The ISME Journal (2015), 1–5

www.nature.com/ismej

SHORT COMMUNICATION

Metatranscriptomic insights into polyphosphate metabolism in marine sediments

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Microorganisms can influence inorganic phosphate (P_i) in pore waters, and thus the saturation state of phosphatic minerals, by accumulating and hydrolyzing intracellular polyphosphate (poly-P). Here we used comparative metatranscriptomics to explore microbial poly-P utilization in marine sediments. Sulfidic marine sediments from methane seeps near Barbados and from the Santa Barbara Basin (SBB) oxygen minimum zone were incubated under oxic and anoxic sulfidic conditions. P_i was sequestered under oxic conditions and liberated under anoxic conditions. Transcripts homologous to poly-P kinase type 2 (*ppk2*) were 6–22× more abundant in metatranscriptomes from the anoxic incubations, suggesting that reversible poly-P degradation by Ppk2 may be an important metabolic response to anoxia by marine microorganisms. Overall, diverse taxa differentially expressed homologues of genes for poly-P degradation (*ppk2* and exopolyphosphatase) under different incubation conditions. Sulfur-oxidizing microorganisms appeared to preferentially express genes for poly-P degradation under anoxic conditions, which may impact phosphorus cycling in a wide range of oxygen-depleted marine settings.
The ISME Journal advance online publication, 18 September 2015; doi:10.1038/ismej.2015.169

Polyphosphate (poly-P) is a linear phosphate polymer that is produced by organisms from all domains of life. Microorganisms use intracellular poly-P for energy and nutrient storage, metal chelation, stress response and for certain regulatory functions (Rao *et al.*, 2009). The ability to use poly-P as an energy reserve appears to be especially important in episodically anoxic settings. For example, poly-P is thought to act as an energy source during wastewater treatment by the enhanced biological phosphorus removal (EBPR) process. EBPR reactors cycle P-rich sludge through oxygenated and anoxic phases. Certain organoheterotrophic organisms accumulate poly-P in the oxygenated phase, and then hydrolyze that poly-P in the anoxic phase for energy to uptake and store organic carbon (Oehmen *et al.*, 2007). In a similar manner, large marine sulfur-oxidizing bacteria in the family *Beggiatoaceae* also store poly-P, and have been shown to hydrolyze their poly-P stores upon exposure to anoxic or sulfidic conditions (Schulz and Schulz, 2005; Brock and Schulz-Vogt, 2011). Poly-P hydrolysis by large sulfur-oxidizing bacteria has been implicated in phosphate mineral formation in seasonally anoxic upwelling zones through the supersaturation of sediment pore waters, and might constitute an important sink in the global P cycle, both in modern sediments (Schulz and Schulz, 2005; Goldhammer *et al.*, 2010; Brock and Schulz-Vogt, 2011) and in the past (Bailey *et al.*, 2013; Crosby *et al.*, 2014).

However, marine microbial communities are diverse, and beyond studies of large sulfur-oxidizing bacteria, little is known about the diversity and activity of poly-P utilizing microbes in marine sediments that experience fluctuating redox conditions. Therefore, we used a metatranscriptomic approach to compare the expression of poly-P-related genes in sediments that were experimentally exposed to anoxic and oxygenated conditions. We collected sediments from two marine environments: (1) sediments from a deep methane seep near Barbados (13°46.65′ N, 57°32.25′ W, 4743 m water depth) that included a white sulfur-oxidizing bacterial biofilm, and (2) sediments from the Santa Barbara Basin (SBB) oxygen minimum zone (34° 14.493′ N, 120°06.932′ W, 573 m water depth) with no visible sulfur-oxidizing mat. Concentrations of authigenic phosphatic minerals do not occur in sediments at either site, and weight percent phosphorus is below 0.2% (Supplementary Table S1; Reimers *et al.*, 1996). (For contrast, phosphorites are commonly defined as sediments with $>18\%$ P_2O_5 (Föllmi, 1996).) After collection, Barbados sediments were stored in plastic centrifuge tubes at 4 °C with 0.5 mM P_i added to core top water, whereas SBB sediments were stored at 4 °C in unamended core top water. Bulk sediments likely remained anoxic prior to incubation (see Supplementary Information and Supplementary Figure S1). Upon return to the laboratory, sediments were incubated in duplicate under oxic and anoxic conditions for 6–9 h, using the

