**Supplementary document for**

**Microbial diversity in an early earth analogue: From ancient novelty to modern commonality**

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**Supplementary Text**

**1. Detailed phylogenomic analysis of Zodletone spring sediments and water communities.**

***Anoxic sediments.***

*Overall sequencing overview and diversity patterns.* Metagenomic sequencing of the spring sediments yielded 281 Gbp, 79.54% of which assembled into 12 Gbp contigs, with 6.8 Gbp contigs longer than 1Kbp. 1,848 genomes were binned, 683 of which passed quality control criteria, and 516 remained after dereplication (Table S1). These MAGs represented 64 phyla or candidate phyla (53 bacterial and 11 archaeal), 127 classes, 198 orders, and 300 families (Figure 1a-b). Diversity assessment utilizing small subunit ribosomal protein S3 from assembled contigs (n=2079), as well as a complementary 16S rRNA illumina sequencing effort (n=309,074 amplicons), identified a higher number of taxa (82 phyla and 1679 species in the ribosomal protein S3 dataset, and 69 phyla and 1050 species in 16S rRNA dataset) (Figure S2). Nevertheless, the overall community composition profiles generated from all three approaches were broadly similar.

*Detailed phylogenetic analysis of Zodletone sediment MAGs.* The Chloroflexota (n=69), Planctomycetota (n=47), Bacteroidota (n= 43), Desulfobacterota (n= 43), Spirochaetota (n= 28 genomes), Patescibacteria (n=20 genomes), and the archaeal phylum Nanoarchaeota (n=21) were the most abundant phyla in Zodletone spring sediments, albeit representing only 52.52% of the total number of recovered genomes (Figure 1, 2C). A notable absence, or extreme paucity, of genomes belonging to the Proteobacteria (6 genomes) and Firmicutes (12 genomes), the most successful taxa within current biomes 1, as well as the oxygen-generating oxycayanobacteria (0 genomes) were observed (Figure 1, 2C). Within the Chloroflexota, 38/69 genomes belonged to 3 novel orders, 5 novel families, and multiple LRD orders (Thermoflexales, 4572-78, and UBA2777) and families (E44-bin32, Fen-1058, J111, RBG-13-53-26, RBG-16-64-43, UBA11579, UBA11858, UBA2029, UBA2162, UBA3940, UBA4811, UBA4823, UBA5620, UBA5760, and UBA6092) (Figure S3a). Within the Planctomycetota, 17/47 genomes belonged to 2 novel orders, 8 novel families, and multiple LRD orders (FEN-1346, SZUA-567, and UBA8890) and families (Fen-1342, SM23-30, UBA1845, UTPLA1, and UBA8108, Figure S3b). Within the Bacteroidota, 27/43 genomes belonged to 1 novel family, and multiple LRD families (FEN-979, GCA-2748055, NBLH01, UBA10428, UBA12170, UBA5072, UBA6680, and SZUA-365, Figure S3c). Within the Spirochaetota, 19/28 genomes belonged to one novel class, 2 novel orders, and 9 novel families, as well as multiple LRD families (ARS1246, Marispirochaetaceae, and RPPD01, Figure S3d). Within the Desulfobacterota (Figure S3e), 35/43 genomes belonging to 3 novel classes, 10 novel orders, and 7 novel families were identified, as well as multiple LRD families (B25-G16, BuS5, HGW-15, MLS-D, NaphS2, UBA2210, and UBA3084). Only 6/43 genomes belonged to the well-described families Desulfovibrionaceae, Geopsychrobacteraceae, Smithellaceae, and Syntrophaceae. Finally, an extremely diverse community of Patescibacteria (13 different orders, 3 of which belonging to LRD orders, and 14 different families, including 6 novel and 2 LRD families), and Nanoarchaeota (2 orders including the LRD order CG07-land), and 15 different families, including 5 novel and 10 LRD families) were identified in the spring sediments (Figure S3F). A similar pattern of high proportion of novel and LRD families was identified throughout all other lineages (Figure 1a). Therefore, in addition to expanding the number of novel lineages (classes, orders, and families), and greatly enriching available genomes in rare, poorly represented taxa, our results highlight the uniqueness and distinction of the microbial community thriving in Zodletone spring sediments, compared to all previously studied habitats on the current earth.

