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Salt marsh rhizosphere affects microbial biotransformation of the widespread halogenated contaminant tetrabromobisphenol-A (TBBPA)

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Abstract

Estuarine sediments are the repository for a wide range of contaminants. Anthropogenic impacts and variations in the belowground biomass of salt marsh plants potentially select for different sediment microbial communities with different functional capabilities, including the ability to biotransform anthropogenic contaminants. There are large differences in both root morphology and the amount of fine root biomass of *Spartina alterniflora* and *Phragmites australis*; *Spartina* is the species commonly used to replace *Phragmites* in northeastern US salt marsh restoration projects. Our study compared the effect of these two macrophyte species on sediment microbial communities responsible for the biotransformation of the halogenated flame retardant tetrabromobisphenol A (TBBPA). Sediments were obtained from contaminated and uncontaminated salt marsh field sites in New Jersey. Anaerobic methanogenic sediment microcosms were established and incubated for up to 130 days. TBBPA was reductively dehalogenated resulting in the transient formation of two intermediates, identified as tribromobisphenol A and dibromobisphenol A, and the formation and accumulation of bisphenol A (BPA) as the end product. *Spartina* sediments from both sites were found to dehalogenate TBBPA more rapidly than the *Phragmites* or unvegetated sediments, resulting in greater production of BPA. Microbial community diversity as measured by *in situ* sediment phospholipid fatty acid (PLFA) composition prior to TBBPA exposure, was found to be higher in the uncontaminated sediments; differences in microbial PLFA diversity were not seen in contaminated sediments associated with either the different plant species or unvegetated sediment. The results of this study demonstrate that these two plant species affected sediment microbial community function with respect to dehalogenation capabilities, even though the disturbed and undisturbed sediments varied in microbial community composition.

Keywords: Rhizosphere; Sediment; Microbial community; Microbial diversity; PLFA; *Spartina alterniflora*; *Phragmites australis*; Tetrabromobisphenol A; Bisphenol A; Reductive dehalogenation; Estuarine contamination; Salt marsh restoration

1. Introduction

Estuaries are among the most ecologically sensitive and economically important of all ecosystems (Costanza et al., 1997), and many projects have been undertaken to restore anthropogenically damaged estuarine sites. Restoration goals typically focus on vegetation (species, percent cover), habitat usage, and rare or endangered species (NRC 2001). While aboveground attributes are important, estuarine sediments are the repository for a wide range of contaminants (Hale and LaGuardia, 2002; Scrimshaw et al., 1996; Kennish, 1992), whose degradation presents very different challenges from the restoration of aboveground plant and animal habitat.

The ultimate fate of anthropogenic contaminants is largely determined by microbially mediated biotransformations. Vegetation influenced affects, including nitrogen fixation and sulfate reducing activity, as well as variation in microbial community composition and numbers, have

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been observed in estuarine rhizosphere microbial populations (Burke et al., 2002; Nielsen et al., 2001; Bagwell et al., 2001; Hines et al., 1999). However, few studies of either wetland vegetation or sediment microbial communities have compared functional differences associated with different macrophyte species with respect to contaminant biotransformation. If plant-mediated effects on microbial communities result in changes to the rates and processes of contaminant biotransformations, then efforts to manage and restore the vegetation of coastal marshes may inadvertently affect the fate, transport, and ecological impact of these contaminants. In northeastern US salt marsh restoration sites *Phragmites australis* (Cav.) Trin. ex. Steud. (hereafter *Phragmites*) is commonly removed and replaced with *Spartina alterniflora* Loisel (hereafter *Spartina*). There is currently limited understanding of how this vegetation change affects sediment microbial community structure or functional processes.

Halogenated organic compounds are considered to be some of the most persistent, ubiquitous, and toxic pollutants in estuarine sediments (Hale and LaGuardia, 2002; Häggblom et al., 2000; Kennish, 1998). Marine environments are known to be a rich source of natural biogenic halogenated compounds produced by native plants, animals and microorganisms (Neori et al., 2000; Cowart et al., 2000; Gribble, 1994; Manley and Dastoor, 1987; Fenical, 1981), and it is thus likely that dehalogenating microbial communities are common in estuarine ecosystems.

Tetrabromobisphenol A (TBBPA) [4,4′-isopropylidene-bis (2,6-dibromophenol)] is a brominated flame retardant, whose worldwide production is greater than 500,000 metric tonnes annually (Alaee et al., 2003). Due to its low water solubility TBBPA sorbs onto particles and organic matter, and has been detected as a sediment contaminant worldwide (for reviews see Wantanabe and Sakai, 2003; Santillo and Johnston, 2003; Alaee et al., 2003). The final product of microbial reductive dehalogenation of TBBPA (Arbeli and Ronen, 2003; Voordeckers et al., 2002; Ronen and Abeliovich, 2000) is bisphenol A (BPA) (4,4′-isopropylidenediphenol), a known endocrine disrupter (Markey et al., 2003; Hunt et al., 2003). BPA has not been found to degrade under anaerobic conditions (Voordeckers et al., 2002; Ronen and Abeliovich, 2000) typical of wetland sediments. Understanding the effect estuarine vegetation has on TBBPA dehalogenating microbial populations is necessary to predict the fate of TBBPA, as well as the production and accumulation of BPA in estuarine sediments.

The purpose of this study was to examine the effect of two salt marsh macrophyte species on the sediment microbial community responsible for the biotransformation of TBBPA (microbial community function). We also examined differences in dehalogenation potential between sediments that had prior exposure to anthropogenic contaminants versus sediments without such exposure. A second goal of the study was to relate the dehalogenation function to microbial community structure through analysis of the phospholipid fatty acid (PLFA) biomarkers. The proportion of individual membrane PLFAs is distinct for different bacterial species, and a description of the total PLFAs extracted from a given environmental sample forms a PLFA profile describing the *in situ* bacterial community at the time of sampling. These molecular biomarkers have been used extensively to describe microbial communities in soils and sediments (Ravit et al., 2003; Kourtev et al., 2003; Ibekwe and Kennedy, 1998; Borga et al., 1994; White et al., 1979).

