 10 g wet wt. sediment was placed into a 40 ml amber glass vial; amber vials were used to prevent photosynthesis (and a corresponding loss of CO_2) from occurring during the incubation period. Addition of 1 ml of each substrate and headspace gas sampling were done though a rubber septum in the vial cap. Substrates were individually added to 5 replicate vegetation samples and 3 replicate unvegetated controls ($N = 30$ substrates × 3 or 5 replicates × 3 vegetation treatments × 2 sites = 780). The vials were then vortexed to ensure complete mixing of the substrate and the samples were incubated at 20 °C for 3–4 h. After incubation the samples were again vortexed and the headspace gases were analyzed for CO_2 and CH_4 using a Shimadzu 14C Gas Chromatograph (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a Porepak Q 4 m 1/8″ analytical column (Supelco); at a column temperature of 55 °C and H_2 carrier gas (total flow rate 30 ml min^{-1}). The retention times were 2.5 and 3.2 min (CH_4 and CO_2, respectively). Headspace gas concentrations were calculated by comparison to three point external standard curves.

Sediment controls for all samples received 1 ml of filter sterilized deionized H_2O. To determine the CO_2–C or CH_4–C produced as the result of substrate addition, gas concentrations in the controls were subtracted from gas concentrations after substrate amendment. This resulted in either a positive or negative flux value for CO_2 or CH_4 after substrate addition; flux values are reported in μg C g dry wt sed^{-1} h^{-1}.

2.3. Phospholipid fatty acid analysis (PLFA)—microbial community structure

The method of PLFA analysis described by White et al. (1979) was slightly modified. Samples were thawed and dewatered by centrifugation for 15 min at 2000 rev. min^{-1}. Two replicate 10 g wet wt subsamples ($N = 52$) were processed from each field plot. Fatty acids were extracted with 25 ml of a single-phase chloroform:methanol:0.05 M phosphate buffer (pH 7.5) solvent. The samples were then centrifuged for 15 min at 2000 rev. min^{-1} and the chloroform fraction removed. The sample was then re-extracted for 12 h after the addition of 5 ml of chloroform only. After centrifugation, the second chloroform fraction was removed. The combined chloroform fractions were concentrated to ~1 ml prior to separation into lipid fractions on a silicic acid column (King et al., 1977). The methanol eluted polar lipid fraction containing the PLFAs was retained.

This phospholipid fraction was saponified and methylated according to procedures in the Microbial Identification System (MIDI 1995). The MIDI Sherlock Microbial Identification System (MIS, Microbial ID, Newark, DE.) was used to identify individual fatty acid methyl esters based on their GC retention time. Individual fatty acids were quantified as a percentage of the total fatty acids recovered from the sample. PLFAs that contributed $<1\%$ of the total amount extracted from each sample, or PLFAs that were observed in only one sample were eliminated from the data set, yielding 42 PLFAs for statistical analysis.

2.4. Statistical analysis

The design of the study was a two-factor Model I analysis of variance (ANOVA), in which response variables were tested for differences between species (*Phragmites*, *Spartina*, unvegetated), between sites (MAUR and SMC), and for the interaction of site and species. All ANOVAs and correlation analyzes were conducted using SAS System GLM (SAS Software, Version 8.2). When site × species interactions were found in the 2-way ANOVAs, 1 df contrast analyses were used to test within site differences. Because we employed a multiple series of ANOVAs, a Bonferroni correction factor was used to test the significance of *P*-values associated with the 30 organic substrates ($\alpha = 0.05$ Bonferroni corrected $P = 0.0017$). *Post hoc* means were tested using Tukey's HSD method. Diversity of PLFAs was calculated using the Shannon–Weiner diversity index (PC-Ord, Version 4), and two-way ANOVA, as described above, was used to test for differences in PLFA diversity between samples.

Principal components analysis (PCA) was used to examine patterns among the 26 samples ($n = 5$ for each plant species in each site, $n = 3$ for unvegetated samples in each site) based on the multivariate set of 42 PLFAs and the multivariate set of 30 organic substrates (PC-Ord, Version 4). These analyses provided a reduction of the PLFA and CRP data sets, and also allowed the site and species differences among the multivariate PLFA and CRP data sets to be displayed and tested by using the principal component scores in subsequent correlation analyses.

The relationship between microbial function (CRPs) and diversity (PLFAs) was examined using correlation analysis, which was also used to examine the relationship between environmental variables (moisture, organic matter and pH) and PLFA principal components 1 and 2. Canonical correlation analysis (CANCOR) was used to test the relationship between rhizosphere microbial community function (CO_2- and CH_4-CRPs) and community structure (PLFAs). When applied to two multivariate data sets, CANCOR extracts pairs of canonical functions from both data sets that are correlated with each other. CANCOR requires that the number of samples be greater than the number of variables. To meet this requirement, we used the first 3 principal components extracted from the analysis of PLFAs and CO_2- or CH_4-CRPs (6 variables total) or the Shannon–Weiner diversity index and CO_2-CRP PC 1 and 2 (3 variables total).

3. Results

3.1. Sediment characteristics

Sediment characteristics were quite similar between the sites and species, and the observed values are within the range of variability typically observed both within and between brackish marshes (Table 1). Within the undisturbed MAUR site small, but statistically significant differences, in SOM and pH were observed between vegetated and unvegetated sediments. There were no statistically significant differences between vegetated and unvegetated sediments within the SMC site. As expected, SOM was higher in the vegetated versus unvegetated sediments at MAUR, and a similar trend was observed at SMC. These results correspond with previous studies comparing SMC sediments with sediments from another undisturbed site on the Mullica River (Ravit et al., 2003).

