Short-Term Effect of Capping on Microbial Communities in Freshwater Sediments

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ABSTRACT: Because biogas bubbles can influence cap integrity, the effect of capping and cap material on the ebullition potential in sediments must be studied. The goal of this comprehensive study was to determine the short-term effect of capping regime on the activity, metabolic potential, and community structure of sediment microorganisms. To evaluate the effect of capping (sand, synthetic aggregate, and no cap) on microbial communities (i.e., nitrifiers and methanogens), sediments were collected from the Anacostia River (Washington, D.C.). Microbial communities in sand-capped sediments exhibited the highest activity (tetrazolium redox dye, fluorescein diacetate hydrolysis assay, and biogas production), while communities in uncapped sediments exhibited the highest metabolic diversity. Substantial changes in microbial community structure (denaturing gradient gel electrophoresis) did not occur as a result of capping. Our data showed that the nature and magnitude of the effect that capping can have on microbial activity (biogas production) will likely be dependent on the capping materials chosen. *Water Environ. Res.,* **81,** 441 (2009).

KEYWORDS: capping, sediments, microbial activity, denaturing gradient gel electrophoresis, microbial communities, biogas.

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Introduction

The Anacostia River, which flows through Washington, D.C., has experienced increased pollution as a result of rapid urbanization (Reible et al., 2006). Main pollution sources, including stormwater runoff and combined sewer overflows, contribute contaminants, such as organic compounds (i.e., polychlorinated biphenyls) and metals, which partition into river sediments (Reible et al., 2006). Contaminated sediments continuously release toxic chemicals into the overlaying aqueous system by hydraulic flow, molecular diffusion of solutes absorbed on sediment particles, upward advection by groundwater, localized in-bed advection, bioturbation, and ebullition. Consequently, severe bioaccumulation of toxic material and breakdown of ecosystem function occurs (Reible et al., 2006; Thibodeaux, 2002), which affects water quality and the health of humans and aquatic organisms.

To mitigate the ecological effect of contaminated sediments, capping is gaining popularity over conventional dredging, which can be cost-prohibitive (Thoma et al., 1993). Capping is an effective sediment remediation strategy, because it physically isolates sediments from the water column and slows contaminant release by

(1) Increasing the distance for contaminants to diffuse and advect,
(2) Retarding or decreasing the molecule adsorption to the pore water, and
(3) Preventing resuspension and direct desorption (Thibodeaux, 2002; Thoma et al., 1993).

Cap performance depends on capping materials, cap thickness, and placement methods, which are selected to provide low porosity, cost, and high effectiveness, while maintaining cap durability (Gunnison et al., 1987; Thibodeaux, 2002). While sand is the traditional capping material, recent studies have focused on active capping materials that are more effective at sequestering specific contaminants. For example, synthetic aggregate (SAG) caps consist of granular material with a gravel core and clay coating. The SAG caps are useful for permeability control, as hydration of the clay coating following placement results in swelling and the formation of a unified, cohesive, and impermeable layer (Hull et al., 1999; Quadrini et al., 2003; Reible et al., 2006).

After capping, aerobic microbial activity typically decreases in lieu of anaerobic microbial activity that results in biogas production (Huttunen et al., 2001; Palermo et al., 2002). Anaerobic respiration, including nitrate reduction, sulfate reduction, and methanogenesis, are the main processes that are responsible for anaerobic biogas production (i.e., methane [CH_4], nitrogen [N_2], and carbon dioxide [CO_2]) in sediments (Huttunen et al., 2001; Rudd and Taylor, 1980; Rusmana and Nedwell, 2004; Scholten et al., 2002). Biogas bubbles (ebullition) have been observed in surface waters above contaminated sediments in the months immediately following cap placement in the Anacostia River and elsewhere (Palermo et al., 2002; Reible et al., 2006). Biogas bubbles can damage caps, resulting in increased porosity, pore water pollutant concentration, and chemical flux of contaminants into the water column (Palermo et al., 2002; Richardson, 1998; Rudd and Taylor, 1980). These observations have prompted an increased interest in the analysis of microbial ebullition potential in sediments as it relates to capping applications.

The effects of capping and cap type on microbial communities—specifically the presence and activity of biogas producers in sediments—are not well-characterized. By understanding the effect of capping, cap design, and cap placement on biogas production in sediments, overall cap maintenance can be improved. We investigated the activity and structure of the microbial communities in contaminated sediments 6 months after cap placement, because capping or cap type may affect microbial activity and shifts in the microbial community. Increases in the activity of biogas producing bacteria will result in ebullition, which may damage caps. The sediments studied were subject to two capping treatments—a synthetic aggregate cap; a sand cap; and a control, no-cap treatment. Direct enumeration of active and total bacteria and the fluorescein diacetate (FDA) hydrolysis assay were used to estimate bacteria density and to evaluate the overall activity of bacteria (Green et al., 2006; Proctor and Souza, 2001). Biogas production

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Table 1—Characteristics of sediment samples.

