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Metagenomic analysis provides insights into functional capacity in a hyperarid desert soil niche community

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Running Title: Functional capacity in Namib Hypoliths

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Abstract

Environmental stressors such as low water activity and temperature extremes impose severe limitations on the productivity of soils in hyperarid deserts. In such ecosystems, macroscopic communities are often restricted to cryptic niche habitats, such as hypoliths (microbial communities found beneath translucent rocks), which are widely distributed in hyperarid desert environments. While hypolithic communities are considered to play a major role in the productivity of hyperarid habitats, the functional guilds implicated in these processes remain unclear. Here, we describe the Illumina-based metagenomic sequencing (± 30 Gb), assembly and analysis of hypolithic microbial communities from the south-west African Namib Desert. Taxonomic analyses using Small Subunit (SSU) phylogenetic markers showed that bacterial phylotypes (93%) dominated the communities, with relatively small proportions of archaea (0.43%) and fungi (5.6%). BlastX analysis against the refseq-viral database showed the presence of double stranded DNA viruses (7.8% contigs), dominated by Caudovirales (59.2%). Analysis of functional genes and metabolic pathways revealed that cyanobacteria were primarily responsible for photosynthesis with the presence of multiple copies of genes for both photosystems I and II, with a smaller but significant fraction of proteobacterial anoxic photosystem II genes. Hypolithic community members demonstrated an extensive genetic capacity for the degradation of phosphonates and mineralization of organic sulfur. Our data suggest that Proteobacterial guilds may be more significant in desert niches than previously recognized, as they showed widespread genetic capacity for mediating key stages in all biogeochemical cycles. Surprisingly, we were unable to show the presence of genes representative of complete nitrogen cycles. The diversity of *nif* genes was low, and the metagenome showed no evidence of other key N-cycling genes. Taken together, our analyses suggest an extensive capacity for carbon, phosphate and sulphate cycling but only limited nitrogen biogeochemistry.

Introduction

The mechanisms controlling primary productivity in soil systems, particularly in hyperarid deserts, remain poorly understood (1). Arid soil ecosystems cover over 40% of terrestrial surfaces and therefore contribute a significant fraction of the global soil carbon budget (2). In hyperarid deserts, where the extreme stochasticity of rainfall events generally results in very low plant primary productivity, it is generally accepted that microbial communities are major contributors to the key processes of ecosystem services (1). Hypoliths, cryptic assemblages found on the ventral surfaces of translucent rocks, are a prominent feature of both hot and cold deserts (3, 4). The cryptic hypolithic habitat is known to modulate some of the extreme environmental stressors associated with hyper-aridity, including temperature extremes, high incidences of UVA/B and low water availability (5-8). These communities provide a model system for understanding the factors that control microbial primary productivity (4).

The majority of published studies on hypoliths have focused on understanding the microbial diversity and ecology of these communities (6, 9-12). Environmental DNA sequence-based analyses have demonstrated that hypolithic microbial communities in hot deserts are dominated by cyanobacterial lineages of the order Pleurocapsales, predominantly members of the genus *Chroococcidiopsis* (10, 12-14). In contrast, hypoliths in cold and polar deserts are dominated by Oscillatorian cyanobacterial morphotypes (3). Hypolithic communities also contain diverse groups of heterotrophic bacteria from the phyla Actinobacteria, Acidobacteria, Proteobacteria and Bacteroidetes, many of which belong to the 'so-called' category of microbial "dark matter" (3, 6, 10, 15). It has been shown that hot desert hypoliths selectively recruit microbial taxa from surrounding soils, and that these cyanobacterial-dominated communities may drive community interactions and system functionality in hyperarid deserts where plant biomass is limited and transitory (12).

Acetylene reduction assays have been used to show that hypolithic communities in cold deserts are a vital input source of nitrogen (16). A more recent study by Chan and colleagues applied microarray analysis of functional genes involved in autotrophy, nitrogen metabolism and stress responses in Antarctic Dry Valley soils (17) and showed, for the first time, that hypoliths harbor high metabolic potential for biogeochemical cycling. However, little is currently known of the breadth of functional capacity in hot desert hypolithic communities and their role in edaphic biogeochemical cycles (4).

Here we report a metagenomic analysis of the functional potential of hot desert (Namib) hypolithic communities. The central Namib Desert, on the south-west coast of continental Africa, is designated as a hyper-arid zone with a mean annual rainfall of approximately 25 mm (18). The northern Namib gravel desert zone is rich in quartz reefs, resulting in extensive contributions of quartz pebbles to the desert pavement (4). In this study, we explore the metagenome-derived community structure, assess the genetic capacity for primary productivity and nutrient cycling (including N, C, and P metabolism) and demonstrate the diversity of genes and pathways which may represent adaptations of taxa in the hypolithic niche to environmental stressors in this hot desert environment.

Results and Discussion

Sequence data

Illumina Hiseq-2000 sequencing of bulked metagenomic DNA from multiple (n = 40) Namib Desert hypolithic biomass samples generated 19.5 billion bp of sequencing data (**Supplementary Materials Table S1**). Primary assembly using Velvet resulted in 2,188,786 contigs with a total assembly size of approximately 634 million bp (**Supplementary**

Materials Table S2). All contigs shorter than 500 bases were culled, and the average size of the remaining contigs was 787 bp (**Supplementary Materials Table S2)**.

Phylogenetic analysis

We first explored the microbial community composition by analyzing the reads using two approaches; the ribosomal small subunit (SSU) using Metaxa2 (19) and unique clade-specific marker genes using MetaPhlAn (20). SSU analysis suggested that the Namib hypolithic niche contains very high Bacterial diversity (93% of total phylotypic signals) with a significantly lower proportion of Fungi (5.6%) and Archaea (0.43%). The low archaeal and eukaryotic diversity in the hypolith metagenome is consistent with previous phylogenetic surveys, which indicated that these groups are poorly represented in such microenvironments (5, 6, 9, 21, 22).

