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# Data report: total cell counts and qPCR abundance of Archaea and Bacteria in shallow subsurface marine sediments of North Pond: gravity cores collected during site survey cruise prior to IODP Expedition 336<sup>1</sup>

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## Chapter contents

<a href="#">Introduction</a> .....	1
<a href="#">Materials and methods</a> .....	1
<a href="#">Results and conclusions</a> .....	2
<a href="#">Acknowledgments</a> .....	3
<a href="#">References</a> .....	3
<a href="#">Figures</a> .....	5

## Introduction

The total number of prokaryotes in deeply buried marine sediments decreases with sediment depth and varies over orders of magnitude in different areas of the ocean. It is mainly controlled by the content of organic carbon in the sediment as the microbial substrate (Parkes et al., 1994; D'Hondt et al., 2004; Schippers et al., 2005, 2012; Edwards et al., 2012; Kallmeyer et al., 2012; Lomstein et al., 2012; Hoehler and Jørgensen, 2013). Organic-lean, oligotrophic, and oxic sediments of the Pacific host oxygen-respiring prokaryotes (Røy et al., 2012). Specific archaeal communities for sediments with different trophic states could be detected (Durbin and Teske, 2012). An open question is if Bacteria or Archaea dominate in oligotrophic sediments as previously discussed for eutrophic sediments based on quantitative polymerase chain reaction (qPCR) analysis (Schippers et al., 2005, 2012). Similar to oligotrophic Pacific sediments, oligotrophic (total organic carbon =  $\sim 0.15\% \pm 0.07\%$ ) and oxic sediments from the North Pond area in the 7 m.y. old western flank of the Mid-Atlantic Ridge at 23°N have been shown to contain molecular oxygen downhole to >8 m sediment depth. Aerobic respiration likely dominates organic carbon oxidation (Ziebis et al., 2012). During a site survey cruise prior to Integrated Ocean Drilling Program (IODP) Expedition 336 to North Pond, sediment cores were collected to 8 meters below seafloor (mbsf). We sampled these cores to count total cells and determine the abundance of Bacteria and Archaea by qPCR using modified protocols.

## Materials and methods

Organic-lean, oligotrophic, and oxic sediments from the North Pond area in the 7 m.y. old western flank of the Mid-Atlantic Ridge at 23°N were sampled during the Expedition 336 site survey cruise with the German R/V *Maria S. Merian* in 2009 (MSM 11/1). Sediment cores (12 cm diameter) were successfully retrieved at six locations within the North Pond basin between 4040 and 4480 meters below sea level using gravity corers (Ziebis et al., 2012). These cores extended to 8 mbsf. Once recovered on deck, these cores were quickly cut into 1 m sections that were immediately carried to the cold storage room. Each 1 m section was subse-

<sup>1</sup>Breuker, A., and Schippers, A., 2013. Data report: total cell counts and qPCR abundance of Archaea and Bacteria in shallow subsurface marine sediments of North Pond: gravity cores collected during site survey cruise prior to IODP Expedition 336. In Edwards, K.J., Bach, W., Klaus, A., and the Expedition 336 Scientists, *Proc. IODP*, 336: Tokyo (Integrated Ocean Drilling Program Management International, Inc.).  
doi:10.2204/iodp.proc.336.201.2013

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quently split into half-core sections. From one half, samples for microbiological analysis were taken from the interior portion by excavation with a sterilized spoon, and the outer centimeter layer was left in the core liner to avoid contamination with seawater. In addition to the gravity cores, an additional 21 sediment samples from the shallow subsurface (0–15 cm depth) were taken with the remotely operated vehicle (ROV) *Jason II* (push cores) during another cruise of the *Maria S. Merian* in 2011 (MSM 20/5).