***Hypoxic waters*.**

*Overall sequencing overview and diversity patterns.* Metagenomic sequencing of the oxygen-exposed overlaying water column community yielded 323 Gbp, 80.07% of which assembled into 3.6 Gbp contigs with 3.1 Gbp contigs >1K. 883 genomes were binned, with only 114 remaining after dereplication. Of these, 62 belonged to shared families with the sediment community, and 52 were water specific.

*Detailed phylogenetic analysis of Zodletone water MAGs.* Water-specific genomes (n=52) mostly belonged to well-characterized microbial lineages, e.g. class Alphaproteobacteria (5 genomes belonging to Rhodobacteraceae and Rhodospirillaceae, and 3 belonging to the uncultured lineages NBLK01 and Rs-D84), Gammaproteobacteria (9 genomes belonging to the lineages Thiomicrospiraceae, Halothiobacillaceae, Acidithiobacillaceae, Burkholderiaceae, Chromatiaceae, and Methylothermaceae, and 1 belonging to the uncultured lineage UBA9339), phylum Camplylobacterota (8 genomes, belonging to the families Sulfurimonadaceae, Sulfurovaceae, and Sulfurospirillaceae), Firmicutes/Firmicutes\_A (4 genomes, and 6 genomes belonging to the Bacilli, and Clostridia classes, respectively), and well described families in the phyla Bacteroidota (Flavobacteriaceae, Prolixibacteraceae, Paludibacteraceae, Marinilabiliaceae, Tannerellaceae, Marinifilaceae, Balneolaceae), Desulfobacterota (Families Geopsychrobacteraceae, Desulfuromonadaceae,), and Spriochaetota (Sphaerochaetaceae, Treponemataceae, Spirochaetaceae\_B). Collectively, this demonstrates a pattern where the intrusion of oxygen is associated with a negative impact on novel and LRD lineages that are prevalent in the sediment, and the propagation of communities associated with well-characterized lineages within the bacterial tree of life.

**2. Reductive sulfur processes in Zodletone spring sediment communities.**A total of 149 genomes (28.9 % of all genomes), belonging to 32 phyla, 51 classes, 69 orders, and 97 families were involved in at least one reductive sulfur process (Figure 3, Table S3). By comparison, only 21 sediment genomes (4.06% of all genomes) encoded at least one sulfur oxidation pathway (Figure 3, Table S3). The reductive sulfur-community in the spring exhibited two unique traits: First, a majority of genomes encoding such capacities belonged to novel (47 genomes) or LRD (66 genomes) lineages (Figure 3), and second: sulfite-, polysulfide-, thiosulfate-, and tetrathionate reduction appears to be more prevalent than sulfate-reduction capacities in the sediment genomes.

*Sulfate reduction.* Sulfate-reduction capacity was observed in only 18 sediment genomes (Figure 3, 4a, Table S3), but exhibited a unique community composition, when compared to well-studied marine and terrestrial habitats 2-5. Sulfate-reduction capacities were observed in the Zixibacteria (3 genomes belonging to class MSB-5A5), Acidobacteriota (2 genomes belonging to family UBA6911, equivalent to Acidobacteria group 18), Myxococcota (2 genomes belonging to the order Polyangiales), Bacteroidota (uncultured families UBA10428 and UBA5072 within Bacteroidales), Planctomycetota (1 genome belonging to a novel family within class Phycisphaerae), candidate phylum OLB16 (1 genome), as well as rare and novel lineages within the Desulfobacterota (5 genomes belonging to family HGW-15 within Desulfatiglandales, 1 genome belonging to Order C00003060 within Desulfobacterota). Only 1 genome belonging to the canonical sulfate reducing family Desulfovibrionaceae was identified. Organization of the sulfate reduction genes differed between different phyla, with Myxococcota, Zixibacteria, OLB16, and Acidobacteriota genomes encoding all genes for sulfate activation and reduction, dissimilatory sulfite reduction, as well as energy conservation on one locus, while Desulfobacterota genomes encoded genes for sulfate activation and reduction as well as energy conservation on one locus with genes for dissimilatory sulfite reduction on another, as previously observed in cultured Desulfobacterota 6-8.

