Microbial Communities Associated with Phosphoclast-bearing Sediments of the Benguela Upwelling Zone

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Abstract

Phosphorus is a limiting nutrient in the environment and is an important component of many biological molecules. Calcium phosphate mineral deposits, known as phosphorites, are also the primary source of P for agriculture. Understanding phosphorite formation may improve management of P resources. However, the processes that mediate calcium phosphate mineral precipitation in certain marine pore waters remain poorly understood. Phosphogenesis occurs in sediments beneath some oceanic upwelling zones that harbor polyphosphate-accumulating giant sulfur bacteria (GSB). These bacteria may concentrate phosphate in sediment pore waters, creating supersaturated conditions with respect to apatite. However, the relationship between microbes and phosphogenesis is not fully resolved.

To further study this relationship, we examined microbial communities from two sources: sediment cores recovered from the shelf of the Benguela region, and DNA extracted from washed phosphoclasts recovered from those same sediments. We used iTag and clone library sequencing of the 16S rRNA gene to examine the microbial communities and their relationship with the environment. We found that many of our sediments shared large numbers of phylotypes with one another, and that the same metabolic guilds were represented at localities across the shelf. Sulfur-reducing bacteria and sulfur-oxidizing bacteria were abundant in our datasets. Phylotypes that are known to carry out nitrification and/or anammox (anaerobic ammonia oxidation) were also well-represented.

Our phosphoclast extraction, however, contained a distinct microbial community from those observed in the modern sediments. We observed both an enrichment of certain common microbial classes and a complete absence of others. These results could represent an ancient microbial assemblage that was present when the apatite precipitated. While these taxa may or may not have contributed to apatite precipitation, several groups represented in the phosphoclast dataset have the genetic potential, as determined through the analysis of published genomes, to synthesize, and perhaps accumulate, polyphosphate.

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Introduction:

Phosphorus is a nutrient required by all life. It plays a major role in a wide variety of biological processes as a key constituent in many biological molecules such as phospholipids, nucleic acids, and ATP. Biologically available phosphorus is primarily found in the form of phosphate (PO_4^{3-}) that enters the hydrosphere as a result of the weathering of sedimentary and igneous rocks found around the globe. In most environments, phosphorus is either rapidly assimilated by organisms (Föllmi et al., 1996), or it binds to inorganic particles, such as iron. These processes are so efficient at removing phosphorus from the bioavailable pool that is can become a limiting nutrient in many environments such as lakes and large areas of the oceans (Smith 1984; Tyrrell et al., 1999; Elser et al., 2007; Payton et al., 2007; Moore et al. 2013). Phosphorus is also an important agricultural commodity due to its importance in fertilizer production, with millions of tons of phosphorus mined annually (Cho 2013; Paytan et al.; 2007). Globally, eighty percent of phosphorus comes from sedimentary phosphorite deposits (Kauwenbergh 2010). These sedimentary phosphorites are defined as rocks that contain 6-18% P2O5, principally in the form of apatite group minerals such as carbonate fluorapatite (Herman 2014).

For all of its importance, there are still many questions about the processes that are involved in the formation of phosphorites - a process termed phosphogenesis. The initial precipitation of phosphatic minerals occurs primarily in marine pore waters that are supersaturated with respect to precursors of carbonate fluorapatite (Gunnars et al., 2004; Schulz and Schulz, 2005). Most commonly, this occurs in sediments beneath certain

oceanic upwelling zones, such as the Benguela upwelling region off the coast of southwestern Africa, or the Humboldt current off the western coast of South America (Goldhammer et al., 2011). The precipitation of fine-grained apatite associated with phytodetrital polyphosphate degradation also occurs in other marine settings, but not to the concentrations required for phosphorite formation (Diaz et al., 2008). While we have some understanding of the environmental conditions required for the precipitation and concentration of authigenic phosphatic minerals, these conditions can vary and are not fully understood. Additionally, some research has been conducted to better understand the different ages during which phosphogenesis has occurred (Balson et al., 1990; Compton et al., 2016). However, there are still many questions that need to be formally investigated with regards to where, when, how, and why phosphogenesis occurs.

Oceanic upwelling regions are unique oceanic environments that can vary greatly depending regional factors. In upwelling regions, deep nutrient-rich waters are advected to the mixed layer where they drive increased primary productivity. Remineralization of resulting organic matter increases biological oxygen demands resulting in oxygen depleted waters, termed oxygen minimum zones. Upwelling results, in part, from surface winds and the strength of upwelling can be controlled by atmospheric conditions and seasonality (Fennel et al., 1999). Variability in upwelling-driven productivity and delivery of phytodetritus to the sediments results in variable sediment pore water oxygen content along with differences in concentrations of sulfide, sulfate, ammonia and other biologically important cations and anions. (Fennel et al., 1999; Capone et al., 2013).

In these upwelling settings, the initial precipitation of phosphatic minerals occurs within a few of centimeters of the sediment surface, and is thought to be related to microbial activity (Krajewski et al., 1994; Föllmi et al., 1996; Schulz and Schulz, 2005; Crosby et al. 2012); though subsequent sedimentary reworking processes are thought to be important for concentrating the phosphatic minerals into phosphorites (Filippelli et al., 2011). Accumulations of fluorapatite (Ca5(PO4)3(F), and other apatite group minerals, have been found in sediments collected from the Benguela upwelling zone, as well as from other upwelling zones. In some parts of the organic-rich ooze known as the Benguela mud belt, accumulations of phosphatic minerals and pore waters supersaturated with respect to calcium phosphate minerals, co-occur with the presence of GSB, such as *Thiomargarita* spp. (Schulz and Schulz, 2005; Goldhammer et al., 2010). These Gammaproteobacteria are distinctive for their large sizes and the ability to sequester nitrate in a large central vacuole, as well as store elemental sulfur, glycogen, and polyphosphate as granules in the cytoplasm (Schulz et al., 1999).

As with the polyphosphate-accumulating bacteria that are enriched in wastewater systems, *Thiomargarita spp.* are thought to accumulate polyphosphates under oxic conditions and hydrolyze it when exposed to anoxic or sulfidic conditions. This polyphosphate hydrolysis causes GSB to release phosphate into sediment pore waters that can lead to periodic spikes in local aqueous phosphate concentrations (Schulz and Schulz, 2005; Goldhammer et al., 2010). It is this concentration of phosphate within confined horizons in the sediment pore waters that is thought to drive calcium phosphate mineral

precipitation in these sediments- a hypothesis that is supported by labeling experiments (Goldhammer et al., 2010).

There are, however, upwelling systems where this process is not evident. In sediments beneath the Humboldt upwelling system, for example, the giant sulfur bacteria *Thioploca spp*. were found to correlate with increased phosphate concentration, but not calcium phosphate mineral accumulation (Holmkvist et al., 2010). Additionally, these *Thioploca* did not exhibit polyphosphate granules at the time of collection. Jones et al. (2015) recently conducted a transcriptomic investigation of sediments off Barbados that contain *Thiopilula* sp., a close relative of *Thiomargarita* within the family *Beggiatoaceaea*. In incubation experiments, the *Thiopilua*-containing sediments released phosphate in a manner and magnitude similar to Namibian sediments containing *Thiomargarita*. However, no polyphosphate related gene expression by *Thiopilula* was detected. Instead, diverse taxa exhibited expression of genes associated with polyphosphate accumulation and polyphosphate hydrolysis. These findings suggest that other polyphosphate metabolizing organisms, in addition to *Thiomargarita*, may contribute to the enrichment of phosphate in phosphogenic sediments.

Previously, preliminary molecular characterization of microbial communities from sediments of the Benguela upwelling zone was conducted using DGGE and clone libraries (Schäfer et al., 2007). Here, we continue the study of the Begnuela region by attempting to determine the environmental factors that may be controlling community composition. We do this through the use of a combination of geochemical and iTag 16S rRNA gene sequencing approaches to characterize sediment-hosted microbial

communities from sediments that have previously been shown to host phosphorites and giant sulfur bacteria. We also extracted DNA from washed phosphatic grains. This DNA may represent a relisted microbial community that may have been relevant in past phosphogenic events. We used both 16S rRNA gene methods and clone libraries to compare this past community to the current sediment communities.

Materials & Methods:

Sample Location:

Sediment samples were collected using a multi-corer at nine marine sites located off the western coast of Namibia, in the Benguela upwelling zones during two cruises on the R/V Mirabilis. The first of these cruises took place in March 2014, and the second in May 2015. The samples locations were between latitudes 23° and 26° south and longitudes 12° and 14° west (Figure 1). Samples for DNA sequencing were immediately placed in RNA Shield and stored at -80° C. Phosphoclasts were extracted from one sediment sample as described below. Pore waters for geochemical analysis were collected and filtered (0.15 µm) via Rhizon CSS samplers (19. 21.23, Rhizosphere, Netherlands), acidified, and then stored anaerobically in BD Vacutainer® tubes (5014862, BD) at 4°C. $ZnCl_2$ was used to preserve samples stored at 4° C for sulfide measurements.

Geochemical Analysis:

Concentrations of fluoride, acetate, formate, chloride, nitrite, bromide, nitrate, phosphate, sulfate, oxalate, and thiosulfate were measured via ion chromatography. Chromatography was performed at the Analytical Geochemistry Lab at the University of

Minnesota using a Dionex (Sunnyvale, CA) ICS-200 ion chromatograph system consisting of an AS19 analytical column, AERS 500 suppressor AS40 auto-sampler and integrated dual piston pump and conductivity detector. Sulfide measurements were performed using the Cline assay (Cline 1968).

DNA Extraction from Phosphoclasts:

Phosphoclasts were isolated from a core collected from the continental shelf off Luderitz, Namibia (2600.001°N, 1424.414°E.; Sample name Phos). The phosphoclasts were separated from bulk sediment removed from a depth of 11-12 cm beneath the sediment/water interface using a series of mesh sieves (50 µm, 100 µm, and 250 µm). The separation procedure consisted of placing sediment on progressively larger-sized sieves and washing off smaller particles by rinsing the sediments with sterilized artificial seawater. Once sediment particles smaller than 250 µm had been removed, larger phosphoclast grains were identified under a dissecting microscope and were manually separated from the rest of the bulk sediment. Phosphoclasts for DNA extraction were then placed in a 15 mL falcon tube, and allowed to dry completely overnight.

The separated phosphoclasts were then washed to remove all DNA from their exterior surfaces. The washing was done using a protocol similar to that described in Mason et al. (2015). In brief, the sample was placed in a 50 mL Falcon tube and rinsed in a solution of 1x filter sterilized phosphate buffered saline (PBS). The tube was then sonicated for 45 seconds at 8 W. The tube was then centrifuged at a speed of 4000 x g for 5 minutes and the supernatant was removed. Fresh filtered PBS was then added and

the procedure was repeated twenty-one times. To determine whether or not we had removed all exterior DNA from the phosphoclasts, one mL of supernatant was collected after the third, sixth and ninth, fifteenth, eighteenth, twentieth and twenty-first rinses and was tested for amplifiable DNA via PCR (procedure described below). Once it was confirmed that no amplifiable DNA was present, .101 g of phosphoclasts were manually pulverized in a sterile and nucleotide free bowl. All tools and vessels used for this procedure were rinsed in 70% ethanol, nuclease free water, DNA-off (Takara Bio) and then autoclaved at 121 °C for 30 minutes.