The taxonomic analysis of SSUs of the bacterial fraction revealed that the phyla Actinobacteria, Proteobacteria and Cyanobacteria were highly represented in the metagenome, while other phyla such as Firmicutes, Chloroflexi, Acidobacteria, Bacteroidetes and Planctomycetes were present as relatively minor contributors to total bacterial diversity (**Fig. 1**). MetaPhlAn analysis of the taxonomic prediction using reads also showed a high abundance of Actinobacteria, Proteobacteria, Chloroflexi and Cyanobacteria, although this method yielded slightly different proportions of the taxonomic groups compared to the Metaxa2 (Fig. 1).

These results are in agreement with earlier studies, based on PCR amplification of 16S rRNA genes, which found that hypolithic communities in hot deserts were dominated by the Chroococcidiopsis lineages (order Pleurocapsales) followed by phyla Actinobacteria and

Proteobacteria (6, 12, 23, 24). The results of the taxonomic analysis of reads from the Namib Desert metagenome are generally consistent with the range and relative proportions of phyla present in hypoliths, albeit with slight differences in the relative proportions. The minor inconsistencies between 16S rRNA gene sequence-based diversity from previous studies and the results from the metagenomic analysis are perhaps unsurprising, given the accepted potential for PCR amplification bias (25).

Binning of assembled contigs and functional analysis

Assembled contigs were used to predict ORFs, binned and assigned to taxonomic groups using MyTaxa software which uses the ORF identity in each contig to assign it to the most probable taxon (27). Following contig classification, ORFs were assigned to the following bacterial phyla: Actinobacteria (122362), Proteobacteria (77810), Cyanobacteria (77810), Bacteroidetes (10444), Acidobacteria (6911), Firmicutes (6893), Gemmatimonadetes (3363) and Chloroflexi (3625) (**Fig 2A)**. 36940 ORFs of the most dominant phylum, Actinobacteria, were assigned as unclassified (i.e., no lower order phylogenetic identity) (Fig 2B) and 29805 ORFs of the Proteobacteria were assigned only up to phylum level (**Fig 2C**). Cyanobacterial ORFs were dominated by the order Oscillatoriales (5722 ORFs), with other phyla identified as Nostocales (4808 ORFs), Chroococcales (4360 ORFs), Pleurocapsales (1843 ORFs) and Gloeobacterales (347 ORFs) (**Fig 2D**). A high proportion of the cyanobacterial-assigned contigs (22662: 66%) could not be attributed to specific taxonomic groups. Fewer ORFs (1936) were assigned to archaea (**Fig 2A**), and most archaeal sequences were attributed to the phyla Euryarchaeota (1171 ORFs/648 contigs), Thaumarchaeota (612 ORFs/390 contigs) and Crenarchaeota (146 ORFs/89 contigs). These results were consistent with the results obtained from the classification of reads using Metaxa2. A substantial portion of the total assigned ORFs (80532: 20%) were classified as unknown and were not linked to any known phylum.

Although viruses and bacteriophages are likely to play a major role in microbial diversity and functionality in soil systems (28), very little is known about phage-host associations and processes in desert soils (29, 30). 21,666 contigs (8.5%) matched (blastx) to the RefSeq virus database and most of the sequences were assigned to dsDNA viruses (**Supplementary Materials Fig S1**). Caudovirales, followed by Phycodnaviridae and Mimiviridae, respectively, were the most abundant orders. This result is consistent with findings from a recent hot desert hypolith metavirome sequence analysis (31) and supports the conclusion that these members of the Caudovirales are widespread in hot deserts. Notably, the proportion of the three families (Myoviridae, Siphoviridae and Podoviridae) in the Caudovirales have only slightly different values from those reported by Adriaenssens and coworkers (31) **(Supplementary Materials Table S3)**, perhaps a surprising result given the known biases of the multiple displacement amplification protocol (32) underlying this metaviromics study.

In order to better understand the functional potential of the microorganisms represented in this metagenome, we used MEGAN to assign functions to the predicted ORFs (33). Our analysis showed that of a total of 396,495 genes, an estimated 118,983 (~30%) were successfully assigned to the KEGG orthology (KO numbers) (**Fig. 3A**). A further 57,365 (~14%) were annotated to biological SEED subsystem proteins using the refseq protein database **(Fig. 3B)**. For comparison, in a study of the human gut microbiome metagenome, 47% of the genes were assigned to the KEGG orthology (34).

The most abundant phyla (Actinobacteria, Proteobacteria and Cyanobacteria) were selected for an analysis of shared KEGG pathway modules. From a total of 580 KEGG pathway modules, 358 were incomplete in all three phyla. Interestingly, 83 modules were shared

among these phyla, which suggest that these modules represent core metabolic pathways and are essential for organismal survival. Forty-five pathway modules, shared by the phyla Actinobacteria and Proteobacteria, were mainly involved in heterotrophic metabolism and stress response regulatory systems (**Supplementary Materials Fig S2 & Table S4**). Proteobacteria and Cyanobacteria shared nine pathway modules, including carbon fixation and nitrogen fixation, while Actinobacteria and Cyanobacteria also shared nine pathway modules, assigned to metal transport (**Supplementary Materials Fig S1 & Table S4**). Desert microbial communities have previously been shown to possess multiple genes involved in metal acquisition and the maintenance of metal homeostasis (35). The presence of a high number of common pathway modules implicated in metal homeostasis suggests that these may be essential for survival in hot desert edaphic environments.