Table 1
Soil moisture content (%), soil organic matter (SOM) and pH means and (SE), $n = 5$ for vegetated samples and $n = 3$ for unvegetated controls

Site	Plant	Moisture (ml g dry wt. sed^{-1})	SOM (%)	pH
	2-Factorial ANOVA	NS	$F = 6.16$, $P = 0.001$	$F = 5.8$, $P = 0.0015$
Saw mill creek	*Spartina*	0.54 ± 0.007^{a}	12.5 ± 8.09^{a}	6.28 ± 0.34^{a}
	Phragmites	0.52 ± 0.010^{a}	11.7 ± 1.14^{a}	6.34 ± 0.13^{a}
	Unvegetated	0.56 ± 0.013^{a}	9.8 ± 0.26^{a}	7.12 ± 0.08^{a}
	Contrast ANOVA		NS	NS
Maurice	*Spartina*	0.60 ± 0.005^{x}	15.0 ± 0.22^{x}	$5.88 \pm 0.11^{x,y}$
	Phragmites	0.58 ± 0.006^{x}	13.8 ± 0.43^{x}	5.46 ± 0.34^{x}
	Unvegetated	0.54 ± 0.013^{x}	11.3 ± 1.17^{y}	6.59 ± 0.06^{y}
	Contrast ANOVA		$P = 0.004$	$P = 0.035$

Two-factorial ANOVA df = 2,10. Within site differences were analyzed using 1 df Contrast ANOVAs. Letters indicate significant differences within each site.

Table 2
Substrates used for CO_2 and CH_4 catabolic response profiles (CRPs) that showed significant differences as tested by 2-*factorial* ANOVA (df = 5,20)

Substrate	Type	CO_2	Significant factor	MAUR	SMC	CH_4	Significant factor	MAUR	SMC
H_2O	—	$P<0.0001$	Site***, Veg***	S>P, U	S>U	NS			
α-Ketovaleric acid	CA	$P=0.0004$	Site × Veg**	NS	V>U	NS			
Malic acid	CA	$P=0.0007$	Site × Veg***	NS	V>U	NS			
Oxalic acid	CA	NS				$P<0.0001$	Site × Veg**	S>P, U	NS
Succinic acid	CA	NS				$P=0.0017$	Site × Veg*	S>P,U	NS
Coumaric acid	PA	NS				$P=0.0003$	Site × Veg**	S>P,U	NS
Hydroxybenzoic acid	PA	NS				$P=0.0001$	Site × Veg**	S>P, U	NS
Arginine	AA	$P<0.0001$	Site × Veg*	P>S, U	NS	$P=0.0012$	Site × Veg**	S>P, U	NS
Glutamine	AA	$P=0.0008$	Site***	NS	NS	NS			
Methionine	AA	$P=0.0010$	Site***	NS	NS	NS			
Glycine	AA	$P=0.0017$	Site***	P>S, U	NS	NS			
Rhamnose	SS	$P=0.0002$	Site***	NS	NS	NS			
Fructose	SS	$P<0.0001$	Site***, Veg*	V>U	NS	NS			
Mannose	SS	$P<0.0001$	Site***, Veg**	V>U	NS	$P<0.0001$	Site × Veg***	S>P, U	NS
Lignin	CP	$P=0.0002$	Site***	NS	NS	NS			
Spartina extract	CP	NS				$P=0.0008$	Site × Veg**	S>P, U	NS
Phragmites extract	CP	NS				$P=0.0002$	Site × Veg**	S>P, U	NS

Significant factor(s) are given for each substrate. Significant *P*-value using Bonferroni correction for multiple tests $\alpha = 0.0017$ (asterisks indicate *P*-value associated with the significant factor(s): *** ⩽0.001; ** ⩽0.01; * ⩽0.05). Within site differences (Contrast analysis, df = 1) shown as "MAUR" and "SMC" (S = *Spartina*, P = *Phragmites*, V = vegetated, U = unvegetated). Substrate type: CA = carboxcyclic acid; PA = phenolic acid; AA = amino acid; SS = simple sugar; CP = complex polymer.

3.2. Catabolic response profiles (CRPs)

Although decomposition processes are generally thought to occur at slower rates in anaerobic versus aerobic systems, we observed a rate of CO_2 production (2–20 μg CO_2–C g sed^{-1} h^{-1}), which was equivalent to that seen by Kourtev et al. (2003) and depending on the substrate, somewhat lower than that observed by Degens and Harris (1997) in forested soils (2–15 and 36–200 μg CO_2–C g sed^{-1} h^{-1}, respectively). Unlike the reported terrestrial CRP results, we observed significant production of CH_4 in vegetated samples. Two-factorial ANOVA of sediment catabolic responses to the 30 organic substrates indicated significant differences in production of both CO_2 (11 substrates) and CH_4 (8 substrates) among both the sites and the species (Table 2). No significant differences were observed in sediment responses to putrescine, proline, glucosamine, histidine, tannic acid, ketoglutarate, ketovalerate, malonate, tartarate, citrate, or caffeic, protocatechuic and gallic acids.

The production or consumption of CO_2 and the production of CH_4 varied in response to different substrate categories and by site. Undisturbed MAUR sediments produced measurable amounts of both gases more frequently than did the SMC sediments (78% and 92% of the MAUR tests versus 38% and 74% of the SMC tests, CO_2 and CH_4, respectively). Histograms of carboxylic, amino and phenolic acid CO_2 responses (Fig. 1) illustrate the consistently higher gas production in the MAUR sediments versus the SMC sediments, particularly in response to amino acid and simple sugar substrates. Amino acid substrates elicited the highest CO_2 production of any substrate group, and the MAUR *Phragmites* sediments produced the greatest CO_2 concentrations in response to the amino acids tested (Fig. 1(a)). Conversely, SMC-vegetated sediments produced CO_2 in response to carboxylic acid substrates (Fig. 1(c)). Production of CH_4 in the MAUR sediments was an order of magnitude greater than the CH_4 production in comparably vegetated SMC sediments. This was due almost entirely to high rates of CH_4 production in the *Spartina* sediments at MAUR, including significantly higher CH_4 production in response to the two root extracts; *Phragmites* and unvegetated sediments produced little or no CH_4, respectively (Fig. 2).

