Impacts of Crenarchaeota and Halobacterota on sulfate reduction in the subtropical mangrove ecosystem as revealed by SMDB analysis

Shuming Mo [1, †], Jinhui Li [1, †], Bin Li [2], Ran Yu [1], Shiqing Nie [1], Zufan Zhang [1], Muhammad Kashif [1], Sheng He [1], Jianping Liao [3], Qiong Jiang [1], Peihong Shen [1], Bing Yan [2, *], and Chengjian Jiang [1, 2 *]

[1] State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, Guangxi Research Center for Microbial and Enzyme Engineering Technology, College of Life Science and Technology, Guangxi University, Nanning 530004, China.

[2] Guangxi Key Lab of Mangrove Conservation and Utilization, Guangxi Mangrove Research Center, Guangxi Academy of Sciences, Beihai 536000, China.

[3] School of Computer and Information Engineering, Nanning Normal University, Nanning 530299, China.

† These authors contributed equally to this work.

*: Corresponding Author:

Tel: +86-771-3270736; Fax: +86-771-3237873

Email: jiangcj0520@vip.163.com (CJ); gxybing@tom.com (BY)

Abstract

Motivation

Sulfate reduction is an important process in the sulfur cycle. However, the relationship between this process and the genotype of microorganisms involved in subtropical mangrove ecosystems is poorly understood. Genotyping can identify and is crucial for sulfate reduction in mangrove ecosystems with high sulfur concentrations. A professional and efficient gene integration database of sulfur metabolism has not been established yet.

Results

This work aimed to evaluate sulfate reduction by microorganisms in mangroves by using a sulfur metabolism gene integrative database (SMDB) that was mainly constructed to analyze the sulfur cycle (sub) gene families quickly and accurately. The database achieved high coverage, fast retrieval, and low false positives. Relative enrichment of sulfate adenylyltransferase indicated that environmental factors select for a partial dissimilatory sulfate reduction process. Furthermore, the sulfate reduction community compensates by producing certain sulfate-reduction genes in response to high-sulfur environments in mangrove sediments. Taxonomic assignment of dissimilatory sulfate-reduction genes revealed that Crenarchaeota and Halobacterota are completely responsible for this process. Sulfite reductase can help the community cope with the toxic sulfite produced by these Archaea phyla. Collectively, these findings suggested that Halobacterota and Crenarchaeota play essential roles in dissimilatory sulfate reduction.

42 **Availability and implementation**

43 SMDB is freely available under http://smdb.gxu.edu.cn/ and

44 https://github.com/taylor19891213/sulfur-metabolism-gene-database.

45 **Supplementary information**

46 Supplementary data are available at Bioinformatics online.

47 **Keywords: sulfate-reduction gene families, mangrove sediment, sulfate-reduction**

48 **genotype, sulfur metabolism gene integrative database**

1 Introduction

Dissimilatory sulfate reduction, which results in the conversion of sulfate (SO_4^{2-}) to sulfide (S^{2-}), is an important reaction in the sulfur cycle in numerous environments (Wenk et al., 2018). The study of dissimilatory sulfate reduction provides information about the occurrence of all dissimilatory sulfate-reduction genes in a community. However, sulfate reduction lacks a complete pathway, a condition that might be a common occurrence (Bukhtiyarova et al., 2019; Lun et al., 2018; Xia et al., 2010). The high occurrence of partial sulfate reduction implies that environmental conditions can affect gene occurrence in a pathway. Gene families, including ATP sulfurylase (*sat*), APS reductase (*aprAB*), and dissimilatory sulfite reductase (*dsrABC*), are involved in the canonical dissimilatory sulfate-reduction pathway (Jochum et al., 2018). Recently, the gene family of dissimilatory sulfite reductase beta subunit (*dsrB)* has been applied to study the diversity of sulfate-reducing bacteria (SRB) in various ecosystems (Vavourakis et al., 2019; Wu et al., 2019). Dissimilatory sulfate reduction is primarily driven by SRB, and a complete absence of oxygen (O_2) is vital for SRB to gain energy (Wu et al., 2017). Proteobacteria are the dominant SRB group in mangrove sediments (Varon-Lopez et al., 2014). Sulfate reduction plays an important role in influencing the carbon cycle, atmospheric composition, and climate, and it can mitigate the production of the greenhouse gas methane (Hausmann et al., 2019).

Mangroves are complex environments ideal for studying dissimilatory sulfate reduction. Mangroves are one of the major habitat types at the intersections of tropical and subtropical land and sea, and they are periodically inundated by seawater. Mangrove

sediments are usually characterized as anoxic with high levels of sulfur and salt and rich in nutrients (Ferreira et al., 2010). Under these conditions, microorganisms usually use NO_3^-, Mn (IV), Fe (III), and SO_4^{2-} as electron acceptors (Froelich et al., 1979). Sulfate reduction is one of the most active processes occurring in mangrove sediments because of high sulfate concentrations from seawater (Ferreira et al., 2007; Lin et al., 2019; Sherman et al., 1998). Sulfate reduction is responsible for SO_4^{2-} removal in these ecosystems. Dissimilatory sulfate reduction drives the formation of enormous quantities of reducing sulfide. Sulfide affects cell oxidation and causes cell hypoxia, and the biological conversion of sulfide to nontoxic components could be beneficial (Santos et al., 2015). Sulfide production may have different fates in various ecosystems, and microbial communities in mangrove sediments may primarily convert sulfide to L-cysteine and polysulfide (Lin et al., 2019). However, sulfides may chemically react with metals to constitute insoluble sulfides, such as FeS and FeS_2 (Canfield, 1989; Luther et al., 1986; Wasmund et al., 2017). Therefore, mangrove soil requires a sulfate-reduction community that can adapt to different environmental conditions, thus supporting the occurrence of partial dissimilatory sulfate reduction.

The study of sulfate reduction in mangroves is interesting and important, but this endeavor has several limitations. First, although the diversity of sulfate-reducing bacteria has been studied in mangrove ecosystems, an understanding of sulfate reduction in these ecosystems remains insufficient (Andrade et al., 2012; Wu et al., 2019). Second, researchers have discovered culturable sulfate reduction of single strains via genomic analysis in other ecological environments (Spring et al., 2019), but

similar studies have been rarely conducted in mangrove ecosystems. Finally, the relationship between sulfate reduction and the genotype of microorganisms involved in this process in mangroves are also poorly understood. Furthermore, the environmental conditions that select for dissimilatory sulfate-reduction gene families for a more frequent reliance on sulfate reduction remain unclear. In addition, metagenomics and metatranscriptomics, which avoid cultivation bottleneck, can characterize uncultivated microorganisms (Vavourakis et al., 2019; Zhang et al., 2019).

To the best of our knowledge, a professional sulfur database has not been constructed yet. Different databases, such as Clusters of Orthologous Groups (COG) (Galperin et al., 2015), Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al., 2016), Evolutionary Genealogy of Genes: Non-supervised Orthologous Groups (eggNOG) (Huerta-Cepas et al., 2016), SEED subsystems (Overbeek et al., 2005), and M5nr (Wilke et al., 2012), are widely used for functional assignment of predicted genes. However, the application of these databases in analyzing sulfur cycle via shotgun metagenome sequencing data has several obstacles. First, few of these databases contain a complete database of gene families involved in sulfur cycle. Second, the use of these databases obtains a wrong relative abundance of gene families because of similar functional domains of the genes. For example, certain gene families, such as polysulfide reductase chain A (*psrA)* and thiosulfate reductase (*phsA)*, share high sequence similarity (Stoffels et al., 2012). This condition often leads to the mismatch interpretation of potential ecological processes. Finally, searching for sulfur cycle genes in large databases is time consuming. Therefore, a specific database of genes related to

the sulfur cycle pathway should be established to address these problems. This novel database can also be applied to shotgun metagenomics research.

Therefore, in this study, we created a manually managed database that primarily gathers most of the publicly available sulfur cycle gene families to address the limitations of available public databases in sulfur cycle analysis. An integrated database, namely, sulfur metabolism gene integrative database (SMDB), covering sulfur cycle gene families was established. SMDB was applied to samples from a subtropical mangrove ecosystem to generate functional profiles, and dissimilatory sulfate-reduction genes were then extracted from the functional profiles for further analysis. We also evaluated sulfate reduction in the subtropical mangrove ecosystem of Beibu Gulf in China. This study presents unique microbial features as a consequence of adapting to distinct environmental conditions to dissimilatory sulfate-reduction process.

2 Materials and methods

2.1 Sulfur metabolism gene integrative database development

A manually created integrated database was constructed to profile sulfur cycle genes from shotgun metagenomes. We here used a similar method described in Tu Park et al. to development the database (Tu et al., 2019). The database was expected to bear the following characteristics: all sulfur gene families should be accurate and false positives should be minimized in database searching. The framework is shown in Supplementary Fig. S1.

2.1.1 Sulfur metabolism gene integrative database sources

The universal protein (UniProt) database (http://www.uniprot.org/) was utilized to retrieve core database sequences of sulfur cycle gene families by using keywords. Other databases were used for retrieving nontarget homologous sequences. The other databases included COG (ftp://ftp.ncbi.nih.gov/pub/COG/COG2014/data), eggNOG (http://eggnogdb.embl.de/download/eggnog_4.5/), KEGG (http://www.genome.jp/kegg/), and M5nr (ftp://ftp.metagenomics.anl.gov/data/M5nr/current/M5nr.gz). In addition, the homologous sequences from RefSeq non-redundant proteins (NR) database (ftp://ftp.ncbi.nlm.nih.gov/blast/db/) were also added in the core constructed database. These databases were selected because they are widely used in metagenome research.