DNA Extraction:

DNA extractions were performed on environmental sediment samples, washed and pulverized phosphoclasts, and the rinsate collected from the phosphoclast washing procedure. All DNA extractions were performed using a Powersoil DNA Isolation kit with a modified version of the manufacturer's protocol (Mo Bio Laboratories, Carlsbad, CA, USA). The protocol was modified by adding 3 separate bead beating steps, each of 5 minutes. After the first two bead beating steps, 250 µL of the lysate was removed from the bead beating tube and placed in a separate sterile 1.5 mL tube. The DNA from these tubes was then pooled with the DNA that remained in the bead beating tube after the final bead beating step. The manufacturer's protocol was followed for the rest of the extraction process. The removal of portions of the lysate and the additional bead beating steps were done to reduce potential bias imparted by the bead beating process.

The rinsates were also tested for amplifiable DNA. This was done via PCR (polymerase chain reaction) using a Bio-Rad C1000 Thermocycler. The PCR's were run for 30 cycles

using bacteria-specific primers 27F and 1492R (see Table 1). All PCR products were then run on a 1% agarose gel. No amplifiable DNA was detected in the twentieth and twenty-first rinsate.

DNA Amplification and Sequencing:

DNA extracts from our sediments and phosphoclasts were quantified using a Nanodrop 2000. PCR was then used to confirm that the 16S rRNA gene could be amplified from each sample. The PCR's were performed using 27F and 1492R primers (Table 1). Each reaction totaled 25 μL and was performed using GoTaq® Green Master Mix 2X (Promega, Fitchburg, WI, USA) along with 0.5 μL of each primer at a 10 μM concentration. PCR products were then run on a 1% agarose gel to confirm amplification visually. Once positive amplification had been confirmed, the samples were sent to the University of Minnesota Genomics Center for the generation of Illumina MiSeq paired-end 300 bp reads (itag) amplicon library of the V4 hypervariable region of the 16S rRNA gene.

Amplicon Processing:

Once sequences were generated, data processing was completed using an in-house pipeline. Sickle (https://github.com/najoshi/sickle) was used to trim and filter raw sequences using a quality score of 30 and adaptor sequences were removed using cut-adapt (Joshi et al., 2011; Martin., 2011). PhiX were removed using NCBI- BLASTn (Altschul., 1990). Reads were then assembled using the Paired-END Assembler (Zhang et al., 2013). Uparse was then used to cluster at 97% sequence similarity (Edgar, 2013). Mothur was then used to classify sequences according to SIlva taxonomic assignments

(Silva SSU Ref NR 99 119 database) (Schloss et al., 2009; Quast et al., 2013; Yilmaz et al., 2014). Statistical analysis was then done in the R environment using the Vegan package. Non-metric multidimensional scaling (with 2 dimensions and a Stress value of .044) was done using R. Heat maps were generated in R using the Bray Curtiss distance method and UPGMA Linkage method. Cluster analysis, and rarefaction curves were also created using R(R Core Development team, 2008; Oksanen et al., 2016).

Clone Libraries:

A clone library was generated from the DNA extracted from the washed and pulverized phosphoclasts in order to provide additional phylogenetic resolution of DNA preserved therein. DNA amplification was done using 27F and 1492R primers (Table 1). 3'-A overhangs were repaired using the protocol described in the TOPO TA cloning kit and GoTaq® Green Master Mix 2X (Promega, Fitchburg, WI, USA). The repaired DNA was then cleaned and concentrated using the DNA Clean and Concentrator -5 kit (Zymo Research, Irvine CA USA). Cloning was then performed using the TOPO® TA cloning kit and standard protocol (incl. pCR®4-TOPO®Vector and One Shot® Match1TM T1®chemically competent E. coli cells) (Life Technologies Corporation, Grand Island, NY, USA). Once cloning was complete, 104 colonies were picked and screened using a restriction enzyme digest cut with the HaeIII enzyme. 95 clones were chosen and sent for Sanger Sequencing using 27F and 1492R primers (Table 1). Sanger sequencing was performed by the ACTG Corp using an ABI 3730XL DNA analyzer and BigDye terminator version 3.1 (applied Biosystems, Grand Island, NY USA). Sanger sequences

were assembled using Unipro Ugene (Okonechnikov et al., 2012). All assembled sequences were > 1100 bp in length.

Phylogenetic Analysis:

Assembled 16S rRNA gene sequences were pre-aligned using the SINA aligner (Pruesse et al., 2012). The sequences were then imported into a Silva database V123.1 in ARB V.6 and were manually aligned using the ARB_Edit4 sequence editor tool (Ludwig 2004, Westram 2011, Quast 2013, Yilmaz 2014, Pruesse 2006 & 2012). Neighbor-joining trees were built in ARB using the Jukes-Cantor correction and end-clipped sequences (Jukes & Cantor 1969, Saitou & Nei 1987). A second Neighbor-joining tree was then created using the same full length sequences and select OTUs from the phosphoclast sample. This second tree was created using only the V4 hypervariable region instead of the whole 16S rRNA gene. Sequences were exported from ARB and were then used for Maximum Likelihood analysis that was performed using RAxML v. 8.0.24 with the GTR nucleotide substitution model with a gamma distribution (Stamatakis 2014). A thousand bootstrapping replicates were done using the RAxML's rapid bootstrap equation. Once bootstrapping was complete, the EPA (evolutionary placement algorithm) equation was used to add select OTUs to the final Maximum Likelihood trees (Berger et al., 2011).

Identification of Genetic Potential of Select OTUs:

We examined several of the OTUs classified as Betaproteobacteria, Gammaproteobacteria, and Deltaproteobacteria in our phosphoclast extraction for the genetic potential to accumulate polyphosphate. We did this by analyzing the genomes of

taxa from the same genera as our OTUs. We used the IMG database to search genomes for specific genes (Markowitz et al, 2012). We selected all available genomes from these specific genera and used the IMG search function tool to look for the presence or absence of the *ppx* (exo-polyphosphatase) and *ppk1* (polyphosphate kinase I) genes. We used KEGG to determine the presence or absence of the *ppk1* or *ppx* genes (Kanehisa et al. 2000 & 2016). If *ppk1* or *ppx* genes were present in the genome, we noted that representatives of that genus possess the genes that may allow them to accumulate polyphosphate within the marine sediments (Table 2).

Results & Discussion:

The precipitation of apatite-group minerals that fuel phosphogenesis has long been suspected to be driven by microbial processes. More recently, the metabolism of polyphosphate by giant sulfur bacteria has been linked to the precipitation of apatite in shelf sediments off Namibia's coast. However, other marine bacteria are known to metabolize polyphosphate and may also be important in influencing the saturation state of phosphatic minerals in sedimentary pore waters (Jones et al., 2015). We produced and analyzed Illumina iTag amplicon libraries from ten Namibian shelf sediment samples that occur in the vicinity of phosphorites to better understand the microbial communities that may be contributing to phosphogenesis. Additionally, we extracted DNA from cleaned phosphoclasts in order to compare modern microbial communities from the Namibian shelf with DNA preserved in phosphoclasts. We hypothesized that relict DNA preserved in Namibian shelf phosphoclasts would be similar in composition to extant communities from Namibia shelf sediments that are associated with active phosphogenesis. We also

hypothesized that the microbial diversity of chemolithotrophs and anaerobes would be strongly influenced by variations in local sediment pore water geochemistry due to seasonal changes in the upwelling regime.

Microbial Diversity in Sediment Samples:

iTag libraries produced from our sediment samples ranged in size from 28,400 to 265,406 sequences and revealed both a broad range of microbial diversity within individual sediment samples and a range of diversity between sample sites (Supplementary Figure 5). An examination of our OTU libraries revealed that three classes appeared in all ten of our sediment samples: *Deltaproteobacteria, Anaerolineae,* and *Gammaproteobacteria*. *Deltaproteobacteria* made up between 16% and 28% of individual iTag libraries and consisted mostly of five major orders. OTUs from the order *Desulfobacterales* were the most abundant OTUs in every library, ranging from 29% to 75% of Deltaproteobacterial sequences. Along with Desulfobacterales, the libraries contained varying amounts of OTUs from the orders Myxococcales, Sh765B-TzT-29, Sva0485, Syntrophobacterales and Desulfarculales. There were also large numbers of Deltaproteobacteria OTUs that could not be classified below the class level in all of our libraries. Many of the Deltaproteobacteria found within our samples are putative sulfate reducers. Desulfobacterales, Syntrophobacterales and Desulfarculales are all orders that are known to be completely or partially comprised of sulfate reducing bacteria. These organisms could be contributing in part to the sulfidic conditions found in Benguela sediments (Detmers et al. 2001, Wright et al. 2012, Parson et al. 2015) Along with sulfate reducing bacteria we also found groups whose roles are not fully understood such as

Myxococcales (which are most likely heterotrophs), Sh765B-TzT-29, and Sva0485 (Brenner 2005).

After *Deltaproteobacteria*, OTUs associated with the Anaerolineaceae, and Gammaproteobacteria were the next most abundant classes. Representatives of the Anaerolineaceae made up between approximately 1% and 12% of the community for all samples. All of the detected Anaerolineae sequences were from the family Anaerolineaceae: however, the exact genera represented by these sequences could not be determined using BLAST. Anaerolineaceae and related groups from the Chloroflexi have been previously detected in Namibia and other upwelling sediments. Anaerolineaceae are anaerobic heterotrophs that can use fermentation, though the full range of conditions that they can tolerate are not fully known (Blazejak & Schipper 2012, Nunoura et al. 2013).

OTUs representing the *Gammaproteobacteria* made up between ~4% and 31% of all libraries. The most abundant of the Gammaproteobacterial OTUs found in our libraries were from the orders Xanthomondales (~1-20% of Gammaproteobacteria), Alteromonadales (~6-24%), Oceanospirillales (~2-15%), Thiotrichales (~<1%-24%), Chromatiales (~<1-17%), Sva0071 (~<1-8%), CS-B046 (<1-18%), and Gammaproteobacteria_Incertae_Sedis (~1-26%). There were also many other classes of Gammaproteobacteria that made up smaller portions of the population. There were also Gammaproteobacteria that could not be classified below the phylum level. These unclassified Gammaproteobacteria made up approximately 30-40% of our libraries. Many of the Gammaproteobacteria present were sulfur oxidizing bacteria, many of

which have been found in sediments from other oxygen minimum zones (Wright et al. 2012). OTUs assigned to the order Thiotrichales may represent giant sulfur bacteria such as *Beggiatoa* and or *Thiomargarita spp.,* while the Gammaproteobacteria_Incertae_Sedis and Chromatiales may also represent taxa that oxidize sulfur. Xanthomondales, Alteromonadales, and Oceanospirillales are, however, most likely heterotrophic. The metabolic capabilities of the orders Sva0071 and CS-B046 are not fully understood.

There were also several other classes of organisms that were well-represented in many of our samples. These include representatives of the clades Nitrospira, Planctomyces, Acidimicrobiia, Deferribacteres, BD2-2, the archaeal group Thaumarchaeota, along with others. The class Planctomyces, Deferribacteres, Ignavibacterium and BD2-2 were found in all of our samples and ranged in abundance from around 1% of total sequences to approximately 3.5%. Along with these, Holophagae, Phycisphaerae, Spirochaetes, Sphingobacteriia, Dehalococcoidia, Acidimicrobiia, Clostridia, and Flavobacteriia all made up more the 2% of the community in at least one sample.