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Received 20 March 2015; revised 4 August 2015; accepted 10 August 2015

agar plug method of Schulz and Schulz (2005) to supply sulfide to anoxic incubations. Incubations were performed in low P_i artificial seawater to detect small changes associated with P_i release from cells and sediment, and sediments were gently washed in anoxic artificial seawater to remove excess P_i prior to incubation. P_i changes in the incubations were compared against sediment-free controls. P_i concentrations in the supernatants were measured using the method of Hansen and Koroleff (1999), and total cations in the initial and terminal supernatants were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES). Following incubation experiments, sediments were immediately preserved in RNAlater (Thermo Fisher Scientific, Waltham, MA, USA) for metatranscriptomic analysis. cDNA libraries were generated from one of the oxic and anoxic incubation replicates from each location (Supplementary Table S2) using the Ovation RNA-Seq system V2 (NuGen Technologies, San Carlos, CA, USA) as in Orsi *et al.* (2013). A library was also generated from Barbados sediments that were preserved before the incubation experiments, after the P_i addition and collected immediately prior to the washing step (library 'T_0'). Paired-end sequencing was performed via HiSeq and MiSeq platforms (Illumina, San Diego, CA, USA), and reads were assembled *de novo* using IDBA-UD (Peng *et al.*, 2012) following quality screening and rRNA removal by the methods of Jones *et al.* (2015). Complete details are provided in the Supplementary Methods. Raw metatranscriptomic and rRNA amplicon data sets have been deposited in the Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) under accession SRP056300 and assembled contigs were uploaded to the MG-RAST server (http://metagenomics.anl.gov/) under Project ID 5305.

During all anoxic sulfidic incubations, P_i concentrations increased in the supernatant (Figure 1). Conversely, P_i concentrations decreased in oxic incubations and did not change in sediment-free controls (Figure 1). P_i release from SBB sediments was accompanied by a more than twofold increase in iron in the supernatant, but iron concentration did not change during the Barbados incubations (Supplementary Table S2).

Based on rRNA gene and transcript sequencing, Barbados sediments were dominated by *Gamma-*, *Epsilon-*, *Deltaproteobacteria*, *Bacteriodetes* and anaerobic oxidation of methane group II (ANME-II) archaea (*Methanosarcinales*) (Supplementary Figure S2, Supplementary Table S4). The most abundant *Gammaproteobacteria* were close relatives of *Photobacterium* in the *Vibrionales*, and the majority of *Epsilonproteobacteria* were affiliated with clades of environmental sequences related to the genus *Sulfurovum*. The SBB sediment community was dominated by *Gamma-*, *Epsilon-, Deltaproteobacteria*, *Bacteriodetes* and *Planktomyces* (Supplementary Figure S3). SBB sediments also included abundant phytodetrital material as indicated by chloroplast and diatom rRNA sequences.

Differences in mRNA transcripts between anoxic and oxic incubations indicate short-term changes in activity (Supplementary Figure S4) that may have impacted P_i dynamics. Homologues of genes that encode enzymes for poly-P metabolism were differentially represented in the data sets (Figure 2). In prokaryotes, the synthesis and hydrolysis of intracellular poly-P is catalyzed by polyphosphate kinase (Ppk) and exopolyphosphatase (Ppx). Ppk catalyzes the reversible synthesis of poly-P from nucleoside triphosphates (NTP) (Reaction 1), and Ppx catalyzes the sequential hydrolysis of the terminal phosphate residue (Reaction 2).

$$\mathrm{Ppk}: \mathrm{NTP} + \mathrm{polyP_n} \leftrightarrow \mathrm{NDP} + \mathrm{polyP_{n+1}} \quad (1)$$

$$\mathrm{Ppx}: \mathrm{polyP_n} + \mathrm{H_2O} \rightarrow \mathrm{polyP_{n-1}} + \mathrm{P_i} \quad (2)$$

Two types of Ppk are associated with Reaction 1. Ppk1 has a higher affinity for the forward reaction and is typically associated with poly-P synthesis, whereas Ppk2 generally has a higher affinity for the reverse reaction (Achbergerová and Nahálka, 2011).