2. Materials and methods

2.1. Sample collection

Sediment samples were obtained from unvegetated intertidal mudflats and adjacent populations of *Phragmites* and *Spartina* in two New Jersey coastal marshes The site in the Hackensack Meadowlands (Saw Mill Creek, 'SMC') has a 200-year history of anthropogenic disturbance, which includes inputs of heavy metals, chlorinated hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and other toxic compounds (Kennish, 1992), as well as numerous physical disturbances such as ditching and tidal obstruction (Quinn, 1997). The site on the Mullica River (MUL) is bounded on the inland side by the largest protected watershed in the northeastern US (the Pinelands National Reserve), and on the seaward side is part of the Jacques Cousteau National Estuarine Research Reserve; there is virtually no industrial activity within the watershed. Both locations are brackish, with salinities ranging from 12 to 18 ppt (see Ravit et al., 2003 for map of site locations and detailed sediment descriptions including soil organic matter and moisture content, bulk density and belowground plant biomass).

Transects were established at both sites in adjacent unvegetated, *Spartina*-dominated and *Phragmites*-dominated zones ('treatments') parallel to the main surface water channel, and three plots were delineated along each transect. Using a Russian peat corer (Aquatic Research Instruments), three replicate sediment cores were extracted from each plot during July–August, 2001 within 2 h of low tide. Cores were immediately wrapped in gas-impermeable Saran® wrap, and transported on ice to the laboratory, where they were subdivided into 10 cm depth increments, and then composited for each depth. Subsamples from each 10 cm section ($N=3$ treatments$\times 3$ plots$\times 2$ sites$=18$) were immediately frozen at -20 °C for later PLFA analysis; a second series of 50 g subsamples of the composited sediment samples were immediately placed in 250 ml bottles. The bottles were then filled with pre-filtered (Whatman 42 μm filter) site water and tightly capped to maintain anaerobic conditions. The sediment samples were stored in the dark at 20 °C. The site water was decanted inside an anaerobic Coy chamber, where experimental

microcosms were established within 48 h of sampling. The atmosphere in the chamber consisted of 97% N_2/3% H_2.

2.2. Anaerobic microcosms

Enrichments of TBBPA were established in 150 ml serum bottles following the methods of Voordeckers et al. (2002). One gram of dry, sterile sediment (autoclaved at 121 °C for 45 min on three consecutive days) was placed in the serum bottle and 2.58 mM TBBPA in hexane stock (3.87 ml/serum bottle) was added to the dry sediment; the hexane was evaporated, leaving a coating of TBBPA. An inorganic methanogenic medium containing 1.3 g KCl, 0.2 g KH_2PO_4, 1.17 g NaCl, 0.1 g $CaCl_2 2H_2O$, 0.18 g $MgCl_2 6H_2O$ per liter was prepared under a stream of N_2 following strict anaerobic techniques. Sediment enrichment slurries (50% v/v) were prepared in the anaerobic chamber by adding ~50 g of the sediment sample and 50 ml of the anaerobic medium to a serum bottle containing the sterilized TBBPA spiked sediment, yielding a nominal TBBPA concentration of approximately 100 μM. Serum bottles were crimp sealed with Teflon-lined butyl rubber septa. Three sterile sediment controls were prepared by autoclaving the cultures at 121 °C for 45 min on three consecutive days. All anaerobic microcosms were incubated at 28 °C in the dark. TBBPA and BPA of 97% purity were obtained from Aldrich Chemical Co. (Milwaukee, WI). All other reagents for medium preparation, extractions, high performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GC-MS) analyses were obtained from Fisher Scientific.

2.3. HPLC and GC-MS analysis

The methods of Voordeckers et al. (2002) were used to analyze TBBPA, its intermediate metabolites and BPA. Serum bottles were vortexed prior to sampling and aliquots from each microcosm were taken periodically for up to 130 days. During the incubation period 0.2 ml samples were taken using syringes flushed with N_2, and extracted with 0.6 ml methanol by shaking for 1 h at 60 rpm. The samples were then centrifuged for 5 min (11,150*g*). The supernatant was filtered through a 45 μm Millipore filter and analyzed by HPLC equipped with a UV–VIS detector. A Sphereclone ODS (2) (250×4.60 mm; 5 μm particle size; Phenomenex Inc.) analytical column was used at ambient temperature. The running solvent was composed of 80% methanol, 18% ultrapure water, and 2% glacial acetic acid at a flow rate of 1 ml/min. A wavelength of 238 nm was used to detect TBBPA, BPA and the intermediate metabolites. The retention time of TBBPA and BPA were 6.4 min. and 3.3 min respectively. Concentrations with detection limits of 5 μM were calculated by comparison to three point external standard curves (TBBPA $R^2=0.9982$; BPA $R^2=0.998$). The retention times of the two intermediate metabolites were 4.7 and 3.9 min. Extraction efficiency was above 85%.

The metabolites were identified using gas chromatography-mass spectrometry (GC-MS). After extraction with hexane, the samples were analyzed on an Agilent 6890 gas chromatograph with a Hewlett Packard 5973N mass-selective detector, and a Hewlett Packard 5MS (Crosslinked 5% PH ME Siloxane, 30-m length, 0.25-mm inside diameter, 0.25-μm film thickness) column. The injection volume was 1 μL with a Helium carrier gas at a flow rate of 1 mL/min. The injector and detector temperatures were 280 and 350 °C, respectively. The oven temperature was held at 100 °C for 1 min, ramped to 200 °C at 20 °C per minute, then ramped to 300 °C at 15 °C per minute and then held at 300 °C for 10 min.

2.4. Phospholipid fatty acid analysis (PLFA)

The method of PLFA analysis described by White et al. (1979) was slightly modified. Samples were thawed and dewatered by centrifugation and two replicate 10 g wet weight subsamples were processed for each sample. Fatty acids were extracted using a single-phase chloroform:-methanol:0.05 M phosphate buffer (pH 7.5) solvent. The concentrated chloroform extract was separated into lipid fractions on a silicic acid column (King et al., 1977). The phospholipid fraction was eluted with methanol, saponified and methylated according to protocols for 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 44 PLFAs for statistical analysis.

2.5. Statistical analysis

All ANOVAS and MANOVAS were conducted using SAS System GLM (SAS Software, Version 8.2). Two-way analyses of variance (ANOVA) were conducted to test for differences in sediment organic matter (SITE factor SMC, MUL)×(SPECIES factor *Phragmites*, *Spartina* and unvegetated) with $n=3$ for each combination of site and species. Formation of BPA was analyzed using Repeated-measures two-factor MANOVA (SITE and SPECIES factors as above plus TIME = days of incubation).