PCA ordination of the CO_2 catabolic responses (Fig. 3(a)) demonstrated a clear separation within the ordination space between the undisturbed MAUR site and the disturbed SMC site; unvegetated sediments from both sites clustered together and were separated from their vegetated counterparts. Two-factor ANOVA of PCA axis 1 scores were significantly different between sites ($F_{5,20} = 38.1$, $P<0.0001$). However, there were no significant differences between the two plant species within a given site. The ordination of CH_4 responses resulted in separation of only the MAUR *Spartina* within the ordination space (Fig. 3(b)). In PCA CO_2 loading plots of the substrates tested (Fig. 4(a)), carboxylic acids clustered at one end of the ordination space, while amino acids clustered at the opposite end. These results correspond to the separation of the two sites in the site ordination (Fig. 3(a)), suggesting that the carbon substrate is an important factor in differentiating microbial community functions between the two sites. The ordination of substrates with respect to CH_4 production clearly separates the substrate responses as noted above.

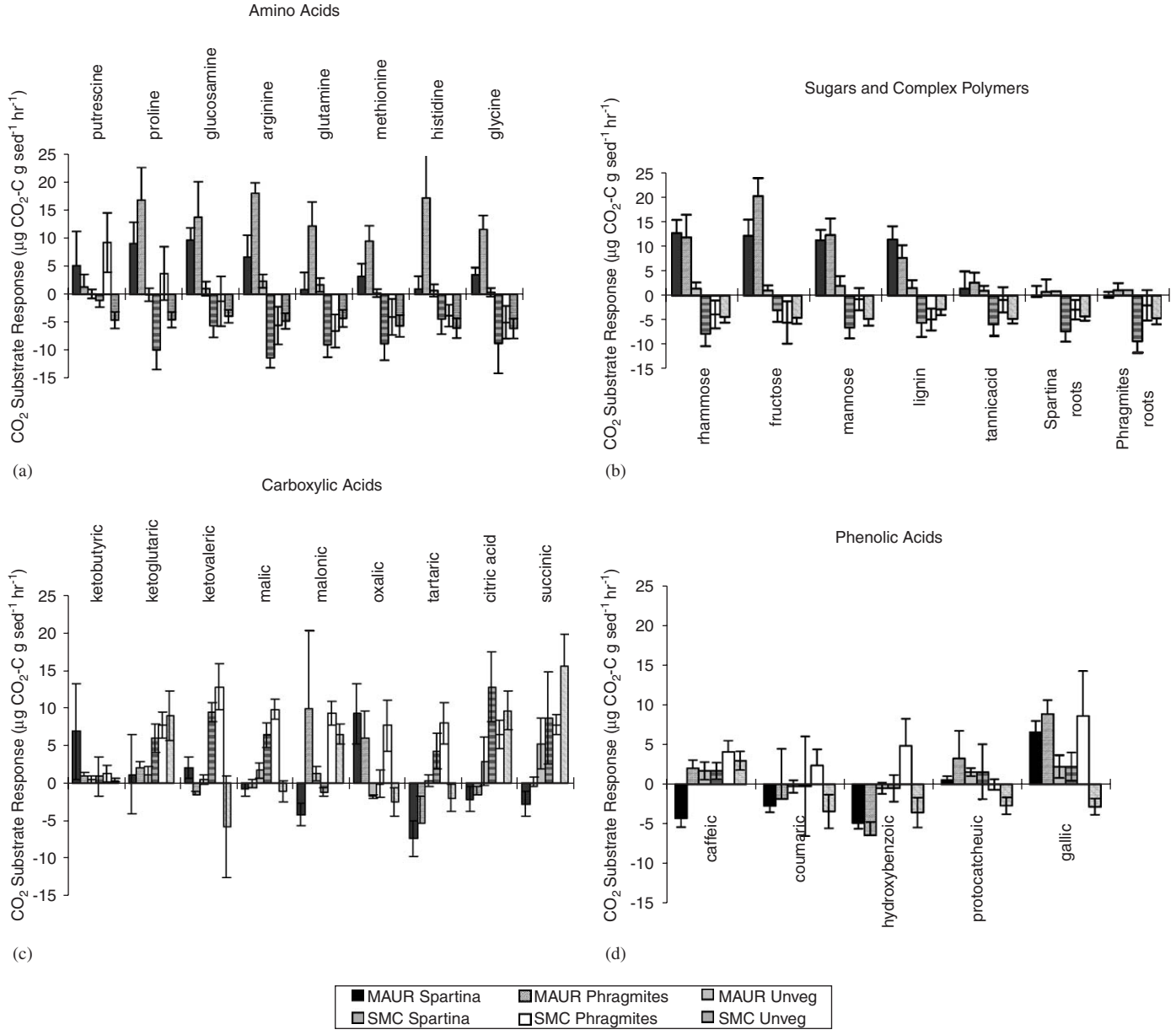

Fig. 1. CO_2 catabolic response profiles describing production or consumption of CO_2 (µg-CO_2-C g sed^{-1} h^{-1}) in response to the addition of carboxylic, amino, phenolic acids, simple sugars and complex polymers. Error bars denote (SE).

3.3. Phospholipid fatty acid (PLFAs)

The composition and relative abundance of PLFAs in the MAUR sediments were distinct from those in the SMC sediments (Tables 3 and 4). PLFA diversity, as measured by the Shannon-Weiner Diversity Index, was significantly different between sites ($F_{5,20} = 7.24$, $P = 0.0005$), and the significant variable was a site × species interaction (Table 3). PLFA diversity was significantly greater in vegetated versus unvegetated MAUR sediments, but PLFA diversity was not significantly different in the SMC samples. Total PLFAs recovered were higher in *Spartina* sediments at both sites (49) and the number of sediment PLFAs recovered from MAUR *Phragmites* were ~30% greater than from SMC *Phragmites* (Table 3).

The PCA ordination of the PLFA data clearly separates the two sites (Fig. 5). Two-factor ANOVA of PCA axis 1 scores demonstrated significant differences between sites ($F_{5,52} = 17.65$, $P < 0.0001$). Microbial communities in the vegetated sediments were clearly distinct from unvegetated communities within the ordination space. Vegetated microbial communities are separated within the MAUR site, but the data suggest that the SMC microbial communities are similar among all samples. This pattern corresponds to previously reported PLFA patterns from SMC versus undisturbed sediments (Ravit et al., 2003).