*Sulfite-reduction.* Sulfite (but not sulfate) reduction via the DsrAB+DsrC+DsrKMJOP system was identified in only 8 genomes belonging to 7 families within the phyla Planctomycetes (1 genome belonging to the uncultured family UBA11386 within the Pirellulales), Chloroflexota (2 genomes belonging to the uncultured family EnvOPS12 within Anaerolineales and the uncultured Family UBA5629 within Dehalococcoidia), Spirochaetota (1 genome belonging to the uncultured class UBA4802), and Desulfobacterota (2 genomes belonging to family HGW-15 within Desulfatiglandales, and 2 genomes belonging to one novel family and the uncultured family UBA2210 within Syntrophales) (Figure 3, 4a, Table S3). Gene organization of the *dsr* locus in the above 9 genomes differed between different phyla, with Chloroflexota, and Spirochaetota genomes encoding all genes for dissimilatory sulfite reduction (dsrABC plus dsrKMJOP) on one locus, while Planctomycetota and Desulfobacterota genomes showed a split *dsr* locus with dsrABC on one locus and dsrMKJOP on another.

On the other hand, sulfite-reduction capacity within Zodletone spring sediment solely via the Asr/Hdr system was rampant, being encountered in 104 genomes belonging to 28 phyla, 43 (8 novel and 9 LRD) classes, 56 (18 novel, and 12 LRD) orders, and 72 (31 novel and 25 LRD) families (Figure 3, 4b, Table S3), with a gene organization of the *asr* locus adjacent to the *hdr* locus in the majority of genomes. Asr-encoding lineages in the spring included the Chloroflexota (15 genomes all from uncultured lineages belonging to novel (6 genomes) and LRD (6 genomes) families EnvOPS12, UBA11858, UBA4823, UBA6092, UBA4811, Fen-1058 in addition to the families UBA1429, UBA3254, UBA5760), Desulfobacterota (14 genomes belonging to novel (3 genomes) and LRD (7 genomes) families B25-G16, HGW-15, MLS-D, UBA3084, in addition to the families Smithellaceae, UBA2185, UBA5619), Planctomycetota (11 genomes belonging to novel (2 genomes), and LRD (3 genomes) families SM23-30 and UBA1845 in addition to the family Anaerohalophaeraceae), Spirochaetota (11 genomes belonging to novel (8 genomes) families in addition to the uncultured family UBA5550), and Bacteroidota (7 genomes belonging to the LRD (5 genomes) family BA5072, in addition to the family VadinHA17). The capacity was also rampant in the yet-uncultured bacterial phyla, many of which have fairly limited distribution on the current earth, e.g. the candidate phyla CSSED10-310, FCPU426, RBG-13-66-14, SM23-31, SZUA-182, UBP14, Aureabacteria, Sumerlaeota. Interestingly, all genomes belonging to the novel phylum Krumholzibacteriota, recently described from the spring sediment 9, encoded complete anaerobic sulfite reductase systems.

Phylogenetic analysis of dissimilatory sulfite reductase Dsr (concatenated dsrA and dsrB) (Figure 4a) showed clustering of Zodletone Desulfobacterota sequences with canonical sulfate-reducing Desulfobacterota as well as the sequences from the Firmicutes genera *Moorella* and *Desulfotomaculum* shown before to have acquired these genes through lateral gene transfer 10. In addition, sequences from Zodletone Chloroflexota, Spirochaetota, Myxococcota, Acidobacteriota, Planctomycetota, Zixibacteria, and candidate division OLB16 clustered sister to the Desulfobacterota clade with no evidence for LGT between phyla.

Phylogenetic analysis of AsrB sequences clustered into four distinct clades (Figure 4B) governed by their organismal phylogeny; (1) Sequences from the Chloroflexota families UBA6092, UBA11858, and EnvOPS12 clustered sister to sequences from Actinobacteria genus *Streptomyces,* (2) sequences from the phyla Desulfobacterota, Myxococcota, Acidobacteriota, Verrucomicrobia, Fibrobacterota, Delongbacteria, UBP14, Sumerlaeota, and Actinobacteriota (class RBG-13-55-18), in addition to the Chlorobia class within Bacteroidota, the Chloroflexota orders UBA1429, Dehalococcoidales, Thermoflexales, B4G1, SBR1031, Promineofilales, novel orders ZNO10, and ZNO11, the Spirochaetota class UBA4802, and the novel family ZNF102 within Spirochaetales all clustered close to sequences from the genera *Desulfocapsa*, *Syntrophocurvum*, *Syntrophomonas*, and *Syntrophobotulus*, (3) sequences from the Firmicutes family Vallitaleaceae clustered with *Acetoanaerobium*, *Petrocella* (Firmicutes) and *Fusobacterium* sequences, and (4) sequences from Fermentibacteriota, the novel Spirochaetales family ZNF103, Krumholzibacteriota, candidate divisions WOR-3, KSB1 and SM23-31, Cloacimonadota, Planctomycetota, and the Bacteroidales families UBA5072 and VadinHA17 clustered close to the single Thermotogae reference sequence from *Kosmotoga olearia*. No evidence for LGT between phyla was deduced from the tree topology. As such, this study greatly expanded the Asr phylogeny beyond the handful of taxa from which it was previously described.

