Impacts of *Desulfobacterales* and *Chromatiales* on sulfate reduction in the subtropical mangrove ecosystem as revealed by SMDB analysis

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Abstract

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. A dedicated and efficient gene integration database of sulfur metabolism has not been established yet. In this study, a sulfur metabolism gene integrative database (SMDB) had been constructed successfully. The database achieved high coverage, fast retrieval, and low false positives. Then the sulfate reduction by microorganisms in subtropical mangroves ecosystem had been evaluated quickly and accurately with SMDB database. Due to the environmental factors, dissimilatory sulfite reductase was significantly high in mangrove samples. Sulfide, Fe, and available sulfur were found to be the key environmental factors that influence the dissimilatory sulfate reduction, in which sulfur compounds could be utilized as potential energy sources for microorganism. Taxonomic assignment of dissimilatory sulfate-reduction genes revealed that *Desulfobacterales* and *Chromatiales* are completely responsible for this process. Sulfite reductase can help the community cope with the toxic sulfite produced by these Bacteria order. Collectively, these findings demonstrated that *Desulfobacterales* and *Chromatiales* play essential roles in dissimilatory sulfate reduction.

Keywords: sulfate-reduction gene families, mangrove sediment, sulfate-reduction genotype, sulfur metabolism gene integrative database

1 Introduction

Dissimilatory sulfate reduction, which results in the conversion of sulfate (SO_4^{2-}) to of HS^- or H_2S, is an important reaction in the sulfur cycle (Wenk et al., 2018). The study of dissimilatory sulfate reduction can reveal the occurrence of all dissimilatory sulfate-reduction genes in a community. However, sulfate reduction lacks a complete pathway in single strains, a condition that might be a common occurrence (Bukhtiyarova et al., 2019). The high occurrence of partial sulfate reduction implies that environmental conditions can affect gene occurrence. Gene families, including adenylyl sulfate reductase (*sat*), adenylyl sulfate reductase (*aprAB*), and dissimilatory sulfite reductase (*dsrABC*), are involved in the canonical dissimilatory sulfate-reduction pathway (Jochum et al., 2018, Anantharaman 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) (Vavourakis et al., 2019). Dissimilatory sulfate reduction is primarily driven by SRB, and a complete absence of oxygen (O_2) or low oxygen condition is vital for SRB to gain energy (Wu et al., 2017).

To the best of our knowledge, a dedicated 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.

Mangrove sediments are usually characterized as anoxic with high levels of sulfur and salt and rich in nutrients (Ferreira et al., 2010). Sulfate reduction is one of the most active processes occurring in mangrove ecosystem (Lin et al., 2019). Dissimilatory sulfate reduction drives the formation of enormous quantities of reducing sulfide. 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 (Wu et al., 2019). Second, researchers have studied culturable sulfate reduction of single strains via genomic analysis in other ecological environments (Spring et al., 2019), but it was not well study 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.

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 the subtropical mangrove ecosystem of Beibu Gulf in China to generate functional profiles. And then the sulfate reduction in the mangrove ecosystem had been evaluated. 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, as the described with Tu et al. (2019), with slight modifications. 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

The KEGG and MetaCyc databases (Caspi et al., 2016) were referenced 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. 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, COG, eggNOG, KEGG, M5nr, and NR databases were used 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. SMDB database had been deposited in https://github.com/taylor19891213/sulfur-metabolism-gene-database on January 8th, 2020. The SMDB website (http://smdb.gxu.edu.cn/) had been online since 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). All the sediment samples were collected on March 27, 2019. At each sample point (0−5 cm), triplicates sediment within an area of 10 m × 10 m were randomly collected using polyvinyl chloride tubes with a diameter of 3 cm and a height of 15 cm. Samples were placed in a sterile bag inside a box filled with ice immediately after sufficient mixing. All samples were stored at −80 °C in the same day. Rhizosphere samples (RS) from near-root were designated as RS1, RS2, and RS3. Nonrhizosphere samples (NRS) from nonrhizosphere 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. Sediment samples were air-dried and sieved through 0.25 mm steel mesh to remove stones and coarse materials, used for the chemical analysis.

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). Available sulfur was determined by Barium sulfate turbidimetry. Available sulfur includes soluble sulfur, absorbent sulfur and partly of organic sulfur. Total nitrogen (TN) was determined by acid fumigation method and followed the method described by Harris et al. (2001). TP was determined by alkali digestion method and followed the method described by Yang et al. (2013). Fe concentration was determined by Atomic Absorption Spectrophotometer (GBC932, Australia) according to the instruction. Sulfide was determined by methylene blue spectrophotometric. TOC were determined with an Automatic Carbon and Nitrogen Analyzer (TOC-TN 1200 The TOC were rmo Euroglas) according to the instruction. A detailed description of the analytical methods used for NH_4^+ and NO_3^-, was previously published (Yang et al., 2017).

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 (https://bigd.big.ac.cn/gsub/).

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. Sequences were assembled using megahit (v1.1.3) with the default parameters (Li et al., 2016). Open reading frames (ORFs) were predicted translated by using Prodigal (v3.02) (Hyatt et al., 2010) and unigenes were identified with CD-HIT (v4.5.6) (Fu et al., 2012). For unigenes, relative abundance is calculated based on the length of genes with same genes assignment in the sum of reads.

For functional annotation, unigenes 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} by using BLASTP. The online website of the SMDB database output was converted to m8 blast format. Gene families of dissimilatory sulfate reduction were screened out. The best hits were extracted for sulfur cycle genes profiling. A correlation heat map was used to visualize the composition of dissimilatory sulfate reduction across all nine samples. Spearman correlation coefficients of abiotic factors and dissimilatory sulfate-reduction genes were calculated by SPSS (George and Mallery, 2013).

For dissimilatory sulfate reduction genes, their taxonomy was assigned based on BLASP results in the NCBI NR database with coverage > 50% and e value < 1×10^{-10}. The methods for gene taxonomy as Hou et al. described (Hou et al., 2020). Taxonomic assignment was summarized at the order level. For each functional gene, taxonomic relative abundance is calculated based on the sum sequencing depth of genes with same taxonomic assignment in the total depth of this gene.