Eight PLFAs (14:0, 15:0 Iso, 15:0 Ante Iso, 16:0, 16:1 ω7c, 16:0 10 Me, 18:1 ω7c, 19:1 ω6c/19 Cy) accounted for 89% of the difference among vegetation treatments based on the variable loading scores. Significant PLFA differ-

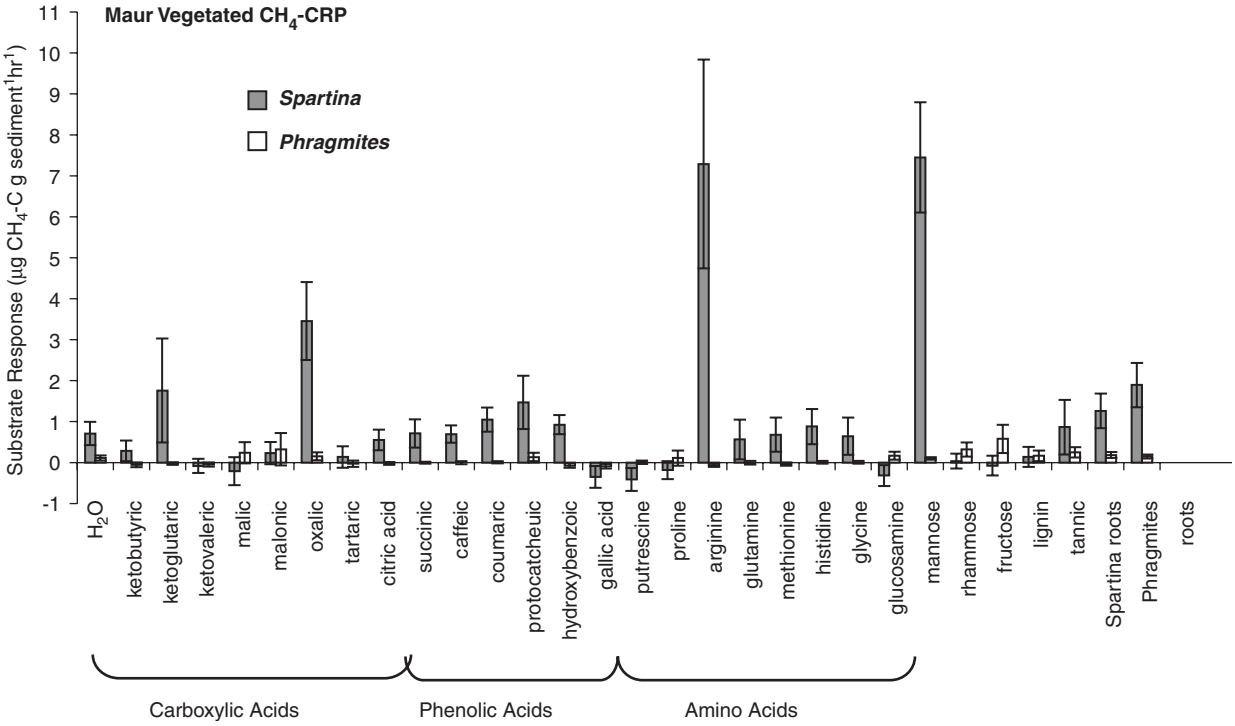

Fig. 2. Catabolic response profiles for MAUR sediments describing the production or consumption of CH_4–C ($\mu g\, CH_4$–C $g\, sed^{-1}\, h^{-1}$). Error bars denote (SE).

ences between sediments in the MAUR samples were observed for all but one PLFA (15:0 Ante Iso), while only one of these PLFAs (19:1 ω6c/19 Cy) showed significant differences between the SMC sediments (Table 4).

3.4. Correlations between CRPs, PLFAs and environmental variables

Microbial community structure (as measured by PLFA), and microbial community function (as measured by CRP) were significantly correlated (Table 5). While the correlation between PLFA PC1 and CO_2-CRP PC 1 was significant (Wilks' Lambda $P = 0.0006$), no correlation was observed between PLFAs and CH_4-CRPs. PLFAs from vegetated sediments exhibited a significant correlation with the soil moisture and organic matter content, but not with pH. Conversely, unvegetated sediments exhibited a significant PLFA correlation with pH (Table 6).

4. Discussion

Contrary to expectation, the sediment microbial communities associated with two estuarine plant species could not be separated by either indices of community structure (PLFAs) or community function (CRPs). We found that sediment microbial community structure and function reflected primarily the conditions of the specific sites that were sampled. Both the structural and functional indices differentiated the vegetated and unvegetated communities at the undisturbed MAUR site, but not at the disturbed SMC site. This result corresponds to our earlier studies analyzing extracellular enzyme activity, which differed in sediments associated with macrophyte vegetation in the undisturbed Mullica River estuary, while 4 of 5 enzymes tested showed no vegetation differences in the disturbed SMC sediments (Ravit et al., 2003). The SMC site has been subjected to multiple and long-term disturbances related to hydrology, sediment composition, and biogeochemistry (both nutrients and contaminants). Our data does not allow us to determine which of these factors is the most important variable(s) affecting the site's microbial community. We also note that our studies have only tested disturbed sediments from one estuarine system (SMC) and additional studies are needed in other disturbed sites to confirm our findings. However, our data does suggest that disturbance of estuarine ecosystems may overwhelm the influences of macrophyte vegetation, resulting in sediment microbial communities whose structure and functional abilities differ from microbial communities in undisturbed systems.