The archaeal classes represented by a large abundance of OTUs were from the Marine Benthic Group B and Thermoplasmata. These OTUs include members of the Thaumarchaeota, which could play a major role in the cycling of nitrogen under anoxic conditions [Do you mean ammonia oxidation? This should be under aerobic or microaerophilic conditions then, correct? Please check and fix.]. OTUs assigned to Marine Benthic Group B were found in all of our samples in low abundance, however,

they were most prevalent in samples C2 and C3, where they made up approximately 3% of the libraries. Thermoplasmata OTUs were also present in all of our samples, but were most prevalent in samples C2, C3 and S1. Along with the classified sequences there were a fraction of organisms that could not be classified at the class level. These were grouped as 'unclassified' organisms and ranged in abundance from approximately 7% to 28% of the sampled communities.

Sites were also examined to determine if there was a relationship between OTU distribution, pore water chemistry, and geographical location. To examine these similarities, NMDS (non-metric multidimensional scaling) analyses were performed using both the entire iTag libraries, as well as subsets of the libraries which contained only species that made up two or more percent of sequences in at least one sample (Figure 2). NMDS analyses showed that sites that more geographically similar sites tended to plot closer in ordination space, representing that they contained more similar OTUs than sites which are more distant geographically. This was true for both the analyses done with full libraries as well for the NMDS analyses performed with subsampled datasets. Samples from our core samples (samples C1-C3) also plotted closely together, indicating that depth may be less of a factor then location. Heat-maps of the most abundant OTUs per site were also generated to better visualize abundance differences between sites (Figure 3). We also attempted to determine what pore water factors controlled the distribution of OTUs [make sure you don't use apostrophes for plural, even with an acronym like OUT, so OTUs not OUT's], However, through both an examination of the data (supplementary table 2) and through several statistical tests we

found that there was not enough variation in our pore water chemistry to explain variations in OTU abundance by differences in water chemistry for those chemical species we measured.

The Benguela Microbial Community:

The compositions of the microbial communities of our sediment samples were similar to those found in previous studies of the Benguela region as well as to those found in other oceanic upwelling zones, such as in the Arabian sea and Humboldt current (Blazejak & Schipper 2012, Wright et al., 2012, Arning et al., 2016). Samples were visually examined for the presence of giant sulfur bacteria, and *Thiomargarita spp.* were found in shallow sediments (<3 cm beneath the sediment/water interface) at all sampling sites. (Schulz & Schulz 2005). Our Illumina-based sequencing, however, only gives a very limited view of GSB diversity within our 16S rRNA libraries. *Thiomargarita spp.* and other *Beggiatoaceae* are difficult to amplify with standard 16S rRNA primers due to factors such as the presence of introns within the 16S rRNA gene (Salman et al., 2011 & 2012). We did detect reads that were classified as *Thiomargarita spp.* in several of our libraries, however, our microscope observations indicated that *Thiomargarita spp.* were present at all of our sampling sites.

Along with GSB, we see a variety of other organisms that are likely playing an active role in the sulfur and nitrogen cycles that could be affecting apatite precipitation in sediment pore waters. There is evidence that a combination of sulfur oxidizing and sulfur reducing bacteria (SRB) may be important to phosphogenesis, though the role of SRB is not well understood (Arning et al., 2016). Our sediment libraries primarily contain OTUs

representing two abundant orders alongside several less abundant orders of SRB. The most well-represented orders in all of our libraries were *Desulfobacterales* and *Desulfarculales.* Both of these orders have been previously detected in sediments from the Benguela region and have links to both sulfur cycling when coupled with sulfur oxidizers, as well as methane oxidation within the sediments (Schäfer et al., 2007, Wright 2012, Arning 2016). There is both 16S rRNA gene and lipid biomarker evidence that SRB are abundant in upwelling sediments both from Namibia and the sediments in other upwelling systems. Evidence of SRB has also been detected within ancient phosphorite deposits, such as the Miocene Monterey Formation in California, USA (Berndmeyer 2012, Arning 2016). Due to their ubiquity in regions where phosphogenesis is occurring, or has occurred, it has been thought that SRB may be contributing in some way to the process of phosphogenesis along with GSB such as *Thiomargarita (*Schulz & Schulz 2005). However, whether this association is purely based on sulfur cycling between SRB and GSB or whether SRB may play some other role is not known (Wright 2012 el al., Arning et al., 2009 & 2016).

Along with GSB and SRB, we have also detected other organisms that may play a role in the nitrogen, phosphorus and sulfur cycles. Members of the genus *Sedimenticola* have the capacity to oxidize a variety of sulfur species (using nitrate as an electron donor under anoxic conditions) and are known polyphosphate accumulators (Flood et al., 2015). OTUs representing several groups of ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA), the most abundant of which belong to the classes Nitrospirales and Thaumarchaeota, are also present in our datasets. Both of these

groups of organisms contain members that have the ability to oxidize ammonia under micro molar oxygen conditions, allowing for an active nitrogen cycle within the suboxic and anoxic OMZ. Both N*itrospirales* and *Thaumarchaeota* have also been found in previous genomic studies of the region, as well as in studies of other upwelling regions and OMZs (Lam & Kuypers 2011, Fussel et al., 2012). Members of the family *Planctomycetes* were also detected in all of our sediment samples. Certain *Planctomycetes* are known to carry out anaerobic ammonia oxidation (ANAMMOX) and have been found, to varying degrees, in other studies of Namibian shelf sediments (Kuypers et al., 2005, Woebken et al., 2007, Lam & Kuypers 2010).

DNA Extracted from Phosphoclasts:

An iTag amplicon library was generated from our phosphoclast extraction that resulted in 79315 sequences, a dataset that was significantly different (state which test here; p=3.62316E-07) from our other Namibian sediment libraries (Figure 4). The phosphoclast extraction library was dominated by members of the *Gammaproteobacteria,* which make up approximately 33% of the total sequences. These *Gammaproteobacteria OTUs*, however, were not well classified below the class level. Sixty-eight percent of the total *Gammaproteobacterial* OTUs were unable to be classified at the order and/or genus levels. Of those OTUs that we could classify below the class level, , those assigned to *Thiohalophilus,* a genus of sulfur-oxidizing bacteria, were most abundant. Representatives of the *Nitrosococcus* and *Sedimenticola* clades were also present. _All three clades may be important to target for examining the genetic potential for phosphogenesis within Namibian sediments, due to their ability to accumulate

polyphosphate, or their close relation to organisms that are known to have this ability (Flood et al.2015, Markowitz 2012).

Deltaproteobacteria made up approximately 20% of the total sequences from the phosphoclast extraction library. The most abundant *Deltaproteobacteria*-associated sequences were from the order Desulfobacterales, which made up 73% of the Deltaproteobacterial OTUs. These were followed by the orders Sh765B-TzT-29 which made up 10% and Myxococcales which made up 4%. Several other orders also made up smaller fractions of the total library. Along with Deltaproteobacteria and Gammaproteobacteria, five other classes were present in our phosphoclast libraries in the following abundances: Nitrospira at 9%, Marine Benthic Group B at 9%, Betaproteobacteria at 8%, Acidomicrobiia at 7%, and Alphaproteobacteria at 2%. Nine percent of the species were unable to be classified at the class level.

A clone library was also generated from the DNA extracted from the phosphoclasts, in order to provide greater phylogenetic resolution for some taxa. Twenty-four nearly full-length 16S rRNA gene sequences were generated from our clone library. Maximum likelihood trees were calculated to determine their phylogenetic position (Figure 5 & Supplementary Figure 1-4). Of the twenty-four clone library sequences, four sequences were placed in the class *Nitrospira* and in the order *Nitrospirales*. Of these four, three were placed in the genus *Thermodesulfovibrio,* and one was placed in the group *4-29*. Fourteen sequences were placed in the class *Deltaproteobacteria*. Of these fourteen, six were placed in the order *Desulfurivibrio,* one was placed in each of the following orders: Bdellovibrionales, Myxococcales,

Desulfobacterales, and 43F-1404R, and four were placed in the order Sh765B-TzT-29. Six clones were also placed in the class Gammaproteobacteria. Of these six, three were placed in the genus *Sedimenticola* and three were placed in the order Ectothiorhodospiraceae. Several of our top amplicon OTUs were also added to our maximum likelihood trees to gain a better phylogenetic context for those sequences. OTUs were placed in the Gammaproteobacterial genera *Nitrosococcus*, Ectothiorhodospiraceae, and Thiohalophilus, as well as in the Deltaproteobacterial genera Bacteriovoracaceae, Desulfobulbaceae, Myxococcales, and Sh765B-TzT-29, and finally, in the class Nitrospira in the order Nitrospirale.

Difference between the Sediment and Phosphoclast Extractions:

The amplicon library from our phosphoclast extraction was significantly different from the ones extracted from our sediment samples. These differences can be seen both in our NMDS (Figure 2) and the radar plot of the top phosphoclast OTUs (Figure 6). There were two primary differences between our phosphoclast library and sediment libraries. First, the phosphoclast extraction contains far less OTU diversity (Supplementary Table 1 & Supplementary Figure 5) than the sediment libraries; second, the phosphoclasts are also greatly enriched in certain OTUs when compared with the sediment samples (Figure 4). We saw a large increase in specific orders of Gammaproteobacteria and Deltaproteobacteria within our phosphoclast libraries that we did not see in the sediment communities. Primarily, we saw enrichment of OTUs from the orders of Desulfurivibrio and Gammaproteobacteria Incertae Sedis (which include the genera *Sedimenticola* and *Thiohalophilus*) along with a large increase in the number of

unclassified Gammaproteobacteria and Deltaproteobacteria at the order level. We also saw increased abundances of OTUs from the classes Marine Benthic Group B, Nitrospira, Alphaproteobacteria, and Betaproteobacteria that are not seen in any of our sediment samples. While the phosphoclast samples were enriched in OTUs from certain classes, they were depleted in OTUs from other classes relative to the extant sediment communities. For example, OTUs from the Deferribacteres, Anaerolineae, and BD2-2, were strongly depleted in the phosphoclast library relative to the sediments. Anaerolineae and Deferribacteres for instance averaged 6.85% and 2.5% in our sediment samples and made up of only 1.2% and .4% of our phosphorite library. (Figure 6).

The difference in community composition between our extant sediment communities and the phosphoclast extraction could result from a variety of different factors. We hypothesize that the DNA extracted from the phosphoclasts represents microbes that were present in the sediment pore waters when apatitic mineral phases precipitated. Previous studies have established that DNA and cells from microbes present in sediment pore waters can be preserved by the precipitation of authigenic phases (Mason et al., 2015; Case et al., 2015; Stevens et al., 2015). In some cases, cells entombed in mineral precipitates can remain active, or may become dormant, or the cellular remains may be protected from degradation. For example, authigenic carbonates and barites have been shown to preserve DNA, and sometimes active cells, entombed in minerals precipitating in their host marine sediment (Stevens et al 2015). While this is, to our knowledge, the first report of DNA extracted from marine authigenic phosphoclasts, experimental results have previously shown that hydroxyapatite can protect DNA from

degradation (Brundin & Figdor, 2013). Additionally, numerous studies have recovered DNA from apatite in ancient teeth and bones (Paabo et al., 2004., Brundin & Figdor, 2013, Lejzerowicz et al., 2013, Weyrich et al., 2015, Huynh et al., 2016).