2.2.4 Annotation of taxonomy

For the taxonomic annotation, the unigenes were aligned against NR database using a local blastp program (E-value set as 10^{-10}). A blastp result was imported into MEGAN Community, and taxonomic classification was performed using LCA algorithm (Huson et al., 2016).

3 Results

3.1 Characteristics of sulfur metabolism gene integrative database

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

215 (sub)families in SMDB with those of existing public databases. Among the 175 gene
216 (sub)families we recruited in SMDB, only 118, 157, 144, 169, and 172 could be found
217 in these gene (sub)families in COG, eggNOG, KEGG, M5nr, and NR databases,
218 respectively (Fig. 1A, Supplementary Table S1). The accuracy of SMDB containing
219 sulfur gene (sub)families exceeded that of public databases (Supplementary Fig. S2).
220 We characterized sulfur metabolism gene (sub)families by searching nine shotgun
221 metagenomic datasets obtained by sampling the mangrove environment against SMDB,
222 COG, eggNOG, KEGG, M5nr, and NR databases. Subsequently, the number of sulfur
223 metabolism gene families detected by searching against SMDB far exceeded that of the
224 five other databases (Supplementary Fig. S3).

225 **3.2 Abundance and diversity of sulfur (sub) gene families**

226 A total of 151 different sulfur gene (sub)families were annotated. The sulfur gene
227 (sub)families in each sample ranged from 142 (sample RS3) to 146 (sample RS3)
228 (Supplementary Table S2). The abundance of pathways showed that the organic sulfur
229 degradation/synthesis pathway in these samples was the highest, followed by sulfur
230 oxidation; the pathway with the lowest abundance was sulfur reduction except for
231 others (Fig. 1B). As shown in Supplementary Table S2, the most abundant genes were
232 sulfonate transport system ATP-binding protein (*ssuB)*, heterodisulfide reductase
233 (*hdrA/D*), arylsulfatase (*atsA*), and dimethylsulfoniopropionate demethylase (*dmdB/A*).

234 **3.3 Microbial diversity based on metagenome**

235 To study the distribution of dissimilatory sulfate reduction in microbial communities,
236 we annotation the taxonomy. The taxonomic assignments indicated that members of

237 *Desulfobacterales* was dominant, with compositions ranging from 16% to 22%, across
238 each sample (Fig. 2A). Other order, such as *Spirochaetales, Cellvibrionales,* and
239 *Gemmatimonadales* comprised apprximately 4% (Fig. 2A). The abundance of
240 *Desulfatibacillum, Desulfobacterium*, and *Desulfosarcina* in MS exceeded that in NMS,
241 while *Desulfobacter, Desulfobulbus*, and *Desulfurivibrio* in RS exceeded that in NRS
242 (Fig. 2B).

243 **3.4 Genes for dissimilatory sulfate reduction**

244 The prevalence of dissimilatory sulfate-reduction genes at the sites was initially
245 analyzed by considering the complete dissimilatory sulfate-reduction pathway.
246 Accordingly, genes encoding adenylyl sulfate transferase (*sat*), adenylyl sulfate
247 reductase (*aprA/B*), and dissimilatory sulfite reductase (*dsrA/B*) were obtained (Fig.
248 3A). In the MS and NMS groups, *dsrA*， *aprA* and sulfite reduction-associated complex
249 DsrMKJOP multiheme protein (*dsrM/P*) were significantly high in mangrove samples
250 ($p < 0.05$, Fig. 3B). While, genes encoding sulfate adenylyltransferase subunit 2 (*cysD*)
251 was significantly low in mangrove samples. *DsrA* catalyzes the reduction of sulfite to
252 sulfide. This is the terminal oxidation reaction in sulfate respiration (Pott et al., 1998).
253 *AprA* which catalyzes reversibly the reduction of adenosine 5'-phosphosulfate (APS) to
254 sulfite and AMP during dissimilatory sulfate reduction (Chiang et al., 2009). In the RS
255 and NRS groups, genes encoding anaerobic sulfite reductase (*asrB*) and *DsrC*-disulfide
256 reductase (*dsrK*) were significantly low in rhizosphere samples ($p < 0.05$, Fig. 3C).

257 Genes *sat* and *aprA* were more frequent than *dsrA/B* (Fig. 3A). The gene of *sat*,
258 accounting for 9.6%–13.3% of the total dissimilatory sulfate-reduction genes in all
259 samples (Fig. 3A).

260 **3.5 Taxonomic assignment of dissimilatory sulfate-reduction genes**

261 *Desulfobacterales* were the predominant in microbial community. Therefore,
262 taxonomic assignments were done at the order level to obtain good process of
263 dissimilatory sulfate-reduction genes families (Table 1). The numbers of dissimilatory
264 sulfate-reduction genes form *Desulfobacterales* and *Chromatiales* exceeded those of
265 other taxonomic assignments, whereas the remaining genes were assigned to
266 *Rhizobiales, Desulfovibrionales, Desulfuromonadales* and *Cellvibrionales*
267 (Supplementary Fig. S5). This finding was consistent with the microbial diversity
268 analysis showed that these samples were as prevalent as Desulfobacterales (phylum
269 *Proteobacteria*) (Fig. 2A). The high abundance of *aprA* was obtained from
270 *Desulfobacterales* and *Chromatiales* (Table 1). In addition, *asr* and *cys* gene families
271 were obtained from *Desulfobacterales* and *Chromatiales* (Table 1). Taxonomic
272 classification of *dsrB* was assigned to *Chromatiales* (11.14%) and *Desulfobacterales*
273 (67.26%) in MS, while it was assigned to *Chromatiales* (22.66%) and
274 *Desulfobacterales* (48.50%) in NMS (Supplementary Fig. S7). However, taxonomic
275 classification of *dsrB* was assigned to *Chromatiales* (9.10%) and *Desulfobacterales*
276 (65.72%) in RS, while it was assigned to *Chromatiales* (12.44%) and *Desulfobacterales*
277 (68.23%) in NRS (Supplementary Fig. S8).

