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ENVIRONMENTAL MICROBIOLOGY

Changes in Community Structure of Sediment Bacteria Along the Florida Coastal Everglades Marsh–Mangrove–Seagrass Salinity Gradient

Makoto Ikenaga · Rafael Guevara · Amanda L. Dean · Cristina Pisani · Joseph N. Boyer

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Abstract Community structure of sediment bacteria in the Everglades freshwater marsh, fringing mangrove forest, and Florida Bay seagrass meadows were described based on polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) patterns of 16S rRNA gene fragments and by sequencing analysis of DGGE bands. The DGGE patterns were correlated with the environmental variables by means of canonical correspondence analysis. There was no significant trend in the Shannon–Weiner index among the sediment samples along the salinity gradient. However, cluster analysis based on DGGE patterns revealed that the bacterial community structure differed according to sites. Not only were these salinity/vegetation regions distinct but the sediment bacteria communities were consistently different along the gradient from freshwater marsh, mangrove forest, eastern-central Florida Bay, and western Florida Bay. *Actinobacteria*- and *Bacteroidetes*/*Chlorobi*-like DNA sequences were amplified throughout all sampling sites. More *Chloroflexi* and members of candidate division WS3 were found in freshwater marsh and mangrove forest sites than in seagrass sites. The appearance of candidate division OP8-like DNA sequences in mangrove sites distinguished these communities from those of freshwater marsh. The seagrass sites were characterized by reduced presence of bands belonging to *Chloroflexi* with increased presence of those bands related to *Cyanobacteria*, γ-*Proteobacteria*, *Spirochetes*, and *Planctomycetes*. This included the sulfate-reducing bacteria, which are prevalent in marine environments. Clearly, bacterial communities in the sediment were different along the gradient, which can be explained mainly by the differences in salinity and total phosphorus.

M. Ikenaga · R. Guevara · A. L. Dean · C. Pisani · J. N. Boyer (✉)
Southeast Environmental Research Center, OE-148,
Florida International University,
11200 SW 8th St, Miami, FL 33199, USA
e-mail: boyerj@fiu.edu

M. Ikenaga
Department of Life Sciences,
Ritsumeikan University,
1-1-1 Noji-Higashi, Kusatsu,
Shiga, Japan

Introduction

Sediment bacterial communities play an important ecological and biogeochemical role in aquatic ecosystems. This is a result of their high abundance (>10^8 cells per gram) [10, 17, 39, 54] as well as their key function in systems by regulating the transformation of biogenic elements such as C, N, P, Fe, O, and S [42]. Moreover, sediment bacterial communities represent a reservoir of genetic variability similar to soil environments showing approximately 10^4 species per gram [63]. Several factors have been described to influence sediment bacterial communities: salinity [3, 16], organic matter quality [2, 44, 65], P content [72], N content [49], and plant cover type [30]. However, determining which factors are most important has been difficult because of the interactions among them.

One approach to addressing this question is to compare community structure changes along an environmental gradient. This approach has been used to investigate the differences of bacterial community structures in river sediments along a downstream reach [73], in sediments away from a source of heavy metals [19], in water column bacterioplankton along a salinity gradient [16], and in Antarctic marine sediments across the shelf [5]. In this work, we investigated how the community structure of sediment bacteria varied along the environmental gradients (salinity, N, and P) of the freshwater

marsh–mangrove forest–seagrass meadows ecotone of the Florida Coastal Everglades (FCE) using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and sequence analyses. To our knowledge, this is the first study to have applied a molecular technique for elucidating the entire sediment bacterial communities in this ecosystem.

Methods

Location and Sampling Sites

The FCE are a hydrologically connected hydroscape of freshwater marsh consisting mostly of sawgrass (*Cladium jamaicense*) and spike rush (*Eleocharis cellulosa*), fringing forests of mostly red (*Rhizophora mangle*) and black (*Avicennia germinans*) mangroves, and *Thalassia testudinum*/*Syringodium filiforme*/*Halodule wrightii* seagrass meadows of Florida Bay (Fig. 1). These three large regions form a semi-enclosed ecosystem with a well-defined oligotrophic gradient from P limitation in the freshwater marsh to N limitation at the marine boundary of the estuary [7, 20]. This ecosystem was designated by National Science Foundation as the Florida Coastal Everglades Long-Term Ecological Research site (FCE-LTER) in 2000 (http://fcelter.fiu.edu/research/). Over the years, much background information on primary production, soil organic matter, nutrient cycling trophic dynamics, plant variation, and abiotic conditions has been collected at these sites [13].

Freshwater marsh sampling sites were located in two distinct watersheds: Shark River Slough (SRS; SRS-2 and SRS-3) and Taylor Slough/Panhandle (TS/Ph; TS/Ph-3, TS/Ph-4, and TS/Ph-5). The mangrove forest sites in both watersheds were SRS-4, SRS-5, and SRS-6, and TS/Ph-6, TS/Ph-7, and TS/Ph-8. The seagrass sites in Florida Bay (FB) included FB-9, FB-13, FB-16, FB-21, and FB-27. Although three FB-9, FB-21, and FB-27 are usually named as TS/Ph-9, TS/Ph-10, and TS/Ph-11 in FCE-LTER maps, the original water quality monitoring site numbers were used instead to distinguish the bay from terrestrial sites.

Equal quantities of sediment were taken using a core (2 cm depth, 3.175 cm diameter). Five random sediment cores from each of the 16 sites with a 10×10-m quadrant were collected in July 2004. Sediment samples were pooled in a sterilized plastic bottle to reduce any spatial variability and transported to the laboratory in the dark on ice. Each composite sample was then homogenized and passed through a 2-mm mesh sieve before being frozen at −80°C until further analysis.