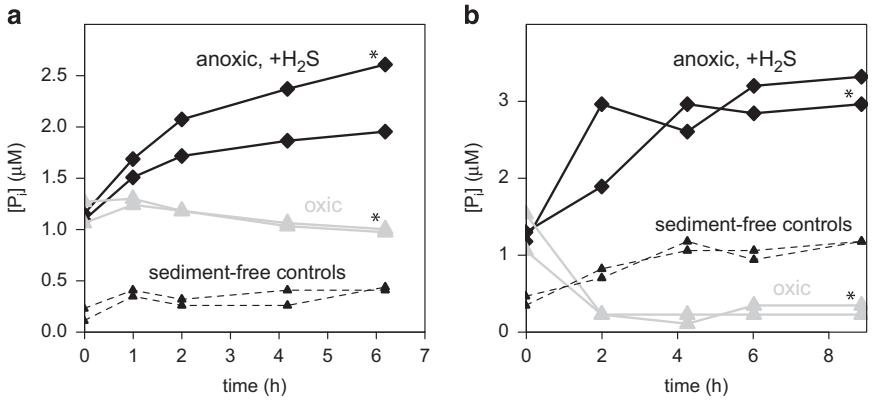

Figure 1 P_i release during (**a**) Barbados and (**b**) Santa Barbara Basin sediment incubations. The (*) symbols indicate samples for metatranscriptomic analysis. Barbados sample T_0 (Figure 2) was collected from the pre-incubation sediment, before the incubations were initiated and prior to washing. Sediment-free controls were incubated under anoxic sulfidic conditions.