*Thiosulfate disproportionation**and reduction.* In Zodletone sediment, the quinone-dependent membrane-bound molybdopterin-containing thiosulfate reductase PhsABC was encoded in 11 genomes belonging to 6 phyla, with Bacteroidota representing the major phsABC-encoding phylum (4 genomes) (Figure 3, S4, Table S3). Within these genomes, only two (a Chloroflexota family UBA6092 genome, and a Desulfatiglandales family HGW15 genome) also encoded a dissimilatory sulfite reductase (the Asr system) akin to the Gammaproteobacteria thiosulfate disproportionating pure culture members, where the final products of thiosulfate disproportionation are expected to be only hydrogen sulfide. On the other hand, 5 of the 11 phsABC-encoding Zodletone genomes also encoded the sulfite dehydrogenase SoeABC system, akin to Desulfobacterota and Firmicutes pure culture members, where the final products of thiosulfate disproportionation are expected to be both hydrogen sulfide and sulfate.

In addition to the phsABC system, 14 Zodletone genomes belonging to 6 phyla (6 Desulfobacterota genomes, 2 Acidobacteriota genomes, 3 Chloroflexota genomes, 1 Bacteroidota genome, 1 Spirochaetota genome, and 1 Myxococcota genome) encoded a rhodanase-like enzyme [EC: 2.8.1.1 or EC: 2.8.1.3] for thiosulfate disproportionation, as well as enzymes for both sulfite oxidation (by means of reversal of sulfate reduction via Sat+AprAB, or the sulfite dehydrogenase SoeABC), and sulfite reduction (via the dissimilatory sulfite reductases Dsr or Asr), where the final products of thiosulfate disproportionation are expected to be both hydrogen sulfide and sulfate. It is worth noting that 113 other Zodletone sediment genomes encoded rhodanase-like enzymes but with no additional mechanisms for sulfite oxidation and/or reduction. We speculate that the function of rhodanase in these organisms could be part of a thiosulfate assimilatory pathway as recently shown in *E. coli* 11.

*Tetrathionate reduction.* Seventy-three Zodletone sediment genomes encoded the octaheme tetrathionate reductase (OTR) enzyme. These genomes belonged to 14 phyla with major contribution from Bacteroidota (30 genomes), Chloroflexota (10 genomes), and Desulfobacterota (10 genomes) (Fig 3, S6, Table S3). In addition to Otr, 68 Zodletone genomes encoded the Ttr enzyme system. These genomes belonged to 14 phyla with major contribution from Chloroflexota (22 genomes) and Desulfobacterota (20 genomes). As shown previously in *Salmonella typhimurium* 12, in presence of means for thiosulfate disproportionation/reduction and sulfite reduction, the thiosulfate produced as a result of tetrathionate reduction could be further reduced to sulfide. Out of the 105 sediment genomes encoding the Otr and/or Ttr enzymes, only 12 genomes also encoded thiosulfate and sulfite reduction enzymes. These genomes belonged to the phyla Acidobacteriota (2 genomes in the family UBA6911), Chloroflexota (3 Anaerolineales genomes), Desulfobacterota (4 in the orders Syntrophales, Desulfatiglandales, and C00003060, and 1 belonging to a novel class), Myxococcota (1 genome in a novel family), and Spirochaetota (1 genome).

*Sulfur (polysulfide) reduction.* Twenty Zodletone sediment genomes encoded *psrABC* genes (Figure 3, S6, Table S3). These genomes belonged to the phyla Bacteroidota (8 genomes belonging to the uncultured Bacteroidales families FEN-979, UBA10428, UBA3824, UBA5072, and UBA6680, and 1 Chlorobia genome), Campylobacterota (2 genomes), Acidobacterota (2 belonging to the order Aminicenantales, and 2 belonging to the family UBA6911), Chloroflexota (2 genomes belonging to the order Dehalococcoidales), Desulfobacterota (2 genomes belonging to the novel class ZNC02), and 1 Myxococcota genome (belonging to the novel family ZNF055). In addition, representatives of the cytoplasmic sulfurhydrogenase I (HydABCD system) and/or II (ShyABCD system) were identified in 119 Zodletone sediment genomes (Figure 3, Table S3). However, as described above, direct involvement of these enzymes in an ETS-associated respiration is not yet clear.

