Microbial Ecology

Distribution of Microbial Communities Associated with the Dominant High Marsh Plants and Sediments of the United States East Coast

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Abstract

Microbial communities in the sediment and associated with the dominant type of standing dead plant were collected from the high marsh zones of 10 sites along the eastern coast of the United States from Maine to Florida. Microbial community composition was examined using T-RFLP, and bacterial and fungal abundance was determined microscopically. Within the sediment, community composition was strongly correlated with latitude, indicating that biogeographical factors are important determinants of sediment community composition, whereas abundance was positively and strongly correlated with sediment organic matter content. A strong biogeographical effect was observed for both bacterial and fungal abundance on standing dead plants, but there was no clear relationship between community composition and latitude. Microbial community composition was more similar among plants of the same type (i.e., related plant species) suggesting that plant type (i.e., substrate quality) is primarily responsible for the determining community composition on standing dead plants.

Introduction

The earth is inhabited by an incredibly diverse assemblage of microorganisms that govern the rates and extents of all the biogeochemical cycles important for the existence of higher life. The prokaryotic biomass is estimated to be as much as half of the total biomass of the earth [71]. Estimates of the numbers of different types of microbes approach 2×10^6 for the oceans, and 4×10^6 per ton for soils [15]. Although Beijerink's dictum that "everything is everywhere; the milieu selects" [3, 54] is still as true as at the time it was presented, the statement foreshadowed, but underestimated, the breadth of variability in both phylogeny and function that we are currently coming to realize. Bacteria may be found everywhere, but they are not uniformly distributed in either space or time. Large numbers of heretofore-undescribed genomes have been discovered using ribosomal DNA sequences as the criteria for identity, although the number of sequences actually recovered thus far is small [28] compared with the estimated total that likely exist [15]. The number of ecosystem functions that microbes perform is large, but the numbers of functions cannot match the number of types of organisms estimated to inhabit the earth [58]. Obviously, different types of organisms must often share similar functions. If the niche concept holds true for microbes (and there is no evidence that it does not), it is easily conceivable that the community selected will not necessarily be the same from place to place even though the environmental conditions are the same. It is clear that on a gross scale, microbial communities from different environments are indeed different: oceanic samples contain a very different suite of organisms from those recovered from agricultural soils [12, 15, 24]. Yet, it is equally conceivable that communities within similar habitat types from spatially distinct locations will be more similar to one another than communities from different habitats that are close together. Thus, variation in community composition within a habitat would be less than variation across habitat types of the same area (e.g., [21]). These ideas lead to the hypothesis that within a habitat type, microbial community composition may exhibit biogeography.

We report here the results of an evaluation of the microbial communities in two salt marsh habitat types, standing-dead plants and sediments in the high marsh

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zones of ten sites from Maine to Florida on the east coast of the United States. The standing-dead materials at these sites are part of a broader study to characterize the development of microbial decay communities within similar habitats and among different habitats to determine whether microbial communities exhibit biogeography. In part because of their variation in latitude, the sites used in this study are characterized by steep gradients in temperature, precipitation frequency and amount, humidity, salinity of sediment and inundating water, and light intensity.

The activities of microbial communities respond to a wide variety of biotic and abiotic factors that include substrate quality (e.g., [25, 57, 62, 65, 67]), substrate particle size [64], edaphic conditions [29, 67], and climate [8, 9, 33, 45]. It is reasonable to hypothesize that these same factors influence not only activity but also community composition. For example, a recent study demonstrated the importance of soil type, itself the product of biotic and abiotic influences, as a major factor in the composition and activity of bacterial communities in arable soils [24]. If similar microbial communities develop on similar marsh plant species or plant types (e.g., grasses vs reeds) across the various sites, substrate quality is implicated as the primary determinant of community composition on standing-dead plants. Conversely, if the microbial communities that develop on standing-dead biomass of the same plant species are compositionally different across sites, then environmental factors (e.g., temperature, precipitation, salinity) would be presumed to be the principal determinants of community composition. If microbial communities of similar composition are detected in the sediment but not on the standing-dead biomass, then environmental factors may constrain the type of microbial communities responsible for litter decomposition. Even though microbial activity and community composition may vary across sites for the same plant type, the decay processes may be very similar because of degeneracy within microbiological systems [17].

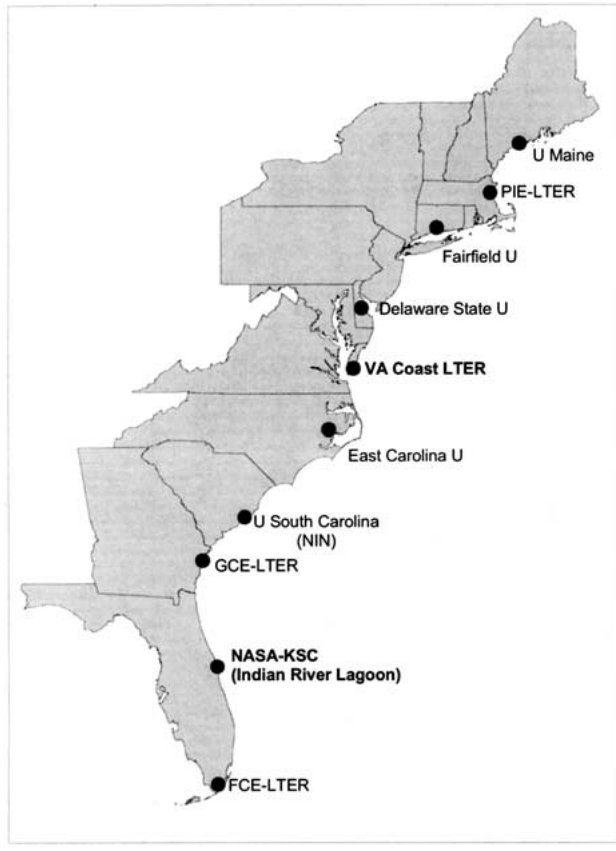

Figure 1. Location of participating sites. Dominant high-marsh plants (standing dead) and sediments were collected from each of the sites in late fall–winter of 2000–2001.

Materials and Methods

Site Descriptions. Ten sites participated in this comparative study (Fig. 1). The sites included all four active Atlantic coastal Long Term Ecological Research sites (Plum Island Estuary, Virginia Coast Reserve, Georgia Coastal Ecosystem, and Florida Coastal Everglades LTERs), two long-term research sites that are not part of the LTER network (North Inlet and Indian River Lagoon sites), and four experimental sites currently being used by other institutions, including the University of Maine, Fairfield (CT) University, Delaware State University, and East Carolina University. The selected sites represent a gradient of climatic conditions and biogeographic regions (see Table 1) that can be categorized into provinces of similar climate, wave climate, and faunal distribution [30, 31]. The northernmost sites have cold winters (the high temperatures are near or below freezing) and cool summers. The southernmost sites are very warm with average high temperatures near or above 30°C. The latter sites also tend to have the most rainfall, with the bulk of the rain coming in the summer rainy season. Tidal ranges are 1–2 m in most areas except the two Florida sites, which are microtidal. In all cases, flooding of the high marshes is accomplished largely by wind-driven storm tides.