2.1.2 Core database for constructing sulfur cycle genes

In addition to our own knowledge base, we also referenced the KEGG database and MetaCyc database (Caspi et al., 2016) of the sulfur cycle pathway to manually retrieve gene families involved in sulfur cycle. Only those genes that had been experimentally confirmed to be involved in sulfur metabolism were collected through an extensive search of the literature. This step was followed by searching the Uniprot database with their keywords to identify the corresponding annotated sulfur metabolism gene families within the databanks, including references. For gene families with vague definitions (e.g., *cysQ* and *MET17*), the full protein name was added in the keywords to exclude sequences containing vague annotations. These datasets were retained as the core database for sulfur cycle genes.

2.1.3 Full sulfur metabolism gene integrative database construction

To reduce false positives in database searching and increase the comprehensiveness of the core database, we used COG, eggNOG, KEGG, M5nr, and NR to retrieve nontarget homologous sequences to construct a full database. The full sulfur metabolism gene integrative database included the core database of sulfur cycle gene families and their nontarget homologous gene families. The sequence files of this integrative database were clustered using the CD-HIT program (version 4.6) (Fu et al., 2012) at 95% and 100% identity cutoffs. Representative sequences from this integrative database were then selected to construct SMDB. Finally, SMDB database had already been depoisted in https://github.com/taylor19891213/sulfur-metabolism-gene-database on Jan. 8th, 2020. The SMDB website had already been online on June 22th, 2020.

2.2 Case study

2.2.1 Sampling sites and sediment collection

The ShanKou mangrove sediment in Beihai City, China (21°29′25.74″N, 109°45′49.43″E) was selected as the sampling location (Supplementary Fig. S4). Rhizosphere samples (RS) from near-root (within 3 cm from the roots) were designated as RS1, RS2, and RS3. Nonrhizosphere samples (NRS) from nonrhizosphere (1.5 m away from these roots) were labelled as NRS1, NRS2, and NRS3. Nonmangrove samples (MS) were assigned as NMS1, NMS2, and NMS3. Mangrove samples (MS) included groups of RS and NRS.

Several sediment properties, such as total organic carbon (TOC), total organic nitrogen (TN) content, nutrients (NH_4^+, NO_3^-), and available sulfur (AS), were determined.

Redox potential (ORP) was measured in situ by using a portable ORP meter (BPH-220, Bell, China). Soil suspension was obtained by centrifugation to determine pH by using a pH meter (PHS-2C; Sanxin, China) and salinity by using a salinity meter (PAL-06S; Atago, Japan). TOC, TN, TP, AS, NH_4^+, sulfide, Fe, and NO_3^- were determined following Chinese standard methods (Ministry of Agriculture of the People's Republic of China, 2006a; Ministry of Agriculture of the People's Republic of China, 2006b; Ministry of Agriculture of the People's Republic of China, 2010; Standardization Administration of the People's Republic of China, 2016; Ministry of Ecology and Environment of the People's Republic of China, 2016; Standardization Administration of the People's Republic of China, 1994.).

2.2.2 DNA extraction and high-throughput sequencing

DNA was extracted from the sediments by using the FastDNA SPIN kit for soil (MP Biomedicals, USA) according to the manufacturer's instructions. At least 6 μg DNA from each sample was submitted to the Shanghai Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) for sequencing on an Illumina platform. The data output from each DNA sample was over 10 Gb. The metagenomic sequencing data were deposited to the NCBI SRA database under the BioProject PRJCA002311.

2.2.3 Shotgun metagenomic sequence processing and analysis

Initial quality assurance/quality control, including trimming sequencing adapters and bar codes from sequence reads, was performed. Adapter sequences were removed using SeqPrep v1.33 (https://github.com/jstjohn/SeqPrep). Moreover, sequences less than 100 bp, sequences with quality of less than 20, and reads containing an N base were

removed using Sickle v1.2 (Joshi NA, 2011). Finally, clean reads were created.

For functional annotation, the clean reads were aligned against the online SMDB database website (http://smdb.gxu.edu.cn/). The blast software of the SMDB database is DIAMOND v0.9.14 (Buchfink et al., 2015) with parameters set as an e-value cutoff of 1×10^{-5} and sequence identity cutoff of 60%. The online website of the SMDB database output was converted to m8 blast format. Then, the reads were assigned as being from a particular gene if the aligned region was >25 amino acids with an identity of >60%. Gene families of dissimilatory sulfate reduction were screened out. A correlation heat map was used to visualize the composition of dissimilatory sulfate reduction across all nine samples.

2.2.4 16S read extraction and characterization

Reads from the 16S rRNA encoding region of bacterial genomes were extracted using SortMeRNA (Kopylova et al., 2012) (https://github.com/biocore/sortmerna/) by running the software in filtering mode. The reference database was compiled by Brian Bushnell from Silva database (https://www.arb-silva.de/no_cache/download/archive/release_138/Exports/). The reads extracted using SortMeRNA were clustered with vsearch (Rognes et al., 2016) with an id = 0.97. The clusters were then matched using vsearch to the Silva SSUref NR99 dataset to assign their taxonomy.

3 Results

3.1 Sulfur metabolism gene integrative database of characteristics

A total of 175 gene (sub)families covering 11 sulfur metabolism pathways, including assimilatory sulfate reduction, thiosulfate disproportionation, sulfide oxidation, dissimilatory sulfate reduction, sulfite oxidation, sulfur oxidation, sulfur reduction, tetrathionate oxidation, tetrathionate reduction, thiosulfate oxidation, and organic degradation/synthesis, were recruited in SMDB (Supplementary Table S1). SMDB obtained 115,321 and 395,737 representative sequences at 95% and 100% identity cutoffs, respectively.

To demonstrate the necessity of building a manually created sulfur metabolism gene database, we first compared the coverage and accuracy of sulfur metabolism gene (sub)families in SMDB with those of existing public databases. Among the 175 gene (sub)families we recruited in SMDB, only 118, 157, 144, 169, and 172 could be found in these gene (sub)families in COG, eggNOG, KEGG, M5nr, and NR databases, respectively (Fig. 1). The accuracy of SMDB containing sulfur gene (sub)families exceeded that of public databases (Supplementary Fig. S2). We characterized sulfur metabolism gene (sub)families by searching nine shotgun metagenomic datasets obtained by sampling the mangrove environment against SMDB, COG, eggNOG, KEGG, M5nr, and NR databases. Subsequently, the number of sulfur metabolism gene families detected by searching against SMDB far exceeded that of the five other databases (Supplementary Fig. S3).

243 **3.2 Abundance and diversity of sulfur (sub) gene families**

244 A total of 165 different sulfur gene (sub)families were annotated. The sulfur gene
245 (sub)families in each sample ranged from 165 (sample RS3) to 160 (sample RS3).
246 These sulfur gene (sub)families exist in 11 pathways. Certain gene (sub) families, such
247 as ATP sulfurylase (*aps*), sulfite dehydrogenase (*sorT*), and adenylyl-sulfate reductase
248 (*APR*), were rarely detected at the current sampling depth (Supplementary Table S2).
249 This finding indicated the low abundance of these gene (sub) families in natural
250 environments, and deeper sequencing depths should be used in shotgun metagenomes
251 to capture these genes. The pathways of sulfur cycle gene (sub) families showed that
252 dissimilatory sulfate reduction and sulfide oxidation were significantly high in
253 mangrove sediments ($p < 0.05$, Supplementary Fig. S9). However, the pathways of
254 assimilatory sulfate reduction, tetrathionate reduction, tetrathionate oxidation, sulfide
255 oxidation, and sulfite oxidation were significantly high in RS ($p < 0.05$, Supplementary
256 Fig. S9). Hence, we focused on the analysis of dissimilatory sulfate reduction in
257 mangrove ecosystems.

258 **3.3 Bacterial community composition**

259 To study the distribution of dissimilatory sulfate reduction in microbial communities,
260 we used SortMeRNA (Kopylova et al., 2012) to extract 16S rRNA reads. The
261 taxonomic assignments of the reads assigned to 16S rRNA genes indicated that
262 members of Proteobacteria and Desulfobacterota were equally dominant, with
263 compositions ranging from 31% to 39%, across all reads from each sample (Figs. 2A
264 and 2B). Members of Desulfobacterota comprised approximately 10% of the reads.

265 Other phyla, such as Crenarchaeota and Chloroflexi, comprised approximately 5% of
266 the total reads. Halobacterota accounted for approximately 1.2% of the total reads (Figs.
267 2A and 2B). In both mangrove and nonmangrove samples, no difference was found in
268 the occurrence of the top three phyla. However, significant differences were observed
269 in the rhizosphere and nonrhizosphere samples ($p < 0.05$, Supplementary Fig. S10). For
270 example, the relative proportion of Proteobacteria was high in the rhizosphere soil,
271 whereas that of Chloroflexi and Thermoplasmatota was low in the rhizosphere soil (p
272 < 0.05, Supplementary Fig. S10).

273 **3.4 Genes for dissimilatory sulfate reduction**

274 The prevalence of dissimilatory sulfate-reduction genes at the sites was initially
275 analyzed by considering the complete dissimilatory sulfate-reduction pathway.
276 Accordingly, reads coming from genes encoding sulfate adenylyl transferase (*sat*),
277 adenylyl sulfate reductase subunit A (*aprA*), and dissimilatory sulfite reductase (*dsrA*
278 *and dsrB*) were obtained. In the MS and NMS groups, adenylyl sulfate reductase-
279 associated electron transfer protein (*qmoA*) and *dsr* subunit C (*dsrC*) were
280 significantly high in mangrove samples ($p < 0.05$, Fig. 3A). However, sulfite reductase
281 (NADPH), flavoprotein alpha-component (*cysJ*), phosphoadenosine phosphosulfate
282 reductase (*cysH*), sulfite reductase (NADPH) hemoprotein beta-component (*cysI*),
283 sulfite reductase (*sir*), and sulfite reduction-associated complex DsrMKJOP proteins
284 *dsrP* and *dsrO* were significantly high in rhizosphere samples, whereas anaerobic
285 sulfite reductase (*asrB* and *asrC*) and *dsrB* were significantly low in rhizosphere
286 samples ($p < 0.05$, Fig. 3B).

