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Microbial Community Response to Seawater Amendment in Low-Salinity Tidal Sediments

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Abstract Rising sea levels and excessive water withdrawals upstream are making previously freshwater coastal ecosystems saline. Plant and animal responses to variation in the freshwater–saline interface have been well studied in the coastal zone; however, microbial community structure and functional response to seawater intrusion remains relatively unexplored. Here, we used molecular approaches to evaluate the response of the prokaryotic community to controlled changes in porewater salinity levels in freshwater sediments from the Altamaha River, Georgia, USA. This work is a companion to a previously published study describing results from an experiment using laboratory flow-through sediment core bioreactors to document biogeochemical changes as porewater salinity was increased from 0 to 10 over 35 days. As reported in Weston et al. (*Biogeochemistry*, 77:375–408, 62), porewater chemistry was monitored, and cores were sacrificed at 0, 9, 15, and 35 days, at which time we completed terminal restriction fragment length polymorphism and 16S rRNA clone library analyses of sediment microbial communities. The biogeochemical study documented changes in mineralization pathways in response to artificial seawater additions, with a decline in methanogenesis, a transient increase in iron reduction, and finally a dominance of sulfate reduction. Here, we report that, despite these dramatic and significant changes in microbial activity at the biogeochemical level, no significant differences were found between microbial community composition of control vs. seawater-amended treatments for either Bacterial or Archaeal members. Further, taxa in the seawater-amended treatment community did not become more "marine-like" through time. Our experiment suggests that, as seawater intrudes into freshwater sediments, observed changes in metabolic activity and carbon mineralization on the time scale of weeks are driven more by shifts in gene expression and regulation than by changes in the composition of the microbial community.

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Introduction

Variation in the freshwater–saline interface (FSI) in coastal ecosystems profoundly influences the distribution and composition of plant and animal communities and the abiotic environment in which they thrive [2, 14, 25, 26, 35]. Because of human activity in coastal areas throughout the world, the hydrodynamics of exchange between fresh and saline water sources have been altered [28]. Increased freshwater storage in reservoirs, construction of impervious surfaces in urban centers, and pumping of groundwater from aquifers has lowered river baseflow and discharge to the coast, particularly during drought periods [21, 38, 51].

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Superimposed upon the direct effects of human activity, climate change influences runoff patterns through changes in regional precipitation patterns, increased rates of evapotranspiration, and sea level rise [34, 44], all forcing the FSI farther inland. Increases in the inland extent of high-salinity waters may change the manner in which prokaryotes cycle nutrients and organic carbon by favoring biogeochemical transformations that dominate in marine environments.

Seawater flux into freshwater sediments abiotically alters the speciation of many ions and displaces some from the surface of sediment particles. Ion exchange can increase NH_4^+ concentrations, decrease pH, and alter metal oxide mineral forms [4, 52, 62, 65]. Seawater also introduces sulfate at higher concentrations than typical in freshwater environments, which may release more P via reductive dissolution of Fe hydroxides followed by FeS mineral formation [10]. These abiotic affects of salinity alter rates of microbial-mediated C mineralization and nutrient transformation [9], causing a decrease in dissimilatory nitrate reductase activity, denitrification, and methane production, while stimulating sulfate reduction [23, 45, 47, 48, 52, 61, 62].

Microbial community structure along naturally occurring salinity gradients has been studied previously. Typically, gamma-, beta-, and deltaproteobacteria and representatives of the Bacteriodetes dominate in freshwater and brackish samples, while alphaproteobacteria and Cyanobacteria dominate in marine samples [5, 7, 13, 24, 32]. However, these trends are not absolute [40, 50, 55, 59]. Community changes in sediments have only been explored in one study, where an experimental increase in NaCl concentration had no effect on terminal restriction fragment length polymorphism (T-RFLP) patterns [4]. Analysis of functional genes, rather than taxonomic markers, have shown negative correlations between salinity and the diversity and abundance of ammonium-oxidizing bacteria [22] and diazotrophs [5, 6, 8, 53, 56]. Concern as to the effect of increased sediment salinity on ecosystem functioning has only begun to be addressed through molecular studies of microbial communities.

Our work evaluated the response of a prokaryotic community to controlled changes in porewater salinity levels in freshwater sediment from the Altamaha River, GA, USA. This watershed has seen a decline in average river discharge of 4.2 $m^3\ s^{-1}$ per year over the past three decades, attributed to long-term change in precipitation [46, 63]. The watershed is also experiencing increases in residential and urban development in the upper portions near Atlanta, GA, USA [63], and water withdrawals have increased since 1950 [19]. Current trends suggest future salinity intrusions into coastal Georgia freshwater sediments, with unexplored consequences to microbial community structure and function.

In our experiments, the biogeochemical results of which have been previously published in Weston et al. [62], rates of microbial metabolism and dissolved and solid-phase geochemistry were monitored for 5 weeks as salinity was slowly increased from 0 to 10 in flow-through bioreactors incubated anaerobically in the laboratory. A decrease in rates of denitrification and methanogenesis occurred, followed by an increase in microbially mediated Fe reduction, and mineralization increased in the salinity amendment [62]. Our goal was to document changes in microbial community structure coincident with these changes in biogeochemical cycling.