The differences in community composition between the phosphoclast extraction and the extant sediment libraries could reflect differences in conditions between the past environment and the present day. For example, all but two of our sediment samples were collected from the sediment/water interface. Our phosphoclasts were collected from a sediment depth of 11-12 cm, but we do not know at what depth those phosphoclasts formed in the past. The model of Schulz and Schulz for Namibia phosphorite formation would suggest that phosphatic minerals initially precipitate near the sediment/water interface where polyphosphate metabolizing *Thiomargarita* are most abundant. But it is possible that these phosphoclasts formed under different conditions in the past, perhaps at a greater depth where geochemical and oxygen conditions may have been different. As sediment depth increases, sulfate is reduced to sulfide by sulfate reducing bacteria. The relative increase in OTUs from the orders *Desulfurivibrio* and *Desulfobulbus* could therefore result from phosphoclast precipitation occurring at a greater depth beneath the sediment/water interface than is represented by our shallow sediment samples**.** We do not see an enrichment of sulfur reducing bacteria in the present core (samples C1-C3), however, this does not preclude a past enrichment if conditions in the sediment were different from their present state.

The differences in abundance within the community may also result from the fact that only a fraction of the community may have been physically entombed within the

precipitating authigenic minerals that comprise the phosphoclasts. This subset could preferentially preserve those organisms that contributed to phosphate supersaturation through polyphosphate metabolism (as described below), but because the supersaturation conditions are thought to be the result of many bacteria in broad zones of marine pore water, it is unlikely that other organisms in the surrounding pore waters would not also be incorporated. It is also possible that some organisms degraded faster than others, and preferential preservation occurred. These factors might explain why the community or communities represented by the phosphoclast extraction is depleted with respect to certain taxonomic groups such as Acidomicrobia and Deferribacteres relative to the modern sediment communities. However, they do not provide an explanation for the relative enrichment of the phosphoclast extraction in certain taxa.

It is also possible that processes that occurred after the precipitation of the minerals may have influenced the DNA assemblage that was ultimately recovered. For example, ancient DNA studies can be complicated by the potential for contamination that postdates mineral precipitation. Our phosphoclast washing process was very stringent and we detected no amplifiable DNA on the exterior surfaces of our phosphoclasts. This is a strong indicator that the DNA we extracted after washing comes from the interiors of the phosphoclasts, which are inaccessible to contamination.

While a sample of a single authigenic precipitate formed over a short period of time may preserve a snapshot of the community at the time of precipitation, in this case, the phosphoclasts themselves likely represent a time-averaged grain assemblage, and thus the taxa represented by the amplicon and clone library results likely include the remains

of organisms that lived at different times. We have not yet dated our phosphoclasts, so they may originate from a wide range of time periods. There is evidence that several episodes of phosphogenesis have occurred in the Benguela region over the last 7 million years (Willerslev & Cooper, 2005; Compton et al., 2016). The phosphoclasts will have to be dated to find an exact age and determine during which period of phosphogenesis they may formed. Taking these complications in understanding the content and context of ancient mineral-hosted DNA into account, the community represented by our phosphorite extraction is very likely composed of one or more ancient microbial sediment communities that lived in conditions that were conducive to the precipitation of authigenic apatite.

While the presence of an organism within an authigenic precipitate is not by itself evidence that the organism contributed to the mineral precipitation, in the case of authigenic marine phosphorites, there is compelling evidence that polyphosphate metabolism plays a role in creating conditions that allow for carbonate fluorapaite (or its precursors) to precipitate. In order to assess the possibility that some of the taxa in our phosphoclast extraction may have contributed to phosphogenesis, we examined the genomes of sister taxa for indications of the ability to metabolize polyphosphate.

Genetic Potential:

In addition to our phylogenetic characterization of the sediment and phosphoclast-associated communities, we also examined the genetic potential of some of the sister taxa of our most abundant OTUs to accumulate polyphosphate. Polyphosphate accumulation, and its subsequent hydrolysis, is thought to be important for phosphogenesis, because the

processes can modulate the concentration of phosphate in localized pore waters in response to environmental stimuli. Giant sulfur bacteria, specifically *Thiomargarita spp.*, have been linked to phosphogenesis via polyphosphate metabolisms in the Benguela region (Schulz and Schulz 2005 Goldhammer et al., 2010).. However, many other taxa, such as Burkholderia, *Sedimenticola,* and Actinobacteria, that have the ability to accumulate polyphosphate under different conditions (Kuroda & Ohtake, 2000; Mullen et al., 2002; Seufferheld et al., 2008 Nakamura et al., 1991, Keller et al., 1999., Flood et al., 2015). Along with these known groups there are others such as *Desulfobulbus* that have the potential to accumulate or use polyphosphate but have not been studied.

To examine the possibility that other bacteria present in our libraries are capable of metabolizing polyphosphate, we looked for the primary gene responsible for polyphosphate formation, polyphosphate kinase I (*ppk1*), and one of the genes responsible for the degradation of polyphosphate, exopolyphosphatase (*ppx*). The presence of both genes in a genome is indicative of the potential to synthesize and hydrolyze polyphosphate (Markowitz et al, 2012, Campbell et al., 2011)). We used the Joint Genome Institute's Integrated Microbial Genomes & Microbiomes (IMG/M) system to search the genomes of organisms related to some the most abundant OTUs that were identified phosphoclast extraction iTag library. Using IMG, we found that several of these genera did have the genetic potential to metabolize polyphosphate (Markowitz et al, 2012). The potential to metabolize polyphosphate is a requirement for polyphosphate accumulation, however, an unambiguous genetic marker for polyP accumulation has yet to be identified

We found the *ppk1* and *ppx* genes in many of genera that we investigated. Both *ppx*. and *ppk1* were present in the genomes of *Nitrosococcus* spp., the most abundant *Gammaproteobacterial* genus in our sediments. In addition, polyphosphate granules have been reported in at least three species of *Nitrosococcus* (Campbell et al., 2011).

The *Thiohalophilus* OTUs in our phosphoclast extraction library are closely related to *Sedimenticola* and may also have the genetic potential to accumulate polyphosphate. Many *Thiohalophilus* reads may also have been miss-classified and may actually represent the closely related *Sedimenticola*. A complete genome for *Thiohalophilus* has yet to be published, but *Sedimenticola thiotaurini* and *Sedimenticola selenatireducens* both contain *ppk1* and *ppx.* genes, and are known to produce polyphosphate granules (Flood et al., 2015). *Sedimenticola* OTUs also abundant in our extant sediment libraries.

Both *ppx.* and *ppk1* were also found in the genomes of several species from the *Deltaproteobacterial* genus, *Desulfobulbus,* and in most of the members of the *Betaproteobacterial* genus, *Burkholderia,* and the *Alphaproteobacteria* genus *Myethylobacterium,* all of which are represented in our phosphoclast extraction library. Both *ppx.* and *ppk1* are also present in species from the orders *Acidimicrobiales* and *Myxococcales*, however, due to lack of phylogenetic resolution we cannot determine if the OTUs in our libraries are likely to represent organisms that would be expected to contain these genes as well. In many cases, sister taxa of the OTUs in our libraries did not possess one or more of the *ppx.* or *ppk1* genes. The genus Desulfurivibrio, Anaerolineaceae, and most members order Nitrospirales have only the *ppx.* Genes, but

lack *ppk* genes. There are also many unclassified OTUs in our datasets that may or may not represent organisms that possess these genes.

The identification of many other organisms with the potential to accumulate polyphosphate may change the way we view phosphogenesis in the Benguela upwelling system. At present, it is thought that the *Thiomargarita spp.* are the main contributors to changing phosphate concentrations that may lead to phosphogenesis (Schulz & Schulz 2005). However, if other polyphosphate accumulating organisms are also present in these sediments, the story may be more complex. These other organisms could also influence sediment pore water phosphate concentrations, making it more difficult to determine the organism (or organisms, as is more likely the case) that are directly involved in mediating phosphogenesis. To better understand the microbial role in the precipitation of phosphatic minerals, more work needs to be done to understand the conditions under which different organisms accumulate, use, and release phosphate and determine whether or not other metabolic processes, such as those that influence pH, or other aqueous chemistry factors may also be important.

Conclusions:

We observed distinct differences between the microbial communities of our longitudinal sediment transect. However, we cannot yet determine which environmental influences might be responsible for these differences. The major anion concentrations are very similar across the sites for which geochemical data are available. In addition, all sites contained giant sulfur bacteria, and all sediment samples were taken from similar water and sediment depths. However, it is known that fluxes of phytodetritus, oxygen

demand, and sediment geochemistry can vary throughout the year; thus, the single snapshot of geochemical data we have does not provide a comprehensive picture of the conditions experienced by these microbial communities. In any case, more environmental data is needed to constrain the variables that might be responsible for the differences we identified. To better constrain these differences, will need oxygen profiles of benthic waters and sediments, a more detailed examination of the local carbon content and a detailed examination of the compositional differences between sediments from different sites. This would also need to be done periodically to constrain seasonal changes during different periods of upwelling. Alternatively, or additionally, the composition or fluxes of organic matter into the sediment could influence the composition of the microbial communities.

Our phosphoclast sample also shows distinct differences from our sediment communities. However, we only had enough phosphoclasts to perform a single series of washings and DNA extraction. Additional DNA analyses of phosphoclasts will need to be performed to better understand the differences seen between the community composition in our phosphoclasts and those present in extant sediments. Phosphoclasts from our sampling region also need to be dated to determine whether they fall within the age range where we could reasonably expect ancient DNA to remain intact. We also need to better classify the large number of unclassified OTUs found in our library. We do not know why so many of these OTUs could not be classified, but the generation of additional clone libraries from current and future phosphoclast extractions could help further elucidate the microbial community represented by DNA recovered from within

the phosphoclasts. However, the presence of DNA within these phosphoclasts presents us with an initial opportunity to compare modern and ancient communities to better understand phosphogenesis, both in modern times and during past phosphogenic events. These comparisons may allow us to better understand the totality of the organisms that may be contributing to the cycling of phosphorus and generation of the critical natural resource of phosphatic rock material.

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Tables:

Primer	Sequence	Reference
EUB 27F	AGAGTTTGATCMTGGCTCAG	Lane 1991
EUB 1492R	GGTTACCTTGTTACGACTT	Lane 1991
V4_515F	GTGCCAGCMGCCGCGGTAA	
V4_806R	GGACTACHVGGGTWTCTAAT	

Table 1: Table 1: Primers used for PCRs and for Illumina sequencing

Organisms	Presence of *ppx*	Presence of *ppk1*	Search Date
Nitrosococcus:			
N. oceani	Yes	Yes	6/13/16
N. halophilus	Yes	Yes	6/13/16
N. qatsonii	Yes	Yes	6/13/16
Desulfobulbus:			
D. propionicus	Yes	Yes	6/13/16
D. mediterraneus	Yes	Yes	6/13/16
D. japonicus	Yes	Yes	6/13/16
D. elongatus	Yes	Yes	6/13/16
tol-SR	Yes	Yes	6/13/16
Sedimenticola:			
Selenatireducens:	Yes	Yes	6/13/16
CUZ	Yes	Yes	6/13/16
SIP-G1	Yes	Yes	6/13/16
***Desulfurivibrio*:**	No	Yes	6/13/16
Burkholderia:			
(Number of Genomes with specific gene vs Number of genomes searched)	Yes (586/613)	Yes (599/613)	6/13/16
Methylobacterium:			
(Number of Genomes with specific gene vs Number of genomes searched)	Yes (45/83)	Yes (83/83)	6/28/16
Acidimicrobiales:			
(Number of Genomes with specific gene vs Number of genomes searched)	Yes (7/9)	Yes (9/9)	6/28/16