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. S6). 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 ($p < 0.05$, Fig. 4, Supplementary Table S3). pH was negatively correlated with *cysD, dsrP,* and *asrB*. Salinity content was significantly correlated with *aprA,* and *dsrB/M*. Sulfide content was significantly correlated with *sat*, Sulfite reduction-associated complex DsrMKJOP (*dsrO*), and sulfite reductase (NADPH) hemoprotein beta-component (*cysI*). Fe content was significantly correlated with *dsrA/B/C/M*, and *aprA*. AS content was significantly correlated with *dsrA/M/P*. 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. Fe was positively correlated with the sulfur reduction (Supplementary Table S4).

4 Discussion

Sulfur cycle is one of the most important biogeochemical cycles in Earth ecosystems. 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 the previous public databases. First, SMDB has a precise definition for sulfur metabolism gene families. Unlike other database generations (Huerta-Cepas 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. 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 display considerable sequence similarity in KEGG, but they are distinct in two sulfur transferase subfamilies. Finally, SMDB considers the problem of false positives. Herein, this issue was addressed by including homologous gene families from multiple public databases. Most importantly, the small size of SMDB reduces the computational cost required to output the functional profiling of files of sulfur metabolism gene families. The database achieved high coverage and accuracy (Supplementary Fig. S1). The discrepancy due to these databases have their own advantages as well as limitations owing to different designing concepts associated with each database. For example, the COG database mainly relies on complete microbial genomes (Galperin et al., 2015).

The KEGG database holds advantages of linking genes to pathways in data interpretation (Kanehisa et al., 2016).

We applied SMDB in characterizing sulfur metabolism gene (sub)families in mangrove sediment environment to demonstrate its potential applications in microbial ecology studies. Most studies have used experiments for microbial. The activity of microbial metabolism can be determined by (radio-)tracer experiments. Polymerase chain reaction (PCR), a technique used to make numerous copies of a specific segment of DNA quickly and accurately. However, PCR usually produces bias, resulting in inaccurate experimental results because of lack of perfect working primers for many of the gene families involved (Peng et al., 2018). In the present study, shotgun metagenome sequencing technology was used to investigate the complete sulfur cycle in mangroves. The metagenomics survey described herein provided strong evidence that in neutral pH, anoxic, high-sulfur soil, the environment in the Beibu Gulf of China biases the dissimilatory sulfate-reduction genotype. AS and Fe concentrations were consistently higher in the mangrove zones than in the nonmangrove zones ($p < 0.05$, Supplementary Table S5), suggesting that the concentrations of AS and Fe determine the abundance of dissimilatory sulfate-reduction genes. Sulfide, Fe, and available sulfur were found to be the key environmental factors that influence the dissimilatory sulfate reduction genes ($p < 0.05$, Fig. 4). However, our current understanding of dissimilatory sulfate reduction suggests that Sulfide, Fe, and available sulfur contents are insufficient to explain gene trends, and available O_2 is also an important factor. Mangrove sediments are usually characterized as anoxic (Ferreira et al., 2010). Mangrove conditions favoring dissimilatory sulfate reduction were probably of sufficient duration to contribute to the patterns observed herein, but a more detailed study of sediment

properties should be conducted for confirmation.

This pattern supported the hypothesis that environmental conditions act on genes in the pathway and biases the dissimilatory sulfate-reduction genotype. In all samples, *aprA* occurred at higher levels than that from dissimilatory sulfate-reduction genes (*dsrA/B*) and anaerobic sulfite reductase (*asrA/B*) (Fig. 3A). This consistent with the previous studies, the occurrence of inorganic sulfur cycle genes in sequence genomes found that *aprA* occurs at higher frequencies than the other gene-encoding proteins required for dissimilatory sulfate-reduction genes (Vavourakis et al., 2019). This condition is probably due to the simultaneous sulfate reduction and sulfide oxidation processes (Watanabe et al., 2013). *DsrA* and *aprA* were significantly high in mangrove samples ($p < 0.05$, Fig. 3B). Owing to the unique ecological environments of subtropical mangrove ecosystems, the class *Proteobacteria* of *Betaproteobacteria* is the dominant SRB group in mangrove (Varon-Lopez et al., 2014). However, our results show that *Deltaproteobacteria* (order *Desulfobacterales, Chromatiales)* was the dominant SRB group in mangrove (Supplementary Fig. S5). In addition, *Desulfobacterales, Chromatiales* had relatively complete gene families of dissimilatory sulfate reduction in this study (Table 1). The *aprA* were basically obtained from *Desulfobacterales* and *Chromatiales* (Table 1, Supplementary Fig. S7), suggesting that the function of *aprA* in mangrove ecosystems may be mainly performed by these *Bacteria orde*r. Microbial sulfate reduction is important for anaerobic degradation of organic matter, and is often mediated by *Deltaproteobacteria*, specifically, *Desulfobacterales* (Meyer et al., 2007).

372 Taxonomic classification of *dsrB* found that *Desulfobacterales* was high in MS than
373 NMS. These findings showed that unique ecological environments selected these
374 microbial communities for dissimilatory sulfate reduction.
375
376 Notably, sulfite is cytotoxic above a certain threshold if not rapidly metabolized and
377 can wreak havoc at the cellular and whole-plant levels. By providing the gene families
378 of *asrC, asrA*, *asrB, cysI, cysJ*, and *cysH* that reduces sulfite levels to protect the
379 members of the local community (Bender et al., 2019). These activities are required for
380 the biosynthesis of L-cysteine from sulfate (Zeghouf and Chemistry, 2000). If sulfite
381 reductase is absent, most organisms in the community would be inhibited and grow
382 slowly. The sediment properties (e.g., OPR, Fe) results in the release of sulfides from
383 sediments (Shi et al., 2016). Other studies have shown that sulfides in mangrove
384 sediments are eventually converted to L-cysteine (Lin et al., 2019).
385 We depicted the process of sulfur metabolism in mangrove sediments. Sulfur
386 metabolism in mangrove sediments includes biological and abiotic processes (Fig. 5).
387 For abiotic processes, Fe(III) is buried and acts as an oxidant for sulfide in deeper
388 sediment layers where it partly binds the produced sulfide as iron sulfide (FeS) and
389 pyrite (FeS_2) (Wasmund et al., 2017). In this study, the concentration of Fe was
390 consistently higher in the mangrove zones than that in the nonmangrove zones ($p < 0.05$,
391 Supplementary Table S5). H_2S and FeS can be oxidized into various oxidation states of
392 sulfur. For biological processes, the role of microorganisms includes both oxidation and
393 reduction of sulfur compounds. Dissimilatory sulfate reduction involves the reduction