Primary productivity: photosynthesis and carbon metabolism

Photosynthetic microorganisms are keystone taxa in hypolithic systems (12), and may be the dominant primary producers for long periods in hyper-arid environments (6, 13, 15, 23). We used the MAPLE server, which evaluates KEGG pathway modules based on KAAS assignment of KEGG orthology terms to specific genes/proteins and calculates the module completion ratios (MCR) for each pathway. Results from MAPLE-MCR analysis suggested that photosystem-I and II modules were complete for the phylum Cyanobacteria, with potentially photosynthetically functional members of the orders Oscillatoriales, Chroccocales, Nostocales and Pleurocapsales **(Fig 2D)**. In addition, anoxygenic photosytem-II genes were present in Proteobacteria, including members of the Deltaproteobacteria, Rhizobiales and unclassified Proteobacteria, although no evidence of the anoxigenic photosystem-I pathway could be found for these taxa **(Fig. 4)**. SEED subsystem and KEGG pathway analyses failed to identify any photosynthesis genes belonging to any other non-cyanobacterial or non-

195 proteobacterial phototrophs, such as Chloroflexi, despite the identification of 220 contigs
196 belonging to the genus *Chloroflexus* (known for its phototrophic metabolism).
197
198 KEGG pathway analyses of the key photosynthetic enzymes chlorophyll synthase (*chlG*) and
199 bacteriochlorophyll synthase (*bchG*) showed sequences with homology to unclassified
200 Cyanobacteria and Proteobacteria (genera *Methylobacterium*, *Rhodopseudomonas* and
201 *Brevundimonas*). Interestingly, Methylobacteria have been previously identified as
202 widespread colonists of hypolithons in both the Atacama and Namib Deserts (15, 23). MCR
203 data and analysis of functional genes showed the presence of the complete Calvin-Benson
204 cycle attributed to the phyla Cyanobacteria and Proteobacteria (**Fig. 4** and **Fig.5**).
205
206 We identified subunits of the gene *acl* (one copy of *acl*A and two copies of *acl*B), which
207 encode the key enzyme (ATP citrate lyase), required for the reductive TCA cycle (rTCA
208 cycle) were all assigned to phylum Aquificae (**Fig 5**). We could not identify the other two key
209 genes (oxoglutarate synthase and fumarate reductase) involved in this cycle. However, these
210 *acl* genes have only been reported from prokaryotes (using the rTCA cycle) and can thus be
211 considered as 'indicator genes' for this pathway (36, 37). We suggest that the Namib Desert
212 hypolithic community may harbor novel taxa affiliated to the phylum Aquificae which may
213 have the capacity to drive anaerobic carbon fixation (**FIG 5**). The proposed capacity for both
214 aerobic and anaerobic carbon fixation in hot desert soil communities may be a consequence
215 of the limited C in these systems (38).
216
217 ORFs with homology to genes encoding formyltetrahydrofolate synthetase (FTHFS), a key
218 enzyme in the Wood-Ljungdahl anaerobic acetogenesis pathway (17), were related to those of
219 Actinobacteria, Proteobacteria, Gemmatimonadetes and Firmicutes. The key enzyme

responsible for methanogenesis (methyl-coenzyme M reductase (*mcr*A)) was not detected in any of the archaeal contigs, possibly due to the low abundance of methanogens in the largely aerobic desert soils (4, 39).

The extent of carbon fixation by members of the Proteobacteria, which are ubiquitous soil colonists, may have previously been underestimated in hypoliths. Proteobacteria possess the capacity for anoxigenic photosynthesis, but this contribution to C fixation is often largely ignored in comparison to cyanobacterial oxygenic photosynthesis (17). In cold desert systems, Cyanobacteria and Proteobacteria both appear to drive carbon fixation (17), and the identification of the relevant genes in the Namib Desert hypoliths metagenome suggest that similar processes may occur in hot deserts soils.

Genes for the heterotrophic utilization of complex carbohydrates (such as starch, cellulose, pectin and xylan) were largely associated with Actinobacteria (*Rubrobacterales*) (**Supplementary Materials Table 5**). Aromatic compound degradation genes were also identified: genes encoding the *ortho*- and *meta*-catechol ring cleavage enzymes (catechol-1, 2-dioxygenase and catechol-2, 3-dioxygenase) with similarity to those of the order *Actinomycetales*, unclassified Actinobacteria and unclassified Proteobacteria were identified (**Fig. 4**). This suggests that Actinomycetales in the hypolithic consortia may play a key role in detoxification of naturally occurring aromatic organics (40).

Nitrogen fixation and metabolism

Hyperarid desert environments are typically nitrogen limited (1), thereby enhancing the importance of diazotrophic microorganisms. Surprisingly, the hypolith metagenome sequence dataset showed very few *nif*H genes, encoding the first and rate-limiting step in the nitrogen

cycle (41). MAPLE server analysis yielded no *nif*H genes, while only one *nifH* gene variant, belonging to the phylum Cyanobacteria, was identified via MEGAN analysis. However a *hmmer* search performed against the pre-aligned *nifH* gene database (42) yielded at least five copies of the *nif*H gene, belonging to the phyla Cyanobacteria and Proteobacteria (Alphaproteobacteria and unclassified Proteobacteria) **(FIG 5B and Supplementary Materials Table S6).** This finding is congruent to previous studies, which have shown that heterocystous cyanobacteria were largely responsible for nitrogen fixation in depauperate edaphic systems (43).

A recent study reported the presence of ammonia-oxidizing bacteria in semi-arid soils (44). However, genes implicated in nitrification (ammonia monooxygenase (*amo*)) could not be detected in our metagenomic contigs. It has been suggested that the relative abundance of these genes may be related to 'rain events' (45). Our samples were collected during the late summer, following a period of months with zero precipitation, which may explain the absence of these genes from our metagenome and suggests that nitrification processes may be severely constrained for extended periods in hyper-arid soils. Genes for nitrate reduction and nitrite oxidation (*nar*GH/*nxr*AB) were identified and showed homology to those previously identified from Actinomycetales and unclassified Actinobacteria (**FIG 5B**). We also found signatures for genes implicated in denitrification (*nor*B), primarily affiliated to members of the phylum Actinobacteria **(FIG 5B)**. Nitrate reduction (*napA*) and ammonification (*nrfA*) genes were mostly affiliated to Actinomycetales and unclassified Proteobacteria, respectively (**FIG 5B**). We also identified the capacity for nitrogen (and ammonia) assimilation, based on the presence of marker genes such as glutamate synthase (*gltA* and *gltB*), assimilatory nitrate reductase (*nas*B) and glutamine synthase (*glnA*)). These genes were linked to a wide range of taxa, including members of the Actinomycetales, Rubrobacterales, Cyanobacteria,

unclassified Actinobacteria, Caulobacterales, Deltaproteobacteria, Acidobacteria, and Firmicutes (**FIG 5B**). However, genes for the anaerobic ammonium oxidation (Anammox) pathway, which converts ammonia directly into free nitrogen, were not identified. This finding is in contrast to other terrestrial systems (46, 47), particularly nutrient rich agricultural soils. *AmoA*-containing microorganisms are more common in aquatic or marine habitats, but the *amoA* gene also been identified in hypersaline microbial mats associated with desert springs (48). Genes for anammox have also been detected in the Antarctic hypolith microbial communities (17), which are known to have higher water contents than the largely aerobic 'Dry Valley' desert soils (49). The absence of anammox genes in the Namib hypolith metagenome may also reflect the limited capacity for anaerobic niches in hot desert soils. ~~It has also been shown that anammox rates in biological soil crusts (BSCs) from the Colorado Plateau were below detection rates (50).~~