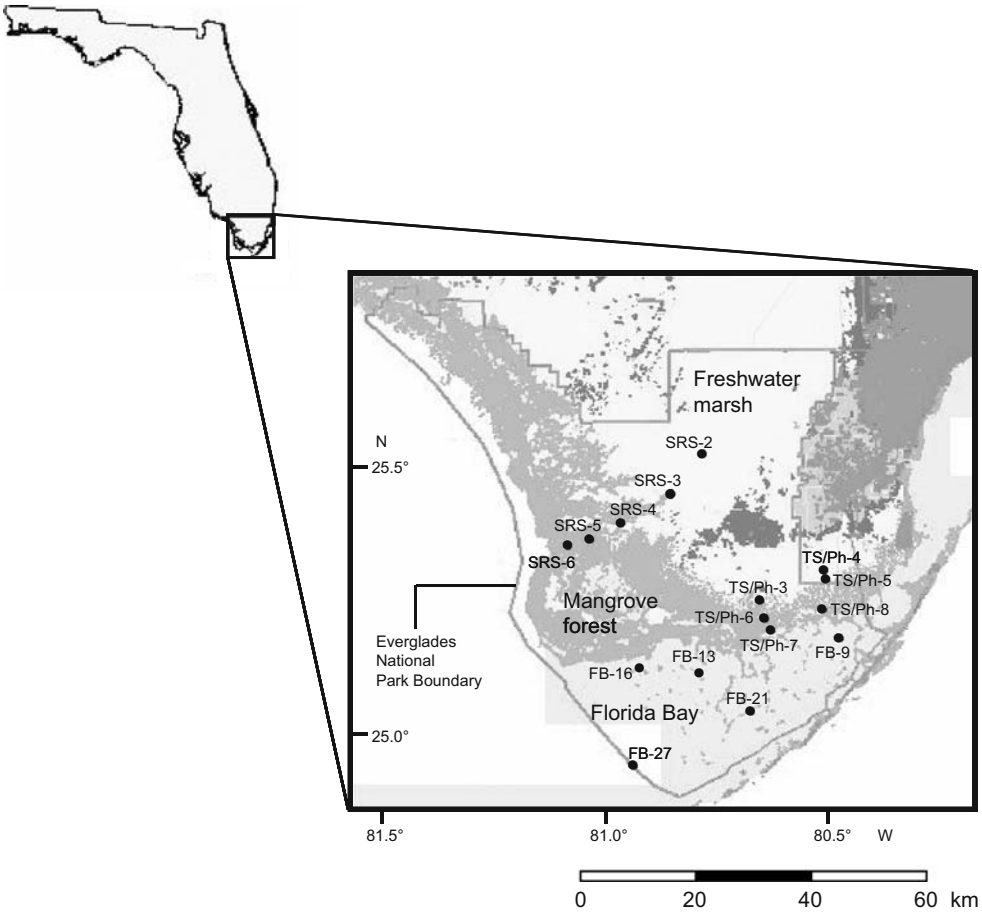


Figure 1 Map of Florida coastal Everglades showing sampling sites in Shark River Slough (*SRS*), Taylor Slough/C-111 canal basin (*TS/Ph*), and Florida Bay (*FB*)

Environmental Data

Surface water samples were collected as outlined by the FCE-LTER protocol [13]. Analytical details are available via the "online datasets" link at http://fcelter.fiu.edu/data/core/index.htm. Briefly, salinity and temperature were measured with an YSI conductivity meter. Water was collected and filtered through a Whatman GF/F filter (0.7 μm) immediately upon return to the lab and the filtered samples analyzed for ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), and soluble reactive phosphorus (SRP). These dissolved nutrients were measured using standard rapid flow autoanalyzer techniques. The unfiltered fraction was analyzed for total phosphorus (TP), total nitrogen (TN), and total organic carbon (TOC). TN was determined by high-temperature combustion with chemiluminescence detection with an Antec TN analyzer; TP was ashed, digested, and analyzed via modified Solorzano and Sharp technique [57]; and TOC was quantified using high-temperature combustion with a Shimadzu TOC-5000.

DNA Extraction, PCR Amplification, and DGGE

DNA extraction was carried out on duplicate ca. 500-mg wet sediment samples using Fast DNA spin kit for soil (Qbiogene, Carlsbad, CA, USA), based on bead beating according to the manufacturer's instructions. Extraction blanks were processed in parallel throughout the full procedure as negative controls to evaluate potential DNA contamination from reagents.

Variable region 3 (V3) of the 16S rRNA gene was amplified by the primer set for bacteria (357f-GC (*Escherichia coli* position, 341–357, 5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′, underline of sequence denotes GC clamp) and 517r (*E. coli* position, 517–533, 5′-ATTACCGCGGCTGCTGG-3′)) [41]. The 50-μl PCR mixture contained 0.5 μl of the primer set (25 pmol each), 0.25 μl (1.25 U) of *Ex Taq* DNA polymerase (Takara Bio, Otsu, Japan), 5 μl of *Ex Taq* buffer (20 mM $MgCl_2$), 5 μl of deoxyribonucleotide triphosphate mixture (2.5 mM each, Takara Bio, Otsu, Japan), 1.5 μl of DNA template (approximately 50 ng), and 37.75 μl of sterilized ultrapure water. PCR amplification was performed by using a PTC-2000 Peltier-effect Thermal Cycler (MJ Research, Watertown, MA, USA). Amplification conditions were as follows: 94°C for 3 min (initial denaturation), followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min with the final extension step at 72°C for 8 min. Negative controls (without DNA) were run in all amplifications.

DGGE was performed with 8% (wt/vol) acrylamide gel containing a linear chemical gradient ranging from 30% to 70% denaturant (100% denaturant, 7 M urea, and 40% (vol/vol) formamide) [41]. Aliquots of 1,200-ng concentrated PCR products were loaded into wells of the DGGE gel and electrophoresed in 0.5× Tris-acetate–ethylenediaminetetraacetic acid buffer at 60°C and 100 V for 14 h, using a DCode universal mutation detection system (BioRad Laboratories, Inc., Hercules, CA, USA). After electrophoresis, the gel was soaked in SYBR Green I nucleic acid gel stain solution (Molecular Probes, Eugene, OR, USA) for 30 min and photographed with FOTO/Analyst Express Electronic Documentation and Analysis System (Fotodyne, Hartland, WI, USA) under Dark Reader Transillumination (Clare Chemical Research, Denver, CO, USA).

Diversity and Statistical Analyses

DGGE bands were divided into five categories (1–5) according to their intensities, with the strongest intensity given a value of 5. NIH image software was used for classification (http://rsbweb.nih.gov/nih-image/). All the DGGE bands of bacterial 16S rRNA gene amplified by the 357f-GC and 517r primer set were used. Shannon–Weiner index (H') [1] was used as an estimation of bacterial diversity. The H' was determined with the following equation: $H' = -\Sigma P_i \ln P_i$. The term P_i was calculated as follow: $P_i = n_i/N$, where n_i is the band intensity for individual bands and N is the sum of the intensities of bands in a lane. Hierarchical cluster analysis was performed using similarity matching data (Morisita's similarity index) produced from the DGGE profiles of 16S rRNA genes. The tree topology was determined by unweighted pair-group method with arithmetic mean (UPGMA), using the MultiVariate Statistical Package (MVSP) v3.12h (GeoMem, Blairgowrie, UK). A cutoff similarity value was used to define the clusters in the UPGMA dendrogram. This value was assessed from original matrix resampling using the Monte Carlo test (999 permutations) with the Pop Tools v3.0 [25].