Table 2: IMG search of the top 20 OTUs and Sedimenitcola genomes with Exopolyphosphatase (PPX) and Polyphosphate Kinase I (PPK I) genes (Markowitz et al, 2012)

Figures:

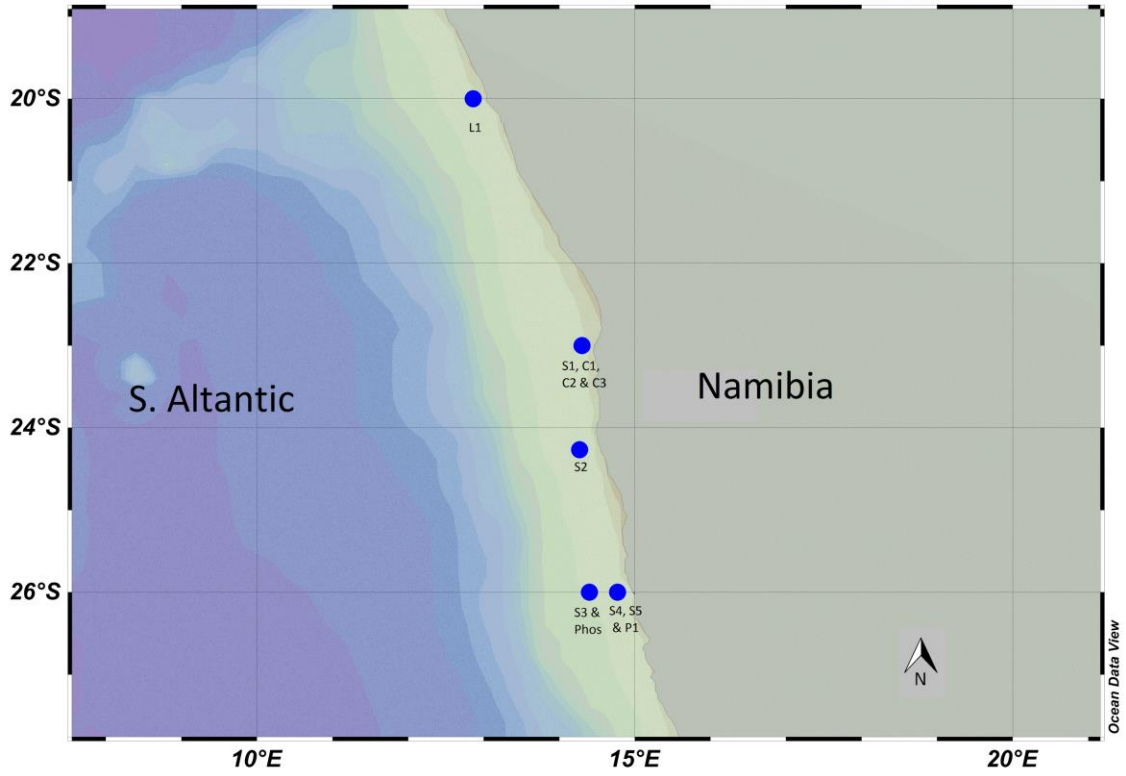


Figure 1: Map of Namibia sample locations. Samples represent cores taken from 2 cruises. Samples S1-5 along with the Phos (the Phosphoclast Extraction) represent samples taken in 2015. P1, L1 and C1-3 represent samples from 2014. C1-3 represents a core taken with C1 representing 1-3 cm in depth C2 3-6 cm and C3 6-10 cm.

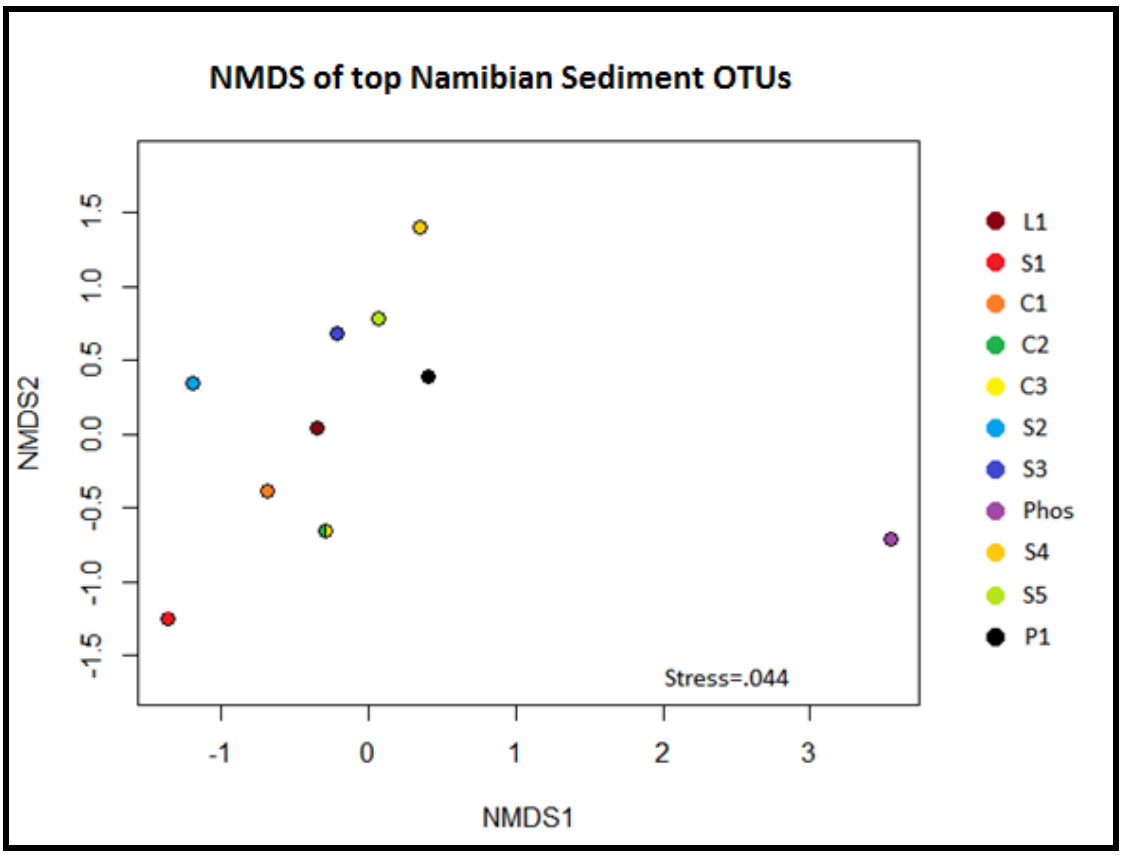


Figure 2: Nonmetric multidimensional scaling ordination of all sediment samples and phosphoclast extractions in this study. Each point represents an iTag library from a single sample. The stress value represents the (dis)similarities in two dimensions between samples and a lower value represents better representation of samples.

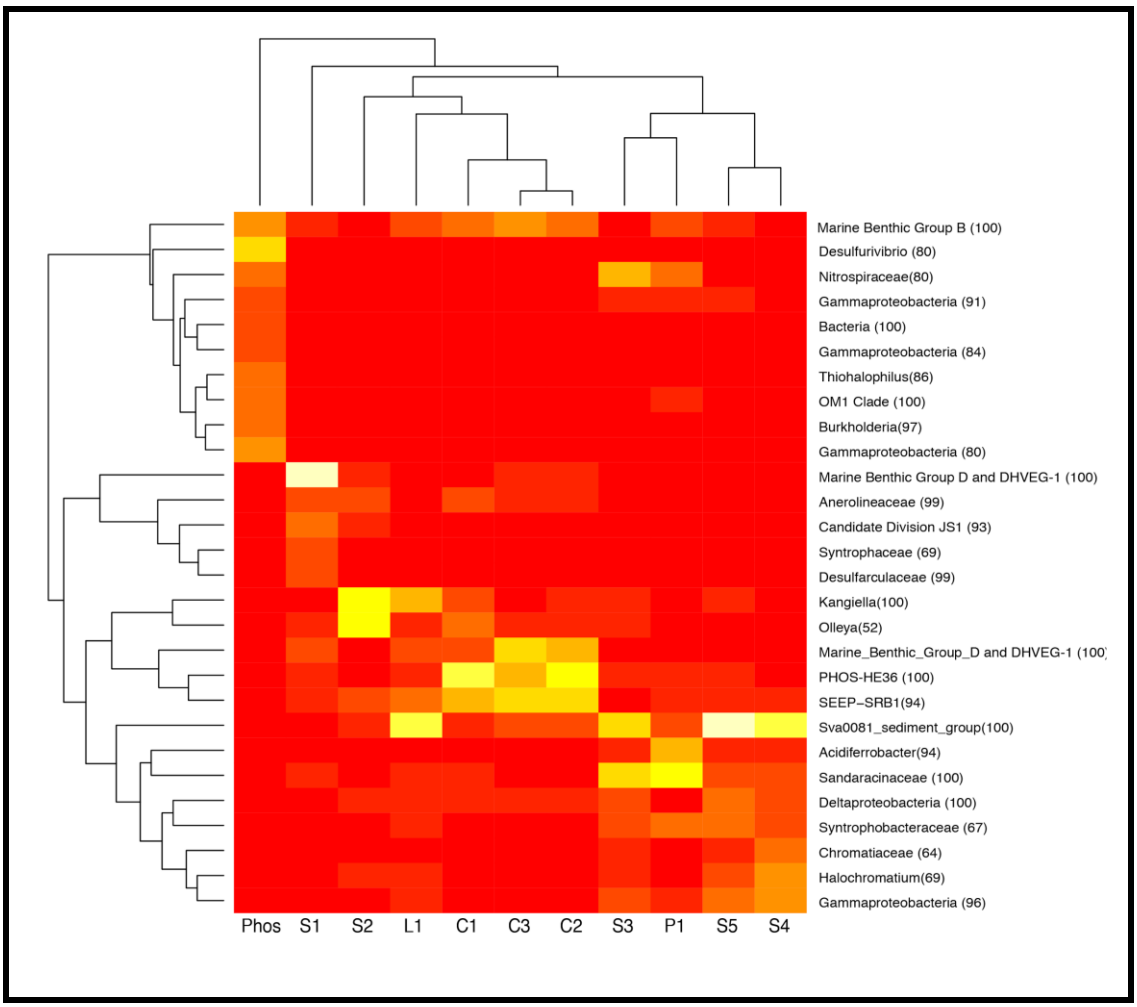


Figure 3: Heat map and cluster analysis of the top OTUs at each site (OTUs that make up 2% or more of any one sample) with brighter orange and yellow representing higher numbers of OTUs assigned to a specific clade at a site. OTUs are defined as with a sequence similarity of 97%. The OTUs have been classified at the lowest known taxonomic level and our sites have been clustered by similarities in the abundance of top OTUs.