Core identification	Sampling date	Depth range (cm)	Easting (m)	Northing (m)	Water depth (m)
No-cap					
CNW-3	9/24/2004	0 to 5	1311065.98	438747.34	14.08
CNE-3	9/24/2004	0 to 5	1311110.85	438769.83	15
CSW-4	9/24/2004	0 to 5	1311100.60	438706.90	15
CSE-4	9/24/2004	0 to 5	1311140.97	438730.67	14.5
SAG-cap					
ABSE-6(4)	9/21/2004	20 to 25	1311097.64	438841.50	12.75
ABSE-6(5)	9/22/2004	25 to 30	1311097.64	438841.50	12.75
ABNE-6	9/22/2004	0 to 5	1311059.63	438905.34	7
AS_5	9/20/2004	0 to 5	1311010.53	438872.22	
Sand-cap					
SES-5	9/23/2004	0 to 5	1311181.11	438869.50	9.75
NWS-5	9/23/2004	0 to 5	1311111.93	438888.49	8.25
NES-4	9/22/2004	0 to 5	1311151.06	438918.34	6.75
LT-3	10/13/2004	0 to 5	1311164.00	438891.00	

was assessed during incubation studies as a surrogate measure for sediment anaerobic activity (Palermo et al., 2002; Richardson, 1998). Community-level physiological profiling (CLPP) was used to estimate the potential of culturable bacteria to use different carbon substrates (Christian and Lind, 2006; Garland and Mills, 1991), while denaturing gradient gel electrophoresis (DGGE) analysis was used to characterize the effect of capping on the structure of the microbial communities inhabiting the contaminated sediment. By using a variety of methods, we tested our hypothesis that capping will have short-term effects on microbiological activity and community structure, resulting in increased biogas production.

Materials and Methods

Sample Collection. In March 2004, a synthetic aggregate (SAG-cap) and a sand cap (sand-cap) were applied to sediments in the Anacostia River (Washington, D.C.) by Horne Engineering Services Inc. (Falls Church, Virginia), under the direction of the Hazardous Substance Research Center/South and Southwest. Six months following cap placement, four sediment cores (2 to 4 m in length) were collected from each capping treatment and from a non-capped area (no-cap) using a vibracore sampler (Table 1). In an anaerobic chamber, cores were cut into 5-cm sections, homogenized, and transferred to sterilized high-density polyethylene bottles (200-mL volume) and sealed. The samples were stored at 4°C until further analysis. The samples processed for this study were core sections collected from 0 to 5 cm below the sediment surface. However, 2 of the 4 synthetic aggregate samples (ABSE-6 [4] and ABSE-6 [5]) represented depths of 20 to 25 cm and 25 to 30 cm, respectively. No other synthetic aggregate samples representing 0 to 5 cm below the sediment surface were available for processing.

Total and Active Bacteria. Florescent microscopy was used to evaluate the effect of capping regime on total and active bacteria in sediment samples. Samples diluted (10^{-1}) in anoxic laboratory-grade water were incubated with 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) (5 mM) at room temperature (25°C) for 16 hours in the dark. Samples were fixed for 5 minutes in formaldehyde (2% v/v) (Kepner et al., 1994), followed by further incubation with Tween 80 (0.1%) for 5 minutes. The samples were sonicated for 5 minutes (1510R-MTH, Branson Ultrasonic Co., Connecticut) (Griebler et al., 2001), to separate bacterial cells from solid particles and decrease background fluorescence. Samples were centrifuged at low speed 50 × g for 20 minutes (Dos et al., 2000), and 600 μL of supernatant was transferred to a fresh tube and incubated for 5 minutes with 0.5% (v/v) PicoGreen (Molecular Probes, Eugene, Oregon). The sample was then filtered onto black polycarbonate filters (polycarbonate, black, 25 mm; pore size = 0.2 μm) (GE Water & Process Technologies, Denver, Colorado) under low vacuum pressure. Slides of samples were prepared and counted using fluorescence microscopy (1000 × magnification). The CTC reduction by enzymatic activity results in red coloration of the cells and an indication of bacteria activity (Gruden et al., 2003; Rodriguez et al., 1992), while the total cells are visualized by PicoGreen treatment, which stains the DNA of intact bacteria (Griebler et al., 2001; Kepner et al., 1994). A minimum of 10 random fields or 200 bacteria were counted for each slide. Each sample was processed in triplicate. All transfers were carried out in an anaerobic chamber.

Fluorescein Diacetate Hydrolysis Assay. The FDA hydrolysis assay was used as an additional method to assess microbial activity in sediment samples. Three grams (wet weight) of each sediment sample were homogenized in 15 mL of 60 mM sodium phosphate buffer (pH 7.6) using a vortex. Fluorescein diacetate solution (100 μL, dissolved in acetone) was added to achieve a final concentration of 10 μg FDA/mL. Two negative controls were included containing (1) sediment and buffer only, and (2) buffer and FDA, to control for the presence of background fluorescence not attributable to microbial hydrolysis of FDA. All samples were incubated for 60 minutes in a shaking water bath (30°C, 200 rpm). The hydrolysis reaction was stopped by adding 15 mL of chloroform and methanol solution (2:1 v/v) to each sample, followed by thorough shaking by hand. The samples were centrifuged for 5 minutes at 2000 × g, and the supernatant was filtered (Whatman no. 3 filter paper, Whatman, Florham Park, New Jersey). The amount of fluorescein produced in each sample was determined by comparing the absorbance (490 nm) of each sample to a standard curve of fluorescein concentration produced, as described by Adam and Duncan (2001). Each sample was processed in triplicate.