394 of sulfate to sulfide where the electron acceptor is sulfate, APS, sulfite, elemental sulfur,
395 etc. By contrast, the reduced sulfide is assimilated in proteins and amino acids in
396 assimilatory sulfate reduction (Fuentes-Lara et al., 2019). Sulfide oxidation by O_2,
397 NO_3^-, and Fe^{3+}as electron acceptors produces sulfur, while Mn^{4+} as electron acceptors
398 produces thiosulfate or sulfate. In addition, sulfur undergoes coupled oxidation and
399 reduction of sulfur compounds (thiosulfate, sulfite, and sulfur) to sulfate and sulfide.
400 DMS was derived from the degradation of organic sulfur (e.g.,
401 dimethylsulfoniopropionate). The dimethylsulfide (DMS) pathway provides a new link
402 between the cycles of organic and inorganic sulfur compounds (Koch and Dahl, 2018).
403
404 The mechanism of dissimilatory sulfate-reduction metabolic pattern in mangrove
405 ecosystem was constructed (Fig. 6). The figure shows the relative abundances of gene-
406 encoding enzymes involved in dissimilatory sulfate reduction. The reduced sulfur
407 compounds were likely oxidized to sulfite by reverse dissimilatory sulfite reductase
408 (encoded by *dsrAB*) and thiosulfate sulfur transferase. EC 2.7.7.4 (sulfate
409 adenylyltransferase) had the highest abundance among the enzymes. EC 1.8.99.2
410 (adenylyl-sulfate reductase) and EC 1.8.99.5 (dissimilatory sulfite reductase) had
411 higher abundance in mangrove samples than in nonmangrove samples. The model of
412 dissimilatory sulfite reductase also supports the hypothesis that mangrove samples
413 prefer dissimilatory sulfate reduction. In rhizosphere samples, the abundance of EC
414 1.8.99.5 was low, whereas that of EC 1.8.1.2 (assimilatory sulfite reductase) was high.
415 This finding indicated that rhizosphere microorganisms are conducive to the

assimilation of sulfate to L-cysteine. Moreover, this result highlighted the importance of mangrove plants in sulfur cycle.

5 Conclusions

In sum, 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. The subtropical mangrove ecosystem biases dissimilatory sulfate-reduction genotype. Sulfide, Fe, and available sulfur were found to be the key environmental factors that influence the dissimilatory sulfate reduction. *Desulfobacterales* and *Chromatiales* 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.

CRediT authorship contribution statement

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

Declaration of competing interest

The authors declare that they have no known personal relationships or competing financial interests that could have influenced the work conducted in this study.

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

taxonomy	RS	NRS	NMS
Chromatiales	*cysJ; dsrK; aprA; cysN; dsrA; sir; sat; aprM; cysQ; cysNC; cysD; cysH; dsrO; asrB; dsrN; dsrP; asrA; nrnA; MET3; dsrB; asrC; HINT4; cysI*	*cysN; aprA; dsrB; dsrO; sir; sat; cysQ; dsrK; cysNC; cysD; MET3; dsrM; cysH; cysJ; asrB; dsrP; dsrN; cysI; dsrA; aprB; nrnA; dsrC; HINT4*	*cysJ; cysN; asrB; cysD; cysNC; dsrK; asrA; sat; cysI; cysH; cysQ; aprA; dsrC; sir; aprM; nrnA; dsrP; MET3; dsrM; aprB; dsrB; dsrA*
Desulfobacterales	*cysJ;cysN; aprA; asrB; cysQ; asrC; dsrK; nrnA; sat; dsrM; asrA; dsrN; cysH; dsrB; sir; dsrP; cysD; SAL; MET3; cysNC; dsrA; aprB; cysI; dsrO; MET22; dsrC;dsrJ*	*dsrK; cysJ; sat; aprA; cysN; cysQ; nrnA; dsrJ; dsrM; dsrC; asrB; cysNC; dsrN; cysH; dsrB; asrC; dsrA; sir; dsrP; aprB; SAL; asrA; MET3; MET22; dsrO; cysI; cysD*	*dsrK; cysJ; sat; asrB; cysN; asrA; cysQ; aprA; dsrB; MET22; nrnA; sir; dsrP; aprB; dsrJ; dsrN; dsrM; cysD; cysNC; dsrA; asrC;cysH; dsrO; cysI; MET3; dsrC*
Rhizobiales	*sat;dsrO; cysI; cysJ; cysH; asrA; cysQ; cysN; sir; nrnA; asrB*	*sat; aprA; cysJ; cysH; cysQ; asrB; HINT4; cysN*	*aprM; sat; dsrA; aprA; cysN; cysI; cysQ; asrB; cysJ; dsrO; sir; dsrK; nrnA*
Desulfovibrionales	*sat; asrC; dsrK; sir; cysN; cysJ*	*aprA; sat; dsrJ; cysN; asrC; dsrK; cysJ*	*sat; dsrK; cysN*
Desulfuromonadales	*cysI; cysN; asrB; nrnA; cysQ; sir; asrC*	*cysN; sir; asrC*	*cysI; cysN; asrB; sat; dsrA; sir; cysQ; dsrP; nrnA*

575

576 **Figure Legends**

577 Figure 1. A) Sulfur cycle gene (sub)families were detected using SMDB, NR, M5nr,
578 KEGG, eggNOG, COG databases. B) Pathway abundances in the samples. The Y-axis
579 is gene (sub)families number in different databases.