In order to establish which DGGE bands characterized the sediment bacterial community in each particular site, a correspondence analysis was performed. Also, to investigate which environmental variables best explained the variability in DGGE profiles, a canonical correspondence analysis (CCA) was applied. CCA is an ordination technique that seeks the most prominent linear gradients in multivariate data sets, under the constraint that the gradients are linear combinations of a set of explanatory variables. Eight environmental variables related with water quality—salinity, TP, TN, TOC, NH_4^+, NO_2^-, NO_3^-, and SRP—were included. Because the correlation between environmental variables may affect the CCA [60], the lack of correlations was tested by Pearson coefficient according to lineal model using GraphPad Prism v4.0 (GraphPad Software, Inc). The statistical significance (at the 5% level) of relationship between genetic diversity (DGGE profiles) and environmental variables was assessed using the Monte Carlo test (999 permutations under the full model). Both correspondence

analyses were performed using the software package CANOCO for Windows v4.0 [61].

Partial 16S rRNA Gene Sequencing of Excised DGGE Bands

Direct Sequencing

The most intense and predominant DGGE bands were excised from the gel and directly amplified as DNA template. The PCR program was the same as that used for the primer set of 357f-GC and 517r, although the volume of the PCR mixture was reduced to 15 µl. A mobility check of the amplified DGGE band was performed to confirm whether the position of the band was the same as that of the original by replicating DGGE analysis using identical condition. Cycle sequencing was conducted using an ABI PRISM BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. DNA sequencing was performed with the ABI 3100 Automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). Primers 357f and 517r, specific to bacteria, were used to sequence both DNA strands and sense, and antisense sequences were compared.

Sequencing from Clones

When DGGE bands were not directly sequenced, a cloning method was applied. Aliquots of 0.15 pmol of the PCR products that showed the same position as the original by DGGE were cloned into the pSTBlue-1 AccepTor™ vector (Novagen, Madison, WI, USA) using the clonable 2× ligation premix and transformed in competent cells of *E. coli* strain XL1-Blue (Novagen, Madison, WI, USA). Plasmids were extracted using the QIAprep Spin Miniprep kit (Qiagen Valencia, CA, USA). Insert sequences were amplified with the 357f-GC and 517r primer set in a final volume of 15 µl by the same PCR program used for the first amplification reaction. The DGGE mobility check of the PCR product thus obtained was performed to confirm the original position of the band by the DGGE under the same condition. DNA sequencing of the plasmid which inserts showed the same position as the original were determined. In this case, T7 promoter primer (5′-CTAATACGACTCACTATAGGG-3′) and SP6 promoter primer (5′-ATTTAGGTGACACTATAG-3′) were used. At least four clones were sequenced for each DNA band.

Sequence Alignment and Phylogenetic Analysis

The 16S rRNA gene sequences (160 bp) obtained from the DGGE bands were searched against the DNA Data Bank of Japan by BLASTN. It is well known that phylogenetic conclusions based in partial sequences data should not be accepted; however, it is possible to use partial sequences to identify organisms or to assign groups, as long as the database contains sequences of close relatives [34, 56]. In this work, only sequences with a percentage of sequence identities higher than 90% were included in the phylogenetic analysis. The 16S rRNA gene sequences were aligned using ClustalX [62]. A phylogenetic tree was constructed by the neighbor-joining method based upon distances determined by the Jukes and Cantor index [31] using Treecon [68]. The topology of the distance tree was tested by resampling data with 1,000 bootstraps to provide confidence estimates for tree topologies.

Nucleotide Sequence Accession Number

The sequences obtained in this study were uploaded and are available at the DNA data banks under accession numbers AB235958 to AB235996.

Results

DGGE Pattern and Genetic Diversity

Community structure of sediment bacteria in FCE freshwater marsh, fringing mangrove forest, and seagrass meadows were compared based on the DGGE analysis of 16S rRNA gene fragments. The DGGE patterns showed remarkable differences in composition among Florida Bay seagrass sites and Taylor Slough/Panhandle and Shark River Slough (Fig. 2). Except for TS/Ph-3, the patterns of terrestrial samples from upstream freshwater marsh (SRS-2 and SRS-3 sites and TS/Ph-4 and TS/Ph-5 sites) to downstream mangrove forest (SRS-4, SRS-5, and SRS-6 sites and TS/Ph-6, TS/Ph-7, and TS/Ph-8 sites) were consistently different, showing a distance of 0.45 according to Morisita's similarity index. We also found differences in DGGE profiles among the seagrass sites. Overall community diversity did not differ along the gradient, as no significant trend in the Shannon–Weiner index was observed. The range of index value was from 3.71 to 4.00 (mean 3.88). There were no detectable differences in banding patterns between duplicate samples (data not shown).

Community Structure

Hierarchical cluster analysis was used to group sites having similar sediment bacterial community structures (Fig. 3). Monte Carlo random community DGGE patterns simulations showed that 75% of all Morisita's similarity index fell below 0.6, which suggested that the majority of relationships indicated in this dendrogram were not attributable to chance alone. Thus, four well-defined groups were observed. The