Community-Level Physiological Profiling. The effect of capping regime on the metabolism of culturable bacteria was assessed using CLPP. Five grams (wet weight) of each sediment

sample were added to 45 mL of sterile 10 mM sodium phosphate buffer (pH 7.6) and shaken for 1 hour at 200 rpm. The samples were serially diluted, and 100 μL of each dilution was inoculated onto 0.1 × tryptic soy agar and incubated at 25°C for 48 hours, to estimate the number of culturable bacteria in each sample. Based on the numbers obtained using culturable bacteria counts, each sample was diluted to achieve approximately 10^4 bacterial cells per 100 μL. Each diluted sample was loaded (100 μL) into the wells of a Biolog (Hayward, California) Ecoplate (31 carbon-source-containing wells and one control well containing no carbon source, in triplicate). The carbon sources in Ecoplates were selected for microbial community analysis in environmental samples—specifically soils. The plates were incubated aerobically at 25°C for 4 days, and the optical density (560 nm) of each well was measured. Raw data were analyzed using Microsoft Excel (Microsoft Corporation, Redmond, Washington) to estimate the average metabolic response (AMR) and community metabolic diversity (CMD) of culturable bacteria (Garland, 1997; Garland and Mills, 1991). The AMR describes the overall carbon substrate usage (i.e., average degree of purple coloration generated in each well among the 31 carbon sources), while the CMD represents the average number of C-substrates used by bacteria.

Biogas Production. The effect of capping regime on anaerobic biogas production, rate, and composition was assessed using sediment incubation studies and periodic gas collection and analysis. Fifteen grams of each sediment and 5 mL of sterile anoxic water were added to 20-mL serum vials for maintaining anaerobic conditions (allowing 40% by volume headspace) (Zanetti and Fiore, 2007). Samples were amended with 2% (w/v) hydrogen release compound (Regenesis, San Clemente, California) to stimulate and accelerate biogas formation. The vials were shaken (by hand) and then sealed and incubated for up to 14 days at ambient temperature (approximately 22°C). The biogas volume production rate (or milliliters per gram dry weight per day [mL gdw^{-1} d^{-1}]) and the rate of production of individual components (micromoles per gram dry weight per day μmol gdw^{-1} d^{-1} [μmol gdw^{-1} d^{-1}]) were measured by periodically sampling the vials using a 10-mL syringe. Biogas components, including methane, carbon dioxide, and nitrogen, were measured by gas chromatography (GOW-MAC series 400 gas chromatograph with thermal conductivity detector [GC/TCD], GOW-MAC, Bethlehem, Pennsylvania) equipped with 3 m × 3.2 mm (10 ft × 0.13 in.) Hayesep DB column using helium as a carrier gas (30 mL min^{-1}). The temperatures of the column, detector, and injector were 120, 135, and 150°C, respectively. The bridge current was set to 105 mA.

DNA Extraction, Polymerase Chain Reaction, and Denaturing Gradient Gel Electrophoresis. A sample of each sediment (0.8 g wet weight) was added to 0.5 mL of 0.1-mm glass beads (Biospec Products Inc., Bartlesville, Oklahoma) and 1 mL sterile DNA extraction buffer (50 mM sodium chloride, 50 mM Tris-HCl [pH 7.6], and 5% sodium dodecyl sulfate) (pH 8) in a 2-mL centrifuge tube. The tubes were then shaken for 30 seconds at 5.5 m s^{-1} (Fastprep, Bio101, Thermosavant, Waltham, Massachusetts) (Sigler and Zeyer, 2004). DNA was isolated using phenol/chloroform extraction (Sambrook and Russell, 2001) and purified using a filtration column containing 10% polyvinylpyrrolidone (PVPP) (Acros Organics, Morris Plains, New Jersey) (Kuske et al., 1998; Menking et al., 1999). Purified DNA was quantified using spectrophotometry (260 nm), diluted with DNA-grade water to a final concentration of 100 μg mL^{-1}, and stored at −80°C.

The V3 region (192 bp) of the 16S rRNA gene of bacteria was amplified using primers 341f and 534r (Muyzer et al., 1993), while the 16S rRNA gene fragments (456 bp) of archaea was amplified using primers ARCH915-GC and UNI-b-rev, as described in Pesaro and Widmer (2002). Each polymerase chain reaction (PCR) consisted of 1 × PCR buffer (with 1.5 mM magnesium chloride), 0.5 mg mL^{-1} bovine serum albumin, 0.2 mM deoxyribonucleic triphosphate (dNTP) mix, 0.2 μM of each primer, and 0.02 units μL^{-1} of *Taq* (all final concentrations). Each set of reactions included a positive control (consisting of DNA isolated from either *E. coli* DH5α or an archaeal clone [Kassem et al., 2005]) and a negative control containing no DNA template. The cycling conditions for the bacteria PCR were 5 minutes at 96°C, 35 cycles of 30 seconds at 96°C, 30 seconds at 52°C, and 1 minute at 72°C, and then 5 minutes at 72°C. The cycling conditions for the archaea PCR were 5 minutes at 94°C, 35 cycles of 30 seconds at 96°C, 30 seconds at 65°C, and 1 minute at 72°C. The proper size of the PCR products was confirmed by agarose gel electrophoresis and comparison with a DNA size ladder.