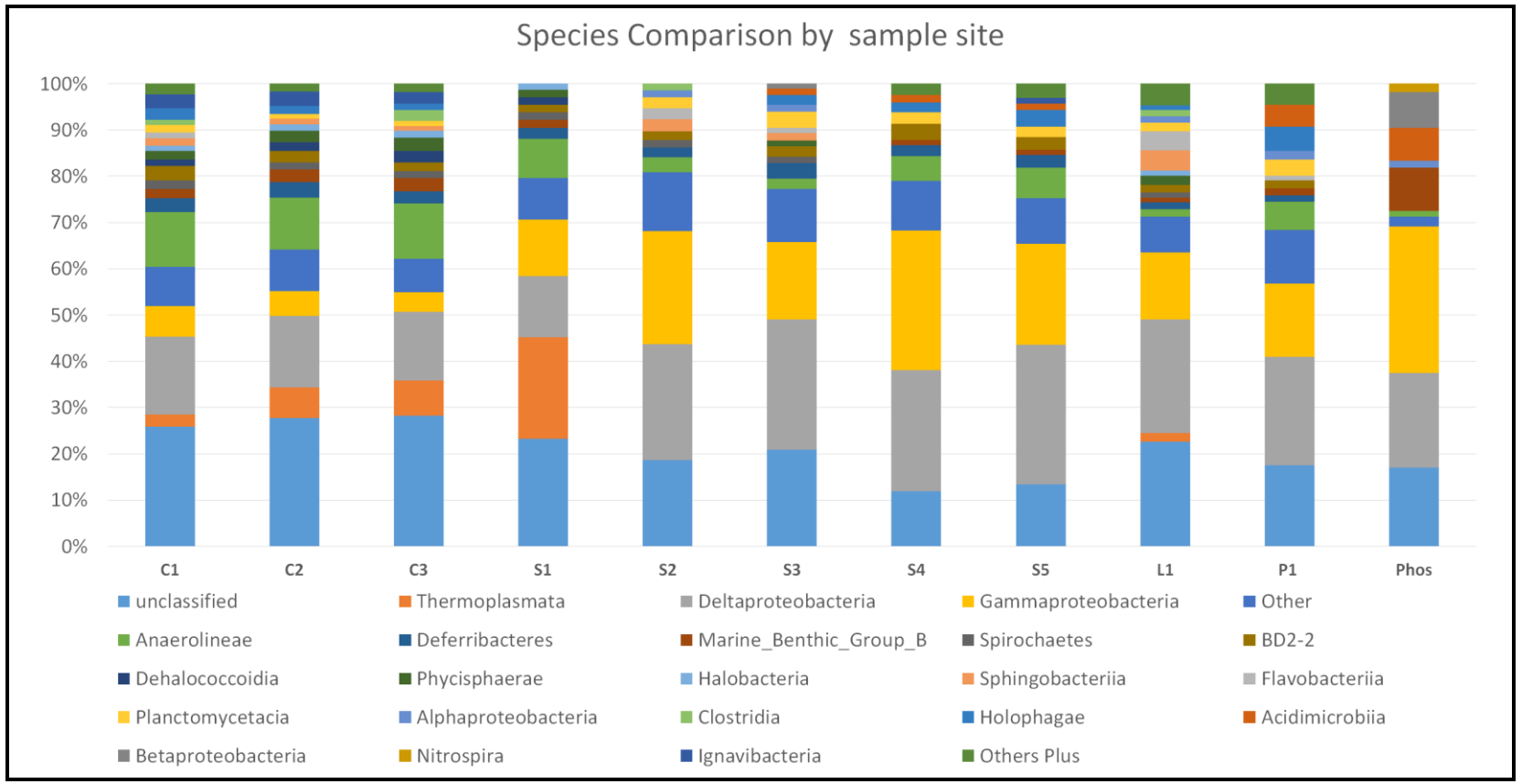

Species Comparison by sample site
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
C1
C2
C3
S1
S2
S3
S4
S5
L1
P1
Phos
unclassified
Thermoplasmata
Deltaproteobacteria
Gammaproteobacteria
Other
Anaerolineae
Deferribacteres
Marine_Benthic_Group_B
Spirochaetes
BD2-2
Dehalococcoidia
Phycisphaerae
Halobacteria
Sphingobacteriia
Flavobacteriia
Planctomycetacia
Alphaproteobacteria
Clostridia
Holophagae
Acidimicrobiia
Betaproteobacteria
Nitrospira
Ignavibacteria
Others Plus

Figure 4: The phylogenetic makeup of each Namibian sediments (C1-C3, S1-S5, L1 P1) and phosphoclast (Phos) extractions at the phylum level. All phyla that made up 1% or less of the sample have been placed in the category "Other".

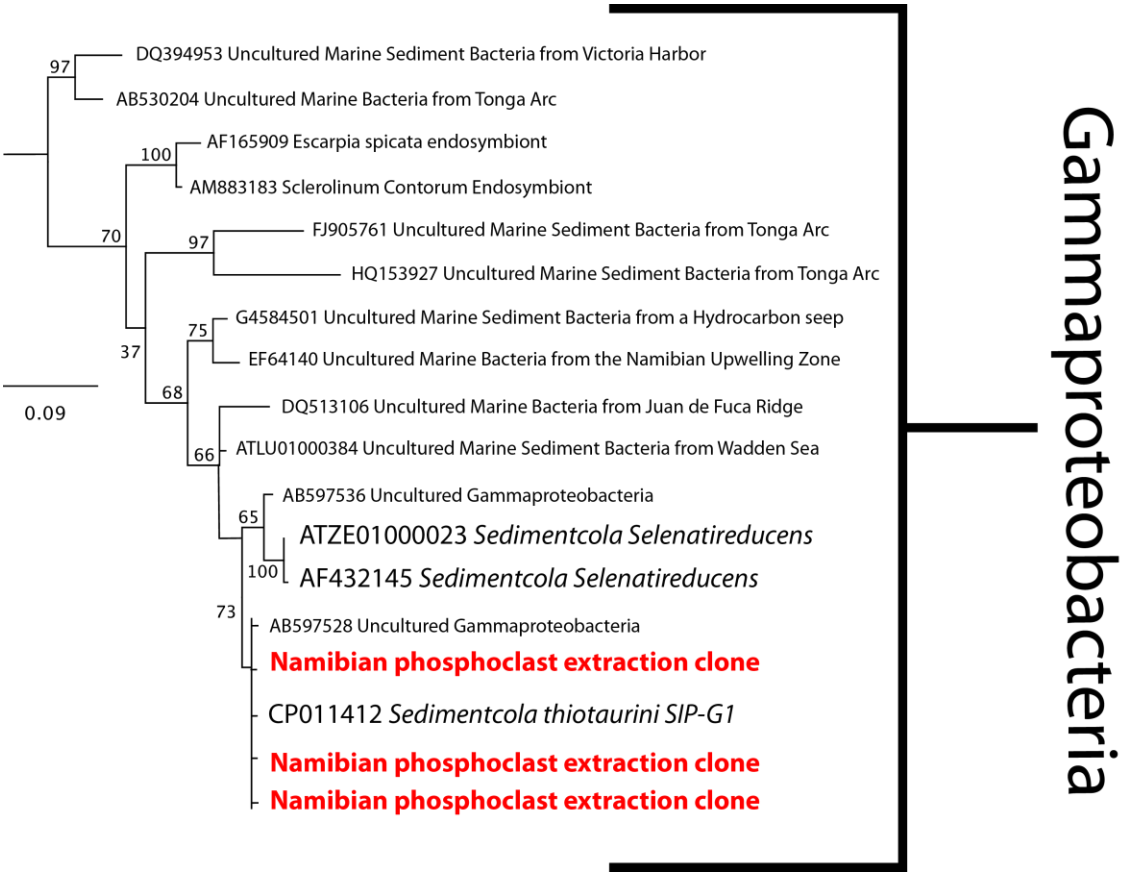


Figure 5: Maximum likelihood phylogram of 16S rRNA gene clone library sequences and select amplicon OTUs sequences from Namibian phosphorite extraction showing Gammaproteobacteria clones. Sequences from our clone library are labeled "**Namibian phosphorite extraction clone"**, and bootstrap values are shown.