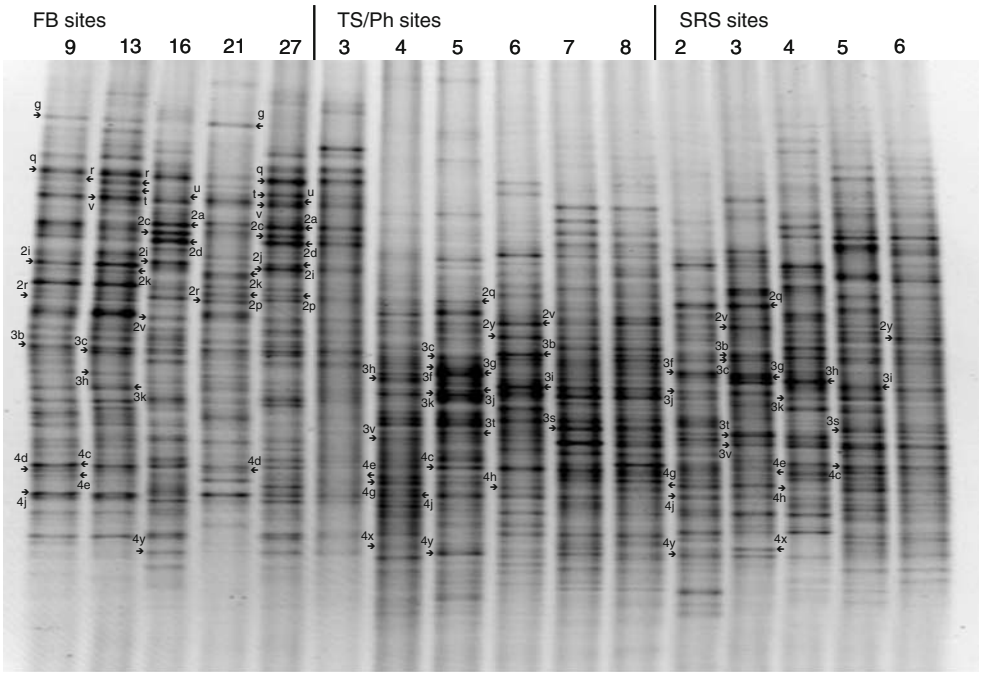


Figure 2 DGGE patterns of amplified 16S rRNA gene fragments of sediment bacterial communities in the sampling sites at Florida Bay (*FB*), Taylor Slough/C-111 canal basin (*TS/Ph*), and Shark River Slough (*SRS*). *Arrows* indicate DGGE bands for which the DNA sequence was determined

first separation occurred between terrestrial and Florida Bay sites with the exception of TS/Ph-3, which grouped with the Florida Bay cluster. The terrestrial sites were further divided into two subclusters consisting of freshwater marsh (SRS-2 and SRS-3 sites and TS/Ph-4 and TS/Ph-5 sites) and mangrove forest (SRS-4, SRS-5, and SRS-6 sites and TS/Ph-6, TS/Ph-7, and TS/Ph-8 sites). The Florida Bay cluster was also divided into two subclusters consisting of eastern-central Florida Bay (FB-9, FB-21, and FB-13 sites) and western Florida Bay (FB-16 and FB-27 sites).

Correspondence analysis established the DGGE bands characterizing the sediment community structures in these particular sites (Table 1). The DGGE bands characterizing Florida Bay sites and freshwater marsh–mangrove forest sites were found in first axis, which had high scores positively and negatively. In addition, the bands characterizing eastern-central Florida Bay sites and western Florida Bay sites were found in second axis, and those characterizing freshwater marsh and mangrove forest were found in third axis, respectively.

The CCA of the 16S rRNA gene DGGE data explained 48.7% of the variation in the first two axes (Fig. 4). CCA confirmed the clear separation of sites according to environmental factors. Axis 1 (CCA1) separated the terrestrial

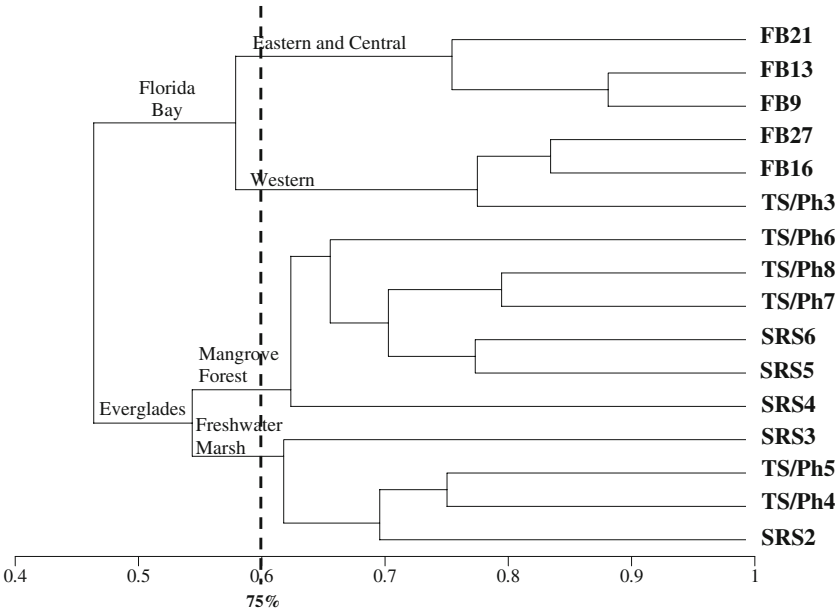


Figure 3 UPGMA dendrogram constructed from the similarity matching data (Morisita's similarity index) obtained from the DGGE profiles of 16S rRNA gene partial sequences amplified from the sampling sites at Shark River Slough (*SRS*), Taylor Slough/C-111 canal basin (*TS/Ph*), and Florida Bay (*FB*). The dendrogram was generated by using MVSP version 3.12h. The *scale bar* represents percent similarity. The cutoff similarity value is indicated by a *dash line*

Table 1 DGGE bands with the highest scores in the correspondence analysis

Axis	Contribution percent	Vector sign	DGGE band (group)	Region
First	22.2	+	v (*γ-Proteobacteria*) q, 2j (*δ-Proteobacteria*)	Florida Bay
			2p, t (*Spirochetes*)	
			2k (*Cyanobacteria*)	
		−	3g, 3j, 3v, 3t, 4g (*Chloroflexi*) 4x (candidate WS3)	Freshwater marsh–mangrove forest
Second	14.4	+	4d (*Planctomycetes*) g (*γ-Proteobacteria*)	Eastern-central Florida Bay
			2r (*Cyanobacteria*)	
			r (*Bacteroidetes*/*Chlorobi*)	
		−	u, 2d (*δ-Proteobacteria*) 2a (*γ-Proteobacteria*)	Western Florida Bay
			2i (*Bacteroidetes*/*Chlorobi*)	
			2c (*Cyanobacteria*)	
Third	10.9	+	2q (*Nitrospirae*) 3f (*δ-Proteobacteria*)	Freshwater marsh
		−	2y (*γ-Proteobacteria*) 3s (*Chloroflexi*)	Mangrove forest
			3i (*Nitrospirae*)	
			4h (candidate OP8)	

sites from the more saline seagrass sites in Florida Bay. CCA2 established a separation between mangrove and freshwater marsh sites. Based on CCA, axis 1 was positively correlated with salinity and negatively correlated with NO_2^- showing intraset correlations coefficient values of 0.662 and −0.641, respectively. Conversely, axis 1 had a positive correlation with TP and a negative correlation with TOC (0.694 and −0.504, respectively). However, according to Monte Carlo analysis, only salinity (F=1.89, P=0.008) and TP (F=1.68, P=0.032) showed a significant correlation to general community structure.