Materials and Methods

Experimental Manipulations

Biogeochemical results from the experiment have been published previously [62]. Briefly, intact replicate sediment cores were collected from the tidal freshwater portion of the Altamaha River on July 12, 2004 (Supplemental Material Figure 1). The cores were collected in an unvegetated area without visible infauna and with a surface water temperature of 29.4°C. Discharge at the site was significant the year prior to sampling, keeping salinity low (Fig. 1). The 3–5 cm depth was sectioned from each intact core and placed in flow-through reactors inside an anoxic chamber at 26°C. This depth was chosen based on the likelihood of having an active microbial community utilizing anaerobic pathways, which was later confirmed with porewater chemistry profiles made down to a depth of 18 cm in two of the collected cores (published in Weston et al. [62]). Inflow reservoirs were bubbled with N_2 to remove oxygen, and they remained anoxic throughout the experiment, verified using an oxygen microelectrode. Artificial freshwater (AFW) consisted of appropriate levels of major ions, dissolved organic carbon (DOC), and nutrients based on average Altamaha River chemistry as measured by the Georgia Coastal Ecosystem Long Term Ecological Research project (http://gce-lter.marsci.uga.edu/lter/) [62]. Sediment porewater pH was an average of 7.1 at this depth, and the pH of the inflow reservoirs was 7.9 [64]. C,

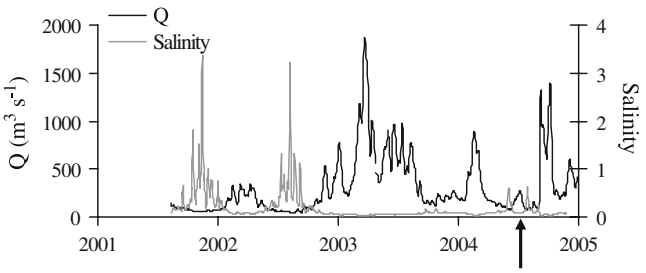

Figure 1 Discharge (Q) and salinity in Altamaha River waters the 2 years preceding sample collection. *Arrow* indicates sampling date for this experiment

N, and P were added to alleviate limitation of the sediment microbial community.

After a 5-day equilibration, the salinity of the inflow water was gradually increased in six of the reactors using artificial seawater (ASW), which included concentrations of dextran and nutrients identical to those in the AFW, with the exception of nitrate (0.2 μM in ASW) [62]. As described in Weston et al. [62], ASW was mixed with the AFW in increasing ratios to generate a salinity increase from 0 to 10 over a 2-week period. Salinity was then held constant at 10 for three additional weeks. Control reactors received AFW for the duration of the experiment. Water exiting the reactors was sampled for a suite of water chemistry parameters [62]. Duplicate reactors from both the control and salinity treatments were sacrificed for sediment solid-phase measurements and molecular analyses on days 9 and 15 and upon termination of the experiment (day 35). Sediments from the reactor were immediately frozen until subsequent molecular analyses, including replicate cores sacrificed initially (day 0). Rates of metabolism and microbial denitrification, iron reduction, sulfate reduction, and methanogenesis were estimated [62].

DNA Extraction

Genomic DNA was extracted from 1–2 g of frozen sediment using MoBio Soil DNA Extraction Kits (MoBio, Solana Beach, CA, USA). DNA was precipitated with ethanol and resuspended in 30 μL of sterile water. As appropriate, the DNA preparations were diluted to reduce the concentration of inhibitory compounds for polymerase chain reaction (PCR) and stored at −20°C.

16S rDNA T-RFLPs

16S rRNA genes were amplified with general Bacterial primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1522R (5′-AAGGAGGTGATCCANCCRCA-3′) [27] and, in a separate PCR reaction, Archaeal primers 21F (5′-TTCCGGTTGATCCYGCCGGA-3′) and 958R (5′-YCCGGCGTTGAMTCCAATT-3′) [15]. Forward primers were fluorescently labeled on the 5′ end with FAM (carboxyfluorescein). All PCR amplifications were carried out with Ready-To-Go PCR beads (Amersham Pharmacia, Piscataway, NJ, USA). PCR reactions contained 0.2 μM concentration of each primer and 50–100 ng of DNA, with a total reaction volume of 25 μl. Thermal cycling conditions for 16S rRNA gene amplification began with an initial 3 min at 95°C, followed by 30 cycles of 1 min at 95°C, 1 min at 55°C, and 1.5 min at 72°C. A final step of 10 min at 72°C was included to complete any partial polymerizations. PCR amplicons were confirmed by electrophoroetic analysis and purified from ethidium bromide-stained 1% agarose gels with the QIAquick gel extraction kit (Qiagen). For terminal restriction fragment length polymorphism analysis [41], restriction enzyme digestion of the PCR products were carried out in a 20-μl volume containing 100 ng of purified PCR product and 10 U of CfoI at 37°C for 6 h in the manufacturer's recommended reaction buffers. Digested DNA was precipitated in ethanol and suspended in 12.5 μl of deionized formamide with 1 μl of DNA fragment length standard Gene-Scan-2500 TAMRA (tetramethylrhodamine; Applied Biosystems). The terminal restriction fragments were separated by electrophoresis on an ABI Prism 310 genetic analyzer using GENESCAN analysis software (Applied Biosystems).