The structure of the bacteria and archaea communities was examined by DGGE analysis using the Dcode Universal Mutation Detection System (Bio-Rad, Hercules, California) according to the method of Sigler et al. (2004). Specifically, electrophoresis was performed at 60°C and 200 V for 5 hours using 8% acrylamide gels containing a denaturing gradient of 40 to 60%. Following electrophoresis, gels were stained for 15 minutes with a 1:10 000 dilution of SYBR Green dye (Molecular Probes Inc., Eugene, Oregon) in 50 mL of 1× tris-acetate ethylenediaminetetraaceticacid buffer (TAE). Images of the gels were archived using a Kodak Gel Logic 200 Imaging System (New Haven, Connecticut). Fingerprints were analyzed using GelCompar II software (Version 3.0, Applied Maths, Austin, Texas) with a band position tolerance of 1% and optimization of 3% to account for electrophoretic shifts between any two identical bands/patterns (Sigler and Pasutti, 2006). The community structure of bacteria and archaea associated with the sediments was assessed by comparing fingerprint patterns. Specifically, a similarity matrix that quantified the relationships among the resulting DGGE fingerprints was calculated by the software using the Pearson's product correlation coefficient (Jobson, 1991). Fingerprint comparisons were facilitated by using a customized DGGE marker, which was developed from several bacteria strains commonly used in our laboratory (Kassem et al., 2005). A DGGE marker was loaded such that a maximum of four samples separated each marker lane. Cluster analysis, using the unweighted pair group method with arithmetic means (UPGMA), was performed on the similarity matrix, resulting in a dendrogram that graphically displayed the relationships among the bacteria or archaea communities. To further validate relational inferences represented by clustering in the dendrograms, GelCompar II software was used to calculate the cophenetic correlation coefficient associated with each dendrogram node.

DGGE Band Excision and DNA Sequencing. The DGGE bands (n = 15) representing bacteria or archaea phylotypes of interest were excised from the gel and incubated in sterile water at 4°C for 24 hours to elute the DNA. In our design, we attempted to select descriptive bands that were unique in each lane and those that were common across the gels. The DNA was amplified (as described above) using primers without guanine-cytosine clamps, purified, and cloned using the pGEM-T vector system (Promega, Madison, Wisconsin) (Kleikemper et al., 2002). One clone carrying a DNA insert of the correct size from each band (as determined by gel electrophoresis) was commercially sequenced (MWG Biotech Inc., High Point, North Carolina). Each resulting sequence was subject to analysis using the BLASTN algorithm to determine the

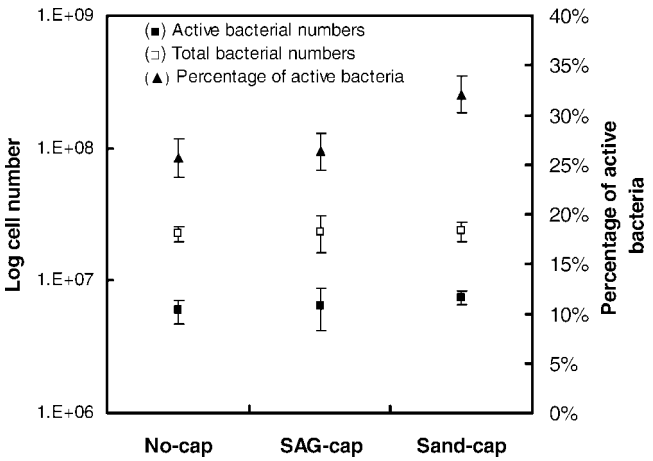

Figure 1—Total and active bacterial counts (number per gram of dry sediment) and percentage of active bacteria cells (number of active bacterial cells per total number of cells) as a function of capping regime (average ± standard error, *n* = 12).

identity of the organism that exhibited the closest related DNA sequence (Altschul et al., 1990). The sequences were deposited in Genbank under the accession numbers EU394216 to EU394232.

Data Analysis and Statistics. To determine statistically significant differences between sediment samples, data were averaged and tested using analysis of variance (ANOVA), followed by the Tukey's test ($p \leq 0.050$).

Results

Enumeration of Total and Active Sediment Bacteria as a Function of Capping Regime. Sediment samples collected from under the different cap treatments exhibited a similar number of total bacterial (2×10^7cells) per gram dry weight of sediment ($p = 0.762$) among treatments (Figure 1). However, sand-cap samples contained a higher number of active bacteria (7.41×10^6 gdw^{-1}) than no-cap samples (5.92×10^6 gdw^{-1}) ($p = 0.047$). The SAG-cap samples exhibited a similar number of active bacteria as no-cap

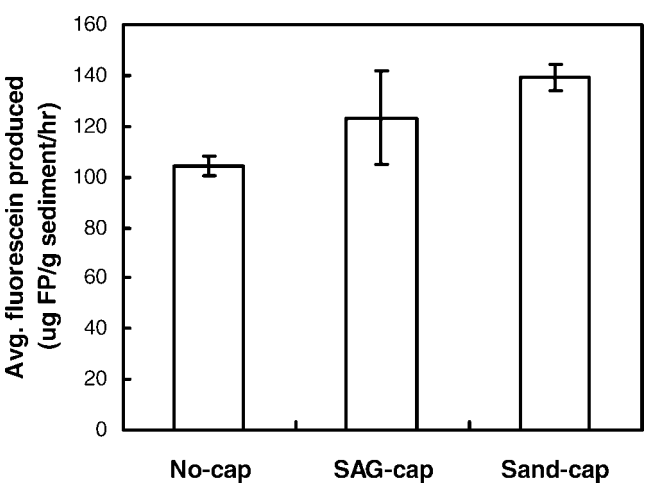

Figure 2—Comparison of overall microbial activity as a function of capping regime, measured using FDA assay (average ± standard error, *n* = 8).

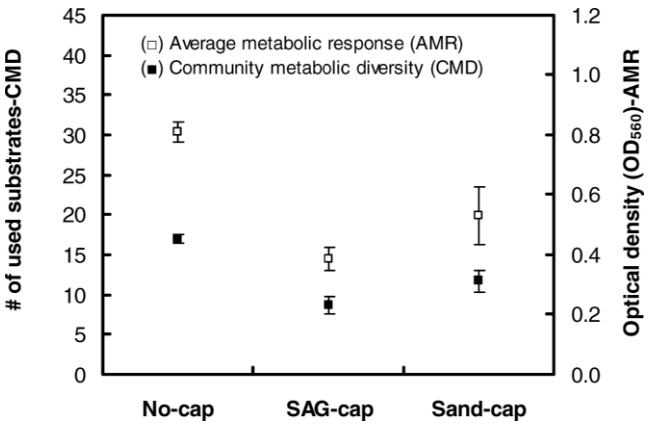

Figure 3—Comparison of the CLPP results (AMR and CMD) at 96 hours of incubation at 25°C as a function of capping regime (average ± standard error, *n* = 6).