Principal components analysis (PCA) was used to examine patterns among the nine site-species combinations based on the multivariate set of 44 PLFAs. Diversity of PLFAs within each site was calculated using the Simpson Index of Diversity (PC-Ord, Version 4). This Index was chosen due to its low sensitivity to sample size

(Magurran, 1988). Two-way ANOVA as described above was used to test for differences between samples in the PLFA Simpson Diversity Index score. Proportions of the 11 PLFAs contributing to >90% of the difference in ordination of the PLFA loading plots (PCA axis 1) were arcsine square root transformed (Pc-Ord, Version 4) prior to using two-factorial ANOVA to test for site and vegetation differences. When Site×Species interactions were found, single factor ANOVA was used to analyze within site vegetation differences. *Post hoc* means were tested using Tukey's HSD method.

3. Results

3.1. TBBPA biotransformation

Under methanogenic conditions, TBBPA was reductively debrominated in all sediment cultures and a stoichiometrically equivalent amount of BPA was produced (Figs. 1a and b and 2a and b). Sterilized sediment controls did not exhibit TBBPA loss or BPA formation. GC-MS analysis indicated that the first metabolite (Figs. 3 and 4a) was a tribrominated compound, with a fragmentation pattern expected for a tribromobisphenol A (TBPA) isomer.

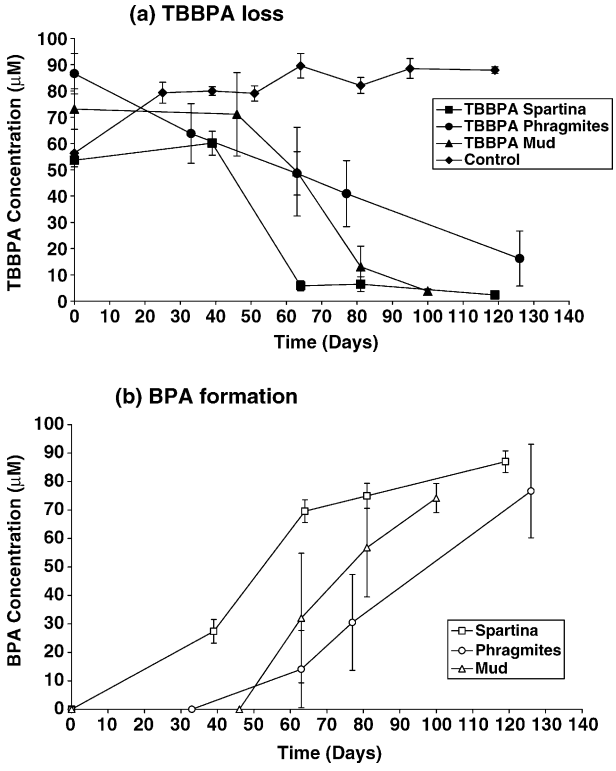

Fig. 1. (a) Saw Mill Creek sediment concentrations in μM of (a) TBBPA and (b) BPA during the 130 day incubation period. TBBPA concentration was below the detection limit after day 61 in *Spartina* sediment and after day 100 in unvegetated sediment. No formation of BPA was observed in sterilized sediment controls.

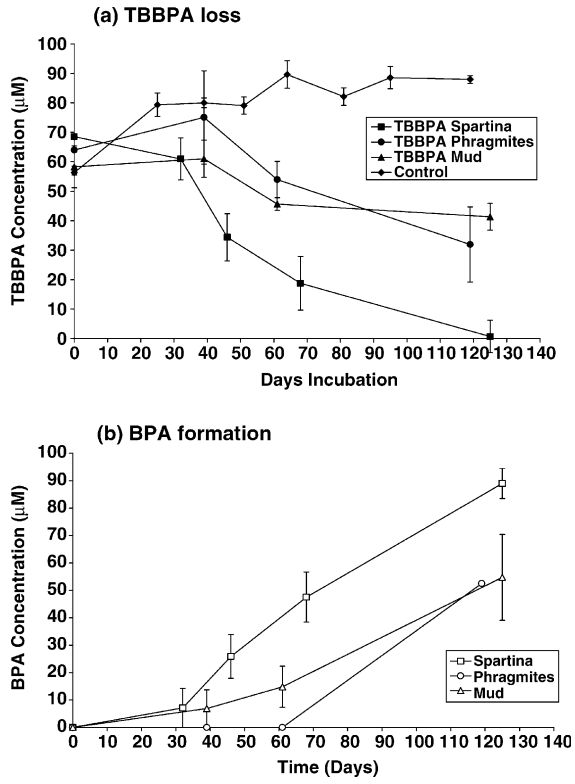

Fig. 2. (a) Mullica sediment concentrations in μM of (A) TBBPA and (b) BPA during the 130 day incubation period. TBBPA concentration was below the detection limit at day 115 in *Spartina* sediments. No formation of BPA was observed in sterilized sediment controls.

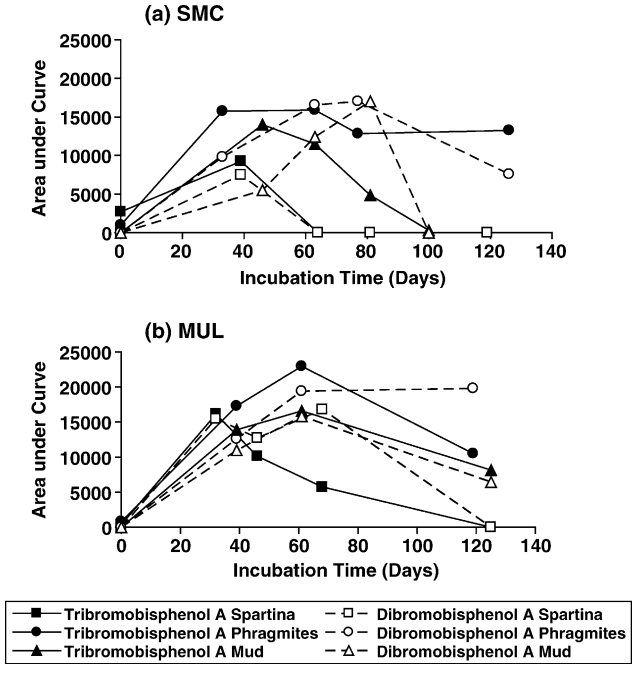

Fig. 3. Production of two secondary metabolites during (a) SMC 130 day incubation; (b) MUL 130 day incubation. The *Y*-axis corresponds to the area under the curve as measured by the HPLC chromatograph. Actual concentrations could not be calculated because the intermediate compounds were unknown and so no standards were available.