4.1. Catabolic response profiles (CRPs)

This study demonstrates that CRPs can differentiate microbial community functionality in anaerobic estuarine sediments. While CRPs have been used to test plant-associated effects in upland soils, this is the first study we are aware of that has used CRPs to test the effect of macrophyte vegetation on sediment microbial communities under estuarine conditions. The analysis demonstrates that

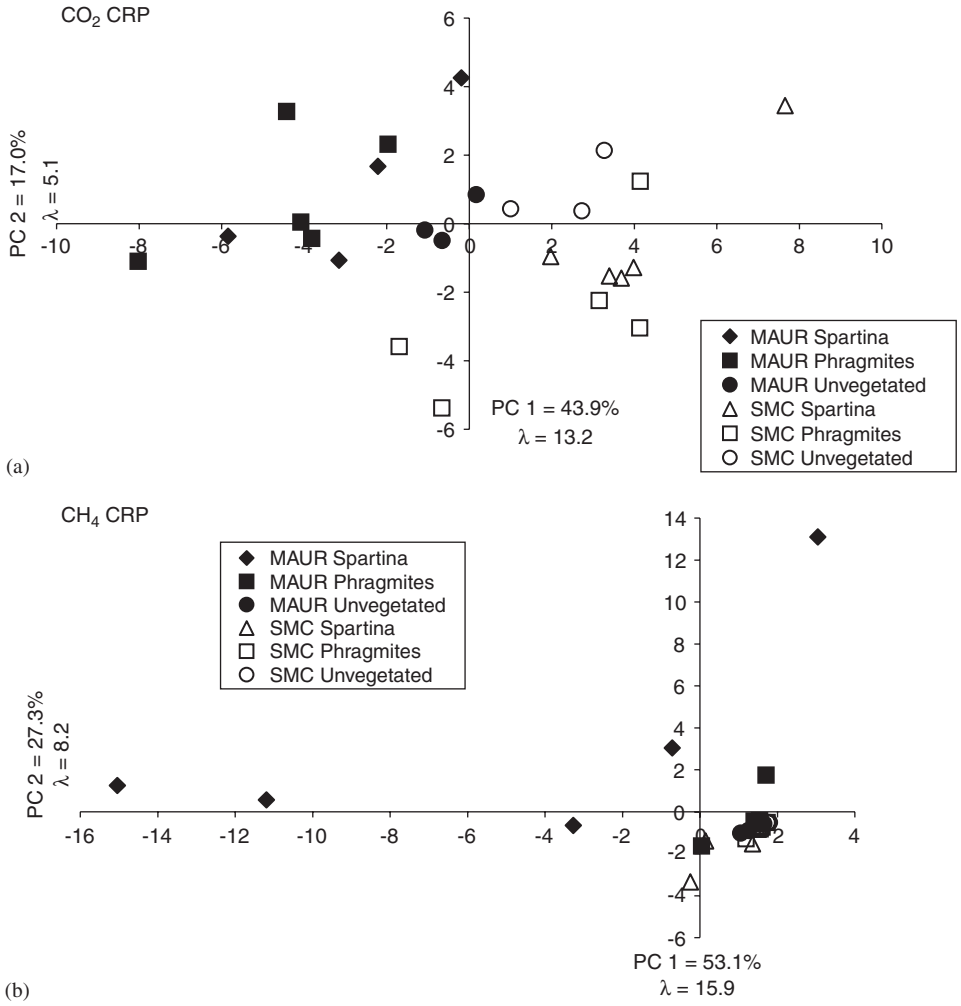

Fig. 3. Principal components ordination diagrams for (a) CO_2 and (b) CH_4 Catabolic response profiles for Saw Mill Creek and Maurice River sediments. For each diagram, the percent of variation explained and the Eigenvalue are given along each axis.

different microbial communities respond differently to the various classes of substrate (carboxylic acids, amino acids, phenolic acids, simple sugars, complex polymers). In both the production of CO_2 and CH_4, the type of substrate acted differentially in stimulating microbial communities in the two sites. Thus we conclude that catabolic response profiles are a useful technique in understanding microbial community function in estuarine sediments.

Plant species are known to differ in both the quantity and quality of carbon transport to the root zone, and macrophyte root exudates vary depending on plant species and environmental factors (Neori et al., 2000; Grayston et al., 1998; Lynch and Whipps, 1990). There are large differences in both root morphology and the amount of fine root biomass of *Spartina* and *Phragmites*, as well as differences in the vertical distribution of roots within the sediments (Ravit et al., 2003). *Spartina* produces dense, fibrous masses of fine roots in the surface sediments (predominately 0–10 cm depth). *Phragmites* fine roots are sparsely distributed along primary roots that emanate from large rhizomes, which extend vertically (depths >70 cm) and horizontally (multiple meters) though the sediments. Therefore it is surprising that despite these differences in root systems, there were few indications of microbial differences associated with the two plant species from each site. It is possible that our inability to identify plant-associated differences may be an artifact due to our anaerobic coring technique used to sample. When extracting sediment cores, material can be obtained that is not in direct contact with the root. We may therefore have sampled a mixture of rhizosphere and bulk sediment microorganisms, which masked the functional abilities of the rhizosphere community alone.

4.1.1. Methane production

The most striking result of the CRP analysis was the high rate of methanogenesis in the MAUR *Spartina* sediments, suggesting that this rhizosphere microbiota was quite distinct from that of the other sediments. This result strongly suggests that in addition to the measurement of CO_2 production, methanogenesis should be included in methodologies using metabolic response