($p = 0.702$) and sand-cap sediments ($p = 0.555$). Because of limitations in available samples, the SAG-cap samples processed for this research included two from the surface (0 to 5 cm) and two from depths of 20 to 25 cm and 25 to 30 cm (ABSE-6 [4] and ABSE-6 [5]) (Table 1), which resulted in increased variability in the number of total and active bacteria measured in the SAG-cap samples (Figure 1). The sand-cap samples showed a significantly higher percentage of active cells than both no-cap and SAG-cap samples for the surface sediments ($p = 0.042$).

Estimation of Sediment Microbial Activity Using Fluorescein Diacetate. Microbial communities in sand-cap samples exhibited higher hydrolysis activity (2.3 μg fluorescein produced per hour [FP h^{-1}]) than those in the no-cap samples (1.8 μg FP h^{-1}) ($p = 0.05$). The microbial FDA hydrolysis activity in SAG-cap samples (1.9 μg FP h^{-1}) was not significantly different than in no-cap and sand-cap sediments ($p = 0.32$) (Figure 2).

Assessment of Sediment Bacteria Metabolism Using CLPP. The effect of capping regime on the metabolic diversity of sediment bacteria was assessed by determining the number of different carbon sources that were used by various sediment samples (no-cap, sand-cap, and SAG-cap). Following 96 hours of incubation, the bacteria in the no-cap samples exhibited greater AMR (measured as the average optical density [OD_{560}] of all carbon source-containing wells) and CMD (the total number of carbon sources used) compared with both sand-cap ($p = 0.009$) and SAG-cap ($p = 0.001$) (Figure 3). Because CLPP (AMR and CMD) was developed for heterotrophic (aerobic) microbial communities, the CLPP response was likely inhibited in capped samples when compared with uncapped sediments. No significant differences in AMR and CMD were detected between sand-cap and SAG-cap samples ($p = 0.182$).

Anaerobic Activity as Measured by Biogas Production. The sediment samples were incubated for 14 days to determine if biogas production (rate and composition) was affected by capping regime. There were no significant differences in the pH (neutral) or the redox potential (range 150 to 200 mV) of the surface sediment samples. It is recommended that future studies measure relevant sediment geochemistry, including sulfate and nitrate concentration. Sand-cap samples exhibited the highest rate of total biogas production (0.27 mL gdw^{-1} d^{-1}) compared with no-cap (0.20 mL gdw^{-1} d^{-1}) ($p = 0.002$) and SAG-cap samples (0.24 mL gdw^{-1} d^{-1}) ($p = 0.023$) (Figure 4). The composition of the biogas included

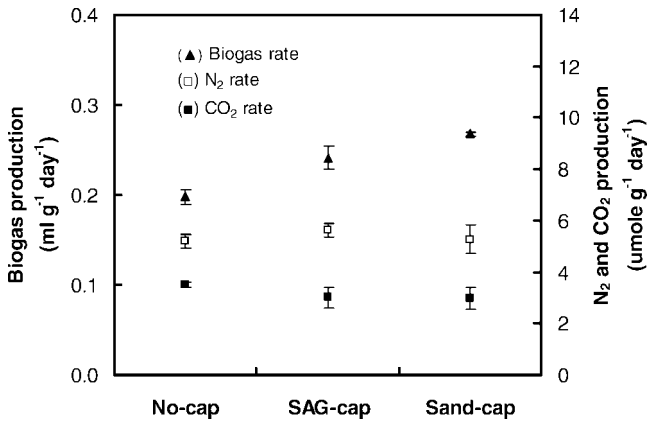

Figure 4—Biogas and composition (N_2 and CO_2) rate for 14-day incubation experiment with 2% hydrogen release compound (HRC) at room temperature (22°C) as a function of capping regime (average ± standard error, n = 12).

measurable quantities of nitrogen and carbon dioxide. However, there were no statistical differences in the rate of production of either nitrogen or carbon dioxide as a function of sample type. Rates ranged from 5.20 to 5.63 μmol gdw^{-1} d^{-1} for N_2 and 2.95 to 3.48 μmol gdw^{-1} d^{-1} for CO_2. Other anaerobic gases that are commonly produced in sediments, including methane (CH_4), hydrogen sulfide (H_2S), and nitrous oxide (N_2O), may have been present, but were not at detectable levels (5×10^{-5} moles) during the period of incubation.