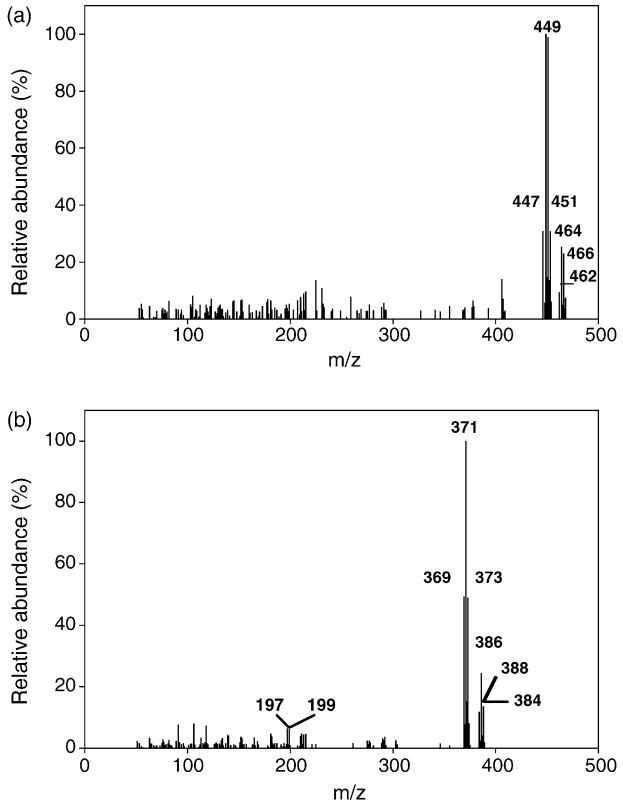

Fig. 4. Mass spectra of (a) tribromobisphenol A (TBPA) and (b) dibromobisphenol A (DBPA).

The second metabolite (Figs. 3 and 4b) was a dibrominated compound, with a fragmentation pattern consistent with that expected for a dibromobisphenol A (DBBPA) isomer. The fragmentation pattern seen in the mass spectrum of DBBPA, particularly the fragments 197 and 199, was characteristic of one bromine per phenyl ring (i.e. 2,2′-DBBPA). A dibromobisphenol A isomer with both bromines on the one aromatic residue (i.e. 2,6′-DBBPA) would yield mass fragments of *m/z* 275 and 119 for the dibrominated and nonbrominated phenyl ring mass fragments, respectively, which was not seen. Complete dehalogenation of the dibromobisphenol A isomer to BPA was confirmed by GC-MS analysis (mass spectrum of BPA not shown).

Debromination was more rapid in *Spartina* sediments than in *Phragmites* or unvegetated sediments from both sites. Debromination was more rapid in the contaminated SMC sediments than in comparably vegetated uncontaminated MUL sediments, and tri- and dibrominated metabolites transiently accumulated to higher concentrations (Fig. 3a and b). Loss of TBBPA was most rapid in the *SMC Spartina* sediments, as was the biotransformation of tri- and dibromobisphenol A, which reached undetectable levels by day 60.

Repeated measures 2-factorial ANOVA showed significant Site×Vegetation BPA concentration differences at all time points analyzed (week 6, $F_{2,1}=20.18$, $p=0.0001$); week 10, $F_{2,1}=11.15$, $p=0.0018$; week 18, $F_{2,1}=10.86$, $p=0.002$). Repeated measures 2-way ANOVA Contrast analysis showed that BPA concentrations in *Spartina* derived sediments were significantly different from concentrations in *Phragmites* sediments (Fig. 5) at all time points (week 6, $p<0.0001$; week 10, $p=0.0006$; week 18, $p=0.0006$), and significantly different from unvegetated BPA concentration at week 6, ($p=0.0003$) and week 10, ($p=0.0088$). The concentration of BPA in *Phragmites* sediments was significantly different from that of the unvegetated sediments at week 18 only ($p=0.0117$). The final MUL *Spartina* concentration of BPA was similar to that observed in SMC sediments; MUL *Phragmites* and unvegetated sediments still contained TBBPA, tri- and dibromobisphenol A after 130 days (Figs. 2a and 3b).

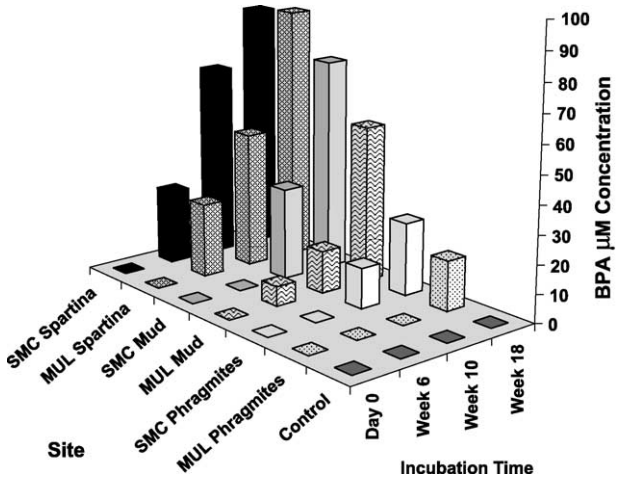

Fig. 5. Production of BPA over the 18 week incubation period.