The combined results from this analysis suggest that N cycling processes may be severely truncated in Namib Desert hyperarid soil niche communities. Denitrification rates in biological soil crusts have also been found to be low, despite the availability of NO_3^- in desert soils (51). Based on the genetic capacity for diazotrophy, we speculate that hypolithons may have a similar role in hyperarid desert systems. In low nitrogen availability environments such as deserts, nitrification is probably restricted to a limited number of low abundance taxa (52). A limited number of contigs assigned to nitrifying taxa such as *Nitrosomonas* (56 contigs/44702 bases), *Nitrobacter* (65 contigs/49915 bases) and *Nitrospira* (80 contigs/66667 bases) were identified, but the low number of sequences implicated in nitrification processes supports a view that these communities harbor a low genetic capacity for both nitrification and denitrification.

Phosphorus and sulfur metabolism

Biologically available phosphorus (P) in soils is mainly derived from rock weathering or from the decomposition of organic matter (53). In deserts, phosphorus-solubilizing bacteria (PSBs) release phosphorus from soil as orthophosphate anions (54). Gluconic acid and 2-ketogluconic acid biosynthesis in the periplasm of Gram-negative bacteria is known to be important for phosphate solubilization activity in soils (55). Gluconic acid biosynthesis is mainly carried out by the enzyme glucose dehydrogenase (GCD) in the presence of the cofactor pyrroloquinoline quinone (PQQ) (56). We identified copies of the *gcd* gene, with homology to members of the orders Rhizobiales, Solibacteriales and Xanthomonadales, and Proteobacteria and Bacteroidetes phyla, respectively, and suggest that these bacteria might be involved in phosphate solubilization in Namib Desert soil communities.

Phosphonates are an alternate source of phosphorus for microorganisms in desert environments, and are produced by protozoa, flagellates, coelenterates, mollusks, fungi and some bacteria (including Actinobacteria, *Pseudomonas* and *Bacillus*) (57). Although phosphonates are widely available in the environment, only microorganisms have the ability to degrade these compounds (58). We identified *phn* genes in the hypolith metagenome which may be implicated in the utilization of alkylphosphonate and phosphonates, ascribed to a wide range of taxa including Alphaproteobacteria (order Rhizobiales, Sphingomonadales and unclassified Alphaproteobacteria), Betaproteobacteria, Gammaproteobacteria and unknown Proteobacteria, Firmicutes, Chloroflexi, Planctomycetes, Cyanobacteria and Actinobacteria (Rubrobacterales, Actinomycetales and unclassified Actinobacteria). We suggest that the presence of diverse *phn* genes in the hypolithon indicates that bacterial utilization of phosphate from phosphonates and alkylphosphonates may be a key factor in ‘P’ turnover.

320 Another important and very common enzyme in phosphate metabolism is alkaline
321 phosphatase ("Alp"), involved in the release of inorganic phosphate (Pi) from the both small
322 and polymeric organic substrates including DNA and proteins (59). 'Alp' genes in this
323 metagenome were associated with Rhizobiales, Caulobacterales, Sphingomonadales,
324 Cyanobacteria, Chloroflexi and Firmicutes, all of which are known to play a role in plant P
325 nutrition (60).
326
327 Genes for assimilatory sulfate reduction (*cys*C, *cys*N and *cys*D) were found in the
328 Rhizobiales, Sphingomonadales, unclassified Proteobacteria and unclassified Actinobacteria.
329 Genes for the mineralization of organic sulfur compounds were detected (**Supplementary**
330 **Materials Fig S3**), with high homology to those of Actinobacteria (Actinomycetales,
331 Rubrobacterales) and Sphinogomonadales (**Fig. 5C**). Although Namib Desert soils are SO_4^{2-}
332 rich (64), genes for the anaerobic process of dissimilatory sulfate reduction and sulfide
333 oxidation (*apr*A, *apr*B and *dsr*A) were not detected in the metagenomic contigs. However,
334 using a conserved domain search (CDD), we identified one partial *sox*B gene assigned to
335 Deltaproteobacteria and *sox*YZ genes in a contig assigned to Alphaproteobacteria (genus
336 *Methylobacterium*). The Sox enzyme system has four principal complexes (*sox*XA, *sox*YZ,
337 *sox*B and *sox*CD) encoding enzymes which catalyze the oxidation of hydrogen sulfite,
338 thiosulfate, elemental sulfur and sulfite to sulfur intermediates or sulfate (61). The *soxB* gene
339 is typically used as a marker gene for the sox system in the environmental bacteria (62). Sox
340 enzymes are commonly associated with the facultative chemolithotrophic
341 Alphaproteobacteria and the *soxB* gene has been found in the chemolithotrophic
342 *Thiobacillus*-like Betaproteobacteria in agriculture soil (63). In the Namib Desert soils, high
343 sulphate concentrations (3242.5 mg/kg) (64) and the presence of SOX system in the hypolith
344 metagenome is suggestive of the presence of chemolithoautotrophic metabolism.