Assessment of Cap Effect on Sediment Community Structure Using DGGE. The DGGE fingerprints were generated from the DNA samples representing each capping regime (no-cap, sand-cap, and SAG-cap). The DGGE analysis of bacterial communities revealed relatively high similarity (88 to 94%) among the fingerprints of the same cap type for most of the samples, with the exception of SAG-cap-1 and SAG-cap-2 (20 to 30 cm depth) sediments (Figure 5). No-cap samples demonstrated the highest similarity (94%) as a group of all capping regimes. No-cap DGGE profiles and those from the capped sediments (SAG-cap and sand-cap) had 88% similarity. Sequencing provided genera and phylotype information, which was useful for investigating the potential effects of capping regime on microbial community structure. The DGGE analysis of bacteria revealed that dominant phylotypes were common to all sediment samples, regardless of the capping regime (Figure 5). Phylotypes, which were either positively or negatively affected by the presence of capping, were also observed (Table 2). For example, bacteria in no-cap samples (represented by bands 10, 11, and 12) exhibited weak intensity or disappeared in fingerprints of capped sediment communities. In contrast, some phylotypes (i.e., band 7) were only detected after capping. The BLAST analysis of DNA indicated that *Sphingomonas* sp. (band 2), *Flavobacterium* sp. (band 9), and *Spirochaeta* sp. (band 7) were the major genera of bacteria detected in all samples, regardless of capping regime. Archaeal populations showed a similarity of higher than 94% for all surface samples (Figure 6).

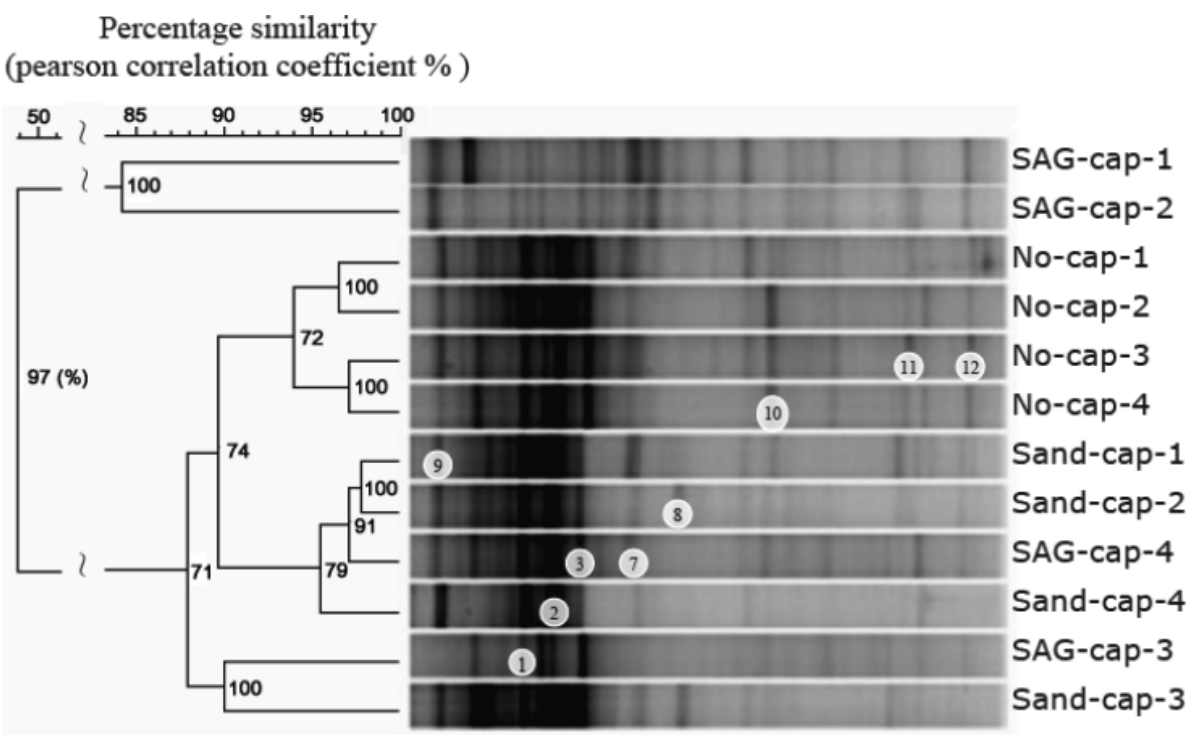

Figure 5—Dendrogram for DGGE analysis of 16S rRNA gene for bacteria communities in sediments as a function of capping regime. The numbered bands were selected for sequencing. No-cap-1 to no-cap-4 = no-cap samples from 4 representative cores; SAG-cap-1 to SAG-cap-4 = SAG-cap samples from 4 representative cores; sand-cap-1 to sand-cap-4 = sand-cap samples from 4 representative cores. Pearson correlation coefficient (similarity between the clusters in DGGE community fingerprint) was shown in the scale above the dendrogram, and cophenetic correlation coefficient (stability of the similarity tree) was shown on the nodes of the similarity dendrogram.

Table 2—Phylogenetic relationships of 16S rRNA genes sequenced in this study. The 16S rRNA gene fragments excised from the DGGE gels were 192 bp (bacteria) and 456 bp (archaea) in size, respectively.

Clone (Genbank accession number)	Number of bp sequenced	Closest identified relative	Sequence similarity (%)	Capping regime
1	120 bp	No matching sequence identified		All
2 (EU394216)	95 bp	*Sphingomonas* sp.	98%	All
3	120 bp	No matching sequence identified		Sand-cap, SAG-cap
7 (EU394220)	120 bp	*Spirochaeta* sp.	92%	All
8	120 bp	No matching sequence identified		Sand-cap, SAG-cap
9 (EU394221)	110 bp	*Flavobacterium* sp.	99%	All
10	120 bp	No matching sequence identified		No-cap, Sand-cap
11 (EU394222)	102 bp	*Sphingomonas* sp.	98%	All
12 (EU394223)	100 bp	*Rhodoferax ferrireducens*	96%	All
13 (EU394224)	403 bp	*Methanobacterium formicicum*	92%	All
14 (EU394225)	370 bp	*Methanofollis formosanus*	95%	All
15 (EU394226)	354 bp	*Methanosarcina barkeri strain*	99%	SAG-cap
16 (EU394227)	362 bp	*Methanofollis liminatans,.*	95%	Sand-cap, SAG-cap
17	300 bp	No matching sequence identified		All
20 (EU394230)	415 bp	Uncultured crenarchaeota	95%	Sand-cap

Dominant phylotypes were *Methanobacterium formicicum* (band 13) and *Methanofollis formosanus* (band 14), which were detected in 10 and 12 samples (of 12 total), respectively.