287 Reads obtained from *sat* and *aprA* were more frequent than reads from *dsrC*, *qmoA*,
288 and *cysI* (Figs. 2C and 2D). The largest number of reads was assigned to *sat*, accounting
289 for 10%–12% of the total dissimilatory sulfate-reduction reads in all samples. In
290 addition, reads assigned to *dsrC*-disulfide reductase (*dsrK)* and *aprA* comprised 17%–
291 20% of the total reads, whereas those assigned to *aprA* were between 8% and 10.5%.
292 Reads assigned to *qmoA* did not exceed 1% of the total dissimilatory sulfate-reduction
293 reads (Supplementary Fig. S8). Reads assigned to *dsrA*, *dsrB*, and *dsrC* were not
294 significantly different in these groups. However, reads from *qmoA* were also
295 significantly high in the mangrove samples ($p < 0.05$, Fig. 3A).

296 **3.5 Taxonomic assignment of dissimilatory sulfate-reduction genes**

297 Proteobacteria were the predominant sources of 16S rRNA reads. Therefore, taxonomic
298 assignments were done at the phylum level to obtain good process of dissimilatory
299 sulfate-reduction genes families (Table 1). The numbers of dissimilatory sulfate-
300 reduction genes of Crenarchaeota and Halobacterota exceeded those of other taxonomic
301 assignments, whereas the remaining genes were assigned to Firmicutes, Proteobacteria,
302 and Desulfobacterota. This finding was somewhat unexpected because the 16S rRNA
303 gene analysis showed that these samples were as prevalent as Proteobacteria (Figs. 2A
304 and 2B). Proteobacteria were the sources of reads assigned to *sat,*
305 dimethylsulfoniopropionate lyase *(cysQ)*, *dsrA, dsrB*, and sulfite reduction-associated
306 complex DsrMKJOP protein (*dsrM)* in the samples (Table 1). The abundance of *aprA*
307 was high in the family of dissimilatory sulfate-reduction genes (Figs. 2C and 2D).
308 However, *aprA* was obtained from Crenarchaeota and Halobacterota in mangrove

samples, but it was obtained from Deferrisomatota, Amorphea, Bacteroidota, Euryarchaeota, and Deferrisomatota in nonmangrove samples (Table 1). *Sat* and *dsrA* were mainly obtained from Halobacterota. However, the reads were also assigned to Bacteroidota, Acidobacteriota, and Verrucomicrobiota. In addition, *asr* and *cys* gene families were obtained from Crenarchaeota, Halobacterota, and Proteobacteria (Table 1).

3.6 Sediment properties

To determine the environmental factors that likely shape the structure and composition of dissimilatory sulfate-reduction genes in mangrove sediments, we generated a correlation heat map (Fig. 4). Certain sediment properties, such as pH, TOC, AS, ORP, NH_4^+, NO_3^-, TN, TP, Fe, salinity, and sulfide content, were determined (Supplementary Fig. S5). The concentrations of AS, Fe, TOC, and TN were significantly higher in the mangrove samples than those in the nonmangrove samples. Moreover, the concentrations of TOC, TN, and TP were significantly higher in the rhizosphere samples than those in the nonrhizosphere samples (Supplementary Tables S5 and S6, $p < 0.05$).

Result showed significant correlations between sediment properties and dissimilatory sulfate-reduction gene (sub) families ($p < 0.01$, Fig. 4). pH was negatively correlated with *qmoA* and *dsrM*. Salinity was positively correlated with bisphosphate nucleotidase (*MET22)*, 3′-phosphoadenosine 5′-phosphosulfate synthase (*PAPSS)*, and inositol monophosphatase 3 (*IMPAD1)*. AS was negatively correlated with bisphosphate nucleotidase (*SAL)*. Sulfide content was negatively correlated with adenylylsulfate

reductase subunit B (*aprB)*. Results of Pearson correlation analysis of the pathways are summarized in Supplementary Table S4. Results showed no significant correlation between environmental factors and the dissimilatory sulfate-reduction pathway. AS was negatively correlated with the organic degradation/synthesis pathway (Supplementary Table S4).

4 Discussion

Sulfur cycle is one of the most important biogeochemical cycles in Earth ecosystems. Characterizing sulfur metabolism at the genetic and species level has become an important approach in understanding sulfur metabolism processes in the current metagenomic era. In this study, we developed a manually created database for the fast and accurate profiling of sulfur metabolism genes from shotgun metagenome sequencing data. The developed SMDB addresses the bottleneck encountered in using public databases. First, SMDB has a precise definition for sulfur metabolism gene families. Unlike other database generations (Galperin et al., 2015; Huerta-Cepas et al., 2016; Kanehisa et al., 2016), gene families in SMDB were manually retrieved by using keywords combined with sequence similarity. Typical examples include the *phsA* and *psrA* genes that encode particle thiosulfate reductase and polysulfide reductase, respectively, but these genes share high sequence similarity (Stoffels et al., 2012). Hence, distinguishing the activities of these two enzymes during genome annotation is difficult (Stoffels et al., 2012). Second, SMDB presents a comprehensive coverage of gene families in sulfur metabolism. We aimed to provide the latest knowledge and research progress in sulfur metabolism studies, such as aerobic dimethyl sulfide (DMS) degradation (Koch and Dahl, 2018). The gene families *TST/MPST/sseA* for K01011

355 display considerable sequence similarity in KEGG, but they are distinct in two sulfur
356 transferase subfamilies (Spallarossa et al., 2003). Finally, SMDB considers the problem
357 of false positives. Herein, this issue was addressed by including homologous gene
358 families from multiple public databases. Most importantly, the small size of SMDB
359 reduces the computational cost required to output the functional profiling of files of
360 sulfur metabolism gene families. SMDB only provides functional assignments of
361 sequencing data into sulfur metabolism gene (sub)families.
362 We applied SMDB in characterizing sulfur metabolism gene (sub)families in mangrove
363 sediment environment to demonstrate its potential applications in microbial ecology
364 studies. The metagenomics survey described herein provided strong evidence that in
365 neutral pH, anoxic, high-sulfur soil, the environment in the Beibu Gulf favored the
366 enrichment and depauperization of dissimilatory sulfate-reduction genotypes. This
367 pattern supported the hypothesis that environmental conditions act on individual genes
368 in the pathway and favor the occurrence of partial dissimilatory sulfate reduction.
369 Moreover, geographical positions can change the occurrence of dissimilatory sulfate
370 reduction in the dissimilatory sulfate-reduction microbial community. When the
371 samples were clustered into mangrove and nonmangrove groups, the occurrence of
372 dissimilatory sulfate-reduction and sulfide oxidation reads was the highest in the
373 mangrove zone and the lowest in the nonmangrove zone ($p < 0.05$, Supplementary Fig.
374 S9). This pattern suggested that conditions allowing dissimilatory sulfate-reduction are
375 more persistent in mangrove zones than in nonmangrove zones. Sulfates are the primary
376 electron receptors in anoxic mangrove sediments (Froelich et al., 1979). AS is an
377 indicator of soil sulfur potentials used to define sulfur-rich soil at concentrations greater
378 than 25 mg/kg (Liu et al., 1981). All AS contents in the sampling sites exceeded 1200
379 mg/kg (Supplementary Fig. S5), indicating that the mangrove sediments of ShanKou

380 were sulfur-rich soil. Similarly, AS concentrations were consistently higher in the
381 mangrove zones than in the nonmangrove zones ($p < 0.05$, Supplementary Table S5),
382 suggesting that the concentrations of available sulfur determine the abundance of
383 dissimilatory sulfate-reduction reads. Spearman correlation coefficients of abiotic
384 factors and dissimilatory sulfate-reduction genes were calculated by SPSS (George and
385 Mallery, 2013). We found that sulfide content limited the abundance of *aprB*
386 (Supplementary Table S3), suggesting that sulfate reduction is inhibited by sulfide
387 content when it exceeds a certain threshold. However, our current understanding of
388 dissimilatory sulfate reduction suggests that AS contents are insufficient to explain gene
389 trends, and available O_2 is also an important factor. Mangrove sediments are usually
390 characterized as anoxic (Ferreira et al., 2010). Mangrove conditions favoring
391 dissimilatory sulfate reduction were probably of sufficient duration to contribute to the
392 patterns observed herein, but a more detailed study of sediment properties should be
393 conducted for confirmation. For example, various nutrients, such TOC, TN, and TP,
394 which serve as energy sources for microbial growth, are often considered in microbial
395 diversity analysis (Wu et al., 2019). In this study, the concentrations of TOC and TN
396 were consistently higher in the mangrove zones than in the nonmangrove zones ($p <$
397 0.05, Supplementary Table S5), suggesting that they determine the microbial diversity.
398 In addition, we found that nutrients were significantly correlated with *dsrO*, *asrA*, and
399 *PAPSS* (Fig. 4). Fe, pH, and OPR were significantly correlated with *qmoA*, *PAPSS*, *cysJ*,
400 adenylylsulfate reductase membrane anchor *(aprM)*, and anaerobic sulfite reductase
401 subunit C *(asrC)* (Fig. 4, Supplementary Table S3).