For total cell counting, 1 mL of each sediment sample was preserved in 9 mL of 0.2  $\mu\text{m}$  sterile-filtered 2% formaldehyde in seawater. In the Federal Institute for Geosciences and Natural Resources (BGR) laboratory, 100  $\mu\text{L}$  of fixed sediment was transferred in a 2 mL Eppendorf tube and suspended in 1 mL of 0.1 M hydrochloric acid (after autoclaving sterile filtered) to dissolve carbonates. Samples were shaken for 5 min and centrifuged for 20 min at 16,000 g. Afterward, 1 mL of supernatant was carefully removed. The pellet was suspended in 1 mL tris-ethylenediaminetetraacetic acid (TE) buffer and centrifuged. This step was repeated. The pellet was suspended with 900  $\mu\text{L}$  TE buffer followed by an ultrasonic treatment for 20 s (Weinbauer et al., 1998). A sample (100  $\mu\text{L}$ ) was stained on filters with SYBR Green as described elsewhere (Lunau et al., 2005). Cells were counted using fluorescence microscopes (Weinbauer et al., 1998).

For qPCR, samples were immediately frozen at  $-20^{\circ}\text{C}$  after shipboard sampling. Samples were transported and stored frozen in the home laboratory. For DNA extraction from thawed samples, a published protocol for DNA extraction (Webster et al., 2003) with an additional preceding acid treatment step with iodic acid was applied to dissolve carbonates and to improve DNA extraction from cells. Kates et al. (1965) published a protocol to break ether bonds in cell walls of microorganisms using iodic acid. According to the described procedure, we applied iodic acid but in a lower concentration and a shorter incubation time (0.1 mol/L versus  $\sim 8$  mol/L and 10 min versus 24 h). We assumed that the modified method dissolved the carbonates and/or partially disrupted the cell walls (in particular archaeal ether bonding) but did not lyse the cells. FAST-Prep tubes without matrix were filled with 0.5 g of sediment and centrifuged for 30 s at 14,000 g. Afterward, 1 mL of 0.1 M filtered 0.1 M iodic acid was added and the pellet was suspended on a shaker for 10 min. The tubes were heated for 10 min at  $80^{\circ}\text{C}$  and centrifuged for 15 min at 16,000 g. A sample of 1 mL supernatant was removed, and 1 mL TE buffer was added to the pellet and vortexed. After centrifugation, this washing step was repeated. The previously removed ma-

trix was added, and the further procedure followed the protocol of the manufacturer with addition of polyadenine (Webster et al., 2003). Empty tubes were used as a negative control. Extracted DNA was amplified in triplicate by qPCR using an ABI Prism 7000 (Applied Biosystems). Published assays for the quantification of the 16S rRNA gene copy numbers of Archaea (Takai and Horikoshi, 2000) and Bacteria (Nadkarni et al., 2002) were applied. 16S rRNA gene copy numbers were converted to cell numbers using conversion factors of 1.5 for Archaea and 4.1 for Bacteria, as previously done (Schippers et al., 2005).

The reliability of our new protocols was tested in spiking experiments in which known numbers of distinct organisms were added to a sediment sample and the recovery of cells was determined. Different numbers of cells of gram-negative (*Escherichia coli*), and gram-positive (*Bacillus subtilis*) Bacteria and Archaea (*Methanohalobium evestigatum*), were suspended in sediment samples, and the recovery of the cells was determined (Fig. F1). The new procedure considerably improved cell number recovery. In addition, the acid dissolution of carbonates was confirmed by scanning electron microscopy of samples before and after acid treatment (Fig. F2).

## Results and conclusions

In agreement with published data for other oligotrophic sediments (Kallmeyer et al., 2012), our data show overall low cell numbers decreasing with sediment depth (Fig. F3). An average  $\sim 1$ – $2$  orders of magnitude higher abundance of Archaea than of Bacteria was found, in contrast to the previously studied, rather organic carbon-rich sediments with a much higher qPCR abundance of Bacteria (Schippers et al., 2005, 2012). The near-surface sediment samples up to 15 cm depth showed mean values (and standard deviation) of  $2.2 \times 10^8$  ( $1.9 \times 10^8$ ) cells/mL for Archaea and  $1.5 \times 10^7$  ( $1.8 \times 10^7$ ) cells/mL for Bacteria. These qPCR data reflect the dominance of Archaea even at the sediment surface. As an explanation for their dominance, Archaea are likely better adapted to low energy flux (Valentine, 2007) and therefore have an advantage over Bacteria in oligotrophic sediments in contrast to eutrophic sediments (Schippers et al., 2005, 2012). Another explanation is the deposition of archaeal cells from the water column and their preservation in the sediment. Karner et al. (2001) counted pelagic cells of Crenarchaeota, Euryarchaeota, and Bacteria by fluorescence in situ hybridization in the open ocean up to 5000 m water depth (station in North Pacific subtropical gyre). In their results, the fraction of Crenarchaeota relative to total DNA containing prokaryotes equaled or exceeded

the bacterial fraction below 1000 m. Further work on the composition of the microbial communities should reveal their origin and function in the oligotrophic subsurface sediments.