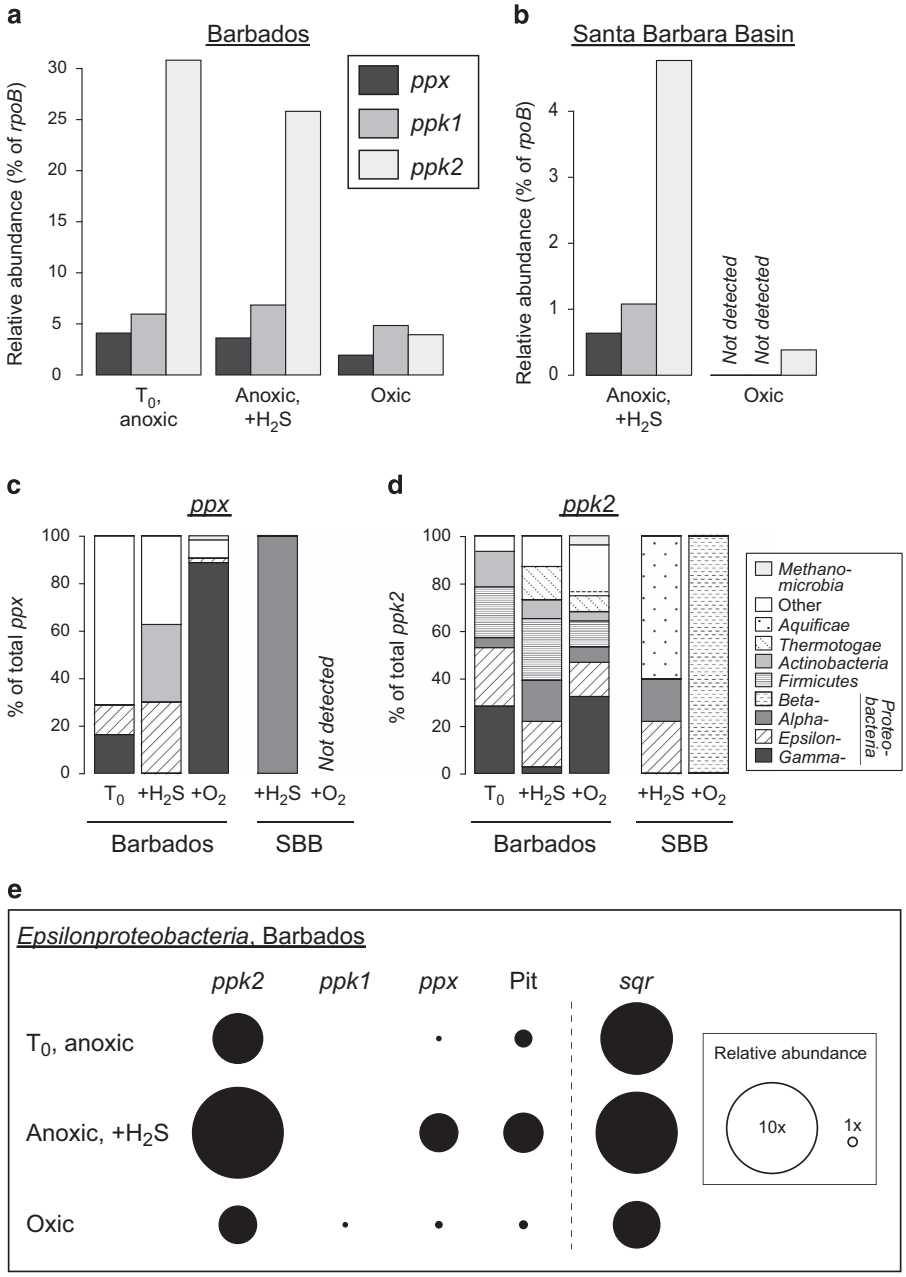

Figure 2 (**a**, **b**) Relative abundance of *ppk1*, *ppk2* and *ppx* homologues in the metatranscriptomes. The relative abundance of each transcript is expressed as a percentage of *rpoB* in each data set. Data set T_0 was generated from the pre-incubation sediment from Barbados. (**c**, **d**) Taxonomic classification of *ppx* (**c**) and *ppk2* (**d**) transcripts in each data set. 'Other' includes unclassified sequences and clades that only occur in that sample (complete information in Supplementary Table S6). SBB, Santa Barbara Basin. (**e**) Relative abundance of *Epsilonproteobacteria* transcripts from Barbados, normalized to epsilonproteobacterial *rpoB*. *sqr* sequences are more abundant than *ppx* and *ppk*, and so are scaled relative to each other in (**e**) (see Supplementary Information for details).

Because Ppx catalyzes the liberation of P_i residues from the poly-P chain, we hypothesized that increased *ppx* expression would be associated with P_i release from cells in sediment pore waters. A role for Ppx in P_i release has been proposed in EBPR (Martín *et al.*, 2006; Zafiriadis *et al.*, 2013), and export of P_i liberated directly from poly-P has been proposed as a mechanism for proton motive force generation in some metabolic models of EBPR organisms (Saunders *et al.*, 2007; Burow *et al.*, 2008; Kawakoshi *et al.*, 2012). Transcripts homologous to *ppx* were detected in the anoxic, but not the oxic, SBB data set, and were 1.4× and 1.25× more abundant in the anoxic sulfidic and T_0 Barbados data sets, respectively, compared with the oxic data set. Furthermore, among the Barbados data sets, certain populations expressed *ppx* differently (Figure 2c). In particular, *ppx* from *Epsilonproteobacteria* were >5.8× more abundant in the anoxic sulfidic Barbados data set than in the other two data sets (Figure 2e).

The low-affinity P_i transport system (Pit) is thought to have a role in energy generation from poly-P

hydrolysis under anoxic conditions, perhaps facilitating the generation of a proton motive force (Saunders *et al*., 2007; Kawakoshi *et al*., 2012). Overall, transcripts homologous to Pit transporters seem to correlate with *ppx* expression ($r^2 = 0.78$, $P = 0.045$) (Supplementary Figure S5,Supplementary Table S5).

Transcripts of genes homologous to *ppk1* were detected in the anoxic, but not the oxic, SBB data sets. In the Barbados data sets, *ppk1* homologues were 1.9× and 2.1× higher in the anoxic sulfidic and T_0 data sets compared with the oxic treatment (Figure 2). However, at both sites, *ppk2* homologues were >6× more abundant in the anoxic as compared with the oxygenated-treatment metatranscriptomes (Figure 2). This suggests that poly-P degradation by the reverse of Reaction 1 may be an important metabolic response to anoxia by certain sediment microorganisms. Accordingly, some models have proposed that poly-P is directly used for NTP generation by Ppk activity under anoxic EBPR conditions, and EBPR organisms often encode multiple *ppk2* (Martín *et al*., 2006; Kawakoshi *et al*., 2012; Motomura *et al*., 2014). Phylogenetic analysis of transcripts homologous to *ppk2* (Supplementary Figure S6) shows that the majority of *ppk2* transcripts expressed in the anoxic treatments are *ppk2* Type I (Motomura *et al*., 2014), which synthesize ATP and other NTPs from poly-P and nucleoside diphosphates (Nocek *et al*., 2008). Ppk2 may represent an important target for future characterization of poly-P metabolism in marine sediments.