Conclusion

Metagenome sequence data can be validly used to assess the functional capacity of microorganisms in poorly studied environments (67). The analysis of metagenome sequence data from Namib desert hypolithic extracts has provided an expanded overview of the taxonomic and functional diversity of hypolithic microbial communities. Our analysis has shown that these communities are predominantly bacterial, but provides evidence of the presence of archaea and eukaryotes, albeit in much lower proportions. While we identified viral sequences affiliated to Caudovirales, Phycodnaviridae and Mimiviridae, the influence of viruses on the diversity of hypolithic systems remains unknown and complementary studies focused on this particular group are urgently required. Our data analyses also provide evidence of novel and unclassified taxa predominantly affiliated to Actinobacteria, Proteobacteria and Cyanobacteria. The analysis of functional gene diversity has implicated a large diversity of genes affiliated with these taxa in primary productivity, with members of the Proteobacteria and Actinobacteria potentially implicated in chemolithotrophic metabolism (P and S) in the desert environment. Overall, our data support the concept that Actinobacteria may be significant in driving productivity in soils, as indicated by the presence of numerous genes and modules implicated in heterotrophic carbon utilization, aromatic compound degradation and (to a much lesser extent) N cycling.

Edaphic ecosystems are key elements of climate-feedback models, due to their extensive capacity for the release and absorption of greenhouse gases (65). Cryptic niches, such as hypoliths, constitute substantial components of desert edaphic ecosystems (1, 30) and may be important drivers of gas exchange and geochemical cycling processes in desert soil ecosystems. Our data suggest that hypolithic communities have a high capacity for C

fixation, as evidenced by the substantial presence of key photosynthetic genes. In contrast, our metagenome sequence analyses suggest a severely limited capacity for N cycling. Given the known interplay between the C and N cycle (66), it is uncertain what the potential imbalance between C and N turnover processes in hypolithic communities means in terms of 'system stability' and ecosystem services.

Materials and Methods

Sequencing and assembly of the hypolith metagenome

Hypolith samples (n=50) were collected from the Namib Desert (S 23°32.031', E 15°01.813') in April 2010 (11). Samples were first processed for the isolation of total DNA and purified metagenomic DNA samples pooled for sequencing. Sequencing of metagenomic DNA was carried out with Illumina Hiseq-2000 using paired-end technology (2 x 101 bases). The metagenomic DNA was sheared into fragments of 300 bases and recovered from agarose gels. Adapters were ligated to the ends of the DNA fragments for bridge amplification and sequencing. The short paired-end reads were used to assess the quality of sequencing data using an in-house custom python script. The reads having ambiguous base (N) and average quality score less than 25 were removed using a custom python script. Assembly of the contigs was performed by Velvet v1.2.10 at hash length (k) 51 (68).

Bioinformatic analysis of metagenome

Metagenomic data were used for all taxonomic and functional gene analyses. ORFs were predicted from the contigs using the program *MetaGeneMark* (69). First, high quality reads were used for the taxonomic assessment by screening for small subunit (SSU) rRNAs with Metaxa2 (19) and for phylogenetic marker genes with metagenomic phylogenetic analysis

(*MetaPhAn*) software (20). Metaxa2 software extracts the SSU sequences from larger sequence datasets and assigns them for archaeal, bacterial, nuclear eukaryote, mitochondrial or chloroplast origins while *MetaPhlAn* predefines unique clade-specific marker genes as species-specific name tags (20). Next, assembled contigs longer than 500 bases were further selected for the binning process using *MyTaxa* (27). Functional annotation of ORFs was based on KEGG pathways and SEED subsystems using a *blastp* search (E-value cutoff at 1e-5) against the NCBI refseq protein database: results were further analysed using MEGAN v5.0.3 (70). KEGG modules were analysed by the Metabolic And Physiological Potential Evaluator (MAPLE) web server (33). The Module Completion Ratio (MCR) for each phylum was calculated using the bi-directional best hit (BBH) algorithm. Venn diagrams were computed by analysing the 100% complete KEGG pathway modules for the three most dominant phyla. Marker genes for the analysis of the Carbon, Nitrogen and Sulfur metabolism were selected and analysed as described by the Llorens-Mares (71).

Nucleotide accession number: The high quality paired end short reads were deposited at the NCBI under the Bioproject ID: PRJNA290687 and SRA accession number is SRR2124832.

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Figure 1. Taxonomic classification of metagenomic reads. Classification was performed by filtered high quality reads using MetaPhlAn and Metaxa2 at Phylum level. Bar graph is showing the percent abundance of the different bacterial phyla in hypoliths metagenome.