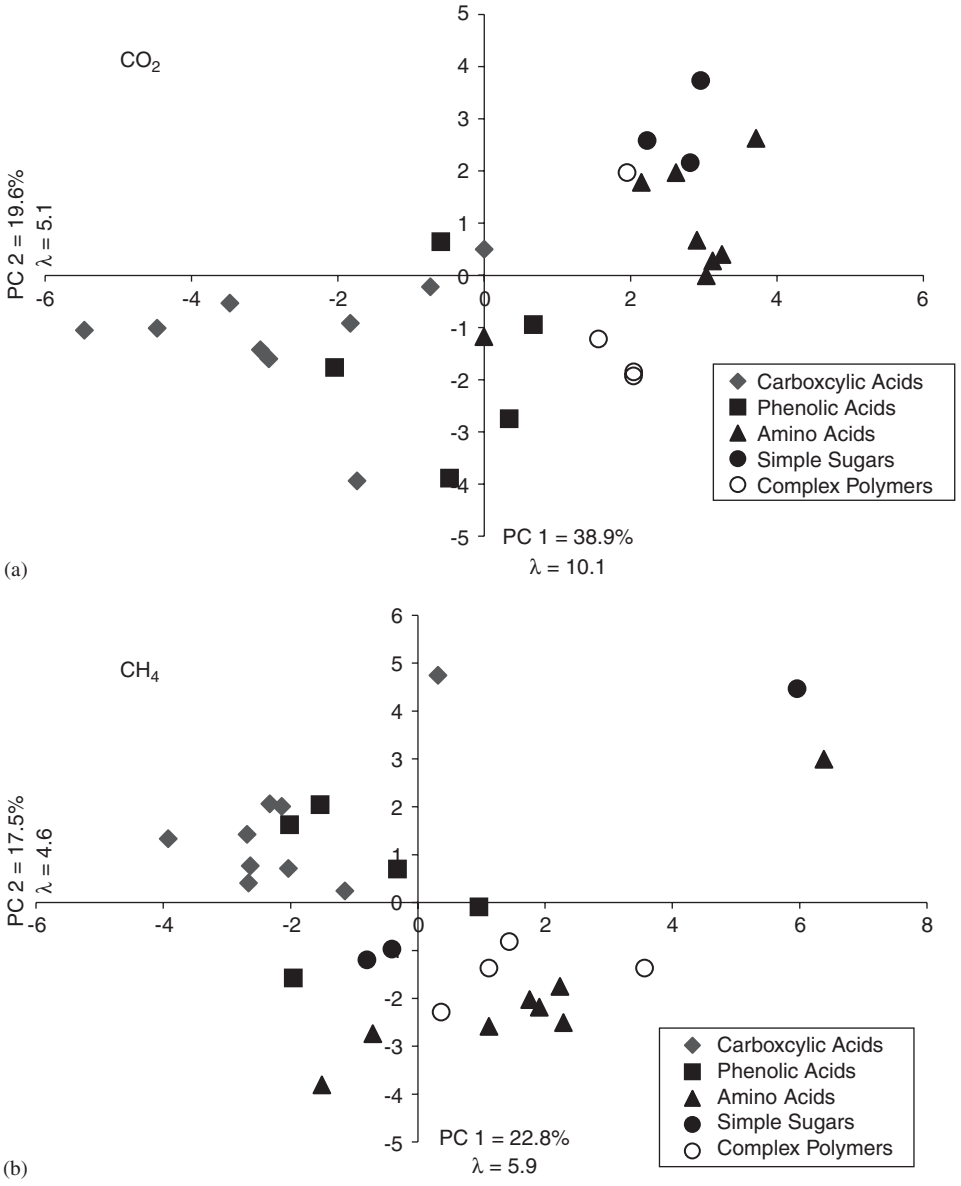

Fig. 4. Principal components ordination diagram for (a) CO_2-CRP and (b) CH_4-CRP loading plots. For each diagram, the percent of variation explained and the Eigenvalue are given along each axis.

profiles in estuarine sediments. The production of CH_4 indicates the presence of active methanogenic archaea. It is possible that the higher than normal precipitation and lowered salinity could be increasing the ability of methogens to compete with sulfate-reducing bacteria (SRB) for available hydrogen (H_2). However, if higher CH_4 production was related to precipitation and salinity levels only, we would expect to see significant CH_4 produced by samples other than the MAUR *Spartina*, but this was not observed.

Under anaerobic conditions, SRB, acetogens, and methanogens compete for H_2 reducing equivalents (Häggblom and Bossert, 2003). However, cluster arrangements between H_2 producers and consumers are common in anaerobic microbial consortia, and the plant or environmental C and nutrient sources may play a role in defining metabolic activity within the root zone community (Alexander, 1999; Moller et al., 1997; Grotenhuis et al., 1991). The release of reduced photosynthates from root surfaces is likely supporting bacteria capable of fermentation or acetogenesis, and the H_2 produced by these processes is an important source of reducing equivalents. While it is likely that root exudates from *Spartina* differ from those of *Phragmites*, the order of magnitude greater CH_4 production by vegetated MAUR sediments suggests that the methanogenic community in this site is more active than in the disturbed SMC sediments. However, our DNA analysis indicates the presence of methanogenic archaea in the vegetated SMC sediments (data not shown) and so further study is required to determine the cause of lower CH_4 production in the disturbed system.

Another potential source of H_2 is as a by-product of N-fixation (Leigh, 2004; Sparling and Gottschalk, 1990).

N-fixing diazotrophs are commonly found in the *Spartina* root zone (Bagwell et al., 2001), although we know of no detailed studies of the functional ability of N-fixers associated with *Phragmites* roots. However, under field conditions, the amount of H_2 produced by N-fixation may be insignificant compared to that produced by fermentation or acetogenic processes.

4.1.2. Disturbed versus undisturbed CRP responses

CRPs differed significantly between disturbed and non-disturbed sites, and the influence of macrophyte vegetation was clearly more important in the undisturbed MAUR sediments. A particularly interesting response is that of the MAUR *Phragmites* sediments to the addition of amino acid substrates, resulting in the highest amounts of CO_2 production observed in this experiment. The rapid microbial mineralization of substrates containing N would indicate that N is a limiting nutrient in the undisturbed *Phragmites* sediments. The MAUR system drains the low nutrient Pine Barrens, while the SMC system has high concentrations of extractable porewater N (Ravit unpublished data). In the low nutrient Mullica system Windham and Ehrenfeld (2003) have demonstrated high *Phragmites* uptake of porewater N, and lower *Phragmites* porewater N concentrations than those found in *Spartina* sediments. As a result of the presence of N-fixing diazotrophs or lower plant uptake of porewater N the MAUR *Spartina* sediments may be less N limited than the MAUR *Phragmites* sediments. If so, this would account for the lower MAUR *Spartina* response to the amino acid substrates.