Sampling and Sample Analysis. During the period from November 2000 to January 2001, standing dead plants were cut at each site, placed on ice, and shipped overnight to the University of Virginia where the samples were frozen at −80°C until analysis. Additionally, at the time the plant samples were collected, samples of the sediment were collected by inserting a detipped 10-mL syringe into the sediment to collect a small core that was 1.5 cm × 2 cm. (Sediment samples were not obtained

Table 1. Environmental characteristics of each of the participant sites

		Climate										
		Coordinates		Temperature (°C)				Cumulative monthly rainfall (mm)		Tide range	Salinity	Dominant high marsh vegetation
				Mean daily high		Mean daily low						
Site	Province	Lat	Long	Jan	Jul	Jan	Jul	Jan	Jul	(m)	(PSU)	
U Maine	Acadian	44.0	69.0	−0.9	26	−11.4	14.6	90	79	2.8	31	*Spartina alterniflora*
PIE-LTER	Acadian	42.4	70.5	2.1	27.7	−5.8	18.4	91	72	1.5	27	*Spartina patens*
Fairfield U	Virginian	41.1	73.6	2.8	26.1	−5.6	21.1	83	90	1.1	20	*Phragmites australis*
Del State	Virginian	38.7	75.1	6.2	29.3	−3.4	19.4	96	101	1.2	27	*Spartina patens*
VCR LTER	Virginian	37.5	74.8	7.7	30.2	−2.2	20	96	109	1.3	28	*Juncus roemerianus*
ECU	Virginian	35.7	75.9	11.3	29.2	2.6	22.1	135	127	0.5	21	*Juncus roemerianus*
USC (NIN)	Carolinian	32.5	80.4	13.2	31.2	4.9	24.1	85	154	1.2	28	*Spartina alterniflora*
GCE LTER	Carolinian	31.4	81.3	15.4	32.8	3.4	22.4	91	162	2.1	28	*Juncus roemerianus*
KSC (IRL)	Floridian	28.5	81.6	22.3	32.6	11.8	23.3	56	191	0.1	28	*Spartina bakeri*
FCE LTER	Floridian	25.0	81.3	24.0	28	11.0	18.0	61	202	0.1	20	*Rhizophora mangle*

from the Massachusetts site.) The cores were frozen and shipped along with the plant material. Three such cores were collected at each site; two were used for analysis of physical characteristics and for determinations of microbial abundance, and one was used to determine the sediment community structure as a representation of the environment in which the plant materials would eventually decay. At all sites except Virginia, three cores were collected randomly within a 10 m × 3 m plot. Two cores were used to determine sediment moisture content, bulk density, and organic matter concentration. At the Virginia site, 10 cores were collected randomly, but at least 2.0 meters apart, within a 20 × 20 m plot. This spacing exceeds the horizontal distance determined by Franklin et al. [18] with a geostatistical approach to be the spacing necessary to ensure independent samples of the microbial community. Three of the Virginia cores were used to determine sediment moisture content, bulk density, and organic matter content. Seven of the cores were used to determine the variability of the sediment microbial community structure at the site.

Abundance of bacteria and fungi in the plant material and sediments was determined by direct counting methods [5]. Abundance (bacterial and fungal) was estimated for three individual plants and from duplicate sediment cores. Portions (1 g, moist weight) of each plant or sediment sample were placed in a blender and homogenized, and dilutions of each suspension were examined for bacterial abundance by acridine orange direct counts (AODC) using the approach of Rublee and Dornseiff [59]. Fungal abundance was estimated as the hyphal length per gram of dry plant material by the hyphal intersection approach of Jones and Mollison [35]. This method is reported to overestimate the abundance of fungal hyphae because of an accumulation of empty (necrotic) hyphae during decomposition [34]. The magnitude of the bias does not appear to be dependent on the plant identity, and it is relatively small, a factor of about two in decaying *Spartina alterniflora* [48]. Given the lack of any pattern in the data obtained with this technique (see Fig. 5), conclusions about those results are not likely to be affected by the bias.

To measure the organic content of the plant materials (or sediments), plants were dried at 70°C to a constant mass, weighed, ignited (450°C, overnight), and reweighed. The carbon and nitrogen contents of the plant materials were determined using a PerkinElmer 2400 series II CHNS/O analyzer. C/N was calculated as the ratio of the masses.

Prior to nucleic acid extraction, ~5–10 g of frozen plant tissue was ground to a fine powder in liquid nitrogen using a mortar and pestle. Macerated plant tissue and sediment samples were transferred in triplicate 0.25-g aliquots into 2-mL screw-top tubes for extraction using the UltraClean Soil DNA Isolation Kit (MoBio

Table 2. Primer sequences used for T-RFLP microbial community profiling in this study

Domain specificity[a]	*Primer*	*Primer sequence (5′–3′)*[b]	*Target and position*[c]	*Melting temp. (°C)*	*Reference*
Bacteria					
Most bacteria	SSU27f	AGAGTTTGATCCTGGCTCAG	16S (SSU rDNA): 08-27	60	41
Most bacteria	SSU1492r	GGTTACCTTGTTACGACTT	16S (SSU rDNA): 1492-1474	59	41
Fungi					
Most fungi	ITS1-F	CTTGGTCATTTAGAGGAAGTAA	18S (SSU rDNA): 455980-456001	55	22
Most fungi	ITS4	TCCTCCGCTTATTGATATGC	25S (LSU rDNA): 455123-455142	53	71

Small-subunit ribosomal DNA (SSU rDNA) and the Intergenic Transcribed Spacer (ITS) region of rDNA were used to target the bacterial and fungal communities, respectively.
[a]Primer specificity within the three phylogenetic domains of life.
[b]IUB nucleotide codes.
[c]Primer positions for the small subunit (SSU) and large subunit (LSU) ribosomal DNA (rDNA) operon using the *Escherichia coli* and the *Saccharomyces cerevisiae* (Ace. No. NC_001144) numbering systems for bacteria and fungal primer sets, respectively.