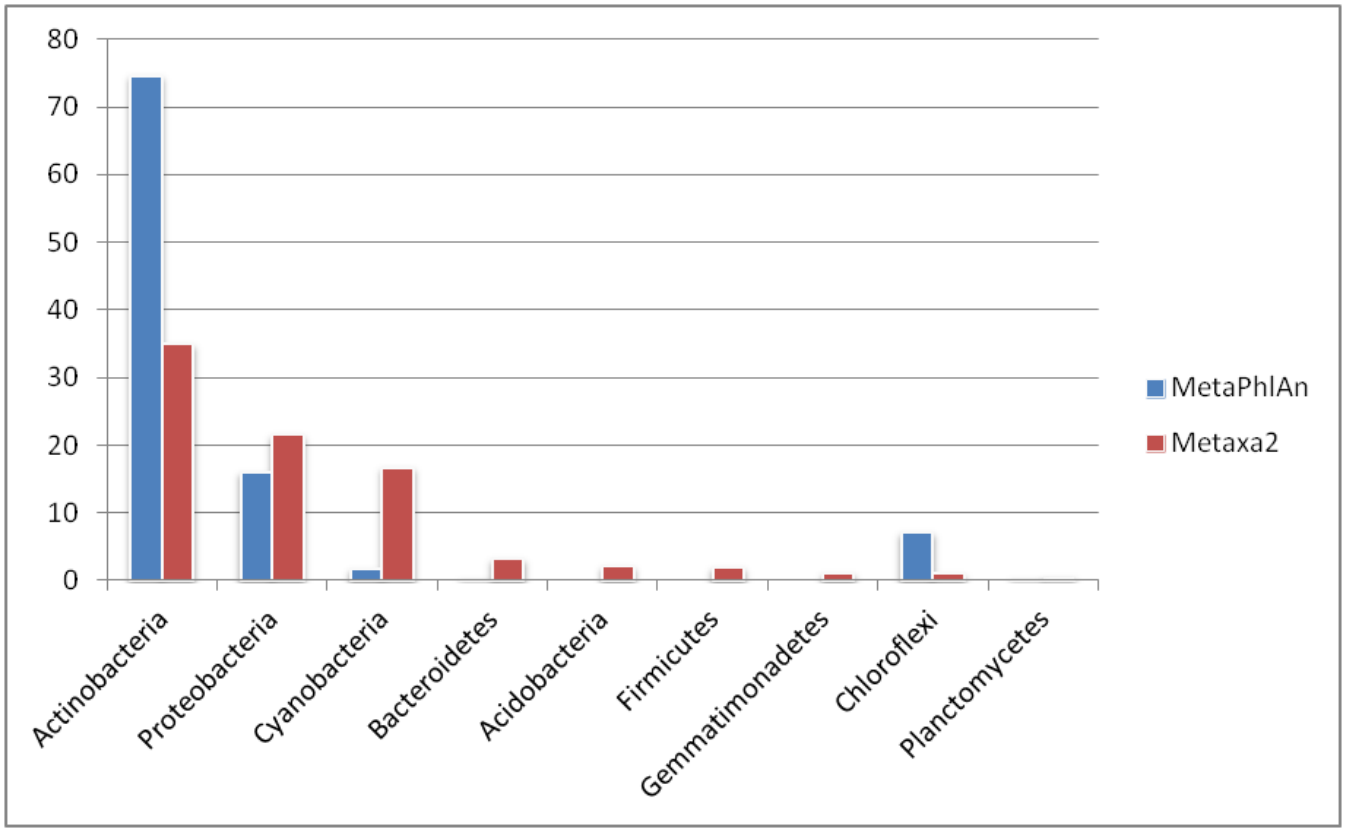


Fig 2. Predicted genes (ORF) and contigs in each taxa(A) Abundance of ORF belongs to different bacterial phya. Distribution of ORFs for the three most abundant bacterial phyla **(B)** Actinobacteria **(C)** Proteobacteria, and **(D)** Cyanobacteria. (Number of contigs for each taxa are written on the top of columns.)

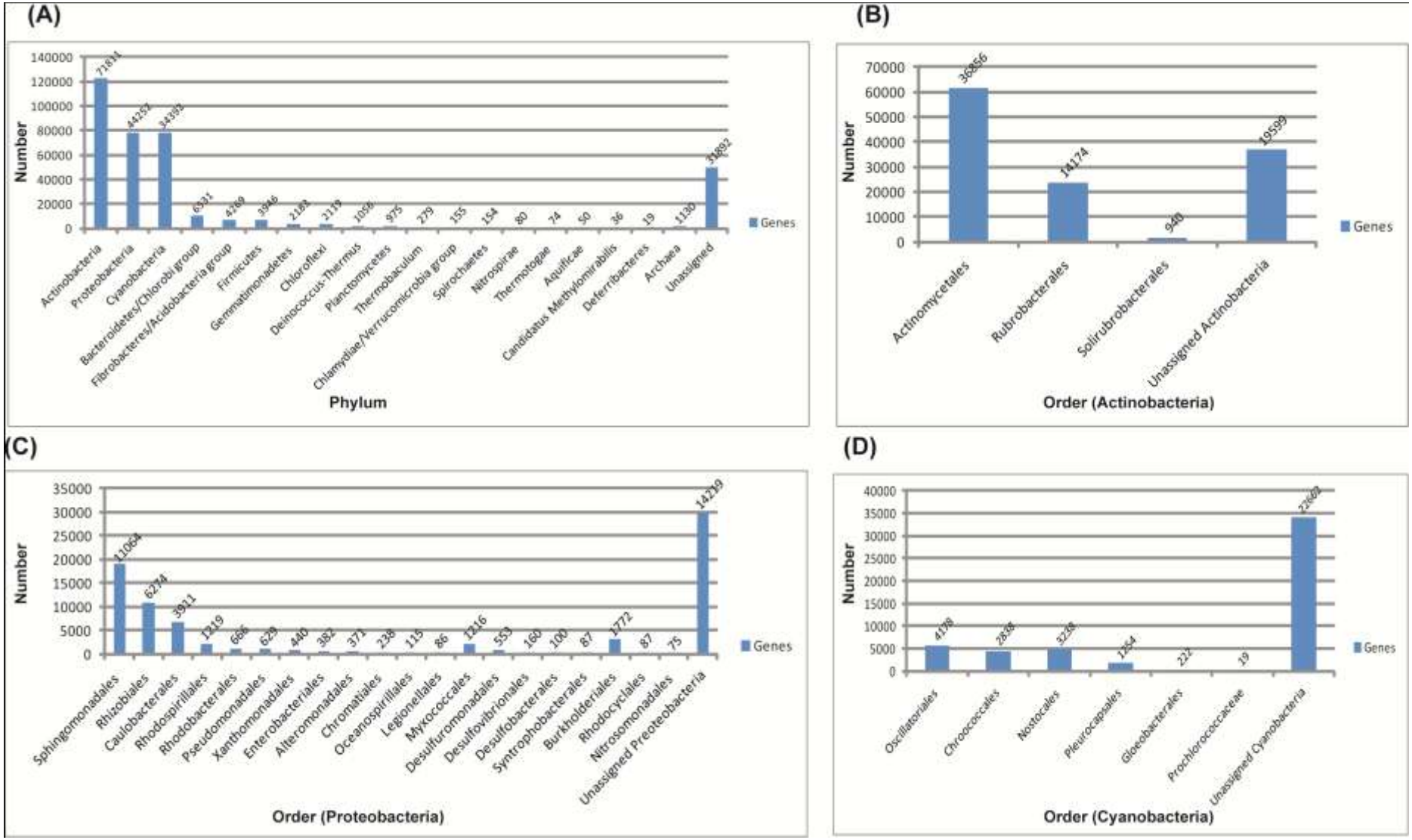


Figure 3. Classification of the genes by MEGAN. Bar graph is showing the number of genes assigned to the each phylum **(A)** KEGG pathway and **(B)** SEED subsystem