*Substrates supporting sulfidogenic capacities at Zodletone spring.* Within lineages mediating reductive sulfur processes in Zodletone sediments (n=98), a wide range of substrates supporting sulfidogenesis were identified (Table S2, Figure 3). These included hexoses (26-87% of sulfidogenic lineages), pentoses (30-41% of sulfidogenic lineages), amino acids and peptides (39% of lineages), short chain fatty acids, e.g. lactate, propionate, butyrate, and acetate (22-73% of lineages), long chain fatty acids (29% of lineages), aromatic hydrocarbons (3% of lineages), and short chain alkanes (6% of lineages). Autotrophic capacities with hydrogen as the electron donor were identified in 28% of sulfidogenic lineages. Of note, the sulfate-reducing lineages also encoded D-lactate oxidation capacity, as well as hydrogen oxidation capacities, similar to canonical sulfate reducers 13,14.

**3. Transcriptomic analysis.** Transcriptional expression of genes involved in S-species reduction/disproportionation was analyzed, and the identity of the active sulfur-reducing community in the spring sediment was examined (Figure S7). All S-species reduction/ disproportionation genes discussed above were identified in the metatranscriptomic dataset, and transcripts belonging to 51 different phyla were identified. Analysis of the spring sediment revealed the transcription of both the Dsr and Asr systems for sulfite reduction with contributions from Chloroflexota, Planctomycetota, Desulfobacterota, Bacteroidota, Fermentibacterota, Acidobacteriota, CSSED10-310, Actinobacteriota, Spirochaetota, WOR-3, and Fibrobacterota (Asr), and Desulfobacterota, Acidobacteriota, Zixibacteria, and Myxococcota (Dsr). Sulfate reduction genes (Sat, AprAB, and QmoABC) were also transcribed with major contribution from Desulfobacterota, Myxococcota, Zixibacteria, and Acidobacteriota. Total transcription levels of the Asr system were 4-times higher than the Dsr system, consistent with the higher number of Zodletone sediment genomes encoding the Asr system compared to the Dsr system. Transcription of the thiosulfate disproportionating rhodanese-like enzyme [EC: 2.8.1.1 or EC: 2.8.1.3] was detected in the phyla Desulfobacterota, Actinobacteriota, Firmicutes\_A, Chloroflexota, Fibrobacterota, Planctomycetota, Spirochaetota, Bacteroidota, Halobacteriota, and Acidobacteriota, while the transcription of the thiosulfate reductase *phsABC* was detected in the phyla Actinobacteriota and Bacteroidota. Transcription of the tetrathionate reduction genes *ttrABC* was detected in the phyla Desulfobacterota, Actinobacteriota, Bacteroidota, Chloroflexota, Acidobacteriota, and Spirochaetota, while the octaheme tetrathionate reductase *otr* transcription was detected in Desulfobacterota, Bacteroidota, Myxococcota, UBP7\_A, and Chloroflexota. Finally, the transcription of *psrABC* for polysulfide reduction was detected majorly in the phyla Bacteroidota, Desulfobacterota, and Campylobacterota, while transcription of the cytoplasmic sulfurhydrogenases I and II (*hyd/shy* systems) was identified in the phyla Actinobacteriota, Chloroflexota, Planctomycetota, Myxococcota, Acidobacteriota, Bacteroidota, and Desulfobacterota.

**4.** **Oxidative sulfur processes dominate Zodletone water community.**

In contrast to sediment communities, reductive sulfur-processes were identified in only 25 (21.92%) water genomes, as opposed to 149 (29%) sediment genomes (Figure 3, Table S3). Dissimilatory sulfate reduction to sulfide capacity was completely absent in water genomes. The capacity for dissimilatory sulfite reduction via the Dsr system was absent, and the Asr system was only encoded in 7 water genomes. Thiosulfate reduction/disproportionation capacity to sulfide and sulfate (PhsABC + SoeABC, and/or Rhodanase + Dsr/Asr + SoeABC/SorAB) was encoded in only four genomes, all of which also encoded the capacity for tetrathionate reduction (via Otr and/or ttrABC). Finally, respiratory polysulfide reduction (via PsrABC) was encoded in 19 genomes. In all cases, the reductive sulfur community in water was a subset of the sediment community.