402 Mangrove sediments have similar pH, temperature, and salinity as tropical marine
403 environments (Abouna et al., 2015). To analyze the effects of mangrove ecosystems on
404 dissimilatory sulfate reduction, we assumed that these ecosystems receive identical

405 sulfur contents at the same place. When total dissimilatory sulfate-reduction reads were
406 grouped into mangrove and nonmangrove samples, no significant difference was
407 observed in normalized read abundance (Supplementary Fig. S6). The most abundant
408 dissimilatory sulfate-reduction genes, such as *sat*, *dsrK*, and *aprA*, had no significant
409 differences in the mangrove and nonmangrove samples ($p < 0.05$, Fig. 3A). However,
410 the reads of *qmoA* and *dsrC* were significantly high in the mangrove samples ($p < 0.05$,
411 Fig. 3A). *QmoA* is a probable electron donor/transfer to *Apr* and appears specific to the
412 sulfate-reduction pathway (Ramos et al., 2012). *DsrC* is involved in terminal oxidation
413 reaction in sulfate respiration, a process catalyzed by sulfate-reducing bacteria (Santos
414 et al., 2015). Results indicated that the highly organic microenvironment improved the
415 abundance of *qmoA* and *dsrC* and promoted the coupling between the dominant
416 microorganisms and dissimilatory sulfate reduction. The correlation heat map showed
417 that pH was significantly negatively correlated with *qmoA* but not significantly different
418 to *dsrC* (Fig. 4). Hence, *qmoA* is easy to express under low pH. The pathways of
419 dissimilatory sulfate reduction form sulfide, leading to sulfide accumulation. Low OPR
420 results in the release of sulfides from sediments (Shi et al., 2016). However, OPR was
421 negative in the present study (Supplementary Fig. S5). This condition led to an increase
422 in sulfide production in the sediments. Sulfides in mangrove sediments are eventually
423 converted to L-cysteine (Lin et al., 2019). L-cysteine production is the main pathway
424 for microorganisms to absorb inorganic sulfur sources from their environment by
425 converting sulfide into organic sulfur via assimilation. This finding was consistent with
426 the abundance of adenylylsulfate kinase (*cysC)* and serine O-acetyltransferase (*cysE)*

(Supplementary Table S2), which are the key genes for cysteine synthesis (Bogicevic et al., 2016). In addition, Fe(III) is buried and acts as an oxidant for sulfide in deeper sediment layers where it partly binds the produced sulfide as iron sulfide (FeS) and pyrite (FeS_2) (Canfield, 1989; Luther et al., 1986). In this study, the concentration of Fe was consistently higher in the mangrove zones than that in the nonmangrove zones ($p < 0.05$, Supplementary Table S5). We found that the amount of sulfide in the sediments was relatively low.

A similar absence of trends in the abundance of total dissimilatory sulfate-reduction reads occurred when the reads were grouped into rhizosphere and nonrhizosphere samples. Sulfite reductase complexes (*cysI*, *cysJ*, and *cysH*) catalyze the reduction of sulfite to sulfide. These activities are required for the biosynthesis of L-cysteine from sulfate (Zeghouf and Chemistry, 2000). The gene families of *asr* and *dsrB* catalyze the reduction of sulfite to sulfide in dissimilatory sulfate reduction (Anantharaman et al., 2018). In soil, SO_4^{2-} is subjected to dissimilatory and assimilatory reduction. However, the pathways of assimilatory sulfate reduction were significantly high in RS in the present study ($p < 0.05$, Supplementary Fig. S9). Prokaryotes, algae, plants, and fungi undergo sulfate assimilatory reduction to produce various organic compounds, such as amino acids (Fuentes-Lara et al., 2019). Several nutrients, such TOC, TN, and TP ($p < 0.05$, Supplementary Table S6), are vital for rhizosphere microorganisms to gain energy. Results indicated that the rhizosphere microorganisms favored the pathway of assimilatory sulfate reduction, which reduces sulfate to L-cysteine. However, sulfate was mainly reduced to sulfide in the nonrhizosphere zones.

449 In all samples, the reads from *sat* occurred at higher levels than that from dissimilatory
450 sulfate-reduction genes (Figs. 2C and 2D). This finding was unexpected because
451 previous analysis of the occurrence of inorganic sulfur cycle genes in sequence
452 genomes found that *aprA* occurs at higher frequencies than the other gene-encoding
453 proteins required for dissimilatory sulfate-reduction genes (Vavourakis et al., 2019).
454 This condition is probably due to the simultaneous sulfate reduction and sulfate
455 oxidation processes (Watanabe et al., 2013). The reads of *aprA* in mangrove zones were
456 basically obtained from Crenarchaeota and Halobacterota, suggesting that the function
457 of *aprA* in mangrove ecosystems may be mainly performed by these Archaea phyla. In
458 this study, the main process of dissimilatory sulfate reduction may be due to anoxia,
459 and unique environmental conditions selected for the gene families of *sat*. The protein
460 product of *sat* is responsible for the activation of sulfate to adenosine 5′-phosphosulfate
461 in the first step of dissimilatory sulfate reduction. Owing to the unique ecological
462 environments of subtropical mangrove ecosystems, the main microbial community
463 structure primarily comprises Proteobacteria (Varon-Lopez et al., 2014). The class
464 Proteobacteria of Betaproteobacteria is the dominant SRB group in mangrove (Varon-
465 Lopez et al., 2014) and estuary sediments (Quillet et al., 2012). In this study,
466 Proteobacteria had the highest abundance in terms of bacterial taxonomy (Figs. 2A and
467 2B). The high abundance of *sat* and *cysQ* gene families were derived from
468 Proteobacteria possibly because special environmental conditions in mangrove
469 sediments choose to convert sulfate to APS (Table 1, Figs. 2A–2D), indicating that the
470 first step of dissimilatory sulfate reduction is mainly performed by Proteobacteria. In

addition, Crenarchaeota and Halobacterota had relatively complete gene families and were responsible for dissimilatory sulfate reduction in this study (Table 1) probably because numerous functional genes and species were disconnected. Crenarchaeota is involved in sulfur metabolism in alkaline hot springs in Galicia (Spain) and in the nitrogen cycle (López-López et al., 2015; Nicol and Schleper, 2006). An order Sulfolobales of the phylum Crenarchaeota , which use elemental sulfur as an electron acceptor or donor generally promote their growth (Sakai and Kurosawa, 2017). Notably, Halobacterota of Methanomicrobiales is primarily involved in carbon metabolism (Anderson et al., 2009). A genus Haloplanus of the phylum Halobacterota, produce H_2S in metabolic product (Cho et al., 2018). These findings suggested that sulfur metabolism may be coupled with carbon metabolism in mangrove ecosystems.

Notably, sulfite is cytotoxic above a certain threshold if not rapidly metabolized and can wreak havoc at the cellular and whole-plant levels. By providing the gene families of *asrC, asrA*, *asrB, cysI, cysJ*, *and cysH* that reduces sulfite levels to protect the members of the local community (Bender et al., 2019; Hensel et al., 1999). If sulfite reductase is absent, most organisms in the community would be inhibited and grow slowly. In this study, we found that microorganisms protect mangrove plants by producing sulfite reductase (Fig. 2D).

We depicted the process of sulfur metabolism in mangrove sediments. The abundance of pathways showed that the organic sulfur degradation/synthesis pathway in these samples was the highest (Supplementary Fig. S7), a finding consistent with that of a previous report that organic sulfur accounts for about 80% of the total sulfur in soil

493 (Tabatabai and Al-Khafaji, 1980). Mangrove forests contain high levels of organic
494 sulfur from dimethylsulfoniopropionate (Nedwell et al., 1994), the main source of DMS.
495 Most bacteria prefer sulfite and sulfate. In the absence of these ions, various types of
496 bacteria utilize organosulfur compounds as alternative sulfur sources. DMS is a major
497 contributor to the total sulfur in the atmosphere. Sulfur metabolism in mangrove
498 sediments includes biological and abiotic processes (Fig. 5). For abiotic processes,
499 sulfur accumulates include pyrite (FeS_2). FeS can be oxidized into various oxidation
500 states of sulfur. For biological processes, the role of microorganisms includes both
501 oxidation and reduction of sulfur compounds. Dissimilatory sulfate reduction involves
502 the reduction of sulfate to sulfide where the electron acceptor is elemental sulfur. By
503 contrast, the reduced sulfide is assimilated in proteins and amino acids in assimilatory
504 sulfate reduction (Fuentes-Lara et al., 2019). Sulfide oxidation by O_2, NO_3^-, Fe^{3+}, or
505 Mn^{4+} as electron acceptors produces sulfur. In addition, sulfur undergoes coupled
506 oxidation and reduction of sulfur compounds (thiosulfate, sulfite, and sulfur) to sulfate
507 and sulfide. The abundance of the dissimilatory sulfate-reduction pathway is
508 significantly higher in mangrove areas than in nonmangrove areas ($p < 0.05$,
509 Supplementary Fig. S9). Sulfate is the major electron acceptor of anaerobic
510 microorganisms in offshore sediments that co-degrade organic matter (Jorgensen et al.,
511 2019). Moreover, sulfate reduction and organic sulfur degradation/synthesis are
512 important processes in mangrove sediments.
513 Considering that the abundance of dissimilatory sulfate reduction was considerably
514 higher in mangrove zones than that in nonmangrove zones (Supplementary Fig. S9),