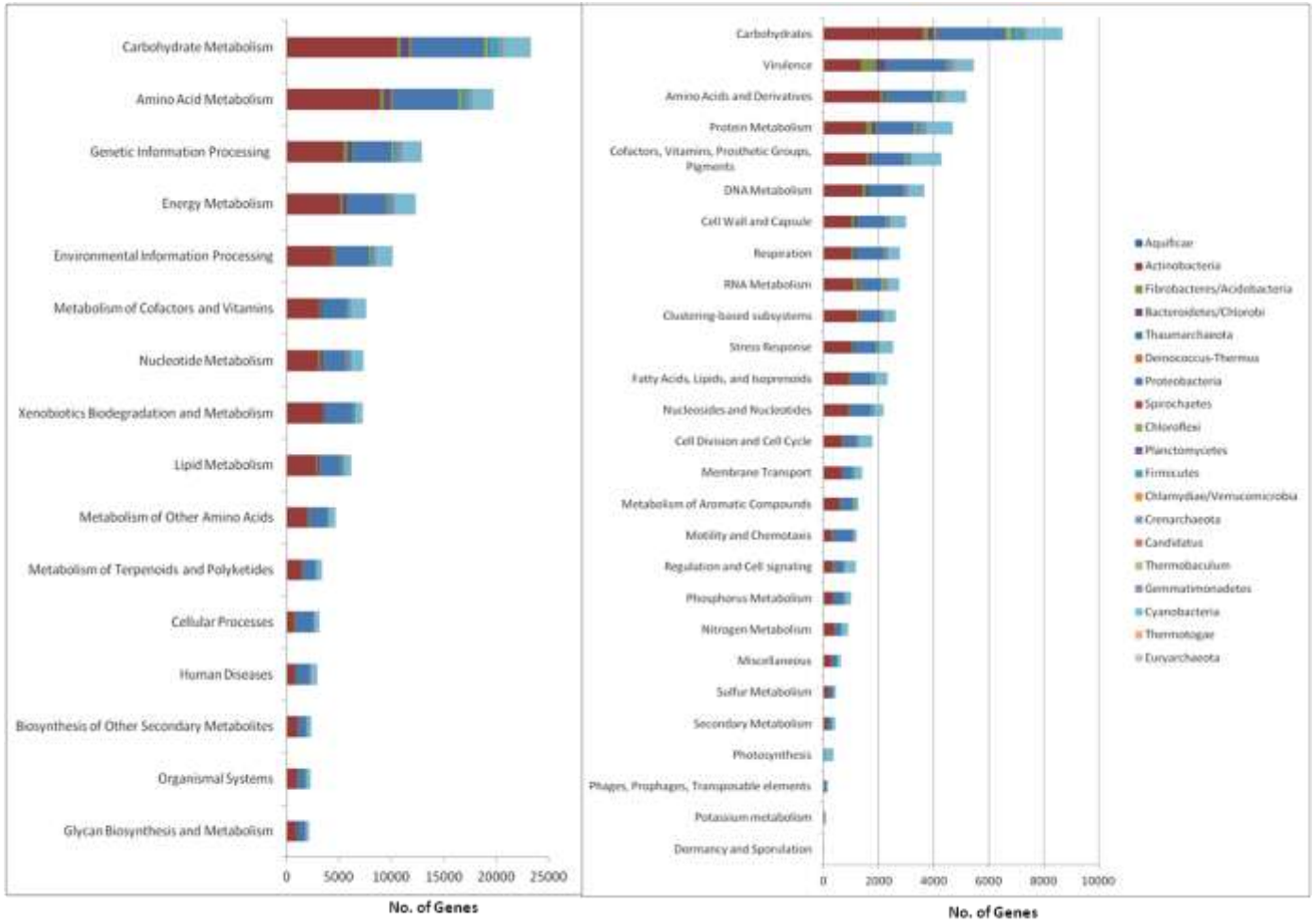


Figure 4. Heat map showing the percentage module completion ration (MCR) for the aromatic compound degradation, energy metabolism and Photosystem apparatus.
Module completion ratio for the pathways was calculated by MAPLE server using KEGG database.