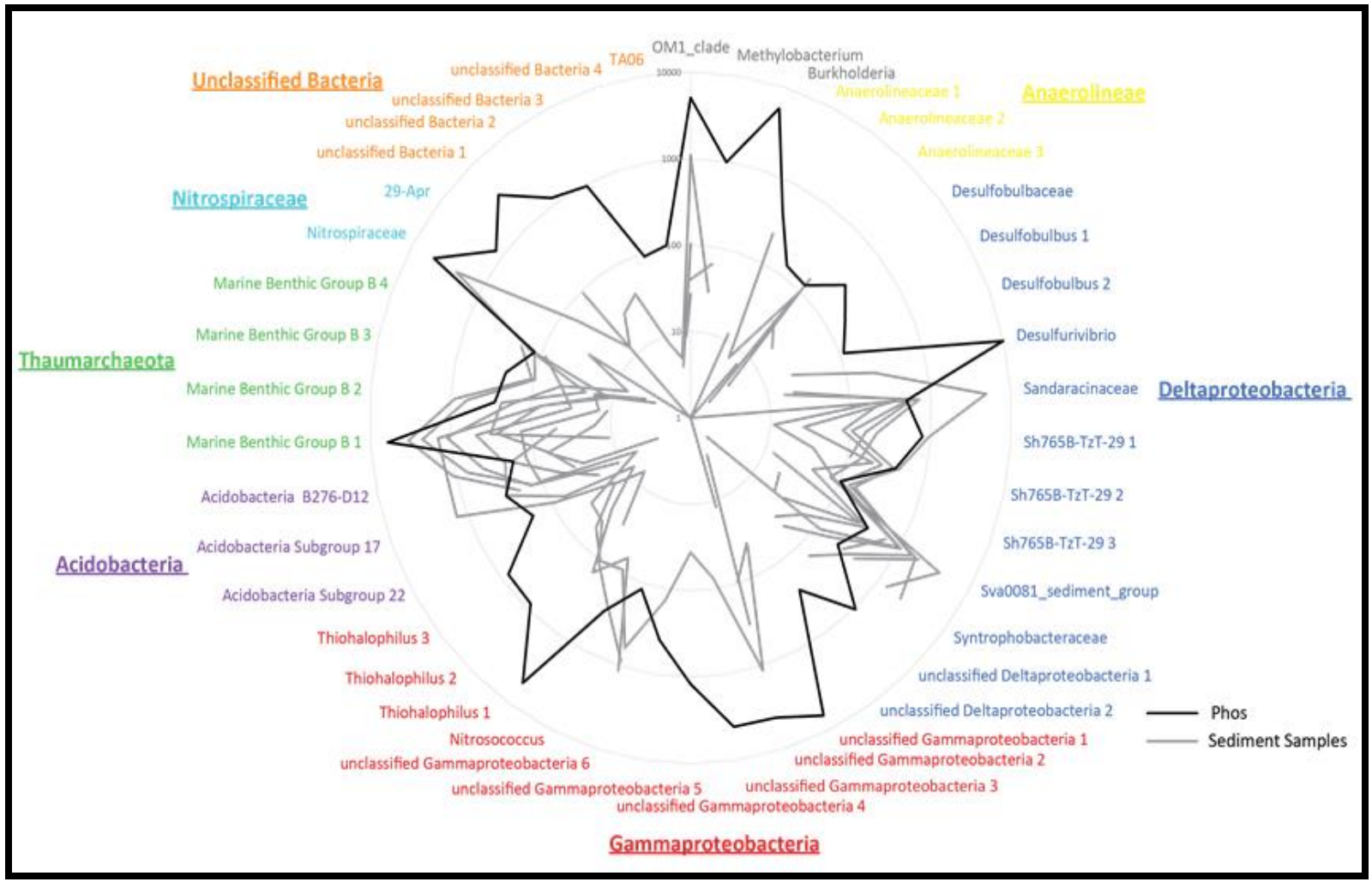
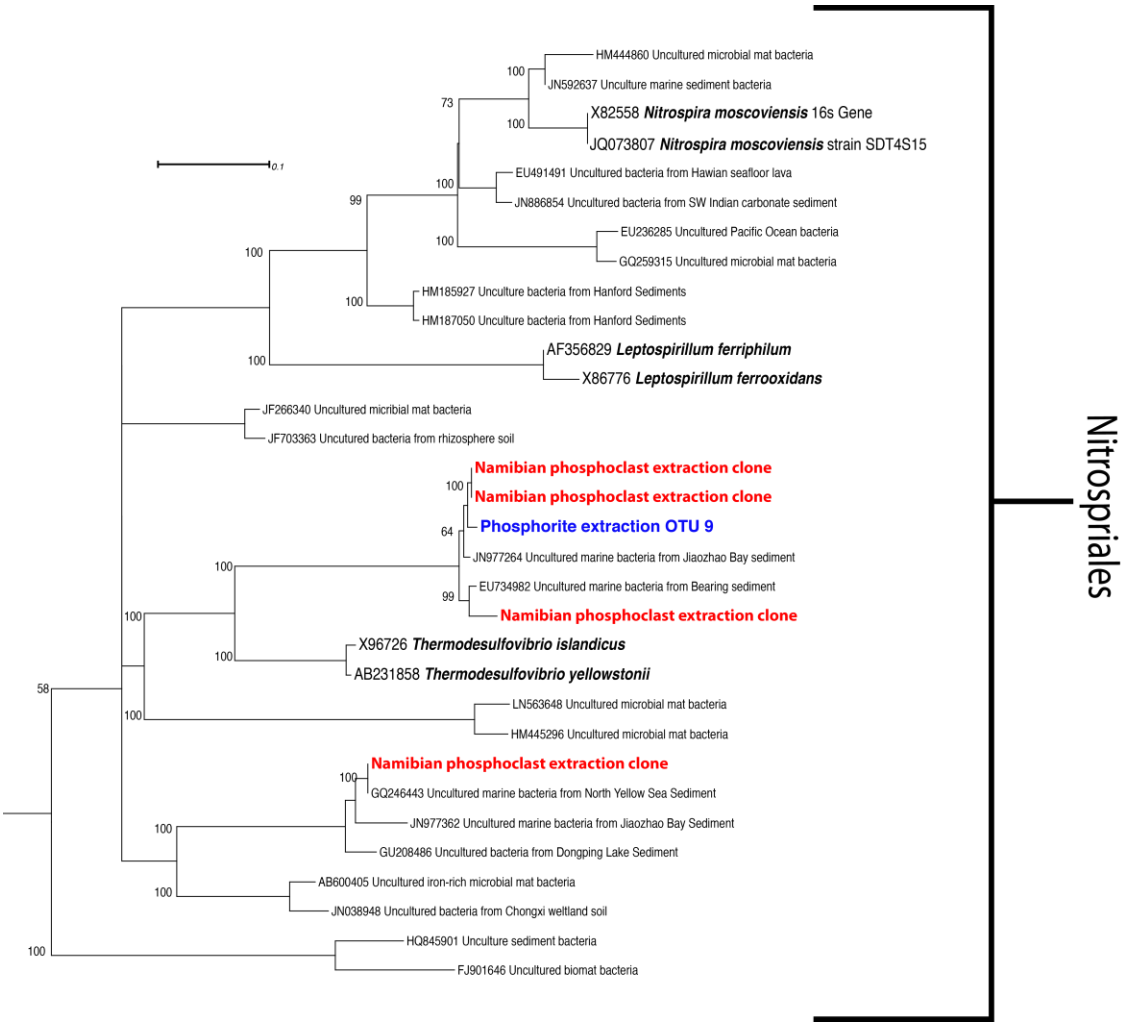
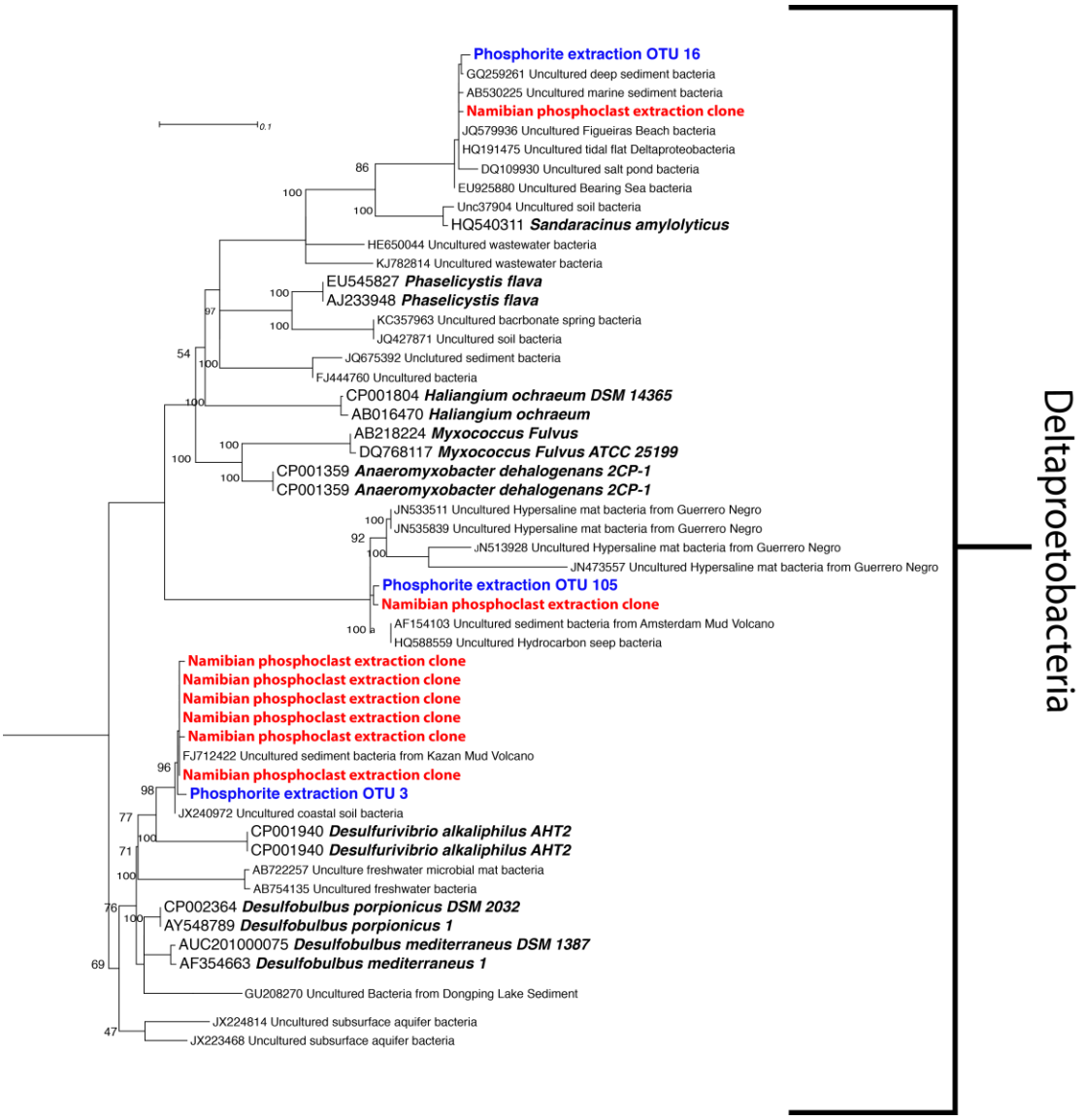

Unclassified Bacteria
unclassified Bacteria 4
unclassified Bacteria 3
unclassified Bacteria 2
unclassified Bacteria 1
Nitrospiraceae
29-Apr
Nitrospiraceae
Thaumarchaeota
Marine Benthic Group B 4
Marine Benthic Group B 3
Marine Benthic Group B 2
Marine Benthic Group B 1
Acidobacteria
Acidobacteria B276-D12
Acidobacteria Subgroup 17
Acidobacteria Subgroup 22
Thiohalophilus 3
Thiohalophilus 2
Thiohalophilus 1
Nitrosococcus
unclassified Gammaproteobacteria 6
unclassified Gammaproteobacteria 5
unclassified Gammaproteobacteria 4
Gammaproteobacteria
unclassified Gammaproteobacteria 3
unclassified Gammaproteobacteria 2
unclassified Gammaproteobacteria 1
unclassified Deltaproteobacteria 2
unclassified Deltaproteobacteria 1
Syntrophobacteraceae
Sva0081_sediment_group
Sh765B-TzT-29 3
Sh765B-TzT-29 2
Sh765B-TzT-29 1
Sandaracinaceae
Deltaproteobacteria
Desulfurivibrio
Desulfobulbus 2
Desulfobulbus 1
Desulfobulbaceae
Anaerolineae
Anaerolineaceae 3
Anaerolineaceae 2
Anaerolineaceae 1
Burkholderia
Methylobacterium
OM1_clade
TA06
10000
1000
100
10
1
Phos
Sediment Samples

Figure 6: Radar plot showing the abundance of OTUs from families with 100 or more reads from our phosphoclast nodule extraction compared to those families found in our sediment samples. Samples are organized by site with the bold line representing the phosphoclast extraction, the light lines representing samples from extant sediment pore waters. C1-C3 represents a sediment core with C1 representing the top 0-3cm, C2 representing 3-6cm, and C3 representing 6-10cm in depth beneath the sediment water interface.

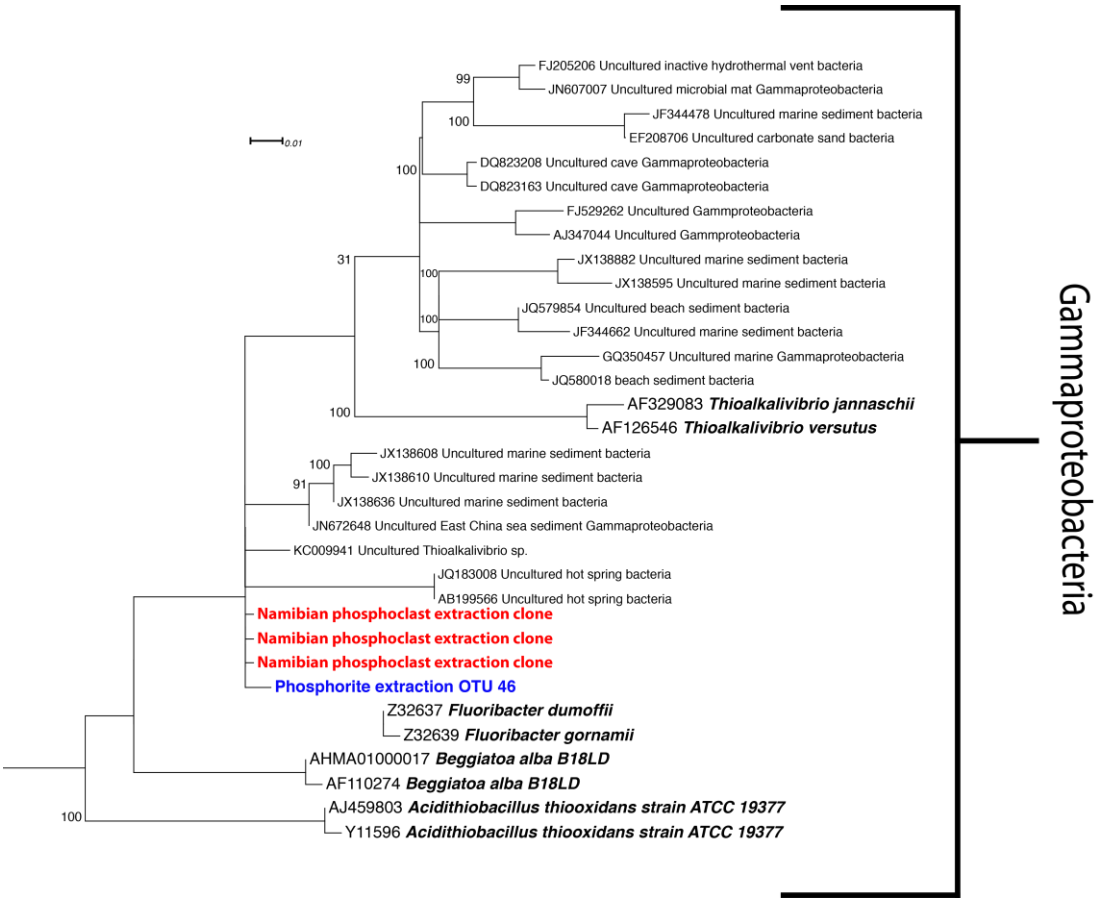
Supplemental Material:



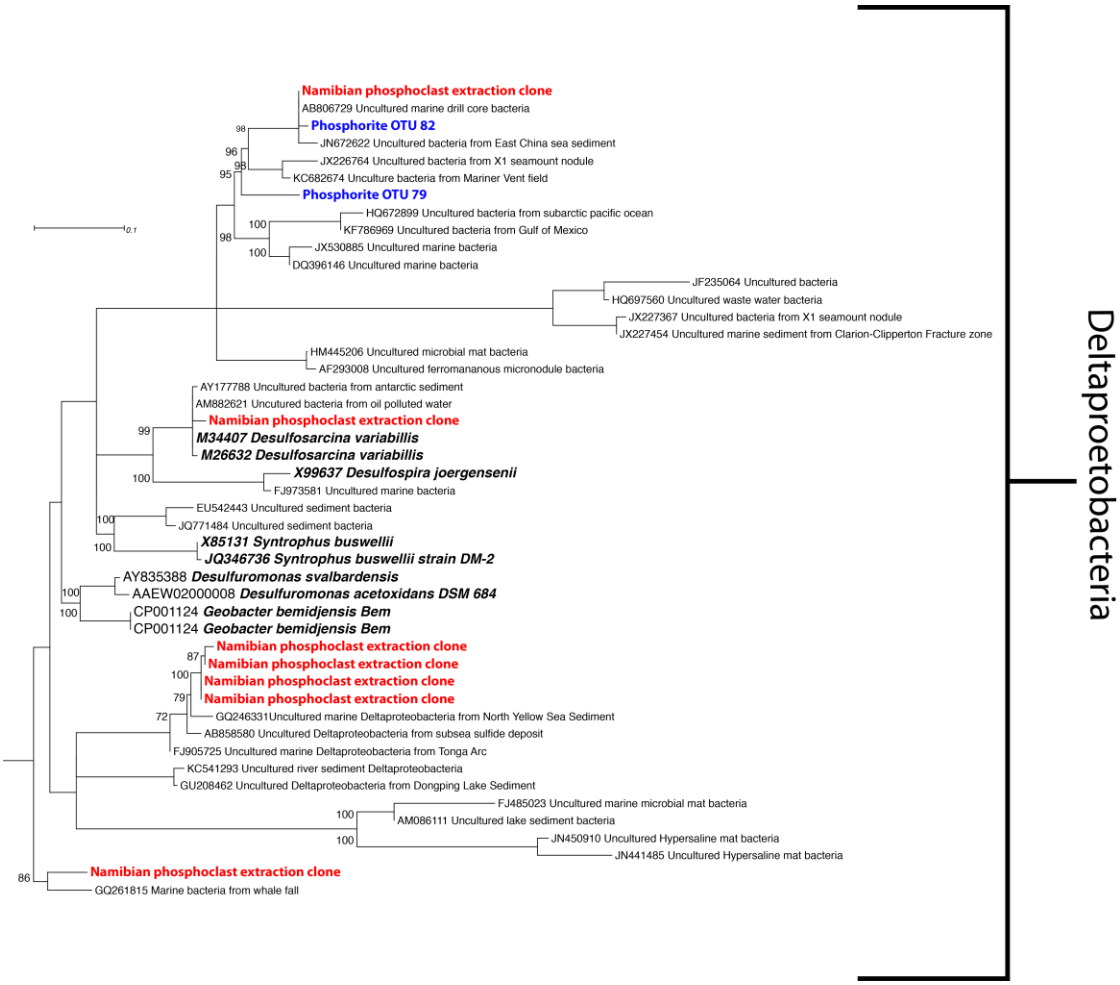
Supplemental Figure S1: Maximum Likelihood phylogram of 16S rRNA gene clone library sequences and select amplicon OTUs sequences from Namibian phosphorite extraction showing possible Nitrospirales clones. Sequences from our clone library are labeled "**Namibian phosphorite extraction clone"**, OTUs are labeled ***"Phosphorite OTU ##"*** and bootstrap values are shown.



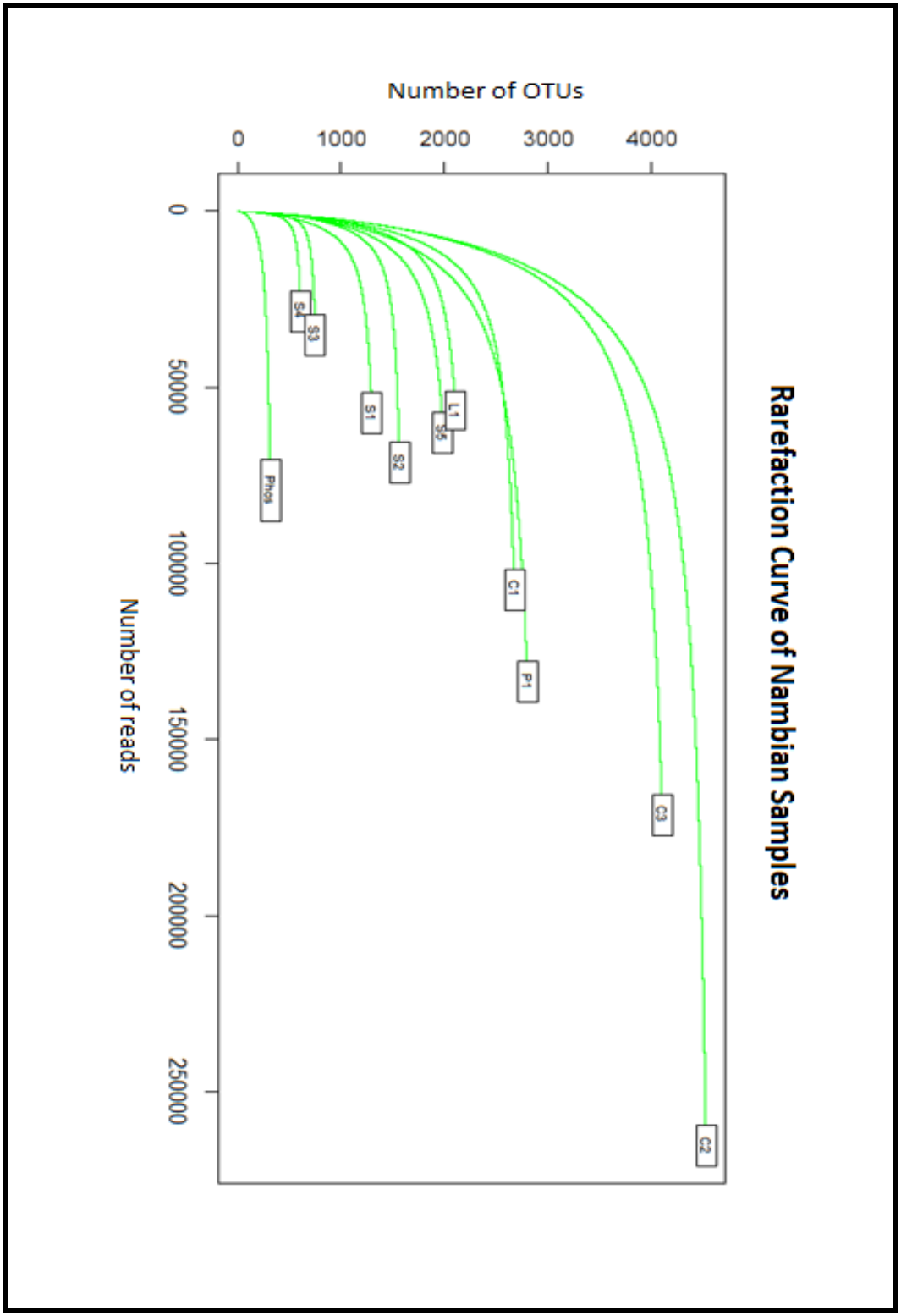
Supplemental Figure S2: Maximum Likelihood phylogram of 16S rRNA gene clone library sequences and select amplicon OTU sequences from Namibian phosphorite extraction showing possible Deltaproteobacteria clones. Sequences from our clone library are labeled "**Namibian phosphorite extraction clone"**, OTUs are labeled **"*Phosphorite OTU ##"*** and bootstrap values are shown.



Supplemental Figure S3: Maximum Likelihood phylogram of 16S rRNA gene clone library sequences and select amplicon OTU sequences from Namibian phosphorite extraction showing Gammaproteobacteria clones. Sequences from our clone library are labeled "**Namibian phosphorite extraction clone"**, OTUs are labeled ***"Phosphorite OTU ##"*** and bootstrap values are shown.



Supplemental Figure S4: Maximum Likelihood phylogram of 16S rRNA gene clone library sequences and select amplicon OTU sequences from Namibian phosphorite extraction showing Deltaproteobacteria clones. Sequences from our clone library are labeled "**Namibian phosphorite extraction clone"**, OTUs are labeled **"*Phosphorite OTU* ##"** and bootstrap values are shown.


Rarefaction Curve of Nambian Samples
Number of OTUs
0
1000
2000
3000
4000
Number of reads
0
50000
100000
150000
200000
250000
S4
S3
S1
S2
L1
S5
Phos
C1
P1
C3
C2

Supplemental Figure S5: Rarefaction curves showing microbial diversity of all sediment samples and our phosphoclast extraction with OTU's being defined as sequences with 97% similarity

Diversity and community structure tables:

	C1	C2	C3	S1	S2	S3	S4	S5	L1	P1
Alpha Diversity (Phos)	.8535 625	.8615 488	.8806 926	.8539 856	.9324 496	.7931 822	.7303 634	.6620 023	.8395 992	.5949 696
B-C (Phos)	.9560 873	.9520 601	.9522 510	.9683 797	.9816 436	.9295 817	.9286 803	.9177 003	.9583 594	.8759 954
Jaccard (Phos)	.9775 507	.9775 507	.9775 507	09775 507	.9775 507	.9635 059	.9775 507	.9570 842	.9787 370	.9338 993

Supplemental Table S1. A comparison of our sediment samples and our phosphorite extraction communities including alpha diversity (choa1), and community structure represented by Jaccard and Bray-Curtis Distance matrices.

	C1	C2	C3	S1	S2	S3	S4	S5	L1	P1	Phos
Simpson	.996	.996	.996	.979	.995	.995	,987	.991	.996	.994	.937
Shannon	6.607	6.874	6.856	5.487	6.221	5.968	5.436	6.197	6.601	6.358	3.278

Supplemental Table S2: Simpson and Shannon Diversity