Differences in treatment conditions between Barbados and SBB (P_i addition to Barbados, slightly different incubation times) may have impacted gene expression, and thus the incubation experiments from the two sites are not directly comparable. Despite these differences, we observe important consistencies between SBB and Barbados. Transcripts associated with poly-P metabolism were more highly expressed under anoxic versus oxic incubations from both sites, and P_i release was observed in all anoxic incubations. P_i flux observed in experiments from both sites could have been generated, at least in part, by poly-P hydrolysis. Indeed, higher abundances of poly-P-related transcripts in Barbados data sets, as well as a lack of increasing iron in Barbados incubation supernatants (Supplementary Table S2) suggests that poly-P hydrolysis may have been an important mechanism for P_i release from the Barbados sediments. Accordingly, P_i release from the reductive dissolution of iron oxides has been proposed as a source of P_i flux under anoxic conditions in the SBB (Reimers *et al*., 1996). Iron release from sediments in anoxic incubations may have also been masked by the precipitation of iron sulfides in our experiments.

Poly-P hydrolysis by large sulfur-oxidizing bacteria (*Thiomargarita* and other members of the family *Beggiatoaceae*) has been linked to the precipitation of apatite in marine sediments (Schulz and Schulz, 2005), and may be specifically triggered by exposure to sulfide (Brock and Schulz-Vogt, 2011). Interestingly, the Barbados sediments contained *Thiomargarita*-like bacteria ('*Ca.* Thiopilula' spp.), but no poly-P-related transcripts were associated with that population, perhaps because despite their large size, the *Thiomargarita*-like microbes are numerically less abundant than other bacteria in these sediments (Jones *et al*., 2015). However, expressed homologues of sulfide-quinone oxidoreductase (*sqr*) were affiliated with the Epsilonproteobacteria. Sqr catalyzes the first step in sulfide oxidation, which indicates that these smaller sulfur-oxidizing bacteria were likely oxidizing sulfide concurrently with poly-P degradation (Figure 2e). Cold seep sediments have steep redox gradients near the sediment surface, and perhaps as in EBPR reactors and marine upwelling zones, poly-P metabolism may also serve as an energy reserve in these environments. Our finding that multiple different taxa including sulfide-oxidizing epsilonproteobacteria (Figures 2c–e; Supplementary Tables S6 and S7) could represent a source of poly-P-liberated P_i under anoxic sulfidic conditions expands the diversity of organisms that are known to metabolize poly-P under sulfidic marine conditions and opens up the possibility that other taxa contribute to the formation of phosphatic mineral deposits. Although phosphorite is not forming at either location sampled here, authigenic calcium phosphate phases could be forming at lower concentrations, as is observed in other ocean environments (Ruttenberg and Berner, 1993), and poly-P hydrolysis could be involved in the formation of these less-concentrated, but more widespread, phosphates. Increased poly-P utilization under anoxic conditions may also indicate that poly-P has a role in phosphorus cycling in other hypoxic marine settings, including those that are expanding in response to eutrophication and climate change.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

We thank three anonymous reviewers for their contributions that improved this manuscript. This work was supported by a Grant-in-Aid from the University of Minnesota and by National Science Foundation (NSF) grant EAR-1057119 to JB. The Barbados cruise was supported by NSF grant OCE-1031050 to C. Van-Dover and C. Cunningham at Duke University, and the SBB cruise was supported by NSF grant OCE-1230900 to C. Reimers. We acknowledge the Agouron Institute for a generous postdoctoral fellowship to DJ. C. Nguyen and the Minnesota Supercomputing Institute provided computing support and the use of facilities. Special thanks to V. Edgecomb for insightful discussion regarding

metatranscriptomics, and to A. Becker, K. Beckman, A. Hague and D. Gohl at the UMGC for assistance and advice with metatranscriptome and amplicon sequencing. We also thank C. Van-Dover and the crew of the R/V *Atlantis* and ROV *Jason* (AT 21-02) for assisting with sample collection in Barbados and to the crew of the R/V *New Horizon*, C. Reimers, and the participants of the UNOLS Early Career Chief Scientist Training Cruise for sample collection in SBB.

References

Achbergerová L, Nahálka J. (2011). Polyphosphate – an ancient energy source and active metabolic regulator. *Microb Cell Fact* **10**: 63.

Bailey J, Corsetti F, Greene S, Crosby C, Liu P, Orphan V. (2013). Filamentous sulfur bacteria preserved in modern and ancient phosphatic sediments: implications for the role of oxygen and bacteria in phosphogenesis. *Geobiology* **11**: 397–405.

Brock J, Schulz-Vogt HN. (2011). Sulfide induces phosphate release from polyphosphate in cultures of a marine *Beggiatoa* strain. *ISME J* **5**: 497–506.