515 we focused on the mechanism of dissimilatory sulfate reduction. A dissimilatory
516 sulfate-reduction metabolic pattern was constructed (Fig. 6). The figure shows the
517 relative abundances of gene-encoding enzymes involved in dissimilatory sulfate
518 reduction. The reduced sulfur compounds were likely oxidized to sulfite by reverse
519 dissimilatory sulfite reductase (encoded by *rdsrAB*) and thiosulfate sulfur transferase
520 (Fig. 6A). EC2.7.7.4 (sulfate adenylyltransferase) had the highest abundance among the
521 enzymes. EC1.8.99.2 (adenylyl-sulfate reductase) and EC1.8.99.5 (dissimilatory sulfite
522 reductase) had higher abundance in mangrove samples than in nonmangrove samples.
523 Hence, the results indicated that the majority of sulfate was converted into APS in
524 dissimilatory sulfate reduction pathway owing to the special environment of the
525 mangrove ecosystem. The model of dissimilatory sulfite reductase also supports the
526 hypothesis that mangrove samples prefer dissimilatory sulfate reduction. In rhizosphere
527 samples, the abundance of EC1.8.99.5 was low, whereas that of EC1.8.1.2 (assimilatory
528 sulfite reductase) was high (Fig. 6B). Significance analysis of the pathways showed that
529 dissimilatory sulfate reduction did not significantly differ, whereas assimilatory sulfate
530 reduction significantly differed ($p < 0.05$, Supplementary Fig. S7). This finding
531 indicated that rhizosphere microorganisms are conducive to the assimilation of sulfate
532 to L-cysteine. Moreover, this result highlighted the importance of mangrove plants in
533 sulfur cycle. Mangrove ecosystems play an important role in maintaining the dynamic
534 balance of sulfur metabolism.
535 In sum, metagenomics can be used to understand better the occurrence of sulfate
536 dissimilation reduction in mangrove zones (Jorgensen et al., 2019; Wasmund et al.,

2017). In this study, a high-coverage database, namely, SMDB, was successfully constructed. This integrative database contains 175 gene (sub)families and covers 11 sulfur metabolism processes. SMDB was able to quickly and accurately analyze sulfur metabolism in the subtropical mangrove sediments of the Beibu Gulf in China. The mangrove ecosystem favored dissimilation sulfate reduction. The gene of *sat* had the highest abundance in all samples because it has been selected for by the mangrove environment. Proteobacteria convert sulfate to APS owing to environmental conditions in mangrove sediments. However, Crenarchaeota and Halobacterota were found to be responsible for dissimilatory sulfate reduction. This study provides a theoretical basis for the mechanism of sulfur cycle metabolism in subtropical mangrove wetland ecosystems.

Acknowledgements

This research was supported by the Science and Technology Basic Resources Investigation Program of China (Grant No. 2017FY100704), the Natural Science Fund for Distinguished Young Scholars of Guangxi Zhuang Autonomous Region of China (Grant No. 2019GXNSFFA245011), the Natural Science Foundation of Guangxi Zhuang Autonomous Region of China (Grant No. 2018GXNSFAA050090, 2017GXNSFBA198101), and the Distinguished Employment Offered Unit of Guangxi for Conservation and Ecological Monitoring of Mangroves and Sea-grasses.

Author Contributions: Conceptualization, C.J. and B.Y.; Methodology, S.M., B.Y. and B.L.; Software, B.Y., S.N., and J.H.; Validation, Z.Z, S.N., R.Y., and J.H.; Formal Analysis, B.Y., C.J. and P.S.; Investigation, M.K. and B.Y.; Resources, C.J. and B.L.;

Data Curation, S.H., J.L., B.Y., and P.S.; Writing—Original Draft Preparation, S.M.; Writing—Review and Editing, C.J. and M.K.; Supervision, Q.J., P.S. and B.Y.; Project Administration, C.J.; Funding Acquisition, C.J. and B.Y.

Conflicts of Interest: The authors declare no conflict of interest.

References

Abouna, S. et al. (2015) First Description of Sulphur-Oxidizing Bacterial Symbiosis in a Cnidarian (Medusozoa) Living in Sulphidic Shallow-Water Environments. Plos One, 10:e0127625

Anantharaman, K. et al. (2018) Expanded diversity of microbial groups that shape the dissimilatory sulfur cycle. The ISME Journal, 12, 1715-1728.

Anderson, I. et al. (2009) Genomic characterization of methanomicrobiales reveals three classes of methanogens. Plos One, 4:e5797.

Andrade, L.L. et al. (2012) Microbial diversity and anaerobic hydrocarbon degradation potential in an oil-contaminated mangrove sediment. Bmc Microbiol, 12, 186-186.

Bender, D. et al. (2019) Impaired mitochondrial maturation of sulfite oxidase in a patient with severe sulfite oxidase deficiency. Hum Mol Genet, 28, 2885-2899.

Bogicevic, B. et al. (2016) Cysteine biosynthesis in Lactobacillus casei: identification and characterization of a serine acetyltransferase. Fems Microbiol Lett, 363:fnw012

Buchfink, B. et al. (2015) Fast and sensitive protein alignment using DIAMOND. Nature Methods, 12, 59-60.

Bukhtiyarova, P.A. et al. (2019) Isolation, characterization, and genome insights into

an anaerobic sulfidogenic Tissierella bacterium from Cu-bearing coins. Anaerobe, 56, 66-77.

Canfield, D.E. (1989) Reactive iron in marine sediments. Geochim Cosmochim Acta 53, 619-632.

Caspi, R., et al. (2016) The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases, 44(D1):D471-D480.

Ministry of Agriculture of the People's Republic of China. (2006a) Method for determination of soil available Sulphur.NYT 1121.14-2006. (in Chinese).

Ministry of Agriculture of the People's Republic of China. (2006b) Method for determination of soil organic matter. 89, 293-304. (in Chinese).

Ministry of Agriculture of the People's Republic of China. (2010) Method for determination of ammonium nitrogen, available phosphorus and rapidly-available potassiumin acid soil.Universal extract-colorimetric method. NY/T 1849-2010. (in Chinese).

Standardization Administration of the People’s Republic of China. (2016) Determination of nitrate nitrogen in soil-Uitraviolet spectrophotometry method. GB/T 32737-2016. (in Chinese).

Ministry of Ecology and Environment of the People’s Republic of China. (2016) Soil-Determination of bioavailable form of eight elements-Extration with buffered DTPA solution/Inductively coupled plasma optical emission spectrometry.

Standardization Administration of the People’s Republic of China. (1994) Determination of sulfide （Methylene blue spectrophotometric method. SL 89-1994.

(in Chinese).

Cho, E.S. et al. (2018) *Haloplanus rallus sp.* nov., a halophilic archaeon isolated from crude solar salt. Int J Syst Evol Microbiol, 68, 3226-3231.

Ferreira, T.O. et al. (2010) Spatial patterns of soil attributes and components in a mangrove system in Southeast Brazil (Sao Paulo). Journal of Soils and Sediments, 10, 995-1006.

Ferreira, T.O. et al. (2007) Effects of bioturbation by root and crab activity on iron and sulfur biogeochemistry in mangrove substrate. Geoderma, 142, 36-46.

Froelich, P.N. et al. (1979) Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis, 43, 1075-1090.

Fu, L. et al. (2012) CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics, 28, 3150-3152.

Fuentes-Lara, L.O. et al. (2019) From Elemental Sulfur to Hydrogen Sulfide in Agricultural Soils and Plants. Molecules, 24:2282.

Galperin, M.Y. et al. (2015) Expanded microbial genome coverage and improved protein family annotation in the COG database. Nucleic Acids Res, 43, D261-269.

George, D. and Mallery, P. (2013) IBM SPSS Statistics 21 Step by Step: A Simple Guide and Reference. Pearson.

Hausmann, B. et al. (2019) Draft Genome Sequence of *Desulfosporosinus sp.* Strain Sb-LF, Isolated from an Acidic Peatland in Germany. Microbiol Resour Announc, 8, e00428-19.

Hensel, M. et al. (1999) The genetic basis of tetrathionate respiration in Salmonella

typhimurium. Mol Microbiol, 32, 275-287.

Huerta-Cepas, J. et al. (2016) eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. Nucleic Acids Res, 44, D286-D293.

Jochum, L.M. et al. (2018) Single-Cell Genomics Reveals a Diverse Metabolic Potential of Uncultivated Desulfatiglans-Related Deltaproteobacteria Widely Distributed in Marine Sediment, 9 :2038.

Jorgensen, B.B. et al. (2019) The Biogeochemical Sulfur Cycle of Marine Sediments. Front Microbiol, 10, 849-.

Joshi NA, F.J. (2011) Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ files [Software]. Available at https://github.com/najoshi/sickle.

Kanehisa, M. et al. (2016) KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res, 44, D457-D462.

Koch, T. and Dahl, C. (2018) A novel bacterial sulfur oxidation pathway provides a new link between the cycles of organic and inorganic sulfur compounds. Isme Journal, 12, 2479-2491.

Kopylova, E. et al. (2012) SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. Bioinformatics, 28, 3211-3217.

Lin, X.L. et al. (2019) Mangrove Sediment Microbiome: Adaptive Microbial Assemblages and Their Routed Biogeochemical Processes in Yunxiao Mangrove National Nature Reserve, China. Microb Ecol, 78, 57-69.

Liu, C.Q., et al., 1981. Sulphur in soils of southern china and the application of sulphur

fertilizer, (in Chinese), 2, 185-193

López-López, O. et al. (2015) Metagenomics of an Alkaline Hot Spring in Galicia (Spain): Microbial Diversity Analysis and Screening for Novel Lipolytic Enzymes. 6 :1291.

Lun, W.H., et al. (2018) Disentangling the drivers of functional complexity at the metagenomic level in Shark Bay microbial mat microbiomes. ISME, 12, 2619-2639

Luther, G.W. et al. (1986) Inorganic and organic sulfur cycling in salt-marsh pore waters. Science, 232, 746-749.

Nedwell, D.B. et al. (1994) Dynamic turnover of organlc carbon, nitrogen and sulphur in the sediments of Jamaican mangrove forest. (in Chinese).

Nicol, G.W. and Schleper, C. (2006) Ammonia-oxidising Crenarchaeota: important players in the nitrogen cycle. Trends Microbiol, 14, 207-212.

Overbeek, R. et al. (2005) The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. Nucleic Acids Research, 33, 5691-5702.

Quillet, L. et al. (2012) Abundance, diversity and activity of sulfate-reducing prokaryotes in heavy metal-contaminated sediment from a salt marsh in the Medway Estuary (UK). Mar Biotechnol, 14, 363-381.