To avoid detection of primers, terminal fragments smaller than 50 bp were excluded from analysis, as were peaks comprising <1% of total chromatogram area. Reproducibility of patterns was confirmed by repeated T-RFLP analysis using the same DNA extract. T-RFLP data were analyzed using a Visual Basic program that reconciles minor shifts in fragment sizes between successive chromatograms [59]. Communities were characterized by the number of peaks and their relative abundance as a percentage of the total chromatogram area.

16S rRNA Gene Clone Library Construction

Bacterial 16S rRNA genes were amplified using the same primers as for T-RFLP analysis. Twenty-one PCR cycles were carried out, with each cycle consisting of 30 s at 94°C, 30 s at 55°C, and 90 s at 72°C, with a final step of 10 min at 72°C [29]. This PCR product was then diluted 1:10, and an additional three cycles were completed to remove heteroduplexes [1]. The single PCR product (~1,500 bp) was recovered from a 1% agarose gel with a QiaSpin gel extraction kit (Qiagen, Valencia, CA, USA), and amplicons were cloned using the TOPO-TA cloning system (Invitrogen, Carlsbad, CA, USA). Individual clones were sequenced using the universal forward primer for Bacterial 16S rRNA genes. Archaeal 16S rRNA gene sequences were not generated, as the biogeochemical results demonstrated that methanogenesis steadily declined to near zero within the first week, and the majority of the variation in C mineralization throughout the experiment was associated with terminal pathways found exclusively in Bacteria.

Phylogenetic Analyses

Cloned sequences were aligned with those of cultured organisms and environmental clones obtained from GenBank using the ClustalW algorithm [60] in the BioEdit software package (v7.0.4.1) [30]. Similarity matrices were generated using unambiguously (>50% of sequences had

the identical nucleic acids) aligned positions. The program DNAdist from the PHYLIP package (version 3.5c) [20] was used to perform Jukes–Cantor distance matrix calculations [36]. Operational taxanomic units (OTUs) were assigned using a distance of 0.03 (97% similarity) for all gene libraries in the DOTUR software package [54]. A bootstrapped, neighbor-joining tree was created using a nonredundant dataset of sequences in the software package Geneious version 3.7.0 (Biomatters Ltd, Auckland, New Zealand). Bacterial 16S rRNA genes were also taxonomically classified using the Ribosomal Database Project (RDP) sequence classifier interface developed by the Sapelo Island Microbial Observatory [17]. Sequences were assigned to taxonomic ranks based on similarity-weighted cut-off limits following local pairwise Smith–Waterman alignments. Percent similarity to reference sequences as required for identification at each level was determined empirically by comparing a large number of aligned sequences for known type species (http://simo.marsci.uga.edu/public_db/bioinformatics.htm). These cut-offs were set at 75% similarity for the phylum level, 85% for the class level, 91% for the order level, 92% for the family level, 95% for the genus level, and 97% for the species level. Only type sequence matches with a minimum alignment length of 250 basepairs and overlap of at least 50% with the query sequence were considered.

Statistical Analysis

The Chao 1 species richness estimate for each sample was generated by DOTUR for a thousand random samplings of the gene library (with replacement) using 32 of the sequences available in each library. This eliminated bias in Chao 1 estimates due to uneven library sizes. Evenness was calculated as Shannon Index/ln(S). We used two independent methods to assess differences in community composition. One method evaluated whether differences between gene libraries were significant using webLIBSHUFF (http://libshuff.mib.uga.edu) [31, 57]. The second method involved a nonparametric analysis of similarity (ANOSIM) and had the advantage of using the replicate data when calculating