Burow LC, Mabbett AN, McEwan AG, Bond PL, Blackall LL. (2008). Bioenergetic models for acetate and phosphate transport in bacteria important in enhanced biological phosphorus removal. *Environ Microbiol* **10**: 87–98.

Crosby CH, Bailey JV, Sharma M. (2014). Fossil evidence of iron-oxidizing chemolithotrophy linked to phosphogenesis in the wake of the Great Oxidation Event. *Geology* **42**: 1015–1018.

Föllmi K. (1996). The phosphorus cycle, phosphogenesis and marine phosphate-rich deposits. *Earth Sci Rev* **40**: 55–124.

Goldhammer T, Brüchert V, Ferdelman TG, Zabel M. (2010). Microbial sequestration of phosphorus in anoxic upwelling sediments. *Nat Geosci* **3**: 557–561.

Hansen H, Koroleff F. (1999). Determination of nutrients. In: Grasshoff K, Kremling K, Ehrhardt M (eds). *Methods of Seawater Analysis*. Wiley-VCH: Weinheim, pp 159–226.

Jones DS, Flood BE, Bailey JV. (2015). Metatranscriptomic analysis of diminutive *Thiomargarita*-like bacteria ("*Candidatus* Thiopilula spp.") from abyssal cold seeps of the Barbados Accretionary Prism. *Appl Environ Microbiol* **81**: 3142–3156.

Kawakoshi A, Nakazawa H, Fukada J, Sasagawa M, Katano Y, Nakamura S *et al.* (2012). Deciphering the genome of polyphosphate accumulating actinobacterium *Microlunatus phosphovorus*. *DNA Res* **19**: 383–394.

Martín HG, Ivanova N, Kunin V, Warnecke F, Barry KW, McHardy AC *et al.* (2006). Metagenomic analysis of two enhanced biological phosphorus removal (EBPR) sludge communities. *Nat Biotechnol* **24**: 1263–1269.

Motomura K, Hirota R, Okada M, Ikeda T, Ishida T, Kuroda A. (2014). A new subfamily of polyphosphate kinase 2 (class III PPK2) catalyzes both nucleoside monophosphate phosphorylation and nucleoside diphosphate phosphorylation. *Appl Environ Microbiol* **80**: 2602–2608.

Nocek B, Kochinyan S, Proudfoot M, Brown G, Evdokimova E, Osipiuk J *et al.* (2008). Polyphosphate-dependent synthesis of ATP and ADP by the family-2 polyphosphate kinases in bacteria. *Proc Natl Acad Sci USA* **105**: 17730–17735.

Oehmen A, Lemos PC, Carvalho G, Yuan Z, Keller J, Blackall LL *et al.* (2007). Advances in enhanced biological phosphorus removal: from micro to macro scale. *Water Res* **41**: 2271–2300.

Orsi WD, Edgcomb VP, Christman GD, Biddle JF. (2013). Gene expression in the deep biosphere. *Nature* **499**: 205–208.

Peng Y, Leung HC, Yiu S, Chin FY. (2012). IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. *Bioinformatics* **28**: 1420–1428.

Rao NN, Gómez-García MR, Kornberg A. (2009). Inorganic polyphosphate: essential for growth and survival. *Annu Rev Biochem* **78**: 605–647.

Reimers CE, Ruttenberg KC, Canfield DE, Christiansen MB, Martin JB. (1996). Porewater pH and authigenic phases formed in the uppermost sediments of the Santa Barbara Basin. *Geochim Cosmochim Acta* **60**: 4037–4057.

Ruttenberg KC, Berner RA. (1993). Authigenic apatite formation and burial in sediments from non-upwelling, continental margin environments. *Geochim Cosmochim Acta* **57**: 991–1007.

Saunders AM, Mabbett AN, McEwan AG, Blackall LL. (2007). Proton motive force generation from stored polymers for the uptake of acetate under anaerobic conditions. *FEMS Microbiol Lett* **274**: 245–251.

Schulz HN, Schulz HD. (2005). Large sulfur bacteria and the formation of phosphorite. *Science* **307**: 416–418.

Zafiriadis I, Ntougias S, Kapagiannidis AG, Aivasidis A. (2013). Metabolic behavior and enzymatic aspects of denitrifying EBPR sludge in a continuous-flow anaerobic–anoxic system. *Appl Biochem Biotechnol* **171**: 939–953.

Supplementary Information accompanies this paper on The ISME Journal website (http://www.nature.com/ismej)