Ramos, A.R. et al. (2012) The membrane QmoABC complex interacts directly with the dissimilatory adenosine 5 '-phosphosulfate reductase sulfate reducing bacteria. Frontiers in Microbiology, 3:137.

Rognes, T. et al. (2016) VSEARCH: a versatile open source tool for metagenomics. Peerj, 4:e2584.

Sakai, H.D. and Kurosawa, N. (2017) *Sulfodiicoccus acidiphilus gen. nov., sp. nov.*, a sulfur-inhibited thermoacidophilic archaeon belonging to the order *Sulfolobales* isolated from a terrestrial acidic hot spring. Int J Syst Evol Microbiol, 67, 1880-1886.

Santos, A.A. et al. (2015) A protein trisulfide couples dissimilatory sulfate reduction to energy conservation. Science, 350, 1541-1545.

Sherman, R.E. et al. (1998) Soil-plant interactions in a neotropical mangrove forest: iron, phosphorus and sulfur dynamics. Oecologia, 115, 553-563.

Shi, J.C. et al. (2016) The Variation Characteristic of Sulfides and VOSc in a Source Water Reservoir and Its Control Using a Water-Lifting Aerator. Int J Env Res Pub He 13:427.

Spallarossa, A. et al. (2003) SseA, a 3-mercaptopyruvate sulfurtransferase from *Escherichia coli:* crystallization and preliminary crystallographic data. Acta crystallographica. Section D, Biological crystallography, 59, 168-170.

Spring, S. et al. (2019) Sulfate-Reducing Bacteria That Produce Exopolymers Thrive in the Calcifying Zone of a Hypersaline Cyanobacterial Mat. Frontiers in microbiology, 10:862.

Stoffels, L. et al. (2012) Thiosulfate Reduction in Salmonella enterica Is Driven by the Proton Motive Force. J Bacteriol, 194, 475-485.

Tabatabai, M., Al-Khafaji, A., 1980. Comparison of Nitrogen and Sulfur Mineralization in Soils1. Soil Science Society of America Journal, 44, 1000-1006.

Tu, Q.C. et al. (2019) NCycDB: a curated integrative database for fast and accurate metagenomic profiling of nitrogen cycling genes. Bioinformatics, 35, 1040-1048.

694 Varon-Lopez, M. et al. (2014) Sulphur-oxidizing and sulphate-reducing communities
695 in Brazilian mangrove sediments. Environ Microbiol, 16, 845-855.
696 Vavourakis, C.D. et al. (2019) Metagenomes and metatranscriptomes shed new light on
697 the microbial-mediated sulfur cycle in a Siberian soda lake. BMC Biol, 17:69.
698 Wasmund, K. et al. (2017) The life sulfuric: microbial ecology of sulfur cycling in
699 marine sediments. Environ Microbiol Rep, 9, 323-344.
700 Watanabe, T. et al. (2013) Diversity of sulfur-cycle prokaryotes in freshwater lake
701 sediments investigated using aprA as the functional marker gene. Syst Appl Microbiol,
702 36, 436-443.
703 Wenk, C.B. et al. (2018) Electron carriers in microbial sulfate reduction inferred from
704 experimental and environmental sulfur isotope fractionations. The ISME Journal, 12,
705 495-507.
706 Wilke, A. et al. (2012) The M5nr: a novel non-redundant database containing protein
707 sequences and annotations from multiple sources and associated tools. Bmc
708 Bioinformatics 13:141.
709 Wu, J. et al. (2017) Oxygen Reduction Reaction Affected by Sulfate-Reducing Bacteria:
710 Different Roles of Bacterial Cells and Metabolites. Indian Journal of Microbiology, 57,
711 344-350.
712 Wu, S. et al. (2019) Depth-related change of sulfate-reducing bacteria community in
713 mangrove sediments: The influence of heavy metal contamination. Mar Pollut Bull,
714 140, 443-450.
715 Xia, S. et al. (2010) Bio-reduction of nitrate from groundwater using a hydrogen-based

716 membrane biofilm reactor. Journal of environmental sciences (China), 22, 257-262.

717 Chemistry, M. et al. (2000) A Simplifed Functional Version of the Escherichia coli

718 Sulfite Reductase. The Journal of biological chemistry, 275, 37651-37656.

719 Zhang, W. et al. (2019) Complete Genome Sequence of Saccharospirillum mangrovi

720 HK-33T Sheds Light on the Ecological Role of a Bacterium in Mangrove Sediment

721 Environment. Curr Microbiol, 76, 231-236.

722 **Table 1. Taxonomic assignments of dissimilatory sulfate-reduction genes.**

723

Phylum	RS1	RS2	RS3	NRS1	NRS2	NRS3	NMS1	NMS2	NMS3
Crenarchaeota	*aprA;asrA ; dsrK; dsrN*	*aprB; cysJ; cysN; dsrB; nrnA*	*dsrN; nrnA*	*aprA;dsrB; dsrK*	*cysJ; nrnA*	*cysN; cysQ*	*asrC; cysH; cysN; nrnA*	*asrB; asrC; cysD; cysH; cysQ; dsrK; nrnA; sat*	*asrB; cysI; nrnA*
Halobacterota	*cysJ*	*nrnA; asrA; asrC; cysJ; dsrA; dsrM; sat*	*aprA;aprB; cysD;dsrA; dsrB; sat*	*aprA;cysQ; dsrA; dsrB;sat*	*aprA; asrA; cysD; cysH*	*dsrA; dsrM; nrnA*	*asrC; cysD; cysN; cysQ;dsrK; dsrM; dsrO; nrnA; sat*	*asrA; asrB; asrC; cysI; cysN; cysQ; dsrA; dsrB; dsrM; dsrP; nrnA; sat*	*dsrP*
Amorphea	NA	*cysN*	cysD	NA	*cysN; cysNC*	NA	NA	*aprA; dsrA*	NA
Firmicutes	NA	*cysN; dsrB*	NA	NA	*cysN*	NA	NA	*cysH; cysNC*	NA
Chloroflexi	NA	NA	*dsrN*	NA	NA	NA	*dsrA*	NA	NA
Iainarchaeota	NA	NA	NA	*asrA*	NA	NA	NA	NA	NA
Proteobacteria	NA	NA	NA	*cysQ*	*sat*	NA	*asrC*	*dsrA; dsrB; dsrM*	NA
Verrucomicrobiota	NA	NA	NA	NA	*nrnA*	NA	NA	NA	NA
Thermoplasmatota	NA	NA	NA	NA	NA	NA	sat	*dsrN*	NA
Actinobacteriota	NA	NA	NA	NA	NA	NA	NA	*asrC; cysD; cysQ; dsrB; nrnA*	NA
Bacteroidota	NA	NA	NA	NA	NA	NA	NA	*aprA; asrA; sir*	NA
Deferrisomatota	NA	NA	NA	NA	NA	NA	NA	*aprA*	NA
Desulfobacterota	NA	NA	NA	NA	NA	NA	NA	*cysQ; sat; sir*	NA
Euryarchaeota	NA	NA	NA	NA	NA	NA	NA	*aprA; dsrK*	NA
Planctomycetota	NA	NA	NA	NA	NA	NA	NA	*aprB*	NA
Spirochaetota	NA	NA	NA	NA	NA	NA	NA	*asrB*	*dsrM*
Zixibacteria	NA	NA	NA	NA	NA	NA	NA	*dsrJ*	NA
Gemmatimonadota	NA	NA	NA	NA	NA	NA	NA	NA	*asrB*

724

725 **Figure Legends**

726 Figure 1. Sulfur cycle gene (sub)families were detected using SMDB, NR, M5nr,
727 KEGG, eggNOG, COG databases.

728 Figure 2. Comparison of taxonomic assignments of 16S reads extracted from the
729 metagenomes according to A) mangrove and nonmangrove or B) rhizosphere and
730 nonrhizosphere groups. Comparison of read numbers of genes associated with
731 dissimilatory sulfate reduction grouped into C) mangroves and nonmangrove and D)
732 rhizosphere and nonrhizosphere. Rates are expressed as the relative frequency of reads
733 for each gene multiplied by the average of the total read counts for all samples. Error
734 bars represent 95% confidence interval, and statistically significant groupings are
735 indicated by different letters above the bars ($p < 0.05$).

736 Figure 3. Significant differences in sulfur (sub) gene families between mangrove and
737 nonmangrove sediments (A) and rhizosphere and nonrhizosphere sediments (B).

738 Figure 4. Correlation heat map according to z-scores of the 20 most abundant
739 dissimilatory sulfate reduction gene (sub) families with significant correlation between
740 sediment properties.