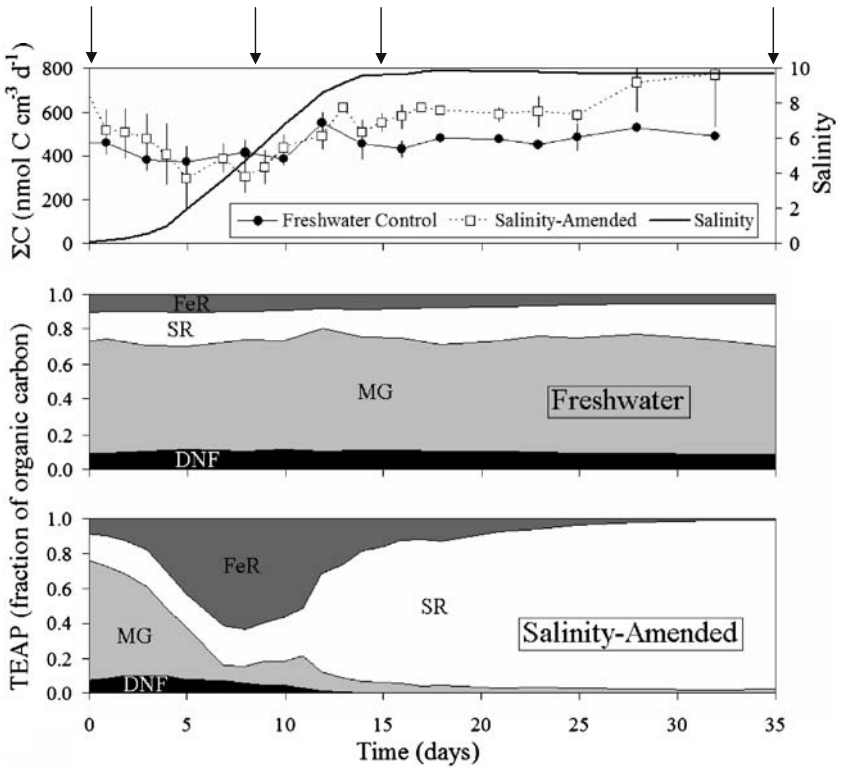

Figure 2 (*Top panel*) ΣC oxidation (sum of DIC and CH_4 production, mean ± standard deviation) in the freshwater control (*closed circles*) and seawater-amended (published as salinity-amended in Weston et al. [62]) reactors (*open squares*) and salinity (*solid line*) in the seawater-amended reactors. (*Bottom two panels*) Estimated contribution of denitrification (*DNF*), methanogenesis (*MG*), sulfate reduction (*SR*), and iron reduction (*FeR*) terminal electron accepting processes (TEAP) to total organic carbon oxidation in control (*middle*) and seawater-amended (*bottom*) flow-through reactors. *Arrows* indicate sampling time points for sediment microbial community analyses. Modified and reproduced by permission of American Geophysical Union. N.B. Weston et al. [62]. Copyright 2006 American Geophysical Union

significance. Using PRIMER 5 for Windows software (Plymouth Marine Laboratory, Plymouth, UK), we generated separate Bray–Curtis similarity matrices [18, 42] using fragment composition of the Bacterial community, and 16S rRNA gene OTU distributions. Differences in community composition among the groups were visualized using nonmetric multidimensional scaling (MDS) of the similarity matrix to produce a two-dimensional ordination figure [11]. The ANOSIM tested whether there were significant differences in prokaryotic community composition between treatments [12].

Nucleotide Sequence Accession Numbers

Sequence data have been deposited with GenBank and assigned the following accession numbers, GQ242269–GQ243201.

Results

Biogeochemical Response to Salinity Increase

Previously published biogeochemical results are summarized here (Fig. 2); for further details, refer to Weston et al. [62]. Elemental cycling of N, P, Fe, S, and C was altered with the progressive increase in salinity in freshwater tidal sediments from the Altamaha River. Increased salinity led to solid-phase ion exchange in the salinity treatment generating a transient pulse of NH_4^+ into porewaters and a subsequent reduction in the total exchangeable NH_4^+ from sediment surfaces. Within this same time period (first 12 days of seawater addition), rates of denitrification decreased to below detection. Porewater phosphate concentrations initially decreased, then increased above control levels, most likely as a function of changes in iron mineral phase composition in the sediments and/or mineralization of organic phosphorus. Increases in bioavailable Fe mineral forms (presumably created by a decrease in pH) likely stimulated microbially-mediated Fe(III) reduction. This resulted in significant increases in soluble Fe^{2+} above control levels during the salinity ramp. Sulfate concentrations slowly increased with the addition of ASW, but hydrogen sulfide production did not increase significantly above control levels until after salinity reached 10, at which time microbially mediated sulfate reduction dominated carbon mineralization pathways. Methanogenesis rates dropped to almost zero within the first 6 days of ASW addition. Total mineralization of organic carbon was 25% higher in the salinity treatment at the end of the experiment as compared to the control (Fig. 2).

