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Tim Ku (Wesleyan University) Suzanne O'Connell (Wesleyan University), Anna Martini (Amherst College)
Students: Erin Algeo, Jennifer Bourdeau, Justin Clark, Margaret Selzer, Ulyanna Sorokopoud, Sarah Tracy

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Keck Geology Consortium: Projects 2007-2008
Short Contributions – Puerto Rico

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CHARACTERIZATION OF SEDIMENT MICROBIAL COMMUNITIES IN THE BIOLUMINESCENT BAYS OF VIEQUES, PUERTO RICO

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BACKGROUND

Examination of pore water and sediment chemistry provides insight into nutrient cycling and the microbial processes that may in turn influence the local abundance of dinoflagellates in the bays of Vieques. Although sedimentary bacteria have little influence on processes in the water column in the open ocean, the extent of benthic-pelagic interaction increases in shallower, more productive coastal marine environments, such as Puerto Mosquito and Puerto Ferro (Capone et al. 1988).

Mangrove ecosystems are rich in organic matter and highly productive, yet they are generally nutrient-deficient, especially of nitrogen and phosphorus (Holguin et al. 2001, Sengupta and Chaudhuri 1991; Holguin et al. 1992; Alongi et al. 1992; Vazquez et al. 2000). This puzzle may be explained by the participation of microbial communities in nutrient transformation in the mangrove ecosystem. Microbial activity in the sediment column may support a very efficient recycling system of nutrients from decomposing mangrove leaves (Holguin et al. 2001).

Previous studies suggest that there is an intimate linkage between the productivity of bay waters and underlying shallow coastal marine sediments (Capone et al. 1988, Kristensen 2000, Alongi et al. 1998). Specifically, studies of intertidal mangrove ecosystems have revealed close couplings among benthic nutrient pools, microbes, and mangrove trees: mechanisms which maximize the use of scarce nutrients (Alongi et al. 1998). Currently, it is established that heterotrophs in the sediment column decompose organic matter supplied by photoautotrophic processes in the water column. Aerobic and anaerobic microbes in the sediments, in turn, affect the production of new organic matter in the water column by the rate at which they oxidize organic material and return inorganic nutrients to the overlying water.

Organic carbon oxidation within the sediment column follows a vertical sequence of microbial respiration reactions starting with the most energetically favorable electron acceptors. The predicted thermodynamic progression is often observed (O_2 >NO^{3-} >MnO_2 > FeO(OH) > SO_4^{2-} > CO_2) (Capone et al. 1988). However, the thermodynamic argument does not fully explain the distribution of microbial activities. Factors such as differential toxicity and substrate specificity and affinity are often involved (Capone et al. 1988).

In near-shore marine sediments, organic content is high and oxygen consumption is rapid. Thus, O_2 is usually depleted only a few millimeters into the sediment column, with the exception of areas where bioturbation spreads the transition from oxic to anoxic conditions throughout a larger depth range (Jørgensen 1982; Jørgensen and Revsbech 1983). Aerobic processes are the most efficient means of breaking down organic matter, since oxygen is the most energetically favorable electron receptor for organic decomposition (Kristensen 2000). From previous incubation and flux experiments in Vieques, rates of organic matter decomposition were found to vary locally within each bay, and seemed to be predominately controlled by availability of organic carbon in the sediments (Palevsky 2007).

Anaerobic microbial decomposition is more complex than aerobic respiration. In anoxic zones, individual microbes are incapable of performing

all the metabolic processes required for complete organic matter decomposition and must form symbiotic communities. The processes involved in anaerobic degradation of organic material may be viewed as sequential in space or in time, as recently deposited material moves deeper into the sediments and through different bacterial zones (Capone et al. 1988).

Sulfate reduction ($2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO^{3-}$) is responsible for most of the organic carbon oxidation in the suboxic-anoxic zones of these mangrove forests due to high concentrations of sulfate in seawater. When sulfate is reduced by sulfate reducing bacteria, soluble sulfur compounds such as H_2S and HS are produced, which then react with iron, reducing Fe (III) to Fe (II) and yielding pyrite (FeS_2). Soluble sulfur compounds may also react with manganese, reducing Mn (IV) to Mn (II), which generally occurs as a microbially-mediated process (Alongi 1998).

Nitrogen-fixing (diazotrophic) bacteria may also play in important role in mangrove sediements. High rates of N2 fixation have been associated with dead and decomposing leaves and with the sediments of mangrove ecosystems (Zuberer and Silver 1978; Potts 1979). A positive correlation has been found between acetylene-reduction rates (a measurement of the rate of N_2 fixation) and the availability of organic matter, despite the high energy cost of N_2 fixation (Holguin et al. 2001).