In contrast, oxidative sulfur processes dominated the water community, with pathways encoding sulfide, sulfur, thiosulfate, tetrathionate, and/or sulfite oxidation to sulfate present in 59/114 genomes (51.8% of all water genomes) belonging to 13 phyla, 16 classes, 25 orders, and 43 families. The oxidative sulfur community in the water belonged to mostly well-characterized lineages (Table S3, Figure 3). Only 8 and 10 genomes involved in oxidative sulfur processes belonged to novel, and LDR families, respectively.

A complete SOX system, putatively mediating oxidation of a wide range of reduced sulfur-species to sulfate was encoded in genomes belonging to well-characterized families within the Proteobacteria (11 genomes total belonging to families Acidithiobacillaceae, Burkholderiaceae, Halothiobacillaceae, Rhodobacteraceae, and Thiomicrospiraceae) and Campylobacterota (3 genomes in the family Sulfurimonadaceae).

*Sulfide oxidation to sulfur and sulfite.* Thirty nine water genomes encoded the sulfide dehydrogenase fccAB [EC: 1.8.2.3] and/or the sulfide:quinone oxidoreductase Sqr [EC: 1.8.5.4] both known to oxidize sulfide to sulfur/ polysulfide. These genomes belonged to the phyla Bacteroidota (14 genomes in the well characterized families Chlorobiaceae, Prolixibacteraceae, and Paludibacteracaeae, as well as the uncultured families NBLH01, UBA1556, DTU049, and F082 in the order Bacteriodales), Proteobacteria (13 genomes in the families Acidithiobacillaceae, Burkholderiaceae, Chromatiaceae, Halothiobacillaceae, Methylothermaceae, Rhodobacteraceae, Thiomicrospiraceae), Campylobacterota (8 genomes in the families Sulfurimonadaceae, Sulfurospirillaceae, Sulfurovaceae), in addition to three genomes in the families Anaerolineaceae, Geopsychrobacteraceae, and UBA2242 within the phyla Chloroflexota, Desulfobacterota, and Marinisomatota, respectively, and one genome belonging to a novel Thermodesulfovibrionales family (Nitrospirota). Only two of the above thirty-nine genomes (one Proteobacteria genome and one Nitrospirota genome) encoded the capacity to further oxidize the sulfur/polysulfide to sulfite via the reversal of the Dsr system (encompassing the full Dsr system *dsrAB*+*dsrC*+*dsrMKJOP*, in addition to the genes *dsrEFH*, *tusA*, and *rhdA*).

*Sulfite oxidation to sulfate*: A total of twenty-six water genomes encoded the capacity for sulfite oxidation to sulfate via the reversal of AprAB+QmoABC system (1 Bacteroidales genome), the sulfite dehydrogenase (quinone) SoeABC [EC: 1.8.5.6] (22 genomes belonging to the order Bacteroidales, and the families Acidithiobacillaceae, Burkholderiaceae, Chromatiaceae, Dethiosulfatibacteraceae, Halothiobacillaceae, Methylothermaceae, Rhodobacteraceae, Thiomicrospiraceae within Proteobacteria, the families Sulfurimonadaceae, Sulfurospirillaceae, Sulfurovaceae within Campylobacterota, the Syntrophales family UBA3084, and a novel Thermodesulfovibrionales family (Nitrospirota)), or the sulfite dehydrogenase (cytochrome) SorAB [EC: 1.8.2.1] (3 genomes total within the families Chromatiaceae, Halothiobacillaceae (Proteobacteria), and UBA12059 (Spirochaetota)).

*Thiosulfate oxidation to tetrathionate, and complete thiosulfate oxidation to sulfate via tetrathionate*: Eight water genomes encoded thiosulfate to tetrathionate oxidation capacities via either the thiosulfate dehydrogenase *tsdA* [EC: 1.8.2.2] (7 genomes belonging to the families Sulfurimonadaceae within Campylobacterota, and Rhodobacteraceae, Burkholderiaceae, Halothiobacillaceae, Thiomicrospiraceae within Proteobacteria), or the thiosulfate dehydrogenase (quinone) *doxAD* [EC: 1.8.5.2] (1 Flavobacteriaceae genome). Two of these 8 genomes (1 Rhodobacteraceae, and 1 Halothiobacillaceae genomes) also encoded tetrathionate hydrolase (*tetH*) 15 that is known to cleave tetrathionate to thiosulfate, sulfur, and sulfate. Simultaneous identification of the SOX system and both forms of sulfide dehydrogenase (fccAB and Sqr) imply that these two genomes encode the capacity for complete thiosulfate oxidation to sulfate.