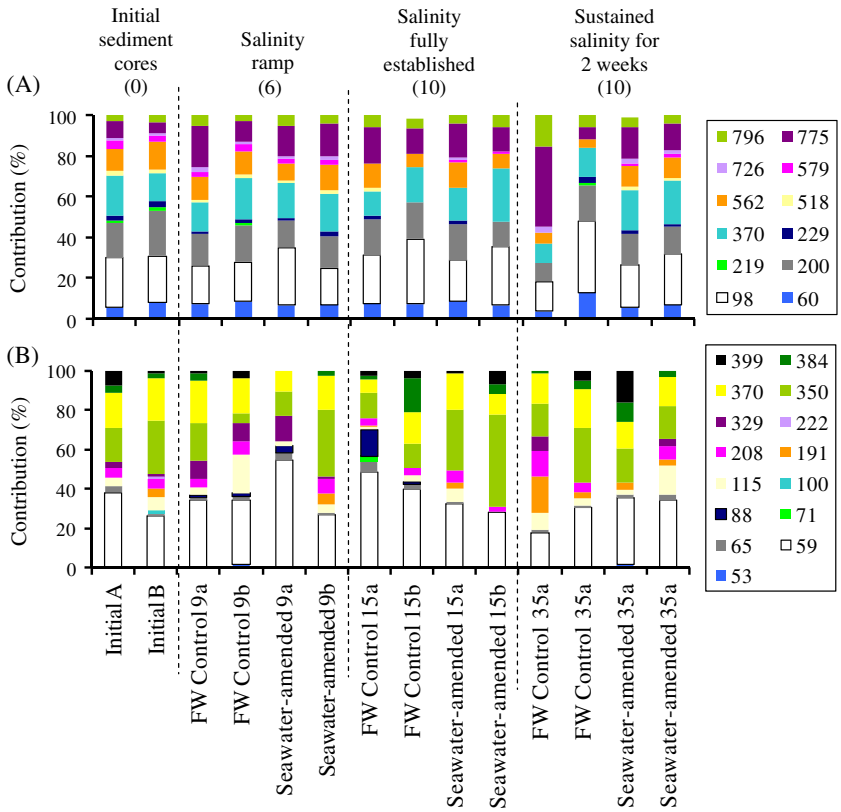

Figure 3 Terminal restriction fragment length polymorphisms analysis of 16S rRNA genes on days 9, 15, and 35, using Bacterial primers (**a**) and Archael primers (**b**). Each fragment area is expressed as a percentage of the summed area of all fragments. Salinity at each sampling point is indicated *in parentheses at the top* of the figure

Table 1 Statistics for Bacterial 16S rRNA gene libraries generated from replicate cores

Sample	Number	OTUs	Chao 1	Evenness	% Coverage (distance=0.03)	% Coverage (distance=0.15)
Initial Core	91, 80	81, 67	328, 236	0.99, 0.99	19, 25	58, 65
Control T=9 days	39, 42	35, 38	283, 155	0.98, 0.99	6, 19	18, 50
Control T=15 days	42, 37	40, 36	185, 256	0.94, 0.97	21, 14	44, 32
Control T=35 days	118, 124	104, 106	257, 188	0.97, 0.95	20, 35	68, 64
Seawater amended T=9 days	34, 32	31, 29	217, 436	0.99, 1.00	15, 9	50, 50
Seawater amended T=15 days	42, 42	39, 41	185, 373	0.98, 1.00	19, 5	57, 52
Seawater amended T=35 days	107, 110	96, 99	225, 253	0.97, 0.97	31, 25	68, 67

All statistics are for OTUs defined at the species level (0.03% distance) except for the final column which shows coverage at the class level (0.15% distance)

Microbial Community Analysis

T-RFLP

There were no significant differences on any date, as assessed by frequency and abundance of T-RFLP profile peaks, between control and salinity treatments for Bacterial and Archaeal sediment communities (ANOSIM, $R<0.5$, $p>0.05$; Fig. 3). Although a few of both the Bacterial and Archaeal T-RFLP profiles had an additional or missing fragment from one time point to the next, their replicate core did not mimic the same pattern (Fig. 3).

Clone Libraries

To check that the T-RFLP profiles were not missing community composition shifts, a more detailed analysis of amplified Bacterial 16S rRNA genes was undertaken. We selected Bacteria for this analysis because of the large increase in sulfate reduction activity measured in seawater-amended sediments [62], an activity known to be mediated primarily by Bacteria in the gammaproteobacteria. A total of 940 Bacterial 16S rRNA gene sequences >250 bp long were generated from the 14 sediment samples, with a median length of 546 bp (Table 1). These sequences were classified into 510 unique operational taxonomic units at a similarity level of ≥97% by DOTUR (Table 1). Estimated richness (Chao 1) varied from 155–436 OTUs, with a median of 245 (Table 1). The presence and abundance of OTUs was used to generate an overall analysis of community composition for Bacterial 16S rRNA genes. Bray–Curtis similarity values were used to identify differences among samples (ANOSIM) and establish clustering patterns (CLUSTER analysis). Both analyses indicated no significant differences between any samples regardless of

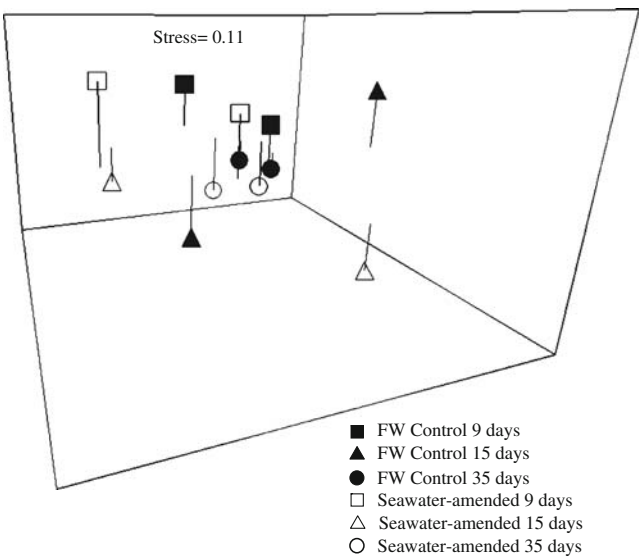

Figure 4 MDS plot using Bray–Curtis similarity values generated from OTU abundance data