Laboratories, Solana Beach, CA). DNA was extracted and purified in accordance with the manufacturer's instructions, as modified by Clement and Kitts [11]. Following extraction, microbial community DNA was amplified by PCR and examined using the terminal restriction fragment length polymorphism (TRFLP) method [42]. As described below, TRFLP of rDNA was used to fingerprint composition within both the bacterial and fungal communities and to cluster each respective community based on shared similarity rather than to assign phylogenetic identities to individual amplicons.

Fungal community PCR was performed in 100-μL reaction volumes using a 6FAM-labeled forward primer (ITS1-F) and a HEX-labeled reverse primer (ITS4) (synthesized by Operon Technologies, Alameda, CA, USA) targeting the intergenic transcribed spacer (ITS) region of the ribosomal DNA operon of the fungi, as described in Klamer et al. [37]. Note that the forward primer is that used by Buchan et al. [7], whereas that group used the ascomycete-targeted reverse primer ITS 4A rather than ITS4 which is reported to target most Ascomycota and Basidiomycota along with some Zygomycota and Chytridiomycota [37]. As shown by Klamer et al. [37], the ITS4 primer amplified Ascomycota with the same efficiency as ITS 4A and also enabled amplification of Basidomycota without nonspecific amplification of plant ITS targets. The primer pair used here has been applied successfully in similar work by Anderson et al. [2].

Bacterial community PCR was performed in 100-μL reaction volumes using a 6FAM-labeled forward primer (Bac-SSU27F) and an unlabeled reverse primer (Bac-SSU1492) targeting the small subunit 16S rDNA region of the ribosomal DNA operon. Primer sequences and optimal annealing temperatures for community PCR in this environment are shown in Table 2. Final PCR reagent concentrations were 2.0 mM $MgCl_2$, 1× Applied Biosystems Buffer II, 200 μM of each dNTP, 1.0 μM of each primer, 0.4 μg/μL BSA (bovine serum albumin), and 1.25 U AmpliTaq DNA polymerase. All amplification reactions were performed in an MJ Research PTC-200 Thermocycler (Waltham, MA, USA) with reagents obtained from Applied Biosystems (Foster City, CA, USA), with the exception of BSA that was obtained from Roche Diagnostics (Indianapolis, IN, USA). Thermocycler reaction conditions for fungal ITS amplification were 5 min initial denaturation at 94°C followed by 35 cycles of 0.5 min at 94°C, 2 min of annealing at 50°C, and 3 min extension at 72°C followed by a final extension step of 5 min at 72°C. Bacterial 16S-rDNA PCR amplifications were identical except that the annealing temperature was 55°C. Reaction yield was determined by 1.5% agarose gel electrophoresis. PCR products were purified and concentrated using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), digested with the appropriate restriction endonuclease at 5 U per reaction according to manufacturer's instructions (New England Biolabs, Beverly, MA, USA). The restriction endonucleases *Hae*III and *Rsa*I were used to digest 16S-rDNA targets while the enzyme *Hinf*I was used for digestion of fungal ITS targets. Following restriction, samples were purified and desalted with QIAquick Nucleotide Removal Kit (Qiagen, Hilden, Germany), denatured at 95°C for 10 min, and separated by capillary electrophoresis in an ABI 310 Genetic Analyzer in GeneScan mode (Applied Biosystems, Foster City, CA, USA).

Electropherogram data from the TRFLP analyses were segregated into size classes of two-basepair increments and converted to binary format representing the presence or absence of a terminal restriction fragment (each TRF ± 2 base pairs representing an amplicon). A minimum cutoff of 100 relative fluorescence units was used to discriminate peak signals from background noise. The matrices (amplicon × sample) were examined with principal component analysis (PCA) to visualize differences among the community fingerprints determined for each site and dominant plant type [19–21, 73]. PCA provides a means of comparing community fingerprints across sites, among plant types, and along environmental gradients. Although PCA ordination is not generally

Table 3. Sediment characteristics at each of the 10 study sites

Site	*Bulk density* ($g\ cm^{-3}$)	*% OM* *(dw basis)*
Maine	0.17 (0.06)	28.48 (0.56)
Mass	0.28 (0.01)	27.65 (1.02)
Connecticut	0.20 (0.04)	40.30 (7.83)
Delaware	0.23 (0.09)	34.91 (10.43)
Virginia	0.12 (0.04)	64.76 (1.53)
North Carolina	1.05 (0.06)	3.42 (0.98)
South Carolina	0.59 (0.01)	9.94 (5.21)
Georgia	0.92 (0.39)	2.48 (1.91)
Indian River	0.25 (0.17)	32.62 (33.84)
South Florida	0.11 (<0.00)	78.62 (1.71)

applied to binary data, its use for the visualization of such data where extraction of factors is not attempted is both acceptable and common [16].

Results

Sediment Characteristics. Sediment bulk density, water content, organic matter percentage, and bacterial abundance for the 10 study sites varied widely but were not atypical of marsh sediments (Table 3, Fig. 2). There was no obvious distributional pattern observed in any of the variables measured with respect to latitude, tide range, salinity, or dominant high marsh vegetation; however, organic matter content was positively correlated ($r = 0.514$) with sediment bacterial abundance (Fig. 2).

Sediment bacterial community composition differed from site to site (Fig. 3). At the Virginia site, seven sediment samples were extracted and analyzed to obtain an estimate of the variance associated with the bacterial communities. Error bars representing 1 standard error of the mean (SEM) are included in Fig. 3, indicating that the magnitude of uncertainty associated with these analyses is such that the separation observed among the data points reflects differences among the sites and not with-site variance.

Of the sediment bacterial communities available for analysis, the northernmost sites (Maine and Connecticut) were separated from the rest of the sites on the first PC axis. The second PC axis separates the remainder of the sites along a general latitudinal gradient from Maine to South Florida with samples from the Virginian and Carolinian provinces (Table 1) forming a cluster midway between the Maine and South Florida sites. The exceptions to this pattern are the Virginia and Connecticut sites, which are located outside of that cluster. However, the strength of the relationship between latitude and sediment bacterial community composition is clear when the second PC scores are plotted versus site latitude (Fig. 4, $r = 0.583$).