3.2. PLFA Bacterial community analysis

The composition and relative abundance of 44 PLFAs found in samples from the undisturbed MUL site resulted in a distinct separation of *Spartina* from *Phragmites* and unvegetated sediments within the PCA ordination space (Fig. 6a). There was no comparable separation of PLFAs in the SMC samples, which actually clustered with the MUL *Spartina* within the PCA ordination space. There were significant differences in the diversity of PLFAs as measured by the Simpson Diversity Index and as evidenced by the total number of PLFAs recovered from the samples (Table 1). Two-factorial ANOVA of sediment PLFA diversity showed significant differences ($F_{5,25}=11.91$, $p<0.0001$), both with respect to site ($F_1=46.57$, $p<0.0001$) and vegetation ($F_2=4.45$, $p=0.0222$). No significant differences in PLFA diversity were found between plant species in SMC sediments. PLFA diversity was similar in *Spartina* sediments from both sites, which were significantly less diverse than MUL *Phragmites* and MUL unvegetated sediments (Table 2).

While there were overall differences in the PLFAs recovered from the two sites, a Loading Plot (Fig. 6b)

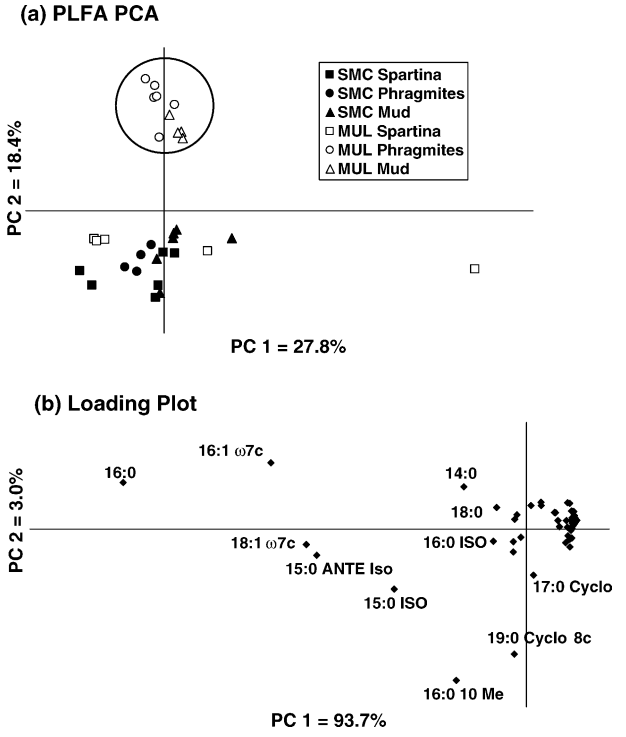

Fig. 6. Principal components ordination diagrams for (a) PLFA variables and (b) loading plot. For each diagram, the percent of variation explained is given along each axis.

indicates eleven PLFAs were responsible for >90% (PCA axis 1) of the variation among vegetation treatments. Two-factorial ANOVA of these eleven PLFAs showed significant differences between *Spartina* and *Phragmites* sediments in eight PLFAs (Table 2). Within site ANOVA shows significant MUL vegetation differences in PLFAs 14:0, 15:0 Ante Iso, 16:0 Iso, 16:1 ω7c, 16:0 10 Me and 19:0 Cy ω8c; the only PLFA showing significant vegetation differences in SMC was 19:0 Cy ω8c.

Table 1
Analysis of PLFA diversity as measured by the Simpson Diversity Index. Index range is 0–1.0, with a value of 1.0 the maximum for a PLFA equally abundant in all samples

Site	Vegetation	Simpson Index	PLFAs (number recovered)
Saw Mill Creek	*Spartina*	0.896 ± 0.0057^{a}	32
	Phragmites	0.897 ± 0.0048^{a}	28
	Mud	0.901 ± 0.0038^{a}	50
Mullica	*Spartina*	0.910 ± 0.0052^{a}	37
	Phragmites	0.927 ± 0.0014^{b}	43
	Mud	0.929 ± 0.0012^{b}	52
2-way factorial ANOVA	$F_{5,25}=11.91$	$p<0.0001$	
Species	$F_{2}=4.45$	$p=0.022$	
Site	$F_{2}=46.57$	$p<0.0001$	

PLFAs used to calculate the Simpson Diversity Index did not include those found in only one sample or any PLFA that contributed <1% of the total PLFAs recovered, resulting in an analysis of 44 PLFAs. Samples were analyzed using 2-way factorial ANOVA. Letters indicate significant PLFA diversity differences between vegetation treatments within each site.

4. Discussion

Sediments vegetated by two grasses commonly found in eastern US brackish marshes differed significantly in their capacity to biotransform the halogenated contaminant TBBPA. Differences in the rates of TBBPA dehalogenation indicate that there are functional differences in sediment microbial communities in vegetated versus unvegetated habitats, as well as differences in the microbial communities associated with *Spartina* and *Phragmites* rhizosphere sediments. The presence and identity of intermediate lesser-brominated compounds is consistent with the observations of Arbeli and Ronen (2003) and Voordeckers et al. (2002), and is a verification of the TBBPA debromination pathway proposed by Ronen and Abeliovich (2000).

Spartina sediments from both contaminated and uncontaminated sites showed the same pattern of more rapid TBBPA biotransformation than *Phragmites* or unvegetated sediments. While sites differed slightly in sediment moisture content and soil organic matter, quantitative differences among the site-species combination for all variables were small (Ravit et al., 2003) and the values are within a range of variability typically observed both within and between brackish marshes. The uniformity of more rapid *Spartina* sediment TBBPA biotransformation in both disturbed and undisturbed sediments supports the conclusion that these microbial functional differences are indeed plant-influenced.

Spartina belowground biomass is significantly greater than that of *Phragmites* at both sites, and belowground biomass of both plant species at SMC is greater than that at MUL (Ravit et al., 2003). It is probable that the higher biomass at SMC is the result of differences in nutrient inputs, especially nitrogen, between the two sites; SMC porewater nitrogen concentration was more than two orders of magnitude greater than the N concentration found in MUL porewater (Ravit unpublished data).

There are large differences between *Spartina* and *Phragmites* in both root morphology and the amount of fine root biomass, as well as differences in the vertical distribution of roots within the sediments (Ravit et al., 2003; Armstrong et al., 1996, 1992; Hwang and Morris, 1991). *Spartina* produces a dense, fibrous masses of fine roots in the surface sediments (predominately 0–20 cm depth). *Phragmites* fine roots are sparsely distributed along primary roots that emanate from large rhizomes that extend vertically (depths >70 cm) and horizontally (multiple meters) through the sediments. While it is unknown exactly how these root systems affect the structure of rhizosphere microbial communities, the prominent differences in fine root density and distribution are likely to contribute to differences in the activity of microbial communities.