The rates and pathways of organic matter decomposition in Vieques sediments can be better understood by characterizing the structure of sediment column microbial communities. This study focuses on the microbial activity (using oligonuceotide probes for eukarya, archaea and bacteria) that occurs at sediment faces changes indicated by TOC (total organic carbon) and TIC (total inorganic carbon) in the sediment column. DNA sequencing, T-RFLP (terminal-restriction fragment length polymorphism) analyses and FISH (fluorescent in-situ hybridization) imaging have been used to characterize microbial populations and to gain an understanding of how they differ with location and depth and how they relate to sediment and pore water chemistry. In order to conserve and sustainably manage the Vieques bay ecosystem, especially the unique populations of dinoflagellates, it is necessary to understand the factors regulating the generally high rates of aboveground net primary production, including the extent to which mangrove-derived organic matter is recycled and conserved within the forest floor (Alongi et al 1998).

METHODS

Replicate 20 cm and 100 ± 50 cm cores of the top sediment layers were collected at deep and shallow water depths in each bay (Puerto Ferro and Puerto Mosquito). 20 cm cores were subsequently frozen at -80°C until processed for DNA and phosphate extraction. The 100 cm cores were used for sediment characterization and pore water extraction.
Volumes of overlying and pore water from each sampling site were filtered through a 0.45µm nylon membrane and preserved for later geochemical analysis. Major anion concentrations were quantified on a Dionex 500-series Ion Chromatograph (IC) and major cations were quantified on a Leeman Labs Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICP-AES). Dissolved inorganic carbon (DIC) was measured using a flow injection analysis instrument designed after Hall and Aller (1992).

Volumes of pore water were processed further by filtering through a 0.2 µm polycarbonate membrane, fixed with formalin and stained with 4, 6-Diamidino-2-Phenylindole (DAPI) for direct cell counts. Filters were mounted on slides and viewed and photographed with a Nikon CoolPix 990 digital camera attached to a Zeiss Axioskope epifluorescence microscope. 7 images/filter were used for cell counting with NIH image analysis software.
DNA for clone libraries of 16S rRNA genes and T-RFLP and analysis was isolated from the preserved 20 cm cores using a MoBio UltraClean Soil Extraction Kit. Three different sets of primers were used for PCR (polymerase chain reaction) amplification of bacterial, archaeal, and eukaryotic 16S/18S rDNA. Forward primers were tagged with fluorescent dye

for fragment analysis. PCR products were purified using a QIAquick PCR purification kit (Qiagen) and digested separately with enzymes MspI, HhaI and RsaI. Digested products were separated by gel electrophoresis on a polyacrylamide gel and fragment size measurements were performed on an ABI PRISMTM 310 genetic analyzer (Cornell University genomics facility). T-RFLP analysis allowed for comparisons of microbial community diversity between different sediment sampling sites and between different depths within the sediment column of each sampling site.

RESULTS

The geochemical profiles of [SO_4^{2-}] in the deep and shallow water cores were similar. At the sediment surface, sulfate concentrations with respect to chloride concentrations were ~51mM/M (standard overlying seawater ratio). Beneath the surface-sediment interface, sulfate decreased rapidly in both cores, but the drop in sulfate concentration was lower in shallow environment cores. At approximately 8cm, pore water sulfate concentrations begin to increase in both cores, and then begin to level off at about 18cm, once again approaching overlying seawater values. The sulfate profile broadly correlates inversely with the DIC profile in the uppermost 20cm of both cores (Fig. 1).

Extracted DNA was tested for PCR amplification with universal primers that targeted ~1,000 bp regions of eukarya, bacteria and archaea ribosomal DNA (rDNA). Expected product was obtained under selected PCR conditions with no evidence of other products, which indicates that no non-specific products were amplified (Fig. 2).

Non-specific products are undesirable, since they may lead to overestimations of microbial diversity in rDNA fragment analyses. Purified and enzyme digested PCR products were verified on 2% agarose gel (Fig. 3).