*Tetrathionate oxidation*: In addition to the above two genomes, ten other water genomes encoded tetrathionate hydrolase, but with no other means of thiosulfate and sulfide oxidation capacities. Surprisingly, TetH (without other means of thiosulfate or sulfide oxidation) was also encoded in 100 sediment genomes, belonging to 26 phyla, 37 classes, 49 orders, and 66 families (including 23 novel families). Only nine of these genomes (belonging to 9 families including 4 novel ones) showed *tetH* transcriptional levels above 1. However, the exact function of tetrathionate hydrolase in these organisms is not entirely clear, as the subsequent steps of oxidation could not be identified.

**5. Additional metabolic capacities in Zodletone spring sediments.**

In addition to reductive sulfur processes, strict fermentative capacities were highly prevalent in sediment genomes (Table S2), being identified in 100 of the 291 lineages studied. On the other hand, a dearth of aerobic (only 38 lineages), nitrate (only 65 lineages encoded dissimilatory nitrite reduction to ammonium, with 2 of which also encoding the suite of genes for denitrification), Fe3+ respiration (8 lineages), or chemolithotrophic nitrifying (only 1 lineage encoded the combination of ammonia monooxygenase and hydroxylamine dehydrogenase), and photosynthetic capacities were identified (Figure 1a, Table S2). Strict fermentative lineages mediate the degradation a wide range of substrates, e.g. sugars (89 of the 100 fermentative lineages), amino acids (85 of the 100 fermentative lineages), short chain fatty acids (37 of the 100 fermentative lineages), complex carbohydrates (36 of the 100 fermentative lineages), long chain fatty acid oxidation (2 lineages), and short chain alkanes (1 lineage) (Table S2), producing a wide range of fermentative end products including lactate, formate, acetate, ethanol, succinate, and hydrogen. Primary productivity in the spring sediments appears to be mostly mediated via hydrogen utilization coupled to either sulfur-cycle intermediates (SCI) reduction (27 lineages, Table S2), or to CO2 fixation by hydrogenotrophic methanogens and acetogens using the Wood-Ljungdahl pathway (8 lineages Table S2).

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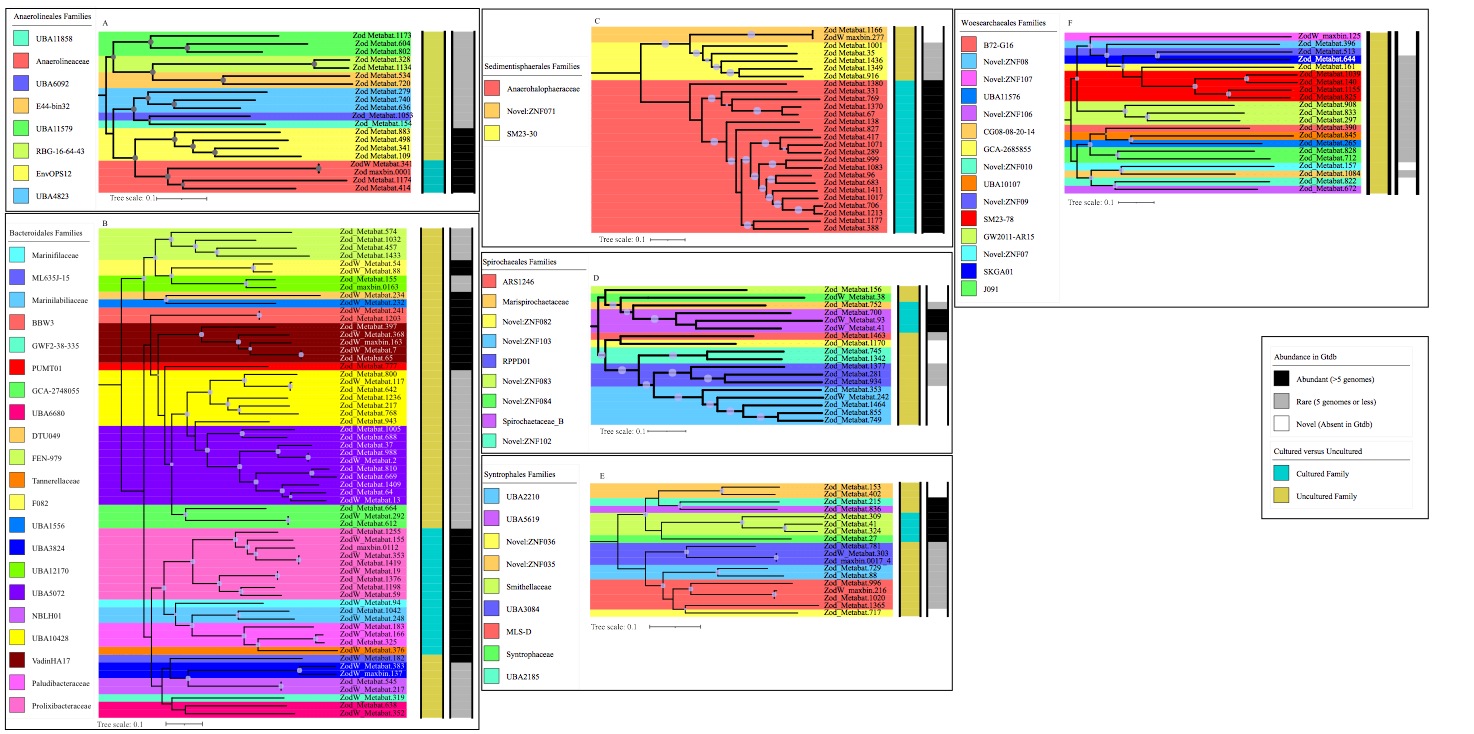
**Supplementary Figures:**