580

581 Figure 2. Comparison of taxonomic assignments from the metagenomes. A) The 13
582 dominant order are shown with their relative abundances; the remaining order are
583 indicated as "Others". B) The heat map of *Desulfobacterales* order.

584

585 Figure 3. A) The bar chart indicates the relative abundances of the 20 abundant
586 dissimilatory sulfate reduction gene families in each sample. Significant differences in
587 sulfur (sub) gene families between mangrove and nonmangrove sediments (B) and
588 rhizosphere and nonrhizosphere sediments (C).

589

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

593 * $p < 0.05$

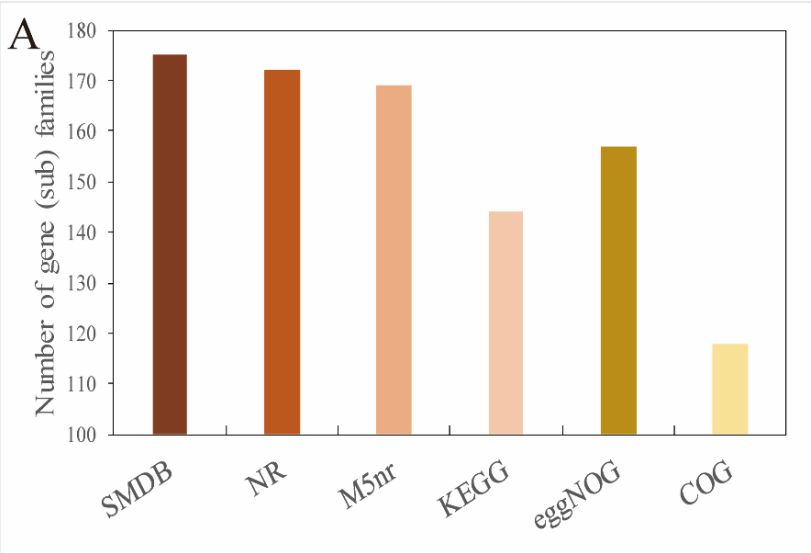
594 ** $p < 0.01$

595

596 Figure 5. Conceptual depiction of sulfur metabolism in mangrove ecosystem, including
597 reactions of inorganic and organic sulfur compounds and transformations of sulfur

598 compounds of intermediate oxidation states (sulfur cycle intermediates, SCI). Inorganic
599 sulfur compounds include oxidation and reduction of sulfur compounds. Black lines
600 depict biological sulfur transformations by microorganisms. Green lines depict abiotic
601 reaction-mediated sulfur transformations to pyrite (FeS_2). Sulfur compounds are
602 depicted within yellow eclipses.
603
604 Figure 6. Model for the pathway of dissimilatory sulfate reduction. Relative abundances
605 of the gene-encoding enzymes involved in dissimilatory sulfate reduction. The EC
606 numbers of enzymes are boxed. The relative abundances of enzymes in the samples are
607 shown in the nearby color bar, in which the four segments from left to right represent
608 MS and NMS, RS, NRS, respectively.

Figure 1


A
Number of gene (sub) families
180
170
160
150
140
130
120
110
100
SMDB
NR
M5nr
KEGG
eggNOG
COG

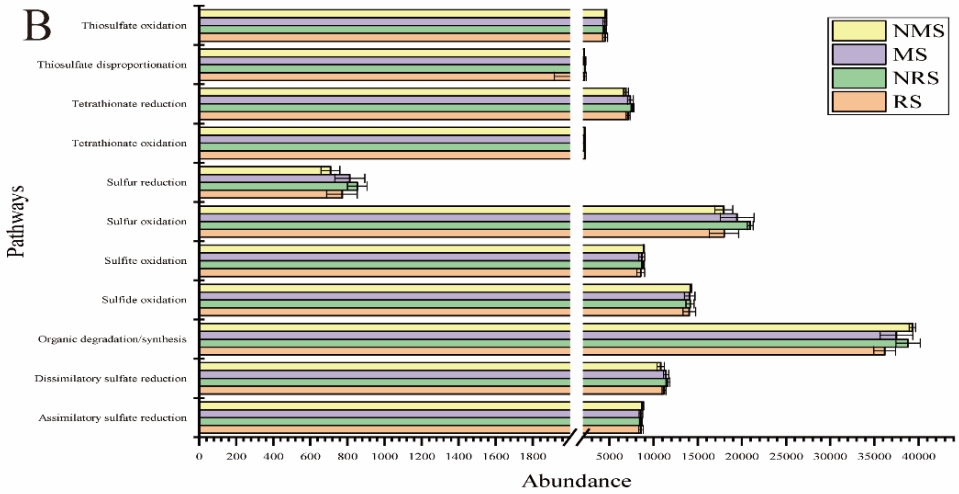

B
Pathways
Thiosulfate oxidation
Thiosulfate disproportionation
Tetrathionate reduction
Tetrathionate oxidation
Sulfur reduction
Sulfur oxidation
Sulfite oxidation
Sulfide oxidation
Organic degradation/synthesis
Dissimilatory sulfate reduction
Assimilatory sulfate reduction
NMS
MS
NRS
RS
0 200 400 600 800 1000 1200 1400 1600 1800
5000 10000 15000 20000 25000 30000 35000 40000
Abundance