741 * $p < 0.05$

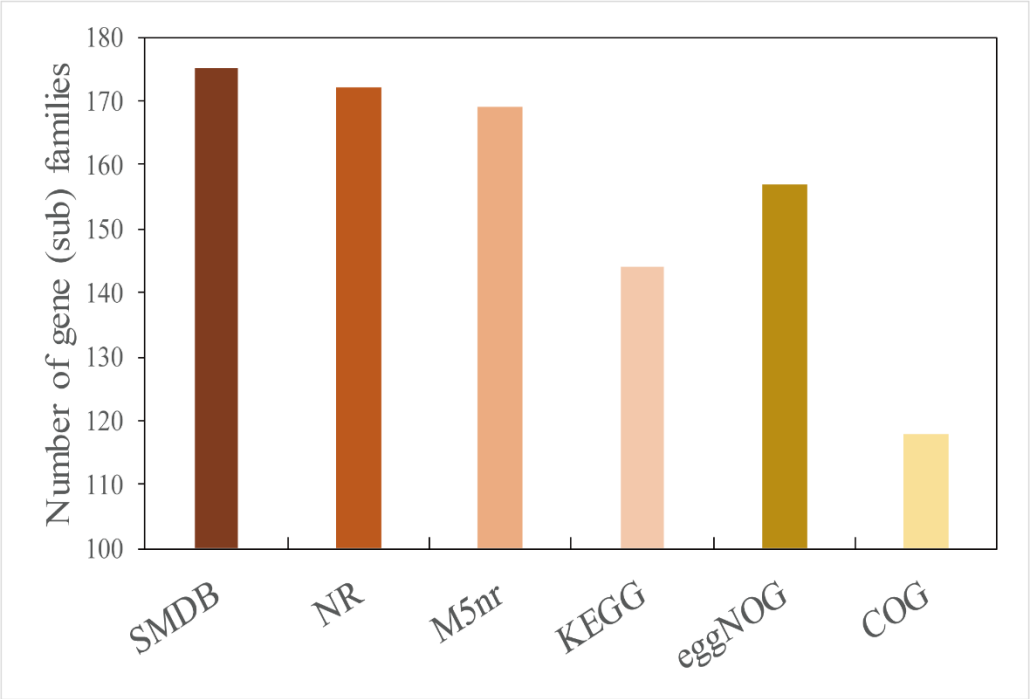
742 ** $p < 0.01$

743 Figure 5. Conceptual depiction of sulfur metabolism in mangrove ecosystem, including
744 reactions of inorganic and organic sulfur compounds and transformations of sulfur
745 compounds of intermediate oxidation states (sulfur cycle intermediates, SCI). Inorganic
746 sulfur compounds include oxidation and reduction of sulfur compounds. Black lines

747 depict biological sulfur transformations by microorganisms. Green lines depict abiotic
748 reaction-mediated sulfur transformations to pyrite (FeS_2). Sulfur compounds are
749 depicted within yellow eclipses.

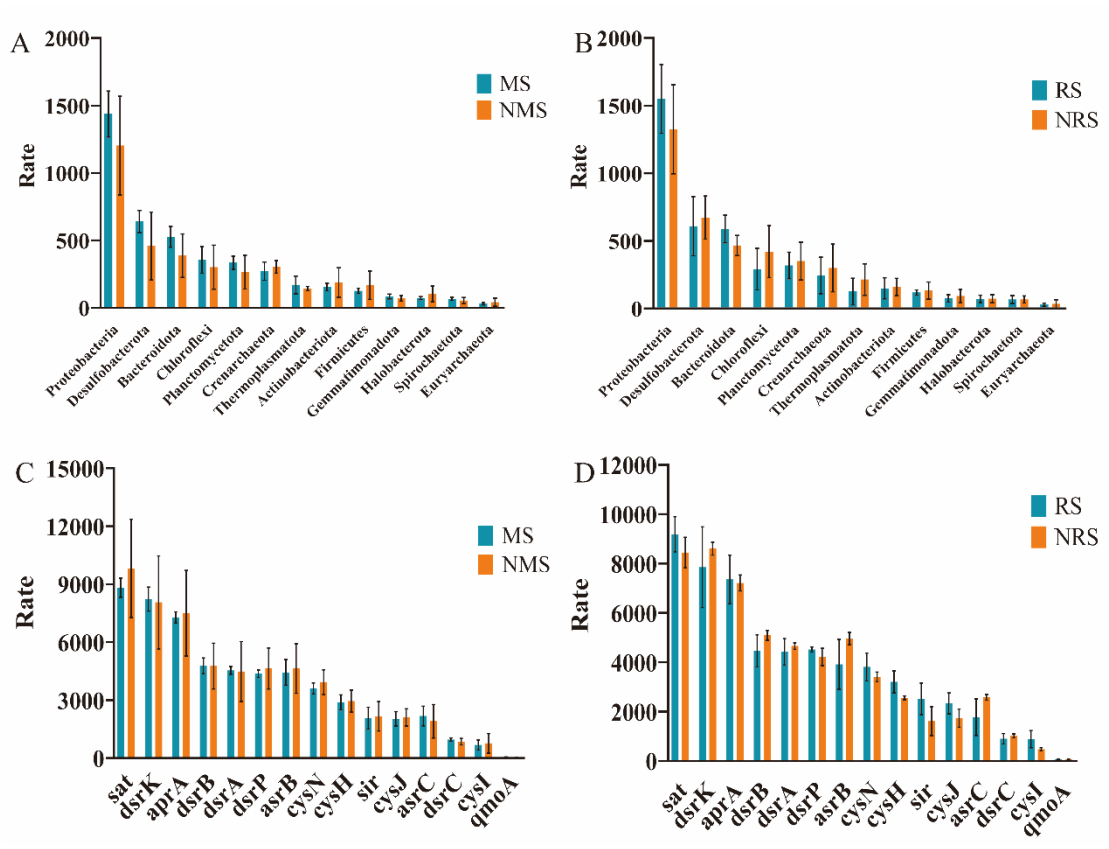
750 Figure 6. Model for the pathway of dissimilatory sulfate reduction. A) Relative
751 abundances of the gene-encoding enzymes involved in dissimilatory sulfate reduction.
752 The EC numbers of enzymes are boxed. The relative abundances of enzymes in the two
753 samples are shown in the nearby color bar, in which the two segments from left to right
754 represent MS and NMS, respectively. B) Relative abundances of the gene-encoding
755 enzymes involved in dissimilatory sulfate reduction. The EC numbers of enzymes are
756 boxed. The relative abundances of genes in the two samples are shown in the nearby
757 color bar, in which the two segments from left to right represent RS and NRS,
758 respectively.

759 **Figure 1**

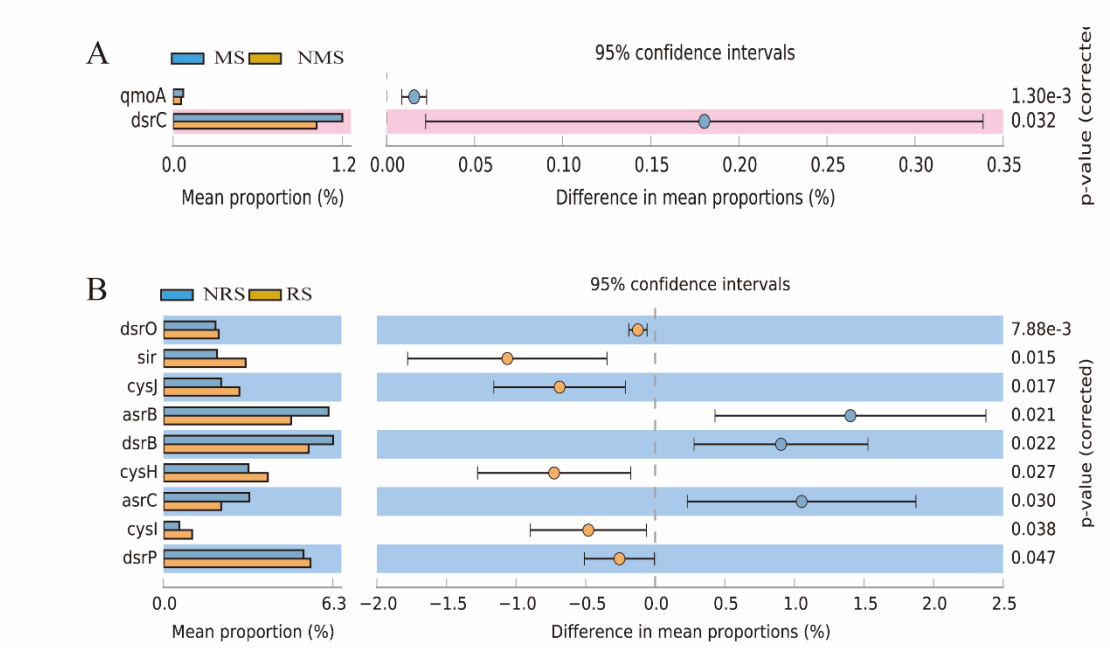


760

Figure 2


A
B
C
D
Rate
MS
NMS
RS
NRS
Proteobacteria
Desulfobacterota
Bacteroidota
Chloroflexi
Planctomycetota
Crenarchaeota
Thermoplasmatota
Actinobacteriota
Firmicutes
Gemmatimonadota
Halobacterota
Spirochaetota
Euryarchaeota
sat
dsrK
aprA
dsrB
dsrA
dsrP
asrB
cysN
cysH
sir
cysJ
asrC
dsrC
cysI
qmoA