Microbial Abundance. Plant-associated bacterial abundance roughly corresponded to the latitudinal gradient; there was a general trend of increasing bacterial abundance from the north to south that ranged widely from a high of over 10^9 to a low of 2×10^6 cells g^{-1} dry mass of plant material (Fig. 5). If the lack of detectable fungi in the South Florida and Indian River samples is considered, fungal abundance also corresponded to the latitudinal gradient but spanned less than an order of magnitude (Fig. 5). In contrast to bacterial abundance, fungal abundance decreases from north to south. There was no clear effect of plant type on either bacterial or fungal abundance. Although the impact of plant type on microbial abundance may have been confounded by the apparent latitudinal effect, the lack of a detectable plant effect may not be surprising given that similar plant tissues were not necessarily used for microbial enumeration. Instead, the entire plant was ground and used to prepare dilutions so that the proportion of leaves, leaf sheath, and stem varied from sample to sample. As Gessner [23] and Newell [48] note, hyphae are unevenly distributed along standing dead *S. alterniflora* and *Juncus roemerianus* leaves. Furthermore, differences in the time between plant senescence and collection of the plant materials used for these analyses may mask the effect of plant type on bacterial and fungal abundance by providing differing lengths of time for standing-dead microbial communities to develop.

Hyphae were not seen in either *Rhizophora mangle* or *Spartina bakeri* samples. Given that the minimum detectable quantity of hyphae for the microscopic technique used in this study is about 170 m of hyphae g^{-1} dry mass of plant material, hyphae could have been present in these samples below this value. For purposes of correlation analysis, a value halfway between 0 and the minimum detectable quantity of hyphae was used for these two sites.

Sediment microbial abundance patterns were different from those for standing dead plants. No fungal hyphae were observed in sediment samples. Bacteria were more abundant in sediments than on standing-dead plant surfaces ranging from a low of 3.9×10^9 cells g^{-1} dry mass sediment in the North Carolina marsh to over 2×10^{10} cells g^{-1} dry mass sediment in the Connecticut location (Fig. 2). Sediment bacterial abundance was not correlated with latitude; but it was correlated with the organic matter content of the small cores (1.6 cm diameter × 2 cm long) used for enumeration of the bacteria (Fig. 2).

Plant-Associated Microbial Communities. A distinct difference in plant-associated community composition was observed for both bacteria and fungi (Fig. 6). Bacterial communities associated with *Juncus* were significantly different on the PC2 axis from the remaining samples which were dominated by three *Spartina* species (ANOVA, $F_{0.05(8,2)} = 7.635$, $p = 0.014$). *Phragmites*- and *Rhizophora*-associated bacterial communities clustered

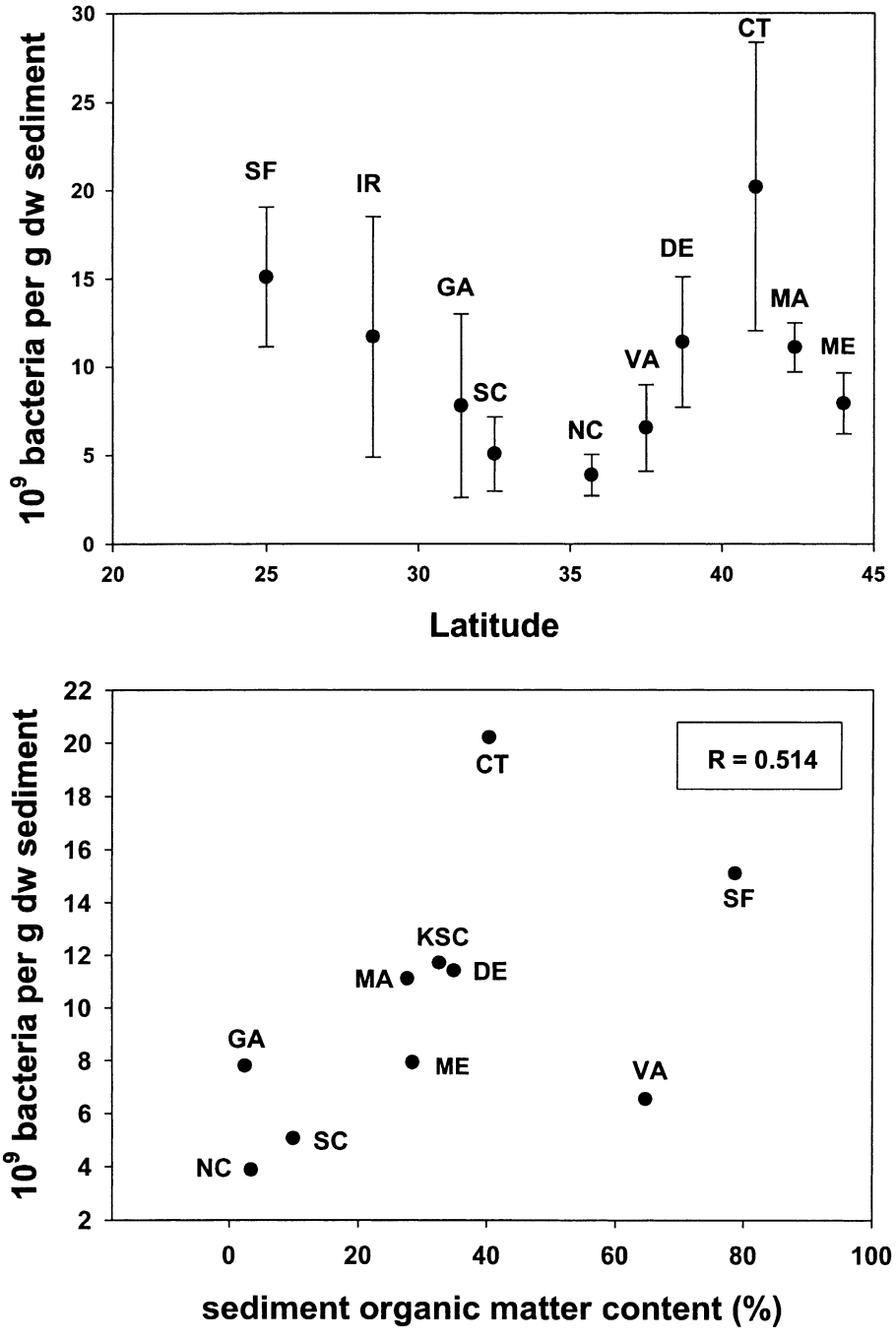

Figure 2. Distribution of the abundance of sediment bacteria by latitude (top) and by the percentage of organic matter present in the sediments (bottom).

along with bacterial communities from the three species of *Spartina*. The *Juncus* datum that is distant from the other two *Juncus*-associated bacterial communities represents vegetation from the Georgia site. This particular microbial community is notable because of the much lower concentration of hyphae on plants from the Georgia site relative to other sites in this study (Fig. 5). The paucity of hyphae on these plant may have influenced the composition of the bacterial community found there or reflect other factors at the Georgia site that affect microbial community composition. The South Carolina *S. alterniflora* bacterial community was also quite different from the other communities found on *Spartina* plants. This might be attributable to the noticeable amounts of fine-grained sediments adhering to the plants from the South Carolina site. Substantially less sediment was observed on plants from other sites. Adhering sediments were not removed from plants before extraction of microbial community DNA. It is interesting to note that if the sediment-contaminated South Carolina sample