**Figure S1. Zodletone spring source sediments and overlaid water.**



**Figure S2.** Zodletone spring phylum-level community composition based on ribosomal protein S3 (RP-S3), binned genomes (MAGs), as well as the gene for 16S rRNA for both the sediment and the water samples.

**Figure S3.** Family-level delineation for orders with 20 or more genomes. The maximum likelihood trees were constructed in FastTree 16 based on the concatenated alignments of 120-, and 122- single copy genes, respectively, obtained from Gtdb-TK 17. Bootstrap support values are shown as bubbles for nodes with >70% support. Families are color-coded. To the right of the trees, tracks are shown for cultured status at the family level (cultured versus uncultured), and abundance in Gtdb based on the number of available genomes (abundant with more than 5 genomes, rare with 5 genomes or less, and novel with no genomes in Gtdb).



**Figure S4.** Phylogenetic affiliation and contig organization of thiosulfate reductase cytochrome b subunit PhsC from Zodletone spring genomes. Alignments were created in Mafft 18 and maximum likelihood trees were constructed in RAxML 19. Bootstrap support values are shown as bubbles for nodes with >50% support. Branches and branch labels are color coded by phylum for Zodletone sequences. Branch labels depict classification to family level followed by the NCBI genome accession number. Reference sequences are shown in black with the Uniprot accession numbers.



**Figure S5.** Phylogenetic affiliation and contig organization of octaheme tetrathionate reductase (Otr) from Zodletone spring genomes. Alignments were created in Mafft 18 and maximum likelihood trees were constructed in RAxML 19. Bootstrap support values are shown as bubbles for nodes with >50% support. Branches and branch labels are color coded by phylum for Zodletone sequences. Branch labels depict classification to family level followed by the NCBI genome accession number. Reference sequences are shown in black with the Uniprot accession numbers.



**Figure S6.** Phylogenetic affiliation and contig organization of polysulfide reductase subunit gamma PsrC from Zodletone spring genomes. Alignments were created in Mafft 18 and maximum likelihood trees were constructed in RAxML 19. Bootstrap support values are shown as bubbles for nodes with >50% support. Branches and branch labels are color coded by phylum for Zodletone sequences. Branch labels depict classification to family level followed by the NCBI genome accession number. Reference sequences are shown in black with the Uniprot accession numbers.



**Figure S7.** Phylum-level distribution for transcribed sulfur cycling genes in Zodletone spring sediment. RNA-seq reads were pseudo-aligned to the S-cycling genes predicted in Zodletone genomes to detect exact matches using Kallisto 20. The transcripts per million are shown on the Y-axis for the gene/ group of genes depicted at the top of the figure.

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**Supplementary tables:**

**Table S1:** List of all genomes analyzed in this study with their NCBI Assembly accession numbers, taxonomic classification, sequencing statistics, and general genomic features.

**Table S2:** Substrates potentially supporting growth, predicted fermentation end products, and energy conservation pathways predicted from genomic analysis.

**Table S3:** S-cycling genes predicted in Zodletone genomes. Genes are shown in the table header and actual gene names are shown in the corresponding cells.