Discussion

Increased biogas production (ebullition) from sediments occurs as a result of increased anaerobic microbiological activity, and the active microbes in the sediment also will determine the composition of the biogas produced. Following cap placement, sediments have been shown to produce substantial amounts of biogas, which threatens cap integrity and, subsequently, ecosystem health (Huttunen et al., 2001; Palermo et al., 2002; Reible et al., 2006). To provide a more comprehensive understanding of this problem, this study assessed sediment microbial activity, metabolic potential,

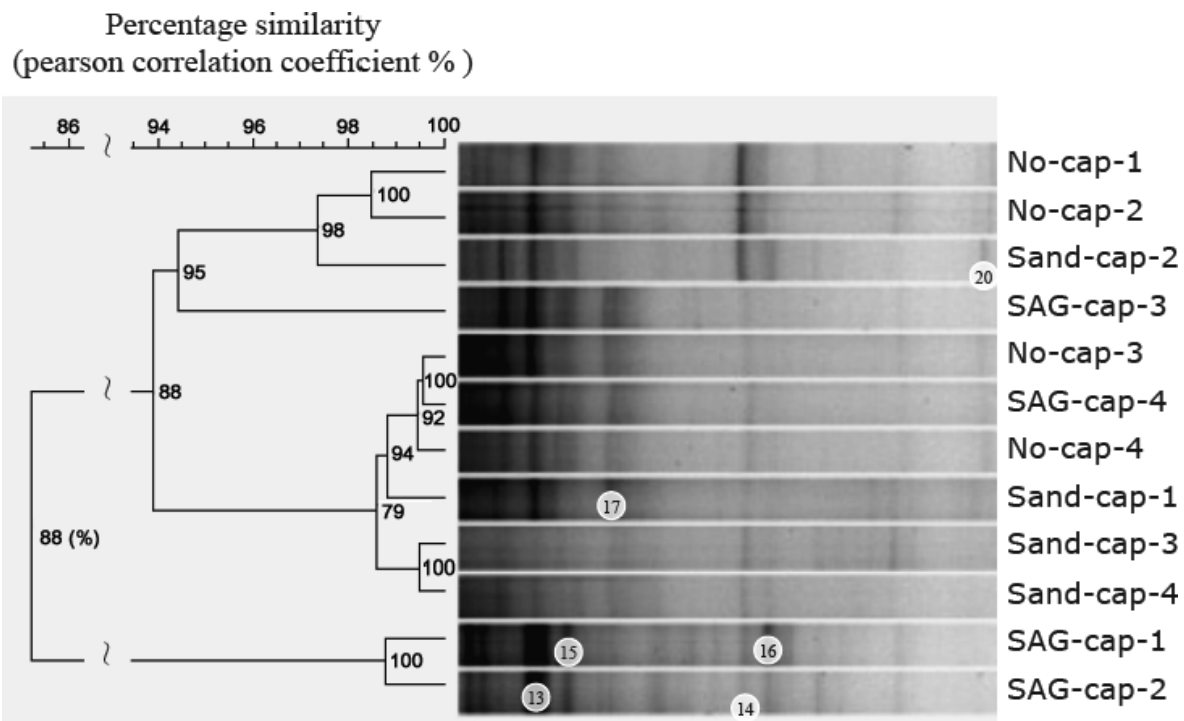

Figure 6—Dendrogram for DGGE analysis of 16S rRNA gene for archaea communities in sediments as a function of capping regime. The numbered bands were selected for sequencing. No-cap-1 to no-cap-4 = no-cap samples from 4 representative cores; SAG-cap-1 to SAG-cap-4 = SAG-cap samples from 4 representative cores; sand-cap-1 to sand-cap-4 = sand-cap samples from 4 representative cores. Pearson correlation coefficient (similarity between the clusters in DGGE community fingerprint) was shown in the scale above the dendrogram, and cophenetic correlation coefficient (stability of the similarity tree) was shown on the nodes of the similarity dendrogram.

and structure as a function of capping regime 6 months following cap placement. In general, sand cap placement resulted in an increase in sediment microbial activity compared with uncapped sediments. The activity assays chosen for this research (direct enumeration and FDA) do not distinguish anaerobic from aerobic activity, as their results incorporate all active microorganisms present (Green et al., 2006; Proctor and Souza, 2001). Therefore, it cannot be inferred that the increased activity observed in these assays resulted from aerobes or anaerobes. However, the biogas production experiments provided a surrogate measure for anaerobic activity, because biogas is composed of the byproducts of anaerobic respiration and fermentation (Rudd and Taylor, 1980; Rusmana and Nedwell, 2004; Scholten et al., 2002). The Sand-cap samples exhibited a statistically higher rate of biogas production (mL gdw^{-1} d^{-1}) than the no-cap samples. These results, combined with the microbial activity and FDA results, suggest that the increased microbial activity following sand-cap placement might be the result of an increase in anaerobic activity.