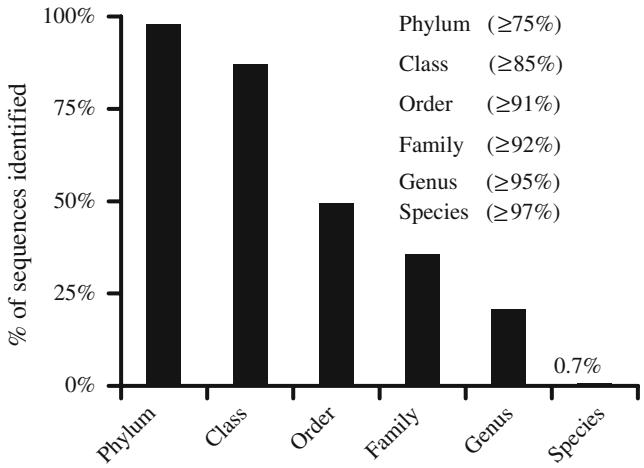

Figure 5 Percentage of Bacterial 16S rRNA genes identified at the indicated taxonomic level using the Ribosomal Database Project sequence classifier. Sequences were assigned to taxonomic ranks based on similarity-weighted cutoff limits following local pairwise Smith–Waterman alignments. Percent similarity to reference sequences as required for identification at each level is indicated *in parentheses*

treatment (ANOSIM, global R=0.003, p=0.503; CLUSTER, treatment and control replicate samples did not cluster together). We additionally analyzed the 16S rRNA gene libraries excluding singletons (rarer species) and similarly found no differences in community composition between control and seawater-amended cores. MDS graphical analysis suggests that differences between samples were greatest at the day 15 sampling period when the salinity maximum [10] had been established for just 2 days (Fig. 4). However, given that there were no statistically significant differences found between treatments, conclusions are tentative, particularly on days 9 and 15 where variability among replicates was high. Analysis of our clone library sequences in a phylogenic tree did not suggest any patterns in taxonomic relatedness with treatment (Supplemental Material Figure 2). The webLIBSHUFF analysis also indicated no differences between the Bacterial communities in the controls vs. the salinity manipulations throughout the experiment [p values >0.05 for control vs. ASW comparisons at all three time points (days 9, 15, and 35)].

When comparing our library sequences to the RDP database, only 50% matched sequences at a high enough similarity level to allow classification at taxonomic levels below class (Fig. 5). Variation between the samples at the