The greater ability of *Spartina* sediments to biotransform TBBPA could be due to a number of factors. Use of fluorescent oligonucleotide probes targeting *Bacteria*, *Archaea* and *Eukarya* have demonstrated that the rhizosphere

Table 2
Mean percent contribution and (SE) of eight significantly different PLFAs in the loading plot responsible for >90% of the variation (PC 1) among vegetation treatments

PLFA	2-*factorial* ANOVA	Saw Mill Creek			Mullica		
		Spartina	*Phragmites*	Mud	*Spartina*	*Phragmites*	Mud
14:0	Site×species	5.0±0.60	4.5±0.28	4.8±0.43	5.1±1.16[a]	3.0±0.22[b]	2.7±0.63[b]
Gram−	14.94***		NS			$F=15.94$, $p=0.0003$	
15 Ante Iso	Site×species	10.4±1.59	10.0±0.55	9.3±0.64	11.1±2.83[a]	9.0±0.67[a]	4.0±0.43[b]
Gram+	16.62***		NS			$F=24.76$, $p<0.0001$	
16 Iso	Site×species	3.2±0.64	2.8±0.09	3.0±0.43	3.3±0.85[a]	3.1±0.12[a]	1.9±0.20[b]
Gram+	5.87**		NS			$F=12.71$, $p=0.0009$	
16:0	Site	20.3±0.80	20.0±1.89	19.6±1.10	15.2±1.74	14.5±0.59	15.3±2.24
Ubiquitous	18.31***		NS			NS	
16:1 ω7c/15 Iso 2OH	Site×species	15.2±2.64	14.1±1.45	12.2±2.45	11.9±3.55[a,b]	8.46±0.65[a]	15.2±2.38[b]
Gram−	6.50**		NS			$F=10.62$, $p=0.0018$	
16:0 10 Me	Site×species	2.8±0.40	3.3±0.55	2.5±1.36	3.3±0.66[a]	8.5±1.26[b]	3.5±0.69[a]
Gram+	32.57***		NS			$F=54.48$, $p<0.0001$	
17:0 Cy	Species $p=0.03$, site $p<0.0001$	0.3±0.73	1.0±0.71	1.1±0.59	2.2±0.77	2.8±0.13	2.4±0.12
Gram−	15.45***		NS			NS	
19:0 Cy ω8c	Site×species	0	2.2±0.21	0.6±0.44	1.6±0.63[a]	5.9±0.61[b]	2.4±0.41[a]
Gram−	115.68***		$F=61.78$, $p<0.0001$			$F=89.54$, $p<0.0001$	

The $F_{5,25}$ values for the two-way ANOVAs are given for each significant factor. Significant differences ($p<0.05$=*, $p<0.01$=**, $p<0.0001$=***) are indicated by asterisks. Species within sites are compared using Contrast ANOVAs. 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 PLFAs 16:1 ω7c/15 ISO 2OH co-elute and cannot be differentiated in the MIDI system.

microbial community associated with *Spartina* species can be 2-fold larger than the community associated with *Phragmites* (Burke et al., 2002). This is probably due to the greater fine root density, which provides a surface area where members of the microbial consortia needed to biotransform halogenated contaminants could be found in close proximity. Higher overall microbial numbers could result in a reduced lag time for dehalogenating microorganisms to reach the biomass required to transform TBBPA and its metabolites.

Variation in plant inputs of organic and inorganic compounds result in variation in rhizosphere microbial densities (Pinton et al., 2001; Marschner, 2001). Plant species are known to differ in both the quantity and quality of carbon transported to the rhizosphere, and root exudates differ depending on plant species and environmental factors (Neori et al., 2000; Grayston et al., 1998; Lynch and Whipps, 1990). *Spartina*'*s* extensive fine root system is potentially releasing greater amounts of organic and inorganic carbon than that provided by the lower fine root density of *Phragmites*. Phenolic compounds released by plant roots have been shown to be viable growth substrates for microbial cultures capable of dehalogenation (Donnelly et al., 1994). One example of a naturally occurring halogenated phenolic compound is found in the salt marsh plant *Juncus roemerianus*, which contains 1,2,3,4-tetrachlorobenzene (Miles et al., 1973), although we know of no reports documenting the production of organohalides by *Spartina* species.

Differences in microbial function were not correlated with differentiation of the in situ bacterial community structure, as indexed by PLFA composition prior to TBBPA exposure. The lowered diversity exhibited in contaminated SMC sediments may be due to microbial exposure to organic pollutants and heavy metals (Thompson et al., 1999; Kuperman and Carreiro, 1997), and it is probable that anthropogenic contaminants have influenced the composition of the microbial populations in the SMC system. Although the *Spartina* sediments exhibited low PLFA diversity, when these sediments were exposed to TBBPA, biotransformation to BPA was more rapid than in the *Phragmites* or unvegetated sediments. This result suggests that it is not diversity per se that determines microbial community functional ability, but the presence of community members with specific metabolic capabilities, in this case the capacity to dehalogenate.

The dehalogenating population is most likely a very low proportion of the total microbial community, and is thus not large enough to make a significant contribution to the total PLFA pool. Conversely, the ability to dehalogenate is found in a diverse group of microorganisms (Häggblom and Bossert, 2003), which includes many bacterial genera that exhibit different suites of PLFAs. We also point out that the PLFA method used to index microbial communities only describes the bacterial component of this biota. Archaea have glycerol ether membrane fatty acids, so are not represented in the glycerol ester PLFA analyses. Under anoxic conditions prevalent in estuarine marshes, archaea

may play an important functional role within rhizosphere microbial communities. For these reasons, total sediment in situ PLFAs may not correlate with differences in microbial dehalogenation ability.