## Acknowledgments

This work is supported by the German Research Foundation (DFG) priority program 527 IODP/ODP grant SCHI 535/7 to A.S. The DFG also provided ship time and financial support for the Cruises MSM 11/1 and MSM 20/5 with the German R/V *Maria S. Merian*. We thank the chiefs Wolfgang Bach, Katrina Edwards, and Heiner Villinger, the crew, and the science party members, especially Anna Blazejak and Beth N. Orcutt for their efforts during sampling.

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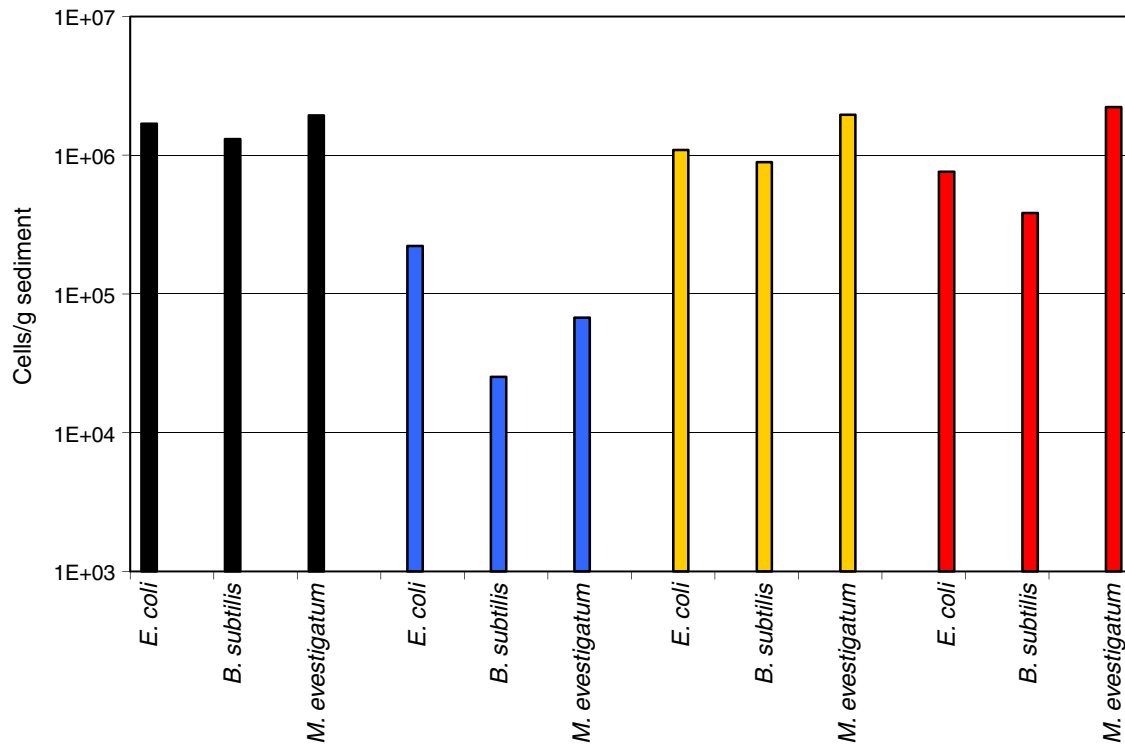
**Initial receipt:** 24 January 2013