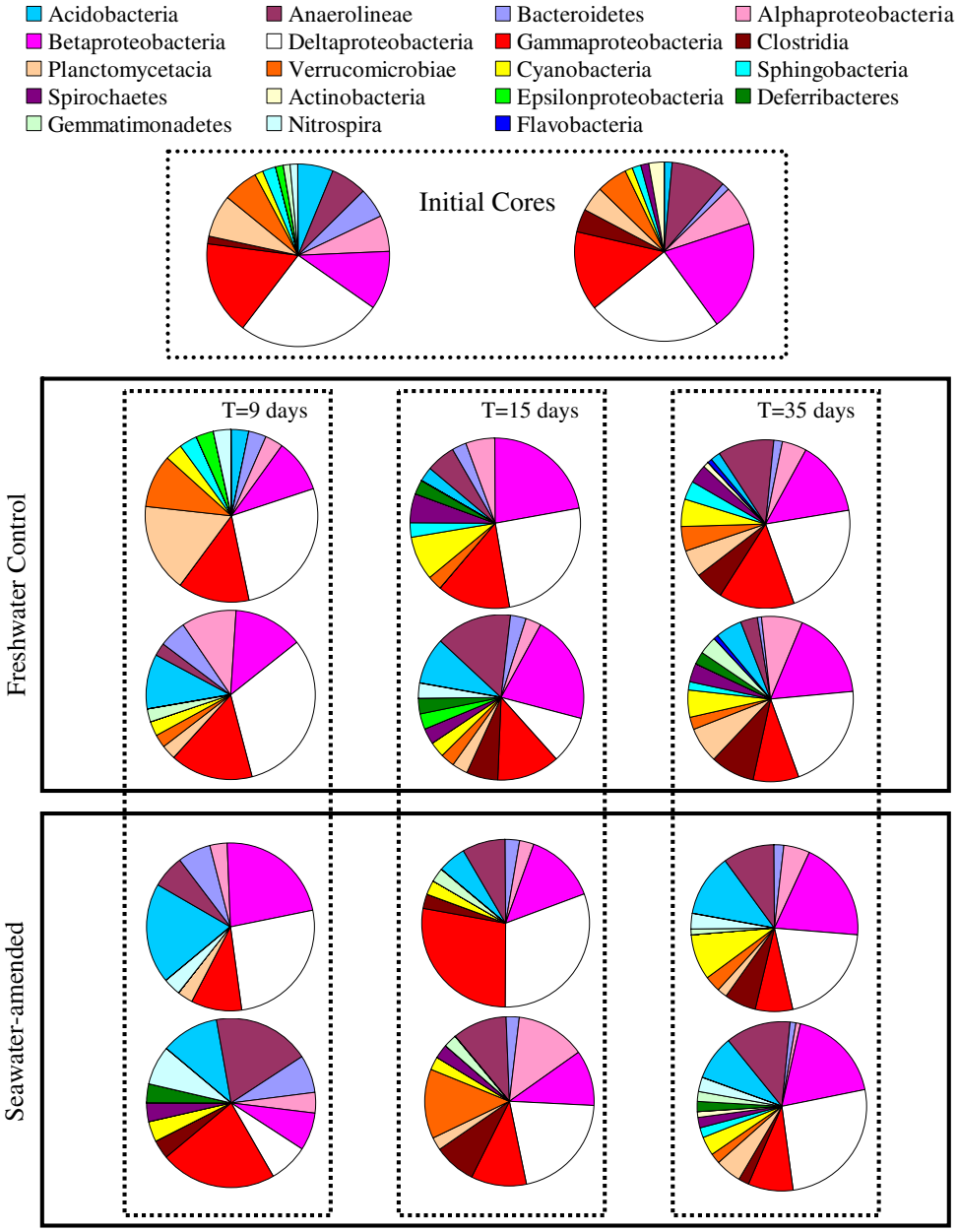

Figure 6 Class affiliations of 16S rRNA gene sequences and percent contribution to the community on each of the three sampling dates (9, 15, and 35 days) and at the initiation of the experiment

class level indicated few differences between control samples and those that received ASW, including classes within the Order Proteobacteria (Fig. 6). Of the 19 classes shown in Fig. 6, nine represented only a small fraction of the total community in our dataset (Cyanobacteria, Nitrospira, Spirochaetes, Actinobacteria, Epsilonproteobacteria, Deferribacteres, Flavobacteria, Gemmatimonadetes, and Sphingobacteria). Overall class-level variation between control and ASW samples was calculated using Bray–Curtis similarity values generated from 16S rRNA gene sequences and statistically evaluated using ANOSIM. Results showed a significant overall result (global R= 0.514, p=0.026), suggesting that one of the many possible pairwise comparisons, either between treatments on a particular day or between two sampling points within a treatment, might be significant. However, further statistical analysis of all combinations of pairwise comparisons (e.g., control vs. ASW on day 9) found no significant results, indicating the marginal statistical significance suggested by the global R and p value may have resulted from low statistical power (high inter-sample variability and low replication).

To assess whether the experimental manipulation caused the microbial community to reflect a more marine-like signature, we compared our sequences to those of marine sediments at a nearby location at Dean Creek, Sapelo Island (sampling site of the Sapelo Island Microbial Observatory; SIMO) using an web tool designed by SIMO researchers (http://simo.marsci.uga.edu/public_db/blastn.htm). The Dean Creek site represents a full salinity endmember, as it is located less than 5 km from Altamaha Sound. The percent of sequences that were ≥97% similar to SIMO sequences (with a minimum of 50% sequence overlap) was almost 30% in the ASW treatment as compared to less than 20% in the control during the initial increase in salinity as measured on day 9 (Fig. 7). However, treatments receiving ASW sampled on days 15 and 35, when compared to the control samples, did not have a higher percent of sequence matches to the SIMO sequences (Fig. 7).

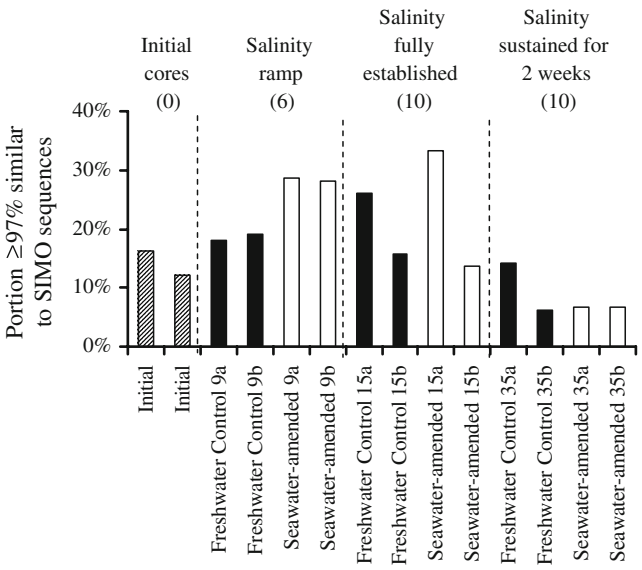

Figure 7 Percent of 16S rRNA gene sequences that were ≥97% similar to those from shallow marine sediments sampled at Dean Creek on Sapelo Island on each of the three sampling dates (9, 15, and 35 days). Dean Creek represents a full salinity endmember for the Altamaha River ecosystem. *Striped bars* are samples taken at the beginning of the experiment, *black bars* indicate freshwater control samples; *white bars* are seawater-amended samples