Figure 2

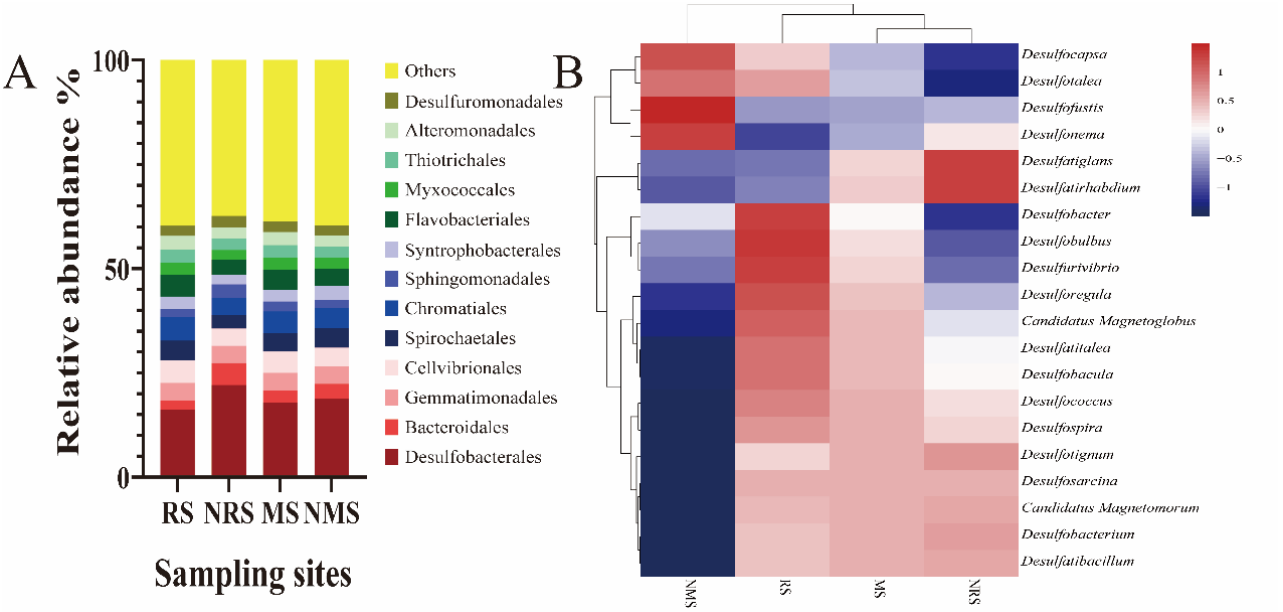

A
Relative abundance %
100
50
0
RS NRS MS NMS
Sampling sites
Others
Desulfuromonadales
Alteromonadales
Thiotrichales
Myxococcales
Flavobacteriales
Syntrophobacterales
Sphingomonadales
Chromatiales
Spirochaetales
Cellvibrionales
Gemmatimonadales
Bacteroidales
Desulfobacterales
B
Desulfocapsa
Desulfotalea
Desulfofustis
Desulfonema
Desulfatiglans
Desulfatirhabdium
Desulfobacter
Desulfobulbus
Desulfurivibrio
Desulforegula
Candidatus Magnetoglobus
Desulfatitalea
Desulfobacula
Desulfococcus
Desulfospira
Desulfotignum
Desulfosarcina
Candidatus Magnetomorum
Desulfobacterium
Desulfatibacillum
NMS
RS
MS
NRS
1
0.5
0
−0.5
−1

Figure 3

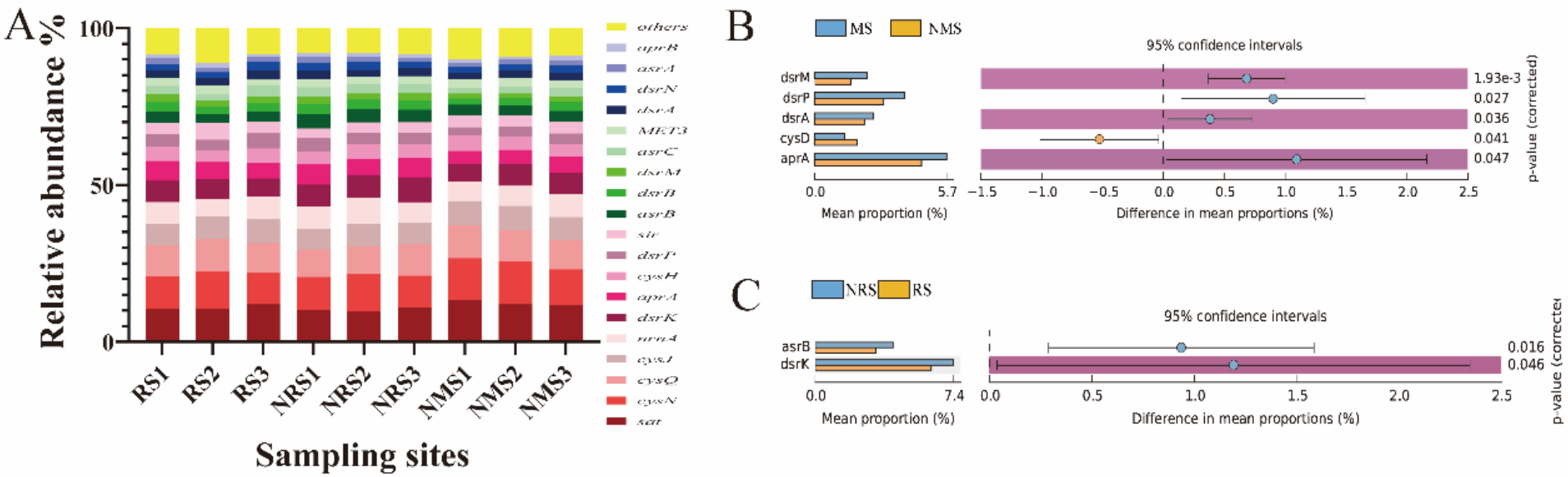

A
Relative abundance %
100
50
0
RS1
RS2
RS3
NRS1
NRS2
NRS3
NMS1
NMS2
NMS3
Sampling sites
others
aprB
asrA
dsrN
dsrA
MFT3
asrC
dsrM
dsrB
asrB
sir
dsrP
cysH
aprA
dsrK
nrnA
cysJ
cysO
cysN
sat
B
MS
NMS
95% confidence intervals
dsrM
dsrP
dsrA
cysD
aprA
1.93e-3
0.027
0.036
0.041
0.047
p-value (corrected)
0.0
5.7
Mean proportion (%)
−1.5
−1.0
−0.5
0.0
0.5
1.0
1.5
2.0
2.5
Difference in mean proportions (%)
C
NRS
RS
95% confidence intervals
asrB
dsrK
0.016
0.046
0.0
7.4
Mean proportion (%)
0.0
0.5
1.0
1.5
2.0
2.5
Difference in mean proportions (%)