Cell populations observed on DAPI-stained pore water filters ranged from 6.6 $\times 10^5$ to 4.3$\times 10^6$ cells/mL in shallow-environment cores and from 1.3 $\times 10^6$

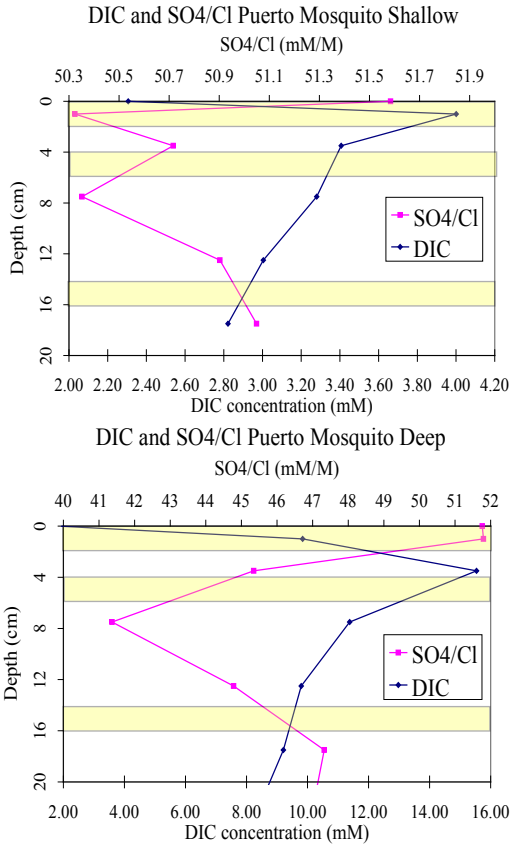

Figure 1: Vertical pore water profiles showing sulfate/chloride and DIC (dissolved inorganic carbon, approximated by [$HCO3^-$]) concentration profiles for A) shallow-water and B) deep-water environment cores from Puerto Mosquito. Shaded zones indicate samples used for molecular analysis.

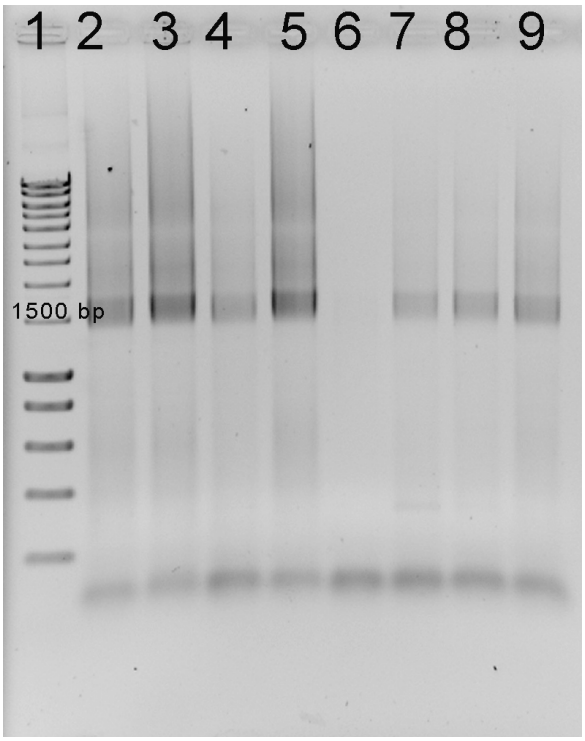

Figure 2: Inverted agarose gel (1%) image of amplified bacterial rDNA from sediment-derived DNA using bacterial-biased PCR primers. Lane descriptions: 1=size marker 200 to 10,000 base standard; 2-5, 7 &8= PF7 0-2, 4-6 and 14-16cm; 6=PM17 14-16cm; 8=PF9 4-6cm.

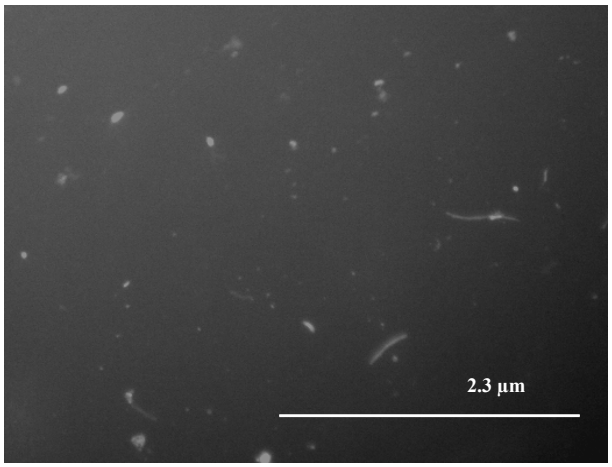

Figure 4: photograph of cells stained with DAPI, viewed on an epifluorescent microscope, 100x objective.

to 4.8 $\times 10^6$ cells/mL in deep-environment cores, with an enumeration uncertainty of ±10%.