Phylogenetic Positions of 16S rRNA Gene Sequences that Mostly Appeared Throughout Sampling Site

A total of 36 DGGE bands were sequenced (Fig. 2). Nine bands (band-2v, band-3b, band-3c, band-3h, band-3k, band-4c, band-4e, band-4j, and band-4y) were observed at least in

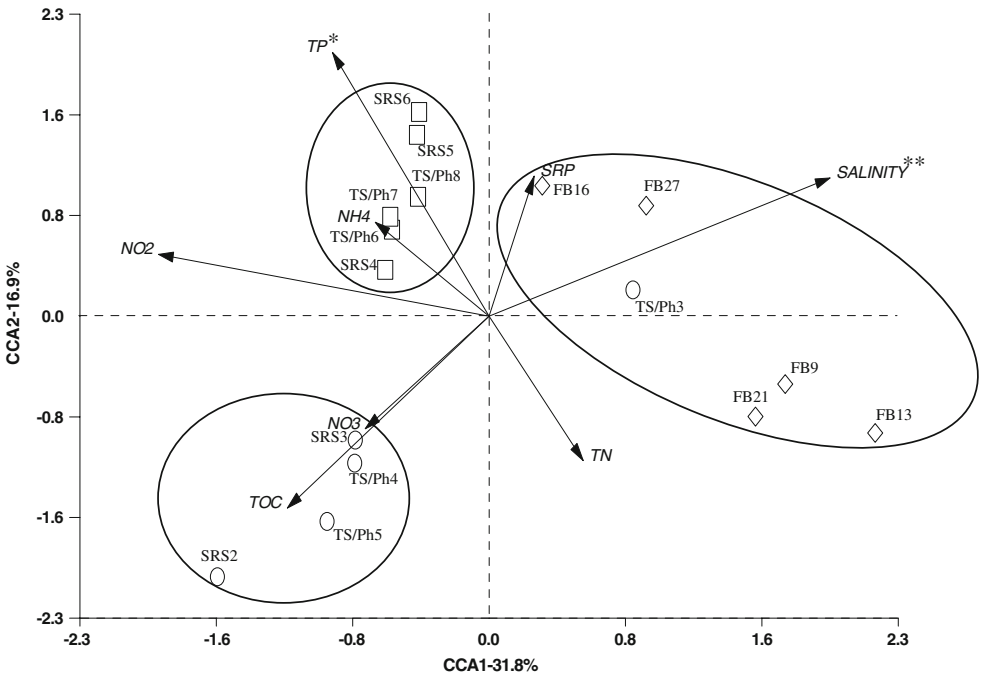
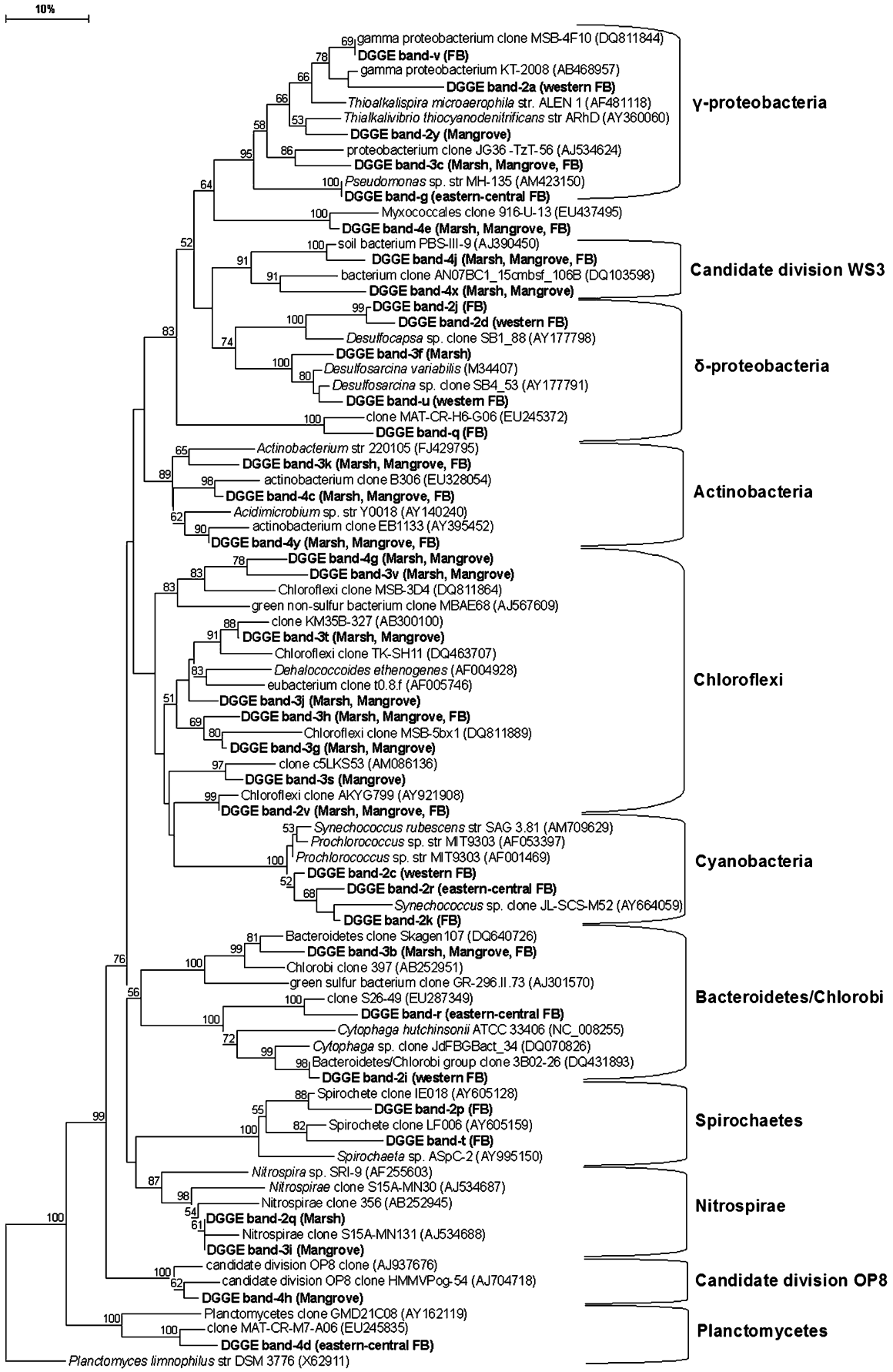