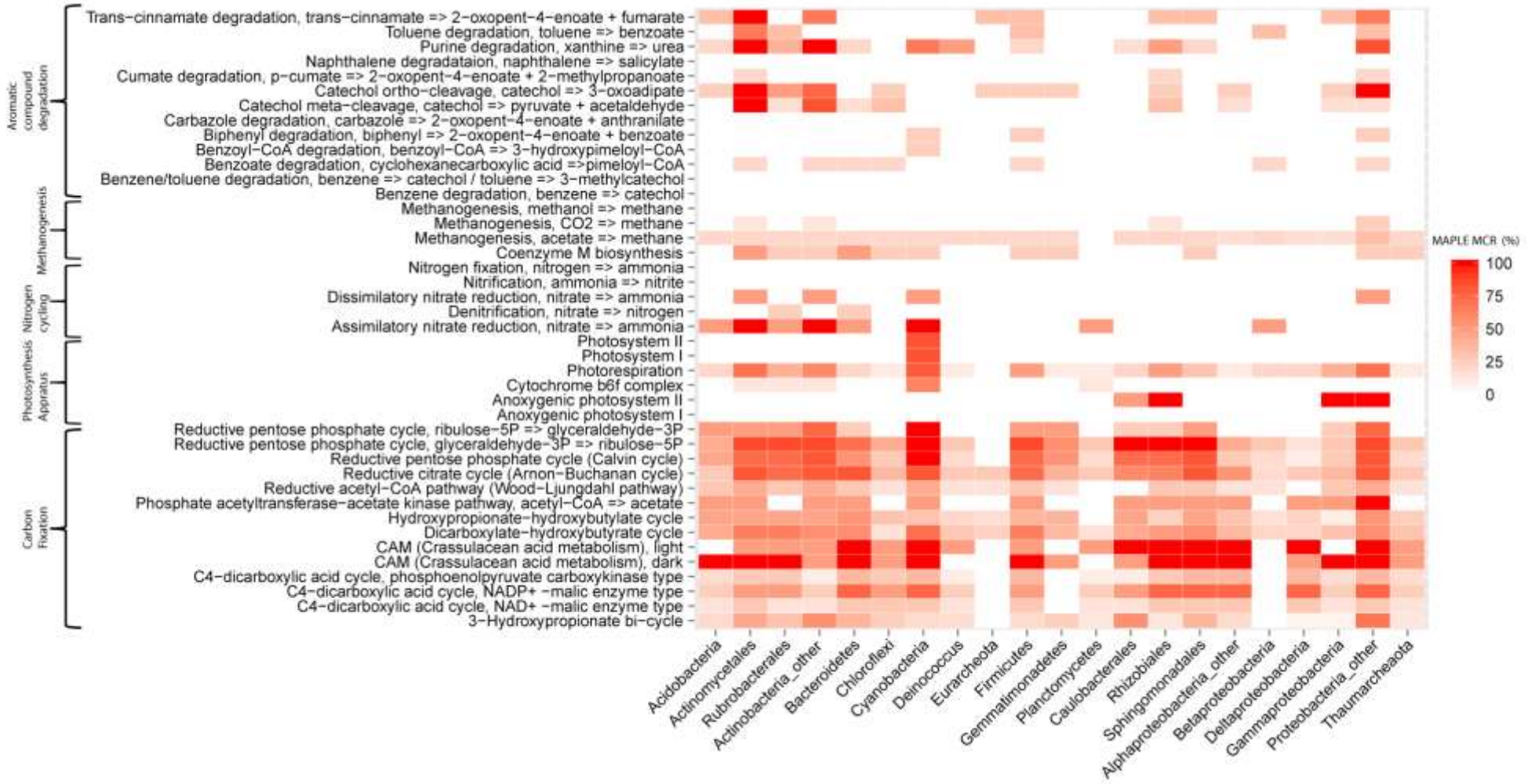
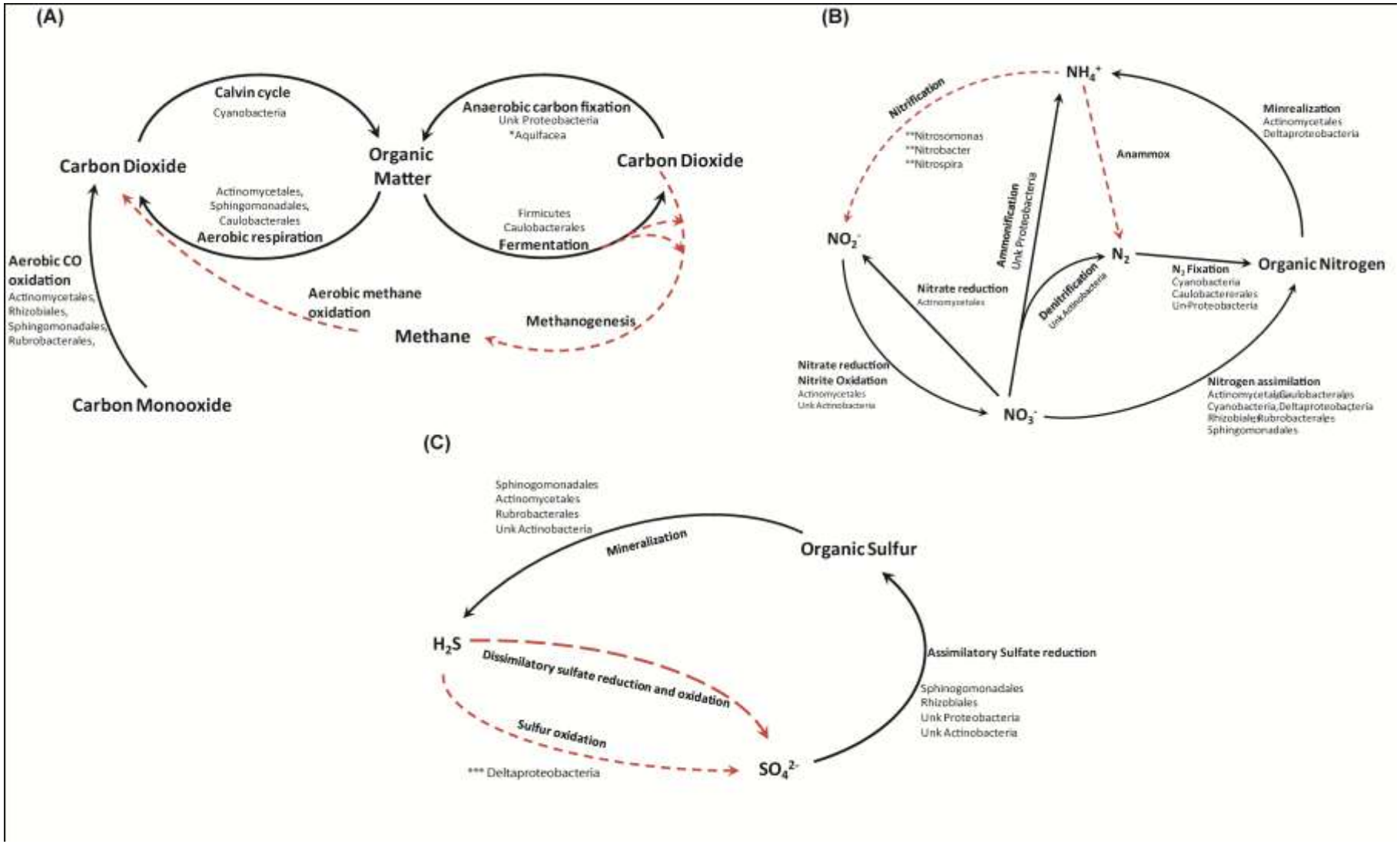


FIGURE 5. Schematic representation of the biogeochemical cycling pathways (based on the analysis of marker genes described by Llorens-Mares et al., 2015) **(A)** Carbon cycling; *Aquifacea potential anaerobic carbon fixation step based on the presence of key enzyme ATP citrate lyase in the phylum **(B)** Nitrogen cycling; **Potential nitrifying bacteria contigs were found in the metagenome but genes for the nitrification were not identified **(C)** Sulfur cycling; ***Deltaproteobacteria *soxB* marker gene for the sulfur oxidation. The dotted lines are representing the absence of the marker genes in the metagenome.



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