PLFA loading plots suggest that a limited number of fatty acids are responsible for the differences observed among samples. Of the eleven PLFAs responsible for >90% of the PC 1 variation, eight showed significant differences between samples. These eight PLFAs are commonly found in three groups of bacteria: Gram negative (14:0, 16:0, 16:0 Iso, 16:1 ω7c, 17:0 Cy, 19 Cy ω8c), Gram positive (15:0 Ante Iso, 16:0 10 Me, 16:0 Iso) and anaerobic bacteria (16:0, 17 Cy, 19 Cy ω8c) (Schuter and Dick, 2001; Waldrop et al., 2000; Zelles, 1999; Ratledge and Wilkinson, 1988). Under the anaerobic conditions common in brackish salt marshes, it is most probable that the Gram negative bacteria contain a substantial number of sulfate reducers (Hines et al., 1999) and that the Gram positive bacteria include acetogens (Leaphart et al., 2003; Drake, 1994).

For reductive debromination of TBBPA to occur, a source of reducing equivalents must be present (Häggblom and Bossert, 2003). Dehalogenating microbial populations function within syntrophic communities, where inducible enzymes catalyze reductive dehalogenation; dehalogenation proceeds in a sequential manner, removing halides from the parent molecule and replacing them with the reducing equivalent, which is typically hydrogen. Fermentation or acetogenic activities are potential sources for the required reducing equivalents. Rhizosphere microbial community members that produce hydrogen, or a reduced presence of competitors such as sulfate reducing bacteria or methanogens that consume hydrogen, may be of critical importance in dehalogenation.

To fully restore salt marsh ecosystem functioning, the production and release of contaminant breakdown products, contaminant mineralization, and/or sequestration must be considered. It is clearly important to understand how changing the plant community during a restoration project may affect the fate of toxic compounds in contaminated wetlands. Restoration management of the plant community should include consideration of plant-driven variations in the sediment microbial community that may result in differences in contaminant fate and transport. Vegetated sediments enhance the biotransformation of PAHs, which are common constituents of estuarine petroleum contamination (Reilley et al., 1996; Kennish, 1992), and PAH degrading bacteria have been found to be abundant in contaminated *Spartina* rhizosphere sediments (Daane et al., 2001). In our study, *Spartina* promoted formation of the metabolite BPA that is stable under anaerobic conditions. However, certain microorganisms can mineralize BPA under aerobic conditions (Ronen and Abeliovich, 2000). If O_2 is released into the rhizosphere by salt marsh plant roots, it is possible that aerobic microorganisms are members of the rhizosphere microbial community. The presence of aerobic microorganisms could increase the possibility of BPA mineralization, and so additional studies under field conditions are needed to further understand the relationship between vegetation, sediment microbial communities and the fate of TBBPA in estuarine sediments.

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References

Alaee, M., Arias, P., Sjödin, A., Bergman, Å., 2003. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. Environment International 29, 683–689.

Arbeli, Z., Ronen, Z., 2003. Enrichment of a microbial culture capable of reductive debromination of the flame retardant tetrabromobisphenol-A, and identification of the intermediate metabolites produced in the process. Biodegradation 14 (6), 385–395.

Armstrong, J., Armstrong, W., Beckett, P.M., 1992. *Phragmites australis*: Venturi-and humidity-induced pressure flows enhance rhizome aeration and rhizosphere oxidation. New phytologist 120, 197–207.

Armstrong, Armstrong, J., Armstrong, W., Beckett, P.M., Halder, J.E., Lythe, S., Holt, R., Sinclair, A., 1996. Pathways of aeration and the mechanisms of beneficial effects of humidity- and Venturi-induced convections in *Phragmites australis* (Cav.) Trin. Ex Steud.. Aquatic Botany 54, 177–197.

Bagwell, C.E., Dantzler, M., Bergholz, P.W., Lovell, C.R., 2001. Host-specific ecotype of rhizoplane diazotrophs of the perennial glasswort *Salicornia virginica* and selected salt marsh grasses. Aquatic Microbial Ecology 23, 293–300.

Borga, P., Nilsson, M., Tunlid, A., 1994. Bacterial communities in peat in relation to botanical composition as revealed by phospholipid fatty acid analysis. Soil Biology & Biochemistry 7, 841–848.

Burke, D.J., Hamerlynck, E.P., Hahn, D., 2002. Interactions among plant species and microorganisms in salt marsh sediments. Applied Environmental Microbiology 68 (3), 1157–1164.

Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, G., Limburg, K., Naeem, S., O'Neil, R.V., Paruelo, J., Raskin, R.G., Sutton, P., Van den Belt, M., 1997. The value of the world's ecosystem services and natural capital. Nature 387, 253–260.

Cowart, J.D., Fielman, K.T., Woodin, S.A., Lincoln, D.E., 2000. Halogenated metabolites in two marine polychaetes and their planktotrophic and lecithotrophic larvae. Marine Biology 136, 993–1002.

Daane, L.L., Harjono, I., Zylstra, G.J., Häggblom, M.M., 2001. Isolation and characterization of polycyclic aromatic hydrocarbon-degrading bacteria associated with the rhizosphere of salt marsh plants. Applied and Environmental Microbiology 67 (6), 2683–2691.

Donnelly, P.K., Hegde, R.S., Fletcher, J.S., 1994. Growth of PCB-degrading bacteria on compounds from photosynthetic plants. Chemosphere 28 (5), 981–988.

Drake, H.L., 1994. Acetogenesis. Chapman & Hall, New York.

Fenical, W., 1981. Natural halogenated organics, in: Duursma, D. (Ed.), Marine Organic Chemistry. Elsevier, New York, pp. 375–393.

Grayston, S.J., Wang, S., Campbell, C.D., Edwards, A.C., 1998. Selective Influence of plant species on microbial diversity in the rhizosphere. Soil Biology & Biochemistry 30 (3), 369–378.

Gribble, G.W., 1994. Natural production of chlorinated compounds. Environmental Science and Technology 28 (7), 310–319.

Häggblom, M.M., Bossert, I.D., 2003. Dehalogenation: Microbial Processes and Environmental Applications. Kluwer, Boston, MA.

Häggblom, M.M., Knight, V.K., Kerkhof, L.J., 2000. Anaerobic decomposition of halogenated aromatic compounds. Environmental Pollution 107, 199–207.

Hale, R.C., La Guardia, M.J., 2002. Emerging contaminants of concern in coastal and estuarine environments. In: Coastal and Estuarine Risk Assessment. CRC Press, Boca Raton, Fl, pp. 41–72.