Figure 4 Canonical correspondence analysis (*CCA*) performed on the sediments of freshwater marsh (*circle*), mangrove forest (*square*), and seagrass (*diamond*) using PCR-DGGE profiles of the 16S rRNA gene and chemical data of samples (salinity, TP, TN, TOC, NH_4^+, NO_2^-, NO_3^-, and SRP). CCA analysis was performed with the MVSP version 3.12h. Significant relationships between environmental variables and canonical axes according to Monte Carlo permutation test (999 permutations) using Canoco program: $^{**}P<0.01$ and $^{*}P<0.05$


10%
gamma proteobacterium clone MSB-4F10 (DQ811844)
DGGE band-v (FB)
gamma proteobacterium KT-2008 (AB468957)
DGGE band-2a (western FB)
Thioalkalispira microaerophila str. ALEN 1 (AF481118)
Thialkalivibrio thiocyanodenitrificans str ARhD (AY360060)
DGGE band-2y (Mangrove)
proteobacterium clone JG36 -TzT-56 (AJ534624)
DGGE band-3c (Marsh, Mangrove, FB)
Pseudomonas sp. str MH-135 (AM423150)
DGGE band-g (eastern-central FB)
γ-proteobacteria
Myxococcales clone 916-U-13 (EU437495)
DGGE band-4e (Marsh, Mangrove, FB)
soil bacterium PBS-III-9 (AJ390450)
DGGE band-4j (Marsh, Mangrove, FB)
bacterium clone AN07BC1_15cmbsf_106B (DQ103598)
DGGE band-4x (Marsh, Mangrove)
Candidate division WS3
DGGE band-2j (FB)
DGGE band-2d (western FB)
Desulfocapsa sp. clone SB1_88 (AY177798)
DGGE band-3f (Marsh)
Desulfosarcina variabilis (M34407)
Desulfosarcina sp. clone SB4_53 (AY177791)
DGGE band-u (western FB)
clone MAT-CR-H6-G06 (EU245372)
DGGE band-q (FB)
δ-proteobacteria
Actinobacterium str 220105 (FJ429795)
DGGE band-3k (Marsh, Mangrove, FB)
actinobacterium clone B306 (EU328054)
DGGE band-4c (Marsh, Mangrove, FB)
Acidimicrobium sp. str Y0018 (AY140240)
actinobacterium clone EB1133 (AY395452)
DGGE band-4y (Marsh, Mangrove, FB)
Actinobacteria
DGGE band-4g (Marsh, Mangrove)
DGGE band-3v (Marsh, Mangrove)
Chloroflexi clone MSB-3D4 (DQ811864)
green non-sulfur bacterium clone MBAE68 (AJ567609)
clone KM35B-327 (AB300100)
DGGE band-3t (Marsh, Mangrove)
Chloroflexi clone TK-SH11 (DQ463707)
Dehalococcoides ethenogenes (AF004928)
eubacterium clone t0.8.f (AF005746)
DGGE band-3j (Marsh, Mangrove)
DGGE band-3h (Marsh, Mangrove, FB)
Chloroflexi clone MSB-5bx1 (DQ811889)
DGGE band-3g (Marsh, Mangrove)
clone c5LKS53 (AM086136)
DGGE band-3s (Mangrove)
Chloroflexi clone AKYG799 (AY921908)
DGGE band-2v (Marsh, Mangrove, FB)
Chloroflexi
Synechococcus rubescens str SAG 3.81 (AM709629)
Prochlorococcus sp. str MIT9303 (AF053397)
Prochlorococcus sp. str MIT9303 (AF001469)
DGGE band-2c (western FB)
DGGE band-2r (eastern-central FB)
Synechococcus sp. clone JL-SCS-M52 (AY664059)
DGGE band-2k (FB)
Cyanobacteria
Bacteroidetes clone Skagen107 (DQ640726)
DGGE band-3b (Marsh, Mangrove, FB)
Chlorobi clone 397 (AB252951)
green sulfur bacterium clone GR-296.II.73 (AJ301570)
clone S26-49 (EU287349)
DGGE band-r (eastern-central FB)
Cytophaga hutchinsonii ATCC 33406 (NC_008255)
Cytophaga sp. clone JdFBGBact_34 (DQ070826)
Bacteroidetes/Chlorobi group clone 3B02-26 (DQ431893)
DGGE band-2i (western FB)
Bacteroidetes/Chlorobi
Spirochete clone IE018 (AY605128)
DGGE band-2p (FB)
Spirochete clone LF006 (AY605159)
DGGE band-t (FB)
Spirochaeta sp. ASpC-2 (AY995150)
Spirochaetes
Nitrospira sp. SRI-9 (AF255603)
Nitrospirae clone S15A-MN30 (AJ534687)
Nitrospirae clone 356 (AB252945)
DGGE band-2q (Marsh)
Nitrospirae clone S15A-MN131 (AJ534688)
DGGE band-3i (Mangrove)
Nitrospirae
candidate division OP8 clone (AJ937676)
candidate division OP8 clone HMMVPog-54 (AJ704718)
DGGE band-4h (Mangrove)
Candidate division OP8
Planctomycetes clone GMD21C08 (AY162119)
clone MAT-CR-M7-A06 (EU245835)
DGGE band-4d (eastern-central FB)
Planctomycetes
Planctomyces limnophilus str DSM 3776 (X62911)

◀ **Figure 5** Phylogenetic tree showing the relationship of the sequences of the 36 predominant 16S rRNA gene-based DGGE bands with GenBank closest sequences determined by BLAST. Sequences were aligned by ClustalW. Neighbor-joining analysis was conducted with the Jukes and Cantor model using TREECON Program version 1.3b with *Planctomyces limnophilus* (X62911) as outgroup and showing bootstrap values as percentages of 1,000 replications. Bootstrap values greater than 50% are indicated at each node. The zones where the bands were predominated are indicated in *parenthesis*. *FB* Florida Bay

14 of the 16 sampling sites. Three of the nine bands (band-3k, band-4c, and band-4y) were *Actinobacteria*-like sequences, and two (band-2v and band-3h) were *Chloroflexi*-like sequences. Band-3b was related to sequences belonging to *Bacteroidetes*/*Chlorobi* group, the latter otherwise known as the photosynthetic green-sulfur bacteria. Band-4e belonged to *Myxococcales* order in the *δ-Proteobacteria* class (Fig. 5). 16S rRNA sequences related to *Nitrospirae*, *γ-Proteobacteria*, *Spirochetes*, *Cyanobacteria*, and candidate divisions WS3 and OP8 (two novel phylogenetic groups containing no cultured members) were also observed throughout sampling sites.