Figure 4

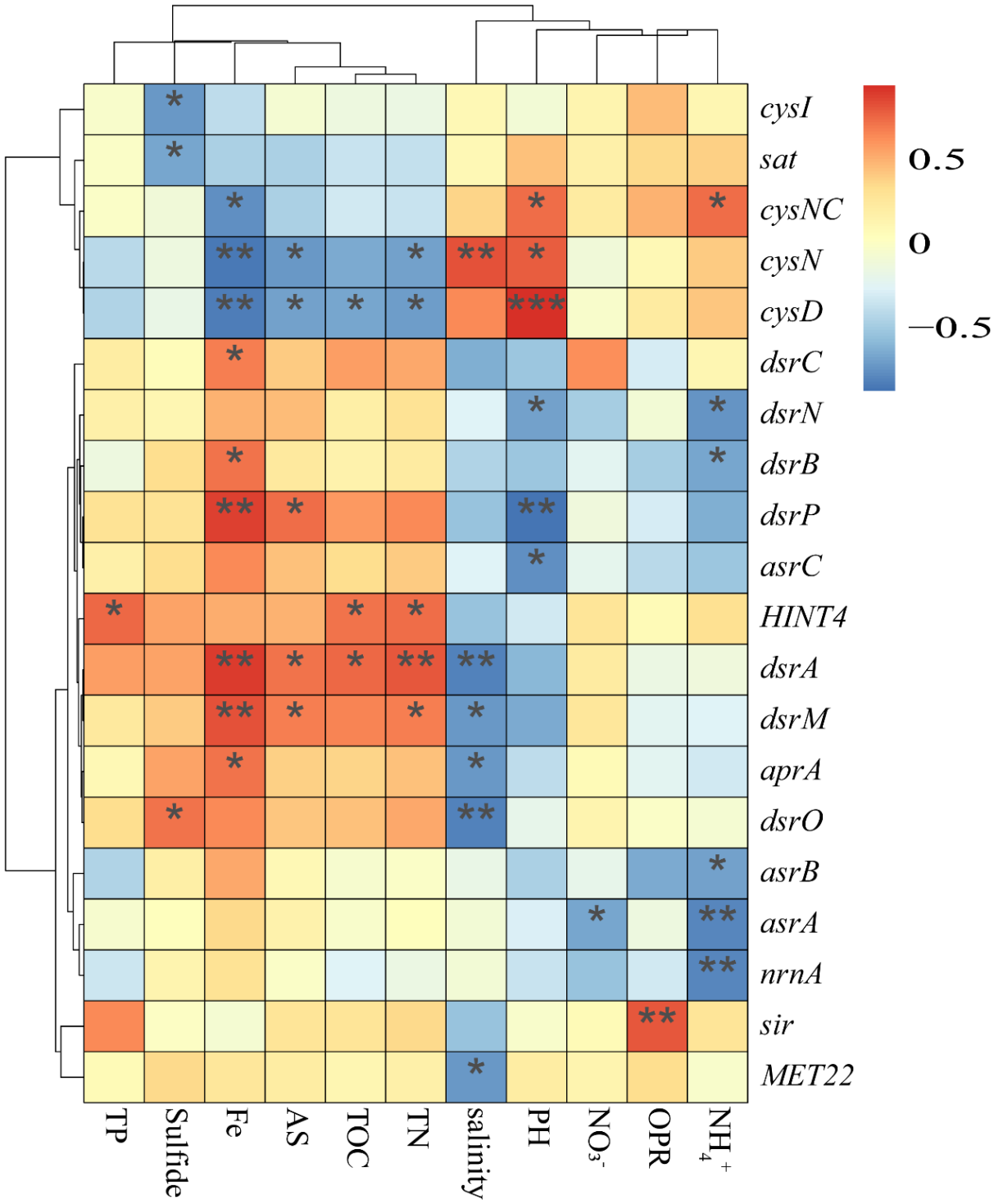
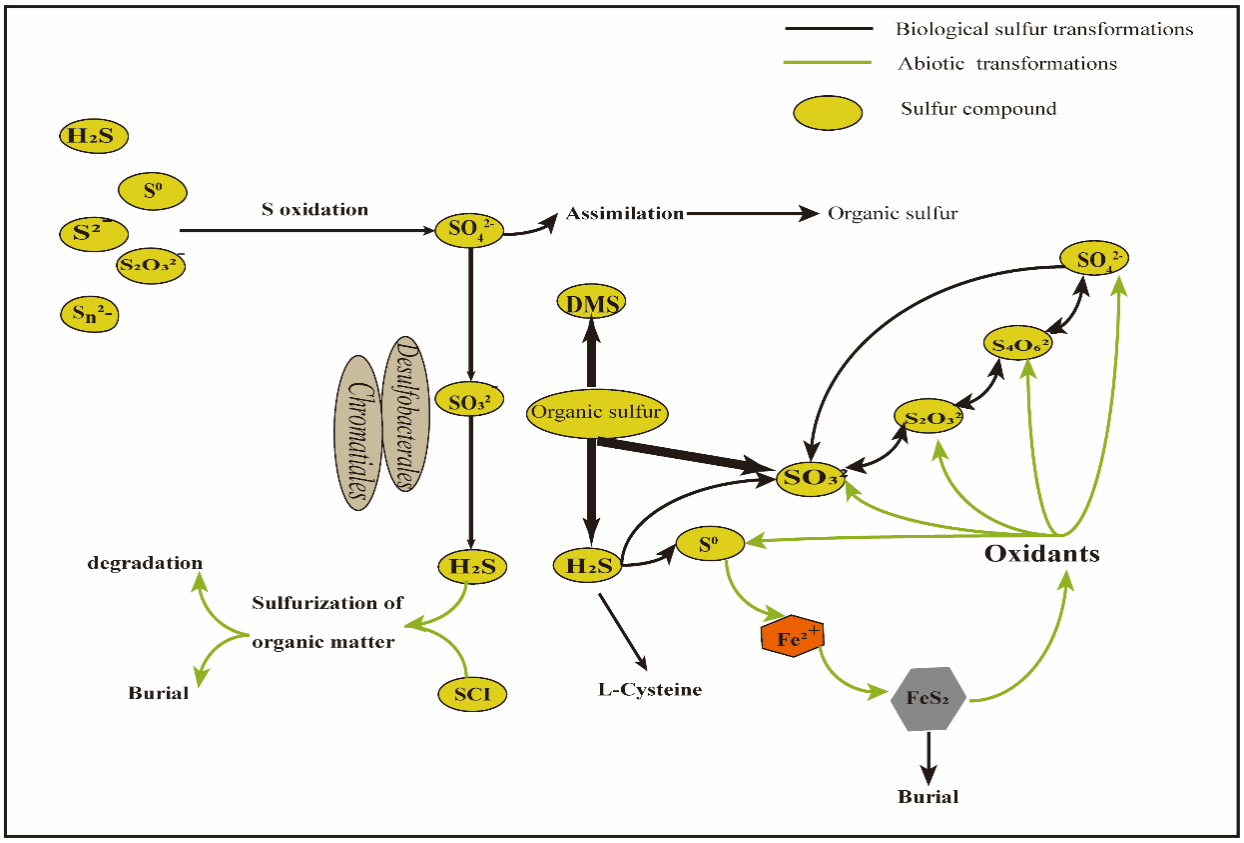
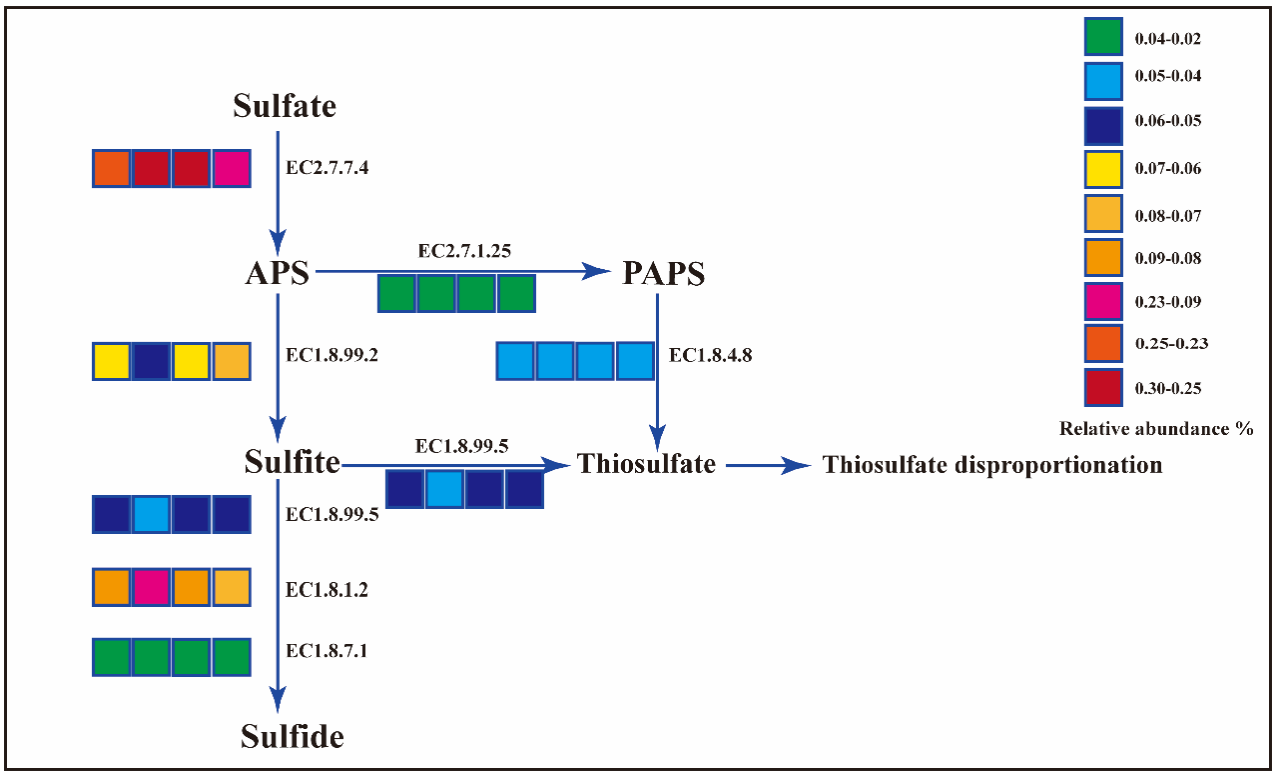

cysI
sat
cysNC
cysN
cysD
dsrC
dsrN
dsrB
dsrP
asrC
HINT4
dsrA
dsrM
aprA
dsrO
asrB
asrA
nrnA
sir
MET22
TP
Sulfide
Fe
AS
TOC
TN
salinity
PH
NO_3^-
OPR
NH_4^+
0.5
0
−0.5

Figure 5



Biological sulfur transformations
Abiotic transformations
Sulfur compound
H2S
S0
S2-
S2O32-
Sn2-
S oxidation
SO42-
Assimilation
Organic sulfur
DMS
Desulfobacterales
Chromatiales
SO32
Organic sulfur
SO42-
S4O62
S2O32
SO32
S0
Oxidants
degradation
H2S
H2S
Sulfurization of
organic matter
Burial
SCI
L-Cysteine
Fe2+
FeS2
Burial

Figure 6


Sulfate
EC2.7.7.4
APS
EC2.7.1.25
PAPS
EC1.8.99.2
EC1.8.4.8
Sulfite
EC1.8.99.5
Thiosulfate
Thiosulfate disproportionation
EC1.8.99.5
EC1.8.1.2
EC1