Hines, M.E., Evans, R.S., Genthner, B.R.S., Willis, S.G., Friedman, S., Rooney-Varga, J.N., Devereux, R., 1999. Molecular phylogenetic and biogeochemical studies of sulfate-reducing bacteria in the rhizosphere of *Spartina alterniflora*. Applied Environmental Microbiology 65 (5), 2209–2216.

Hunt, P.A., Koehler, K.E., Susiarjo, M., Hodges, C.A., Ilagaru, A., Voight, R.C., Thomas, W., Thomas, B.F., Hassold, T.J., 2003. Bisphenol A exposure causes meiotic aneuploidy in the female mouse. Current Biology 13 (7), 546–553.

Hwang, Y-H., Morris, J.T., 1991. Evidence for hygrometric pressurization in the internal gas space of *Spartina alterniflora*. Plant Physiology 96, 166–171.

Ibekwe, A., Kennedy, A.C., 1998. Phospholipid fatty acid profiles and carbon utilization patterns for analysis of microbial community structure under field and greenhouse conditions. FEMS Microbiology Ecology 26, 151–163.

Kennish, M.J., 1992. Ecology of Estuaries: Anthropogenic Effects. CRC Press, Boca Raton, FL.

Kennish, M.J., 1998. Pollution Impacts on Marine Biotic Communities. CRC Press, Boca Raton, FL.

King, J.D., White, D.C., Taylor, C.W., 1977. Use of lipid composition and metabolism to examine structure and activity of estuarine detrital microflora. Applied Environmental Microbiology 33, 1177–1183.

Kourtev, P.S., Ehrenfeld, J.G., Häggblom, M.M., 2003. Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. Soil Biology & Biochemistry 35, 895–905.

Kuperman, R.G., Carreiro, M.M., 1997. Soil heavy metal concentrations, microbial Biomass and enzyme activities in a contaminated grassland ecosystem. Soil Biology & Biochemistry 29 (2), 179–190.

Leaphart, A.B., Friez, M.J., Lovell, C.R., 2003. Applied Environmental Microbiology 69 (1), 693–696.

Lynch, J.M., Whipps, J.M., 1990. Substrate flow in the rhizosphere. Plant and Soil 129, 1–10.

Magurran, A.E., 1988. Ecological Diversity and its Measurement. Princeton University Press, Princeton, NJ.

Manley, S.L., Dastoor, M.N., 1987. Methyl halide (CH_3X) production from the giant kelp. Macrocystis, and estimates of global CH_3X production by kelp. Limnology and Oceanography 32 (3), 709–715.

Markey, C.M., Rubin, B.S., Soto, A.M., Sonnenschein, C., 2003. Endocrine disruptors: from Wingspread to environmental developmental biology. Journal of Steroid Biochemistry and Molecular Biology 83, 235–244.

Marschner, P., Yang, C.-H., Lieberei, R., Crowley, D.E., 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biology & Biochemistry 33, 1437–1445.

Miles, D.H., Mody, N.V., Minyard, J.P., Hedin, P.A., 1973. Constituents of marsh grass: survey of the essential oils in Juncus roemerianus. Phytochemistry 12, 1399–1404.

Neori, A., Reddy, K.R., Ciskova-Koncalova, H., 2000. Bioactive chemicals and biological-biochemical activities and their functions in rhizospheres of wetland plants. The Botanical Review 66 (3), 350–373.

Nielsen, L.B., Finster, K., Welsh, D.T., Donelly, A., Herbert, R.A., de Wit, R., Lomstein, B.A., 2001. Sulphate reduction and nitrogen fixation rates associated with roots, rhizomes and sediments from *Zostera noltii* and *Spartina maritime* meadows. Environmental Microbiology 3 (1), 63–71.

NRC, 2001. Compensating for Wetland Losses under the Clean Water Act. National Academy Press, Washington, DC.

Pinton, R., Varanini, Z., 2001. The rhizosphere as a site of biochemical interactions among soil components, plants, and microorganisms, in: Pinton, R., Varanini, Z., Nannipieri, P. (Eds.), The Rhizosphere. Marcel Dekker, New York, pp. 1–17.

Quinn, J.R., 1997. Fields of Sun and Grass. Rutgers University Press, New Brunswick, NJ.

Ratledge, C., Wilkinson, S.G., 1988. Microbial Lipids, vol. 1–2. Academic Press, San Diego, CA.

Ravit, B., Ehrenfeld, J.G., Häggblom, M.M., 2003. A comparison of sediment microbial communities associated with *Phragmites australis* and *Spartina alterniflora* in two brackish wetlands of New Jersey. Estuaries 26 (2B), 465–474.

Ronen, Z., Abeliovich, A., 2000. Anaerobic–aerobic process for microbial degradation of tetrabromobisphenol A. Applied Environmental Microbiology 66 (6), 2372–2377.

Santillo, D., Johnston, P., 2003. Playing with fire: the global threat presented by brominated flame retardants justifies urgent substitution. Environment International 29, 725–734.

Schuter, M., Dick, R., 2001. Shifts in substrate utilization potential and structure of soil microbial communities in response to carbon substrates. Soil Biology & Biochemistry 33, 1481–1491.

Scrimshaw, M.D., Bubb, J.M., Lester, J.N., 1996. Organochlorine contamination of UK Essex coast. Journal of Coastal Research 12 (1), 246–255.

Thompson, I.P., Bailey, M.J., Ellis, R.J., Maguire, N., Meharg, A.A., 1999. Response of soil microbial communities to single and multiple doses of an organic pollutant. Soil Biology & Biochemistry 31, 95–105.

Voordeckers, J.W., Fennell, D.E., Jones, K., Häggblom, M.M., 2002. Anaerobic biotransformation of tetrabromobisphenol A, tetrachlorobisphenol A, and bisphenol A in estuarine sediments. Environmental Science and Technology 2002;.

Waldrop, M.P., Balser, T.C., Firestone, M.K., 2000. Linking microbial community composition to function in a tropical soil. Soil Biology & Biochemistry 32, 1837–1846.

Wantanabe, I., Sakai, S.-i., 2003. Environmental release and behavior of brominated flame retardants. Environment International 29, 665–682.

White, D.C., Davis, W.M., Nickels, J.S., Kind, J.D., Bobbie, R.J., 1979. Determination of sedimentary microbial biomass by extractible lipid phosphate. Oecologia 40, 51–62.

Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. Biology Fertility Soils 29, 111–129.