Phylogenetic Positions of Partial 16S rRNA Gene Sequences Characterizing Particular Sites

As shown in (Fig. 5), five bands (band-3v, band-3j, band-3t, band-3g, and band-4g) characterizing freshwater marsh and mangrove forest and other band (band-3s) characterizing mangrove forest belonged to *Chloroflexi* cluster. One band (band-3f) observed mostly at freshwater marsh and one band (band-2y) characterizing mangrove forest were sulfate-reducing bacteria-like sequence in *δ-Proteobacteria* and phototrophic purple-sulfur bacteria-like sequences in *γ-Proteobacteria*, respectively.

Phototrophic purple-sulfur bacteria-like sequence (band-v), *Spirochete*-like sequences (band-2p and band-t), and sulfate-reducing bacteria-like sequence (band-2j) were found across the bay. The band-2r and band-2c characterizing eastern-central Florida Bay and western Florida Bay, respectively, and band-2k characterizing Florida Bay were closely related to *Cyanobacteria*. In addition, band-r characterizing eastern-central Florida Bay and band-2i characterizing western Florida Bay were related to *Bacteroidetes*/*Chlorobi* group, suggesting that *Cyanobacteria* and *Bacteroidetes*/*Chlorobi* group are well-represented in Florida Bay. Band-g belonged to *Pseudomonas* cluster which was indicative in eastern-central Florida Bay, while band-2a and band-u and band-2d characterizing western Florida Bay were phototrophic purple-sulfur bacteria-like sequences and sulfate-reducing bacteria-like sequence, respectively. Band-2d also existed in the mangrove forest sites although it was less intense, indicating that sulfate-reducing bacteria-like sequences are detected throughout sediment samples. Finally, band-4d related to *Planctomycetes* was only observed in eastern-central Florida Bay.

Discussion

Bacterial Diversity

Between 46 and 63 DGGE bands were detected in the different FCE sediment samples. These numbers were significantly greater than those found in river and estuarine water samples where similar methodologies for the extraction of DNA and the amplification by PCR were used [11, 55]. However, resolution of the DGGE profiles, in terms of band numbers, is not always sufficient to illustrate the considerable bacterial diversity in indigenous communities and some studies have shown that fragments of different sequences might migrate at the same position [48, 67].

The FCE sediments contain a highly diverse microbial assemblage. The Shannon–Weiner index (*H'*) values for the FCE ranged from 3.71 to 4.00 (mean 3.88) and were similar to those found in other soil and sediment environments—3.66 in primary forest mineral soil [4] and 3.76 in mangrove forest sediments [24], but was higher than the 1.90–2.69 found in contaminated soils [35] and 2.47–2.64 in agricultural soils [23].

Bacterial Community Structure

Bacterial community structure is generally regulated by the ability of individual bacteria to cope with various environmental conditions [9]. According to Prieur et al. [45], these factors may include soil type, plant community type, salinity, nutrient conditions, etc. with the primary factor believed to be salinity. Our results suggest that FCE sediment bacterial communities were primarily influenced by salinity followed by TP concentration. Salinity and TP explained the separation observed throughout CCA1 and CCA2, respectively, and was consistent with other community structure analyses from terrestrial and marine samples using molecular culture-independent and conventional culture-dependent methods [3, 9, 21, 66]. However, other factors such as nitrite could be important. Some studies have suggested that nitrite levels may explain the microbial community fluctuation in nitrogen-limiting estuarine water [52]. There is evidence that nitrification is restricted to the surface of sediment; thus, more oxygen-rich environments are expected to support bacterial populations which are more efficient and competitive at using the carbon supply than under anaerobic conditions, such as denitrifiers [32]. This is in agreement with our findings where were observed DGGE band sequences (band-g, band-2y, and band-2i) which were mainly related to denitrifiers such as *Pseudomonas* sp., *Thialkalivibrio thiocyanodenitrificans*, and *Cytophaga* sp., respectively (Fig. 5).

Cluster analysis of sediment bacterial communities showed that there were major differences among freshwater marsh, mangrove, and seagrass sites (Fig. 3). Seagrass sites clustered independently from the marsh and mangrove sites. The main factor for this separation was salinity (Fig. 4). One exception was site TS/Ph-3, which regularly dries up for long periods of time. As a result of this evaporative process, the site develops high solute concentrations and may help explain the similarity to bay sites.

Sediment bacterial communities from freshwater marsh and mangrove forest sites clustered into two separate groups (Fig. 3). SRS and TS/Ph sampling sites are in separate watersheds which are known to have distinct soil characteristics and hydrological regimes [14, 36, 47]. In spite of this, bacterial communities were more similar within a given plant community type irrespective of watershed. The main environmental factor corresponding to this difference was TP concentration (Fig. 4) although TOC with an intraset correlation coefficient value of −0.504 (F=1.4; P=0.12) and hydrological regime (not tested) may also be important. It has been well documented that mangrove forests in the Everglades have a significantly higher TP concentration than sawgrass marshes [13].

In seagrass meadows, sediment bacterial communities were different between the eastern-central and western regions of Florida Bay (Figs. 3 and 4). This distinction corresponded to a robust nutrient gradient in the bay. The gradient is maintained by a combination of nutrient inputs, tidal advection, and water residence time and results in P limitation in the east and N limitation in the west [7, 20]. In particular, eastern Florida Bay is influenced more by runoff from the mangrove forest, while western Florida Bay is more influenced by the Gulf of Mexico. Although a significant correlation between composition of bacterial community and N content were not observed, the significant relationship between community and TP suggested that environmental factors can alter the bacterial community structures in seagrass communities.

Bacterial Communities Composition Assessed by 16S rRNA Gene Sequences-Based DGGE Bands

Actinobacteria were the closest relative of three predominant DGGE bands detected throughout all sediment samples (Fig. 5). The presence of *Actinobacteria* in marine ecosystems has been attributed by some to runoff from fringing terrestrial habitat [43]; however, other studies have reported a widespread and persistent occurrence of indigenous actinobacterial populations in marine sediments due to their physiological adaptations for growth in marine environments [15, 29, 38, 40, 43, 59, 64]. These results suggest that *Actinobacteria* are adapted to marine environment and may be a dominant group in this ecosystem as they are in soils.