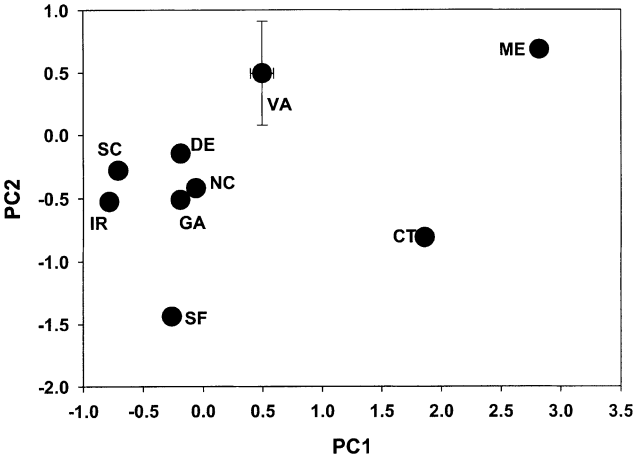

Figure 3. Principal components analysis plot of sediment-bacteria community composition patterns obtained from TRFLP analysis of whole-community DNA extracts amplified with the primer set 27f_6FAM:1492r.

point is excluded from the analysis, the *Juncus* vs other plant comparison on the first PC axis is not significant, but is nearly so at $p = 0.060$ (ANOVA).

Fungal communities (Fig. 6) from each of the *Spartina* species alone and in aggregate were more similar to one another than to those from *Juncus* (which were tightly clustered internally) (ANOVA on PC1, $F_{0.05(5,3)} = 7.404$, $p = 0.027$) or *Phragmites* (a single sample which clustered at a distance from all other plant materials). When the individual *Spartina* species were entered into the analysis separately (*S. bakeri* from the Indian River site was deleted as there was only a single representative of that species), the differences were not quite significant for PC1 ($p = 0.058$), but separation on PC2 was still strongly significant ($F_{0.5(4,3)} = 26.36$, $p = 0.004$) suggesting that different fungal communities develop on standing dead leaves of different *Spartina* species as well as among genera. No amplification products were obtained from *Rhizophora* communities, consistent with the abundance results. Amplification products were obtained from *S. bakeri*, although no hyphae were observed in the enumerations. This result might suggest that fungi were present in *S. bakeri* at a level too low to be seen microscopically. Although the Maine and Connecticut samples did cluster away from the rest of the sites, the lack of overall spatial pattern suggests that plant type affects the structure of the fungal community more than geographic location.

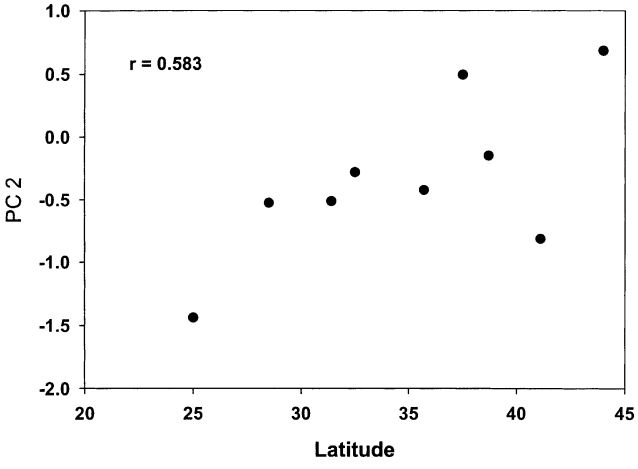

Figure 4. Distribution of PC2 values for bacterial sediment community composition (from Fig. 3) with latitude to display latitudinal gradient in community composition of the sediment. The correlation between the variables was $r = 0.583$.

Comparison of Plant and Sediment Bacterial Communities. Sediment bacterial communities were clearly different from plant-associated communities providing evidence that the differences are real community differences among the standing-dead associated microbial communities (Fig. 7). Only one community (South Carolina's plant-associated community) overlapped with the opposing sediment-associated cluster; however, that sample was very different from its sediment-associated counterpart. This result is consistent with the observation that South Carolina plant materials were coated with sediment particles.

Discussion

T-RFLP analysis of whole microbial community nucleic acid provides information about the presence of the most abundant microbial amplicons in a community. In our previous experience with the quantification of direct nucleic acid extracts from environmental samples, DNA quantity is not always correlated with amplification efficiency because of the presence of PCR inhibitors coextracted in the purified DNA product. Therefore, nucleic acids were extracted from standardized amounts of plant and sediment materials and then amplified directly rather than adjusting the concentration of DNA used in each T-RFLP analysis. Electropherogram patterns reflect the most abundant amplicons per unit of plant or sediment sample but cannot be used to provide specific information about community richness based on similar numbers of individual organisms (i.e., a standardized amount of DNA template in each reaction tube). Although SSU rDNA-targeted T-RFLP analysis of relatively simple communities can be highly reproducible (e.g., [43]), richness estimates derived from direct extracts and PCR amplification of natural samples are at best qualitative measures because of uncertainties in the biases associated with extraction and amplification efficiency from