Interestingly, a statistically significant increase in the microbial activity (active percentage, FDA, biogas production) was not observed with the SAG-cap samples. Synthetic aggregate is a granular material with a gravel core and clay (bentonite and organic polymer) coat. The outer clay layer swells during hydration to form a unified, cohesive, and impermeable layer (Quadrini et al., 2003; Reible et al., 2006). The SAG caps tend to be less permeable and more effective at sequestering organics present in the sediments when compared with sand caps (Baskaran et al., 2005; Gunnison et al., 1987; Reible et al., 2006; Thibodeaux, 2002). Therefore, SAG caps will be more effective at limiting the transfer of nutrients (and oxygen) from overlying water to the sediments and decreasing the availability of electron donors and electron acceptors, which are important factors that affect the distribution and activity of microbial communities in sediments (Luna et al., 2004; Pomeroy, 1984; Wever et al., 2005). Hence, the SAG-cap will likely result in a decrease in microbiological activity when compared with the sand-cap samples. However, there was no statistically significant difference between the microbial activity of the SAG-cap and the sand-cap sediments. This might be attributed to the fact that there was significant variability in the SAG-cap samples. As noted in the Materials and Methods section, two SAG-cap samples were from top surface sediments (0 to 5 cm), while the other two SAG-samples were taken from a depth of 20 to 30 cm, as a result of limitations in available SAG-cap surface sediment samples. On the other hand, the net effect of the SAG-cap on microbial activity might have been masked, because the sequestration of organics or other important nutrients by SAG-cap might have preferentially selected for microbes that functionally persist under these limitations, while drastically decreasing the activity of other microbial populations.

In addition to the effect of capping on microbial activity, metabolic potential appeared to have been affected as well. The CLPP analysis, which was not intended as an absolute measure of total in situ microbial metabolic potential, but as an index by which to compare capping effects, indicated that capping caused a statistically significant decrease in the number of carbon sources used by culturable bacteria. However, DGGE analysis showed that capping did not result in significant changes to the structure of sediment bacteria communities (a 6% shift in similarity). All samples (capped and uncapped) contained archaea communities exhibiting high similarity (>94%). This contrast in findings between activity assays and DGGE is not unexpected and may be attributed to the fact that the activity assays might have reflected a nondominant subset of the microbial community (i.e., CLPP) or increases in abundance of already dominant phylotypes (i.e., active percentage, FDA). For example, CLPP analysis reflects the activity of culturable organisms that can grow on a defined medium, which likely constitute a small proportion of the total microbial community. Alternatively, DGGE characterizes the dominant populations and is not sensitive to changes in nondominant populations (Felske et al., 1998; Konopka et al., 1998). In addition, the time required for functional change (i.e., metabolic potential or activity) for a microbial community is generally considered to be much shorter than that required for structural changes (Chan et al., 2005; Glissman et al., 2004). Thus, although changes in microbial activity were observed, it is possible that a significant change in sediment community structure did not occur during the time since cap placement (6 months), particularly because the organisms present were capable of competing for the available nutrients.

We recognize that DGGE analysis exhibits some inherent limitations. Because the protocol involves the amplification of the 16S rRNA gene, organisms containing multiple copies of the gene with varying sequence might be represented by multiple bands in resulting fingerprints (Nubel et al., 1996). Furthermore, bands of dissimilar sequence (assumed to be from differing organisms) but similar melting behavior can co-migrate, leading to misinterpreted community complexity or structure (Casamayor et al., 2000). The appropriate method for comparing the fingerprints must be selected to effectively interpret DGGE analyses. We used the Pearson's product moment correlation coefficient, instead of band-based methods, because it is insensitive to relative differences in intensity and background and accounts for the entire data set describing each fingerprint (Fromin et al., 2002; Haene et al., 1993). These characteristics are particularly important when processing environmental samples. The cophenetic correlation coefficients showed that the dendrograms (branching and structure of resulting clusters) constructed for bacteria and archaea communities in this study were considerably stable (Figures 5 and 6).

The presence and activity of nitrate reducers was confirmed in the biogas production studies, because nitrogen production was observed in all sample types. These findings suggested nitrate availability in surface sediments. Our molecular analyses also detected potential nitrate reducers (i.e., *Sphingomonas* sp. and *Flavobacterium* sp.) in all sediment types, regardless of capping regime (Eguchi et al., 2001; Fegatella and Cavicchioli, 2000; Tanaka et al., 2006) (Table 2). The detection and observed activity of nitrate reducers (nitrogen production) may explain the absence of methane production in the incubation experiment, because inhibition can result from the toxic intermediates of nitrate reduction (NO_2^-, NO, N_2O, NH_3, and NH_4^+) and the higher uptake threshold of hydrogen and acetate for methane production (Hoehler et al., 2001; Koizumi et al., 2004; Roy and Conrad, 1999; Segers, 1998; Thauer et al., 1977). However, methane production likely will still occur in these sediments as the nitrate concentration or toxic intermediates of denitrification in sediments dissipate (Chan et al., 2005; Scholten et al., 2002; Zepp-Falz et al., 1999).

Conclusions

Our findings suggest that the placement of sand caps may affect the short-term ebullition potential of sediments, by affecting both microbiological activity and metabolic potential in sediments without causing significant changes to the microbial community structure. Because cap integrity is largely influenced by biogas production (microbial activity), the short-term effect on microbial

activity in sediments must be factored into the design of an efficient capping regime. Our data showed that the nature and magnitude of the effect that capping can have on microbial activity likely will be dependent on the capping materials chosen. Additionally, site-specific characteristics, such as available nutrients, redox potential, and sediment community species, which are also important factors affecting the capping efficiency, need further investigation to determine the appropriate capping regime.

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