PF and PM deep environment pore water total cell count profiles were very similar. Cell counts decreased significantly between 0 and 5cm to ~ 1.3 $\times 10^6$ cells/mL, then gradually increased with depth (Fig. 5). PF shallow environment pore water cell counts also decreased to ~1.3$\times 10^6$ cells/mL between 0 and 5cm. The PM shallow pore water cell count profile was slightly different from the rest in that cell counts never increased with depth. PM shallow counts decreased throughout the top 15cm and the most rapid drop in numbers was between 5 and 10cm depth instead of 0 and 5cm (Fig. 5).

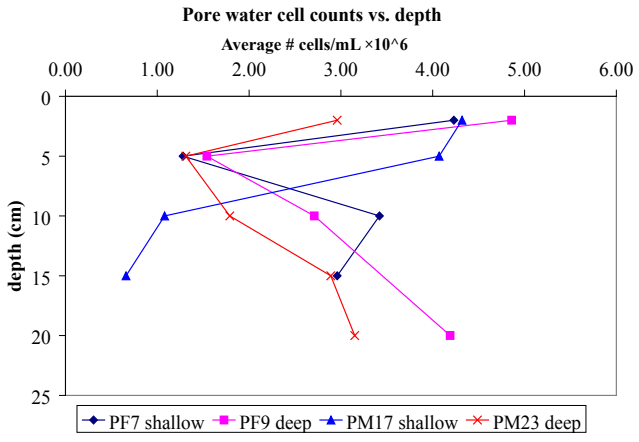

Figure 5: Graphs showing average number of cells counted at pore water intervals with depth for shallow and deep environment cores from Puerto Mosquito (PM) and Puerto Ferro (PF) in the top 20cm.

DISCUSSION

In a typical sulfate reducation profile, sulfate concentration decreases gradually with depth as sulfate reducing bacteria (SRB) use it as an electron acceptor for organic matter decomposition reactions. The typical profile, however, is not observed in most Vieques shallow environment cores. This may be due to the existence of oxic microniches along burrows of zoobenthos or along the living fine roots of mangroves, which allow the oxic zone to extend further into the sediment column and consequently reduce the need for sulfate as a substrate, since O2 is a more thermodynamically favorable electron acceptor in organic matter decomposition reactions.

Cell density in the PM shallow core decreased significantly with depth, while cell density decreased at 2-5cm (relative to 0-2cm) for the deep-environment core from the same bay, then steadily increased with depth to 20cm, possibly as a result of anaerobic microbes thriving and forming symbiotic communities in anoxic sediments. PF shallow and deep average cell count profiles were both similar to the PM deep environment core.

Relative to the shallow core from PF, the shallow core from PM has significantly more cells/mL in the 2-5cm interval, but significantly fewer cells/mL in the 5-10cm interval (2-way ANOVA, $p<0.001$). Pore water cell count fluctuations in PM may be explained by the fact that PF sediment is very homogeneous in the top 16cm, but PM sediment composition becomes significantly more shell-rich at ~6cm, where the pore water cell population decreases. These cell count differences may also be a result of differences in substrate material concentrations in the different bay sediments. For example, perhaps PM shallow sediments have more dissolved NO_3^- than PF shallow sediments at the 2-5cm interval and shallow environment PM sediments have lower heavy metal concentrations than shallow PF sediments at the 5-10cm interval.

Microbial diversity, expressed as ribotype richness, can be estimated by determining the number of unique terminal-restriction fragments (T-RFs)

from bacterial and archaeal 16SrDNA and eukaryal 18SrDNA digested separately with 'targeted' enzymes. This method provides distinct profiles (fingerprints) dependent on the species composition of the communities of the samples and is a helpful tool for comparative community analysis. T-RFLP results may indicate whether populations of bacteria, archaea and eukarya differ significantly between the two bays in Vieques, and how they are affected by vertical redox gradients.

T-RFLP has several shortcomings, however. Results are subject to PCR biases. Thus, the proportion of DNA that is amplified may not be representative of the entire microbial community. Moreover, high humic acid content in near-shore sediment samples may decrease the yield of purified PCR product, since humic acids compete for adsorption sites on purification column binding membranes (Howeler et al. 2003). In this study, purification columns for PCR amplified DNA extracted from near-shore shallow-environment sediments, particularly in Puerto Mosquito, needed to be overwhelmed with more PCR product than deeper-environment sediments to obtain the same amount of purified product. We suspect, therefore, that humic acid content is higher in near-shore sediment than in deep environment sediment.

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