Discussion

In the face of environmental variability, prokaryotic community structure regulates the ability of a habitat to respond through interspecies variation in cellular respiratory pathways, substrate affinity, and resilience to ecological perturbations. The response can include changes in cell-specific activity, either increased gene expression or induction of dormant biochemical pathways, or alternatively, rarer members may become more abundant because they posses unique functional capabilities that allow them to thrive under new environmental conditions. In our experiment, detailed measurements of chemical products and microbial respiration rates as porewater salinity increased provided the opportunity to monitor these two potential fundamental responses by the prokaryotic community: (1) alteration of the gene reservoir as rarer species increase disproportionately relative to the more dominant; or (2) regulation of the existing gene reservoir to shift the biogeochemical pathways mediated by the dominant species. The first mechanism (alteration of community gene reservoir) would result in a more permanent change in the functional capabilities of the prokaryotic community, while the second (fluctuation in gene regulation) would facilitate a quick response by the community in the face of short-lived environmental perturbations. Using the previously published biogeochemical data documenting shifts in key pathways for C mineralization in response to salinity changes, we completed this companion study as an opportunity to investigate these two mechanisms of prokaryotic response.

Based on the conceptual model "everything is everywhere, but the environment selects" [3, 43], we predicted latent bacterial species present initially in this sediment community would increase in number in response to the salinity ramp (mechanism #1). However, despite major shifts in dominant terminal electron acceptors for carbon mineralization, our data showed no significant change in community composition within either the Bacterial or Archaeal communities upon short-term (5 week) increases

in porewater salinity. While the relatively low coverage in some gene libraries may have decreased sensitivity to minor changes, substantial shifts in functional groups would have been detected by our methods.

Because the composition of the communities remained proportionally constant throughout the experiment, the response of the prokaryotic community more likely involved shifts in predominance of metabolic pathways rather than in the community gene reservoir. This suggests that the dominant species in these sediments have diverse genetic capabilities that support multiple possible metabolic strategies in a variable environment. Further, bacterial diversity in these sediment communities remained high throughout (Table 1), indicating widespread tolerance to large sulfate and salinity fluctuations. There were two naturally occurring, short-lived salinity increases (from 0 to 3–4) at our sampling location in the 2.5 years prior to the experiment (Fig. 1). The imprint of these salinity stresses may have selected for taxa capable of maintaining activity under saline conditions. It is likely that given more time (months to years), the gene reservoir in these sediments would eventually respond permanently to the salinity increase.

Very few studies have examined the response of a single, mature sediment community living on natural substratum to environmental change [33, 49]. Baldwin et al. [4] tested the influence of NaCl additions on bottle-incubated anaerobic freshwater sediment slurries by monitoring respiration endproducts and prokaryotic community composition over a 30-day period [4]. Effects from salt additions were only seen in the Archaeal community (30% reduction in methanogenesis and changes in T-RFLP patterns), while Bacterial community composition remained unchanged. Their study corroborates part of our findings, in that we too did not see significant changes in community structure, despite a reduction in methanogenesis and increase in rates of sulfate reduction. Another study followed longer-term (11 month) response to manipulation of both salinity and sulfate levels in microbial mats from solar salterns [58]. They documented significant changes in abundance and composition of the methyl co-enzyme M reductase (*mcrA*) genes harbored by methanogens between treatments with and without sulfate but found no *mcrA* changes directly attributable to lower salinity [58].

The proposed mechanisms through which prokaryotic communities respond to changing environmental conditions do not consider two additional aspects of potential importance for attached consortia: competition for space and cell–cell communication. The increase in salinity in our experimental flow-through reactors may have been insufficient to create large-scale changes in community structure if space for cell growth on sediment surfaces or chemical cues among community members prevented rare species from establishing. These types of interactions have been demonstrated in low species-diversity ecosystems, particularly relating to pathogenic bacteria. For example, experimental work demonstrated two different *Streptococcus* species could coexist in a previously established dental biofilm only if introduced at the same time [39]; if introduced successively, the first species excluded growth of the other through chemical inhibition [39]. Similarly, colonization by sulfate-reducing bacteria in the mouse colon was partially dependent on the presence of a compound produced by an unrelated species [16]. Other work showed compounds used in cell–cell communications by one species were altered by another to interfere with cell division in a competing species [37]. The role of cell–cell communication and availability of colonization sites in structuring prokaryotic communities are relatively unstudied in aquatic sediment communities, where the majority of prokaryotic cells may be members of particle biofilms.

Our experiment documented extraordinary physiological flexibility of microbial communities in freshwater sediments. These species-rich communities were able to alter the predominant biochemical pathways likely within hours of changes in water chemistry, evidenced by the fact that shifts in dominant terminal electron accepting pathways could be readily measured within 1–2 days (Fig. 2, bottom panel). Thus, there is little evidence that changes in biogeochemical cycling at this site resulting from salinity intrusions on the time scale of weeks will influence microbial community structure. Resilience to major changes in porewater chemistry suggests these communities may be structured more by species–species interactions than competition for resources. Finally, documentation of significant increases in metabolic activity and carbon mineralization without significant changes in dominant taxa underscores how understanding prokaryotic community dynamics requires more than a fingerprint of species diversity.

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