A striking difference between anthropogenically disturbed and non-disturbed samples was the consumption of CO_2 by SMC sediments after the addition of various organic substrates. This response may indicate greater activity of acetogens (see below) and/or chemolithotrophs in contaminated versus uncontaminated sediments. Consumption of CO_2 was particularly apparent with the addition of amino acids and simple sugars (Fig. 1(a and b), both of which are substrates utilized by acetogenic microorganisms (Drake, 1994). If acetogens are more active in the contaminated sediments, this may account for the lower activity of methanogens, since these two types of microorganisms compete for H_2.

Table 3
PLFA diversity as measured by the Shannon-Weiner diversity index score (PC-Ord)

Site	Plant	Diversity	PLFAs (number recovered)
2-Factorial ANOVA	$F = 7.24$	$P = 0.0005$	Site × species interaction
Saw mill creek	*Spartina*	2.66 ± 0.107^{a}	49
	Phragmites	2.61 ± 0.035^{a}	31
	Unvegetated	2.59 ± 0.096^{a}	32
Contrast ANOVA		NS	
Maurice	*Spartina*	2.91 ± 0.017^{x}	49
	Phragmites	2.89 ± 0.023^{x}	43
	Unvegetated	2.50 ± 0.008^{y}	28
Contrast ANOVA		$P < 0.0001$	

Diversity indices were analyzed using 2-way factorial ANOVA (df = 5,25). Within site differences were analyzed using 1 df contrast ANOVAs. Letters indicate significant differences within each site.

4.2. Phospholipid fatty acid (PLFAs)

The largest number of PLFAs recovered, and the highest overall PLFA diversity, were found in vegetated sediments from the undisturbed MAUR site. These differences in the composition and diversity of PLFAs were not seen in the disturbed SMC sediments. It is possible that the higher diversity of PLFAs (indicating a more diverse microbial community) in the undisturbed vegetated sediments

Table 4
Percents and (SE) of eight PLFAs responsible for 89% of the ordination space variation (PC 1) in the PLFA loading plot

Site	Vegetation	14:0 Gram −	15:0 ISO Gram +	15:0 Ante Gram +	16:0 Ubiquitous	16:1 ω7c Gram −	18:1 ω7c Gram −	16:0 10ME Gram +, SRB	19:1 ω6c/19:0Cy Gram +/Gram −
2-Factorial ANOVA		$F = 4.24$** Site × Veg**	$F = 7.29$*** Site × Veg*	NS	$F = 5.78$** Site***	$F = 26.77$*** Site × Veg***	$F = 9.2$*** Site × Veg***	$F = 2.59$* Veg**	$F = 50.12$*** Site × Veg**
Saw Mill Creek	*Spartina*	5.0 ± 0.61^{a}	5.3 ± 0.43^{a}	$6.3 + 0.92^{a}$	$31.6 + 4.75^{a}$	$9.7 + 0.91^{a}$	$6.2 + 0.71^{a}$	$3.6 + 0.52^{a}$	0^{a}
	Phragmites	4.7 ± 0.19^{a}	$6.4 + 0.37^{a}$	$7.6 + 0.39^{a}$	$27.5 + 1.91^{a}$	$11.7 + 0.82^{a}$	$7.5 + 0.34^{a}$	$3.8 + 0.53^{a}$	$1.7 + 0.49^{b}$
	Unvegetated	4.6 ± 0.17^{a}	$5.7 + 0.39^{a}$	$6.9 + 0.27^{a}$	$26.3 + 0.49^{a}$	$12.8 + 0.51^{a}$	$7.6 + 0.49^{a}$	$2.7 + 0.24^{a}$	$8.7 + 2.25^{c}$
Maurice	*Spartina*	$3.8 + 0.18^{x}$	$7.0 + 0.18^{x}$	$6.8 + 0.57^{x}$	$16.47 + 0.32^{x}$	$17.2 + 0.87^{x}$	$7.9 + 0.52^{x}$	$3.9 + 0.29^{x}$	$1.8 + 0.33^{x}$
	Phragmites	$4.0 + 0.14^{x}$	$7.3 + 0.15^{x}$	$7.0 + 0.19^{x}$	$19.4 + 0.44^{y}$	$19.4 + 0.44^{y}$	$7.4 + 0.43^{x}$	$5.1 + 0.32^{x}$	$2.5 + 0.16^{x}$
	Unvegetated	$6.1 + 0.67^{y}$	$5.6 + 0.12^{y}$	$6.3 + 0.64^{x}$	$20.5 + 1.33^{y}$	$20.5 + 1.33^{z}$	$3.0 + 0.57^{y}$	$2.5 + 1.46^{y}$	$15.2 + 1.2^{y}$

The *F* values (df = 5,20) for two-factorial ANOVAs and the significant factors are given for each PLFA. Within site differences were tested using Contrast ANOVAs (df = 1) and are indicated by letters. Asterisks indicate significant *P* values ($P < 0.05$ = *, $P < 0.01$ = **, $P < 0.001$ = ***). PLFAs are described using standard nomenclature. Numbering of the carbons begins at the aliphatic (ω) end of the PLFA; the number of double bonds is given after the colon; *cis* configuration is designated with 'c'; 'Iso' and 'Ante' designate iso- and anteiso-branched PLFAs, respectively, ME = methyl; OH = hydroxy; Cy = cyclopropane. Due to identical retention times PLFA 16:1 ω7c/15:0 ISO 2OH and 19:1 ω6c/19:0 Cy co-elute and cannot be differentiated with the MIDI system.