**Acceptance:** 2 May 2013

**Publication:** 28 June 2013

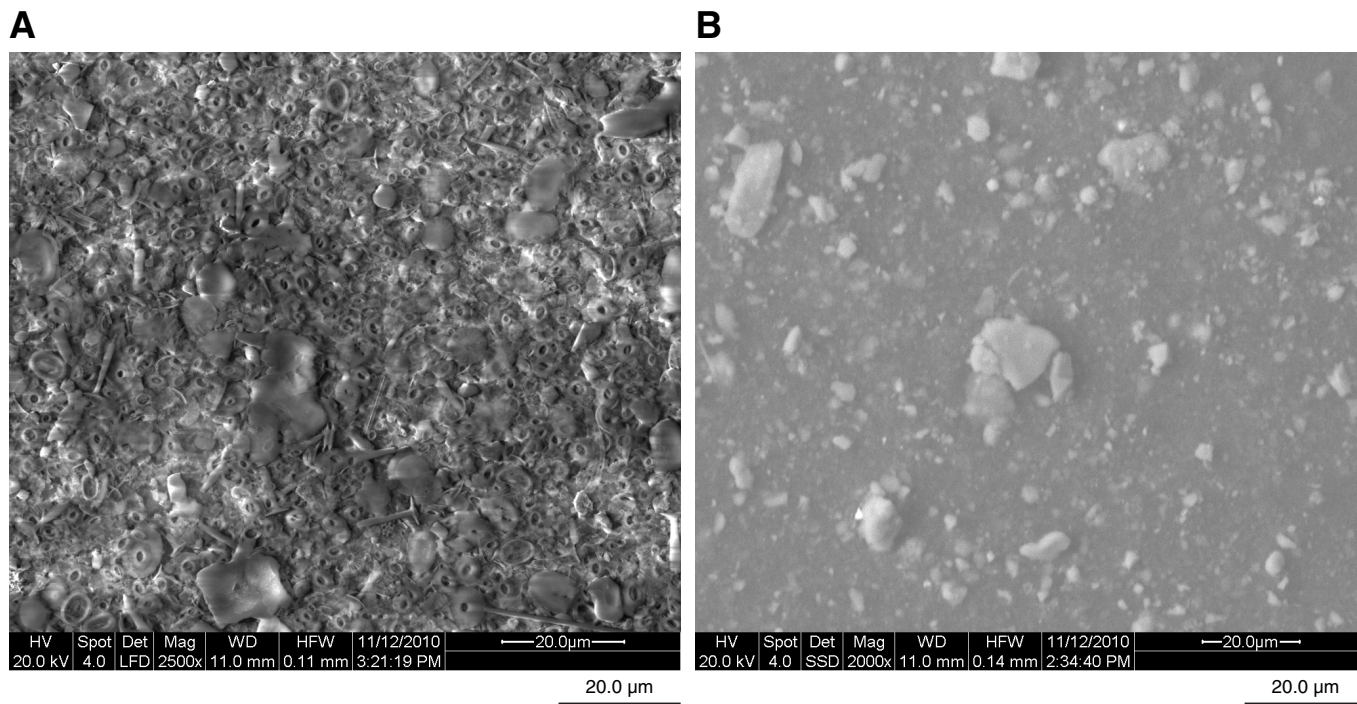
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**Figure F1.** qPCR analysis of sterilized North Pond sediments spiked with  $10^6$  cells/g of different species. Black = total cell counts for comparison, blue = cell numbers with extraction of DNA after Webster et al. (2003) without acid treatment, yellow = cell numbers with HI treatment, red = cell numbers with HCl treatment. 16S rRNA gene copy numbers per cell used for calculation of cell numbers: *Escherichia coli* = 5, *Bacillus subtilis* = 10, *Methanohalobium evestigatum* = 1.5.





**Figure F2.** Scanning electron microscopy images of a North Pond sediment sample (A) before and (B) after acid treatment. On A, residual skeletons of diatoms and foraminifera can be seen; on B, the skeletons disappeared.



**Figure F3.** (A) Total cell counts and (B) qPCR abundance of Bacteria and Archaea in organic-lean, oligotrophic, and oxic North Pond subsurface marine sediments (sampling sites Geob 13501, 13502, 13504, 13507, 13510, 13512 [Ziebis et al., 2012]). Near-surface samples taken with push cores (upper 15 cmbsf) were only analyzed by qPCR.