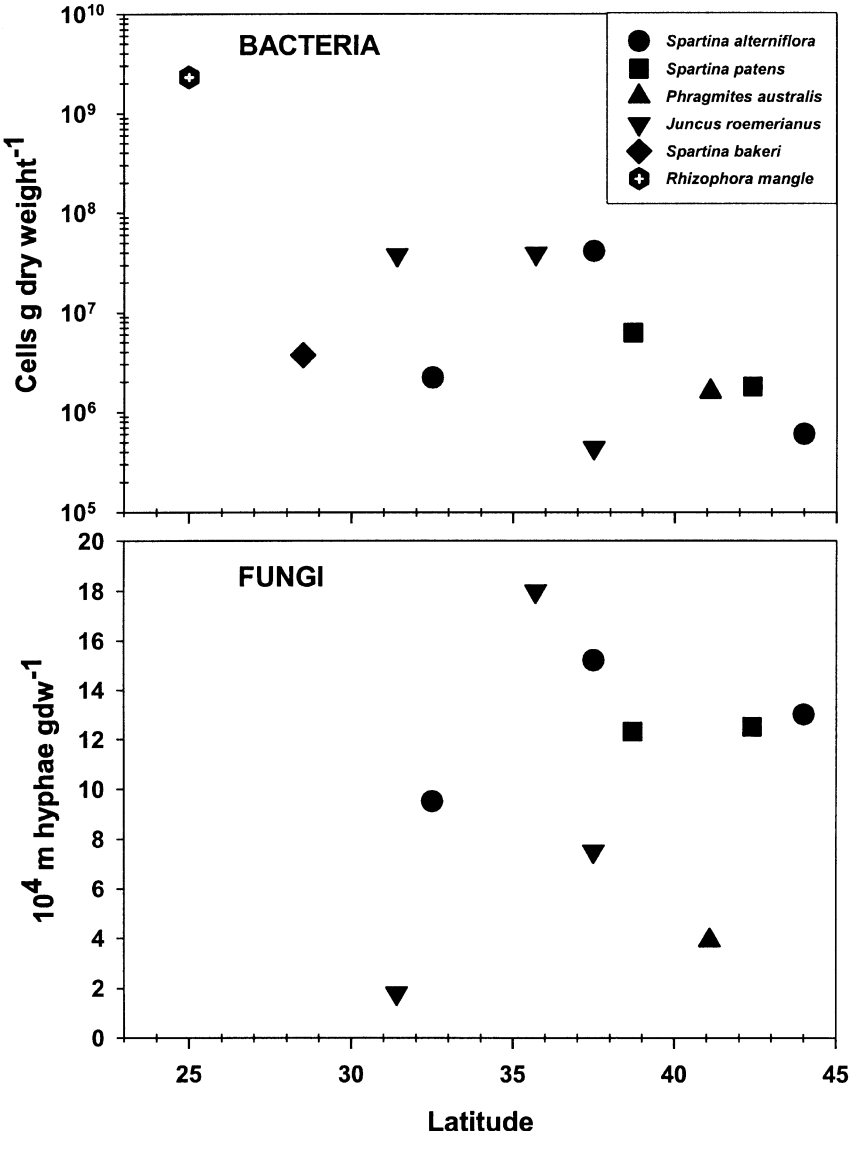

Figure 5. Abundance of bacteria (top) and fungi (bottom) in the plant material collected from the 10 sites. There is a data point for bacteria and fungi associated with *Spartina alterniflora* as samples of this plant from the Virginia site were also included in these analyses. Fungi were below the limit of detection (170 m hyphae g^{-1} dry wt $plant^{-1}$) for *Spartina bakeri* or *Rhizophora mangle*; data points for the hyphae associate with these two plants represent a point halfway between 0 and the minimum detectable quantity.

complex environments [63]. The term *community composition* is used to refer to community fingerprints throughout the remainder of this article.

Microbial communities from different habitats are indeed different: communities associated with standing-dead plants are distinct from sediment communities (Fig. 7). Although the South Carolina plant-associated microbial community appears to be the single exception to this pattern, the observation that plants from South Carolina were coated with visible amounts of fine sediments while the plants from all other sites did not may explain why this microbial community clusters with sediment communities. The separation of the South Carolina point from the standing-dead communities (Fig. 7) indicates that this community was actually a mixture of bacteria typically associated with standing plants and sediments. It is noteworthy, however, that the South Carolina plant and sediment communities are widely separated along the first PC axis, emphasizing that sediment communities and those on standing-dead plants are different from one another. Like the South Carolina samples, biotic and abiotic conditions at the other nine sites are sufficiently different in these two habitat types that microbial communities on standing-dead marsh plants are discrete from sediment communities even when the habitats compared are in the same physical location in a marsh. The difference between the two habitat types is not surprising given that extraction of community DNA includes anaerobes and chemolithotrophs that might be abundant in the sediment but unlikely to be dominant on standing-dead plant material. It does suggest that when the plant material falls to the sediment surface, the litter will be exposed to an entirely new community of decomposers.

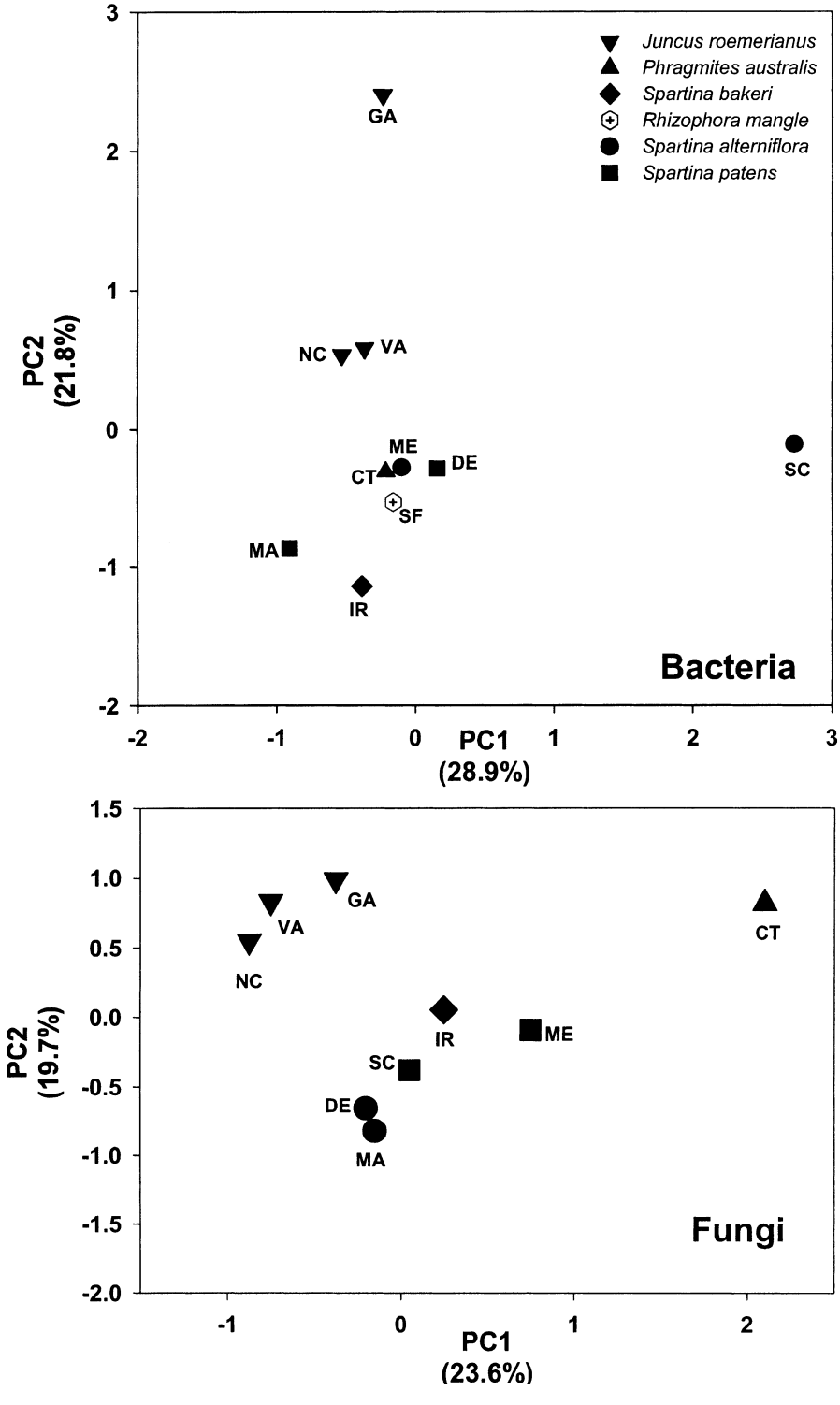

Figure 6. Comparison of bacterial and fungal communities from standing dead marsh vegetation representing the six different plants examined. No products were recovered from the amplification of mangrove-associated fungi.