Sequences related to the *Chloroflexi* were more characteristic of freshwater marsh and mangrove forest sites. Although two DGGE bands detected throughout sediment samples also belonged to *Chloroflexi* cluster, four bands characterizing freshwater marsh and mangrove forest sites were *Chloroflexi*-like sequences. The *Chloroflexi* is recognized as a division-level bacterial group for over a decade [70]. Even today, however, this division is still represented by only a few isolates. The cultured representatives have a wide range of phenotypes, from anoxygenic photosynthesis (*Chloroflexus*) to thermophilic organotrophy (*Thermomicrobium*) [26]. Recent studies reported that *Chloroflexi*-like 16S rDNA sequences were found from such diverse environments as activated sludge [8], freshwater sediment [69], open ocean [22], and high-temperature thermal spring [27]. Piza et al. [43] obtained it as a major bacterial division in Brazilian tropical estuarine sediments. Hugenholtz et al. [26] reported that most environmental sequences of *Chloroflexi* group described to date fall into a different relatedness group of subdivision 1 and suggested that the members of subdivision 1 play significant roles in the environment. Although no sequence fell into subdivision 1 in the *Chloroflexi* phylogenetic tree (Fig. 5), sequences belonged to subdivision 2 were well represented in the freshwater marsh and mangrove forest sediments suggesting that *Chloroflexi* were a dominant bacterial group in these ecosystems.

Phototrophic purple-sulfur bacteria-like sequences were found in mangrove forest and across Florida Bay. The *γ-Proteobacteria* class, which includes the phototrophic purple-sulfur bacteria, is predominant in marine environments [37]. In addition, phototrophic purple-sulfur bacteria and phototrophic green-sulfur bacteria are known to form syntrophic interactions occur between the two groups. Since sequences related to phototrophic green-sulfur bacteria were also amplified throughout the sediment samples, we hypothesize that they both may play an important role in the transformation of sulfur and carbon compounds in the mangrove forest and Florida Bay sediments.

Sulfate-reducing bacteria-like sequences were amplified from both the freshwater marsh and Florida Bay sediments and were a predominant group in western Florida Bay sites. One DGGE band characterizing western Florida Bay was also detected in the mangrove forest although it was not strong band intensity, indicating the presence of sulfate-reducing bacteria-like sequence throughout the sampling sites. Sulfate-reducing bacteria were found in various anoxic environments such as northern Everglades wetland [12], estuarine and coastal marine sediments [46], Antarctic marine sediment [6], saline lake sediment [33], and rice root [28]. The populations of sulfate-reducing bacteria are known to be a major component of bacterial communities in marine environments. Sahm et al. [51] reported that 20% of the total prokaryotic rRNA in coastal marine sediment

originates from the sulfate-reducing bacteria. In addition, the sulfate-reducing bacteria possess high activity within a 2-cm depth range from the sediment surface for active anaerobic decomposition and transformation of deposited organic matter by bioturbation and mixing of sediment [6, 58]. The sulfate-reducing bacteria could play an important role in organic material degradation and subsequent replenishment of nutrients and energy sources, especially in western Florida Bay.

Cyanobacteria-like sequences were found in eastern-central Florida Bay and western Florida Bay, suggesting that *Cyanobacteria* sequences exist across the Bay. The dominance of *Cyanobacteria* in Florida Bay has been demonstrated by pigment analysis, with 86% of samples containing nearly all cyanobacterial pigments that were similar to *Synechococcus elongatus* [50]. However, to our knowledge, the *Cyanobacteria* have never been assessed in this area, using techniques based on DNA. In this study, DGGE bands in *Cyanobacteria* cluster were related to cyanobacterium clones and they were not included into *Synechococcus* and *Prochlorococcus*, which are predominant cyanobacterial groups in seawater environments [18, 53, 71]. Sediment *Cyanobacteria* communities were different from the water column communities (data not shown).

Eastern-central Florida Bay showed a higher band number than western Florida Bay with values of 55 and 50, respectively. Eastern-central zone is surrounded by mangrove forest and the Florida Keys, thus is influenced by the runoff from the Everglades, is generally P limited, and has a long water residence time. These environmental conditions, which are unique in this zone, probably act to form highly variable habitats which help support this diversity.

The primary goal of this study was to characterize environmental factors driving the community structures of sediment bacteria because the bacterial community plays an important role in nutrient cycling throughout the FCE ecosystem. Salinity was the primary driver of sediment bacterial community change; however, salinity also affects changes in vegetation type, which may in turn alter the overall productivity and affect the long-term storage of C, N, and P. The significant relationship between bacterial community structure and P reiterates the possible vegetation effect. Future research should focus on how the interaction between salinity, nutrients, and vegetation affect the structure of sediment microbial communities and their nutrient cycling activities.

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Appendix

Table 2 Summary of environmental factors measured in the study sites

Sample name	Salinity (PSU)	TN (μM)	TP (μM)	TOC (μM)	NH_4^+ (μM)	NO_2^- (μM)	NO_3^- (μM)	SRP (μM)
SRS2	0	59.60	0.28	2,285.83	3.38	0.56	1.16	0.05
SRS3	0	22.65	0.28	1,132.50	1.60	0.08	0.44	0.06
SRS4	3.6	21.55	0.44	1,111.67	0.85	0.16	0.78	0.05
SRS5	14.5	31.43	0.33	1,024.58	2.71	0.38	2.26	0.07
SRS6	8.4	26.60	0.41	698.17	2.09	0.23	0.90	0.13
TS/Ph3	0	59.49	0.17	1,528.33	2.39	0.10	1.52	0.09
TS/Ph4	0	32.78	0.13	911.67	1.28	0.14	1.91	0.02
TS/Ph5	0	21.60	0.10	606.33	1.12	0.07	2.40	0.01
TS/Ph6	42.6	31.77	0.47	821.58	1.94	0.37	0.31	0.04
TS/Ph7	42	41.48	0.25	712.83	4.05	0.31	0.28	0.04
TS/Ph8	40.3	33.10	0.41	727.67	6.90	0.23	0.65	0.03
FB9	39.54	34.53	0.13	528.02	1.40	0.11	0.49	0.05
FB13	48.86	48.56	0.24	942.50	2.51	0.03	1.26	0.06
FB16	38.79	21.20	0.36	238.25	0.18	0.13	0.00	0.03
FB21	47.90	43.11	0.19	823.81	0.00	0.02	0.11	0.03
FB27	37.18	17.54	0.25	166.42	1.86	0.07	0.78	0.02

SRS Shark River Slough, *TS/Ph* Taylor Slough/C-111 canal basin, *FB* Florida Bay, *TN* total nitrogen, *TP* total phosphorus, *TOC* total organic carbon, NH_4^+ ammonium content, NO_2^- nitrite, NO_3^- nitrate, *SRP* soluble reactive phosphorus

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