764 **Figure 3**



765

766

Figure 4

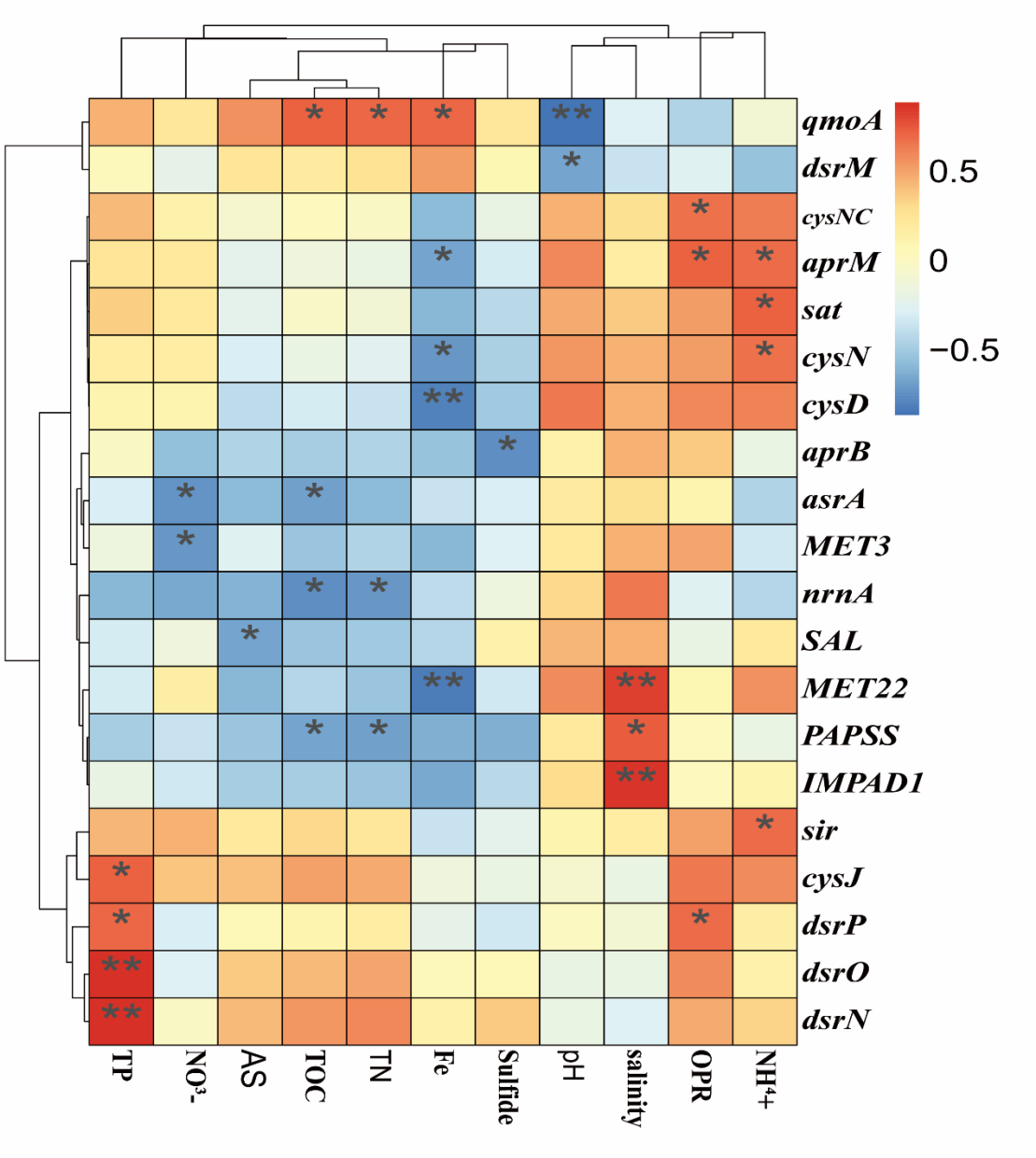
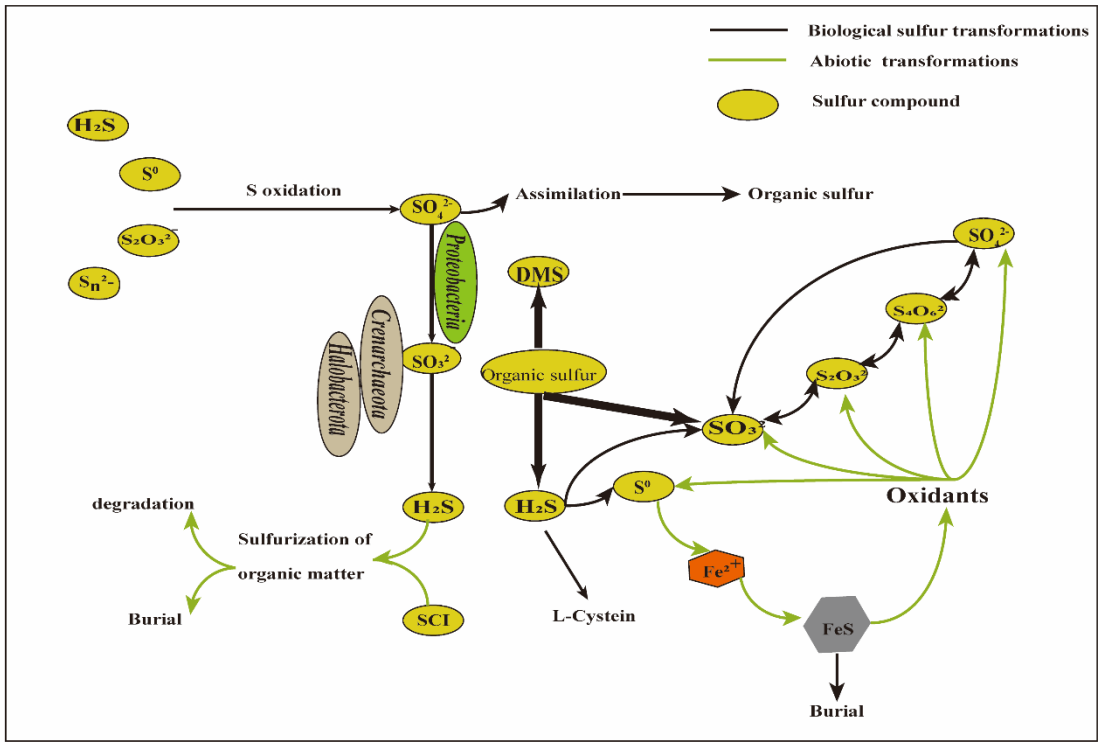
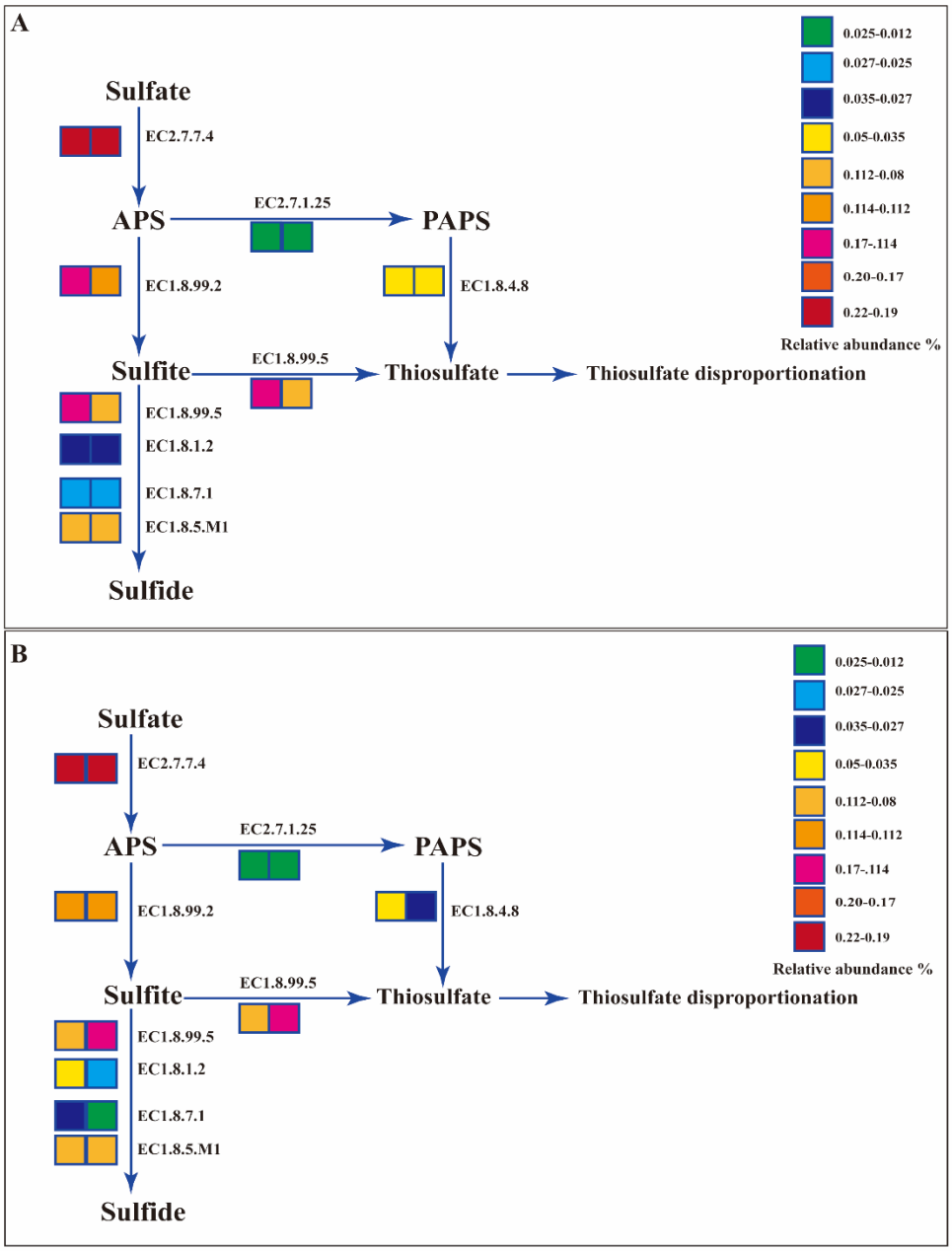

qmoA
dsrM
cysNC
aprM
sat
cysN
cysD
aprB
asrA
MET3
nrnA
SAL
MET22
PAPSS
IMPAD1
sir
cysJ
dsrP
dsrO
dsrN
TP
NO3-
AS
TOC
TN
Fe
Sulfide
pH
salinity
OPR
NH4+
0.5
0
−0.5

Figure 5


Biological sulfur transformations
Abiotic transformations
Sulfur compound
H2S
S0
S2O3 2-
Sn 2-
S oxidation
SO4 2-
Assimilation
Organic sulfur
Proteobacteria
Crenarchaeota
Halobacteroia
SO3 2-
DMS
Organic sulfur
SO4 2-
S4O6 2-
S2O5 2-
SO3 2-
Oxidants
S0
H2S
H2S
degradation
Sulfurization of
organic matter
Burial
SCI
L-Cystein
Fe2+
FeS
Burial

773

Figure 6



774
775
776