Each habitat type (i.e., plant and sediment) was characterized by distinctly different community patterns along the latitudinal gradient. Within sediments, the bacterial community fingerprints correlate with the latitude differences among the sites (Fig. 3 and Fig. 4), while bacterial and fungal communities associated with standing-dead plants form distinct groups based on plant type (Fig. 6). Sediment community fingerprints were not correlated with sediment bulk density or organic matter content. Plant-associated communities were not correlated with latitude. The community patterns observed in sediments across the wide spatial scales examined here

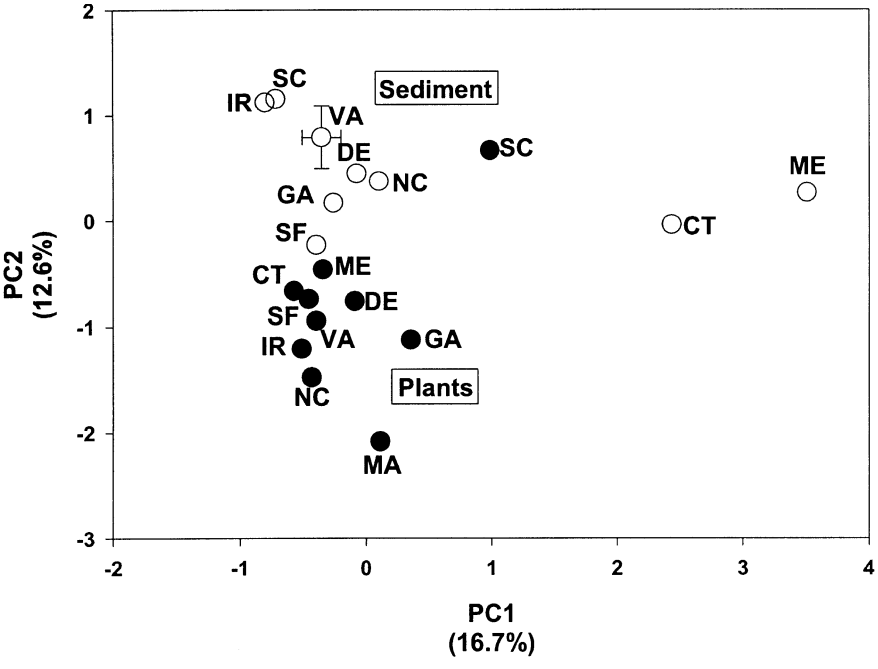

Figure 7. Comparison of plant-associated and sediment-associated bacterial communities showing the distinct differences between the two habitats. Open circles are sediment data; closed circles are plant data.

suggest that in some habitats bacterial communities may exhibit biogeographical distribution patterns. What the factor or factors are that covary with latitude and contribute to these biogeographic patterns is not evident from this survey but could include soil type, temperature, precipitation patterns, seasonality, or light intensity.

In the standing-dead plant habitat, latitudinal effects were not detected. Although climatic factors may contribute to the distribution and composition of microbial communities associated with standing-dead marsh plants, plant type is the most likely primary determinant of microbial community composition. Climate effects were of lesser importance than organic substrate impacts on community composition patterns. Organic substrate effects on microbial community processes have been noted previously; salt marsh plants show greater resistance to decay than mangroves [5, 10, 46, 75]. Support for the importance of organic substrate differences affecting community structure of the standing-dead microbial community comes from direct microscopic observation of fungi and the ability to identify the ascomycete fungi from reproductive structures formed in the standing-dead plant tissues. Work with fungal communities on *Phragmites australis* (K.D. Hyde, personal communication) and the salt marsh plants *S. alterniflora* and *J. roemerianus* [38, 39] clearly shows that there is very little or no overlap among the decomposition communities of ascomycete fungi on different species of marsh grass. Newell's microscopic work is consistent with the rDNA ITS results of Buchan et al. [7] in that all but a couple of minor T-RFLP peaks from naturally decaying smooth-cordgrass blades match to their set of directly observed ascomycetes. Several of the species (especially rarer ones) observed directly have not shown up in the T-RFLP scans, including the ascomycete yeasts that have been reported from smooth cordgrass. Also, the lack of amplification with fungal primers in *R. mangle* leaves is consistent with Newell's findings, at least for leaves decaying in persistently submerged situations. The primary eukaryotic decomposers of mangrove leaves are not true fungi but mycelial straminipiles (oomycotes, species of *Halophytophthora*) that may not be detected with the primers used here [6, 7].

If organic substrates, determined by plant type, have such a strong influence on standing dead microbial communities, why then don't sediment communities segregate by the type of marsh plant contributing the greatest proportion of the sediment carbon or by sediment organic matter content? Perhaps the answer is that salt marsh sediments along the East Coast are more chemically homogenous environments than standing-dead plants so that climate factors such as temperature, or sediment edaphic factors, are the variables that differ the most among the locations we examined. By the time standing-dead plants are deposited on the sediment surface as litter, the litter has been decayed to such an extent that any differences in the living (or recently dead) plant materials have been eliminated. The lack of community composition differences and the positive relationship between sediment organic matter content and bacterial abundance support this hypothesis.

The positive correlation (Fig. 2, $r = 0.514$) between sediment organic matter and bacterial abundance suggests that sediments rich in organic matter have a higher

microbial biomass carrying capacity than low organic matter sediments. Fungi, on the other hand were generally rare in the sediments sampled. The link between organic carbon and bacterial abundance is well established in soils, sediments, and water [1] and has been attributed to a general carbon limitation of bacteria in environmental samples [1]. The absence of fungal hyphae in the marsh sediments examined in this study is not remarkable, as fungi are generally not found in wetland soils [27], although live, active fungi have been observed in surface peat of fresh [27] and salt water marshes [44] or associated with live plant roots as mycorrhizae [13, 14, 32, 68, 72]. Several reports [26, 27, 51] suggest that fungi have the potential to play a significant role in sediment organic matter decomposition, but reports such as these are limited; the distribution and role of fungi in a wider range of (frequently anoxic) sediments remain unclear.