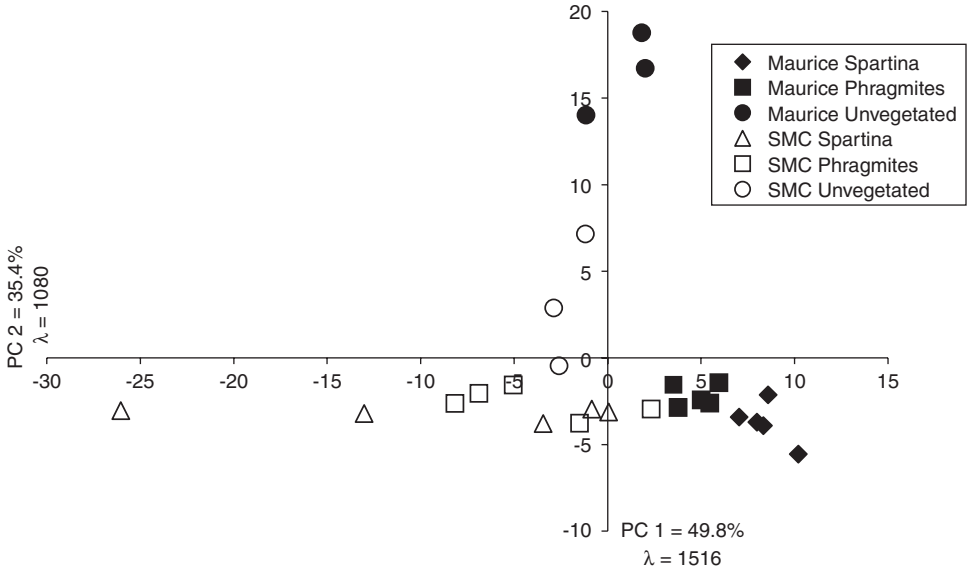

Fig. 5. Principal components ordination diagram for 42 PLFA variables. PFLA values were the mean of two subsamples taken from each sampling location. The percent of variation explained and the Eigenvalue are given along each axis.

Table 5
Canonical correlation analysis (CANCOR) of PLFAs and CO_2-CRPs

Variables	
Canonical variables	
PLFA PC1	1.0
PLFA PC2	0.01
CRP PC1	−0.88
CRP PC2	0.47
Canonical correlation	
CV 1 (PLFA PC1/CRP PC1)	0.68
CV 2 (PLFA PC2/CRP PC2)	0.12

Canonical variables indicate the relationship between the input variables, which were a linear combination of the original data (Principal components 1 and 2) and their canonical variables. The canonical correlation describes the correlation between the PLFA and CRP canonical variables. Wilks' Lambda test statistic $F_{4,44} = 4.15$, $P = 0.006$ and $P = 0.579$ (CV1 and CV2, respectively) indicates a high correlation between canonical variables PLFA PC1 and CRP PC1.

Table 6
Testing of PLFA principal components Axis 1 with the environmental variables of moisture, organic matter, and pH for each vegetation treatment

Variables	*Spartina*	*Phragmites*	Unvegetated
Cross-loading with PLFA–PC 1			
Environmental factors			
Moisture %	0.77	0.71	−0.55
F	0.009	0.022	0.256
Organic matter content	0.67	0.77	0.10
F	0.036	0.009	0.855
pH	−0.45	−0.58	−0.91
F	0.187	0.081	0.012

Pearson correlation coefficient is the *F* statistic, which describes the significance of the association of PLFA PC1 and the specific environmental variable.

resulted in the greater variations in CRP response observed in MAUR sediments. Loading plots suggest that a limited number of PLFAs are responsible for the differences observed between samples. While there were overall differences in the PLFAs recovered from the various sampling sites, eight PLFAs are responsible for 89% (PC 1) of the variation among vegetation treatments. In our previous studies (Ravit et al., 2005) these same PLFAs were observed to account for over 90% of the separation within the PCA ordination space, suggesting that certain bacterial community members may consistently dominate under estuarine conditions of anaerobiosis and brackish salinity.

Six of the eight PLFAs responsible for the separation of PLFAs on the first PCA axis are commonly found in Gram negative proteobacteria (Ratledge and Wilkinson, 1988), which under brackish salt marsh conditions suggest sulfate-reducing bacteria. PLFAs 15:0 ISO, 15:0 Ante ISO, 16:0, 16:0 10Me, and 19:0Cy are also found in Gram positive bacteria (Ratledge and Wilkinson, 1988), which under anaerobic sediment conditions suggest the presence of acetogens (Leaphart et al., 2003; Drake, 1994). Without further molecular analyses it is not possible to determine the active microbial taxa, but we note that a high proportion of acetogens in the SMC sediments would explain the consumption of CO_2 that was observed in the CRP experiments.

4.3. Correlations between microbial community structure (PLFAs) and function (CRPs)

While production of CO_2 was significantly correlated with microbial community structure, there was no sig-

nificant microbial community correlation with CH_4 production. We point out that the PLFA method used to index microbial communities describes the bacterial component of this biota. Archaea do not have glycerol ester membrane fatty acids, so are not represented in these analyses. Under anoxic conditions prevalent in estuarine wetlands, archaea may play an important role in response to variations in plant community composition. In comparing microbial community function and structure in anaerobic systems this archaeal community may be of greater importance than in aerobic terrestrial systems.

4.4. Conclusions

This study demonstrates that under anaerobic wetland conditions the CRP method may provide a useful community descriptor of sediment microbial function. Microbial community composition, diversity and functional abilities were distinctly different in the undisturbed MAUR sediments from those in the disturbed SMC sediments. The lack of a clear differentiation between *Phragmites* and *Spartina*-vegetated sediments at both sites suggests that the paradigm that plant species drive the composition and function of rhizosphere microbial communities may not hold in wetland sediments, particularly in those subject to anthropogenic disturbance. Rather, site-specific factors may override even large differences in the root inputs that structure microbial communities. Although these findings parallel extracellular enzyme results of previous experiments (Ravit et al., 2003) which compared disturbed and undisturbed sites, SMC is the only disturbed system we have sampled, and so further studies are needed to determine the impact of system disturbances on salt marsh microbial function. Further studies are also needed to define the role of methanogens in estuarine rhizosphere communities, to elucidate the role that vegetation plays in structuring these sediment microbial communities, and to explain how the effects of anthropogenic disturbances may overwhelm these macrophyte influences.

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