Bacterial and fungal abundance were correlated with latitude (Fig. 5). The effect is more pronounced for bacteria than for fungi and the correlations are opposite to one another: bacterial abundance decreased with increasing latitude while fungal hyphae became more abundant as latitude increased. Gradients in microbial abundance have been noted previously for bacteria and have been attributed to temperature effects (e.g., [36]). Newell [47] and Samiaji and Bärlocker [60] hypothesized that fungal biomass associated with standing-dead marsh grasses should increase with decreasing latitude, but the present results displayed the opposite effect. Furthermore, these results differ from other literature reports. In a survey of standing-dead *S. alterniflora* collected at a similar time of year from sites ranging from northern Florida to Maine, Newell et al. [49] did not detect a gradient in fungal biomass or activity in the 3 years samples were collected. In that report, 21% of the variation in fungal biomass could be attributed to variation in the C/N ratio of the standing-dead material (the higher the C/N ratio, the higher the fungal biomass). Similar results were not observed for the plant materials used in our study: the relationship between C/N ratio and microbial abundance (hyphal or bacterial) was opposite to that observed by Newell et al. [49], but the correlation was not significant and could explain only 11% of the variation in abundance. The abundance patterns we report are confounded by differences in the plant species collected; however, even when fungi associated with *Spartina alterniflora* or all examined *Spartina* species are considered (Fig. 5), the patterns are still in opposition to that of Newell et al. [49]. The difference in the patterns between these two studies may have resulted from the difference in the techniques used to measure fungal abundance. Newell et al. [49] based their measurements on ergosterol, which is most likely to be associated with living fungi [69]. Ergosterol content may, however, more accurately reflect changes in species composition rather than changes in fungal abundance in some environments [4, 52]. Fungal abundance estimates reported here are based on microscopic examination of fungal hyphae, a method that can overestimate the abundance of living fungi because empty hyphae cannot be distinguished from living hyphae [34, 48].

The inverse relationship between bacteria and fungal abundance (Fig. 5) suggests the potential for a competitive or antagonistic relationship between bacteria and fungi: as the abundance of one of these types of microbes increases, the other decreases. However, limitations of the microscopic techniques used, as noted above for the fungi, make comparisons of bacterial to fungal biomass unreliable. Newell [47] hypothesized that a mutualistic relationship exists between bacteria and fungi on standing dead plants in which fungi create a more hospitable environment for the growth of bacteria citing, for example, Wilson et al. [74], who show that fungi metabolize *S. alterniflora* cinnamic acid, an antiherbivory compound that also exhibits antimicrobial activity. To test Newell's hypothesis about the relationship between bacteria and fungi, measurement of living biomass and metabolic activity would provide greater insight into the nature of the microbial interactions on standing-dead plants than the methods used in our study.

Two of the plant types examined supported very low or no fungal populations (Fig. 5, bottom). The lack of detectable fungi on yellow-but-attached red mangrove leaves by microscopic methods suggests that hyphal fungi were not abundant or were not present on the mangrove leaves. Our inability to amplify fungal DNA from these samples, microscopic examination by other investigators [5], and quantification of ergosterol [5, 50, 55, 56] in red mangrove leaves support the observation that hyphal fungi are not found in association with *R. mangle* leaves. It is important to note that polysaccharides, polyphenolics, which are relatively abundant in sediments, and many leaf tissues including red mangrove, can interfere with PCR amplification [61, 66, 70] and that other investigators have attributed an inability to amplify DNA from similar plant samples to this technical problem [53]. However, our ability to amplify bacterial DNA from these same red mangrove extracts suggests that the DNA used in the PCR reactions was sufficiently purified to avoid polyphenolic inhibition of DNA amplification during PCR. It is possible that the amount of fungal DNA in the extracts was simply below the level of amplification by PCR. Unlike the red-mangrove case, T-RFLP analysis of *S. bakerii* plants revealed that fungi were present in the plant materials below the limit of microscopic detection even though no fungal hyphae were found during microscopic examination. This leads to the conclusion that fungi may be relatively less important in decay of standing dead *S. bakerii* than the abundant bacteria associated with this plant—a conclusion that is counter

to extensive evidence that fungi are critical to decomposition of a wide variety of standing-dead marsh plants. Confirmation of this conclusion would require more extensive study of *S. bakerii* than examination of the three plant leaves and stems included in this study.

There is a strong biogeographical component to the relative abundance of bacterial and fungal communities associated with standing-dead plant biomass, but microbial community composition on standing-dead plants is not strongly influenced by the latitudinal gradient. Conversely, bacterial abundance in the sediments was not associated with latitude but was clearly correlated with sediment organic matter content. Within the sediment, the composition of both bacterial and fungal communities was strongly correlated with latitude, indicating that biogeographical factors are important determinants of sediment community composition. The composition of both the fungal and the bacterial communities on standing-dead plants was more similar among plants of the same type (i.e., related plant species) indicating that plant type (and hence substrate quality) are primarily responsible for the microbial community composition there. The colonization of standing-dead plants is similar in this respect to the colonization of the rhizosphere where there appears to be strong selection for particular microbial types [40]. Although no correlation was evident between latitude and community composition for standing-dead microbial communities, both bacterial and fungal abundance on standing-dead plants varied with latitude.

Whereas bacterial abundance on standing-dead plants and latitude were positively correlated, fungal abundance on standing-dead plants increased with decreasing latitude. Relative microbial abundance on standing-dead plants in the salt marsh is therefore clearly determined by factors relating to the biogeography of the environment. If the bacterial and fungal community composition were considered together, the inverse relationship in abundance might show a latitudinal effect on overall microbial community composition. We have begun to address this question as part of the evaluation of the community succession during decay.

The observed differences in sediment community composition across sites means that different microbial inocula may be available to colonize standing-dead plant material after it falls to the sediment surface and continues the decay process there. If the developing microbial decomposition community is a combination of the resident standing-dead and sediment communities, the mature decay communities may be substantially different among sites even though the fundamental decay process is similar among sites. The balance of natural selection and recruitment in these mature decomposition communities may be strongly influenced by the biogeography of the environment. It will be interesting to observe if the biogeographical component of sediment microbial composition is maintained within the decomposition communities or if its signature is lost through time. Decomposition experiments currently in progress at the 10 sites will enable resolution between these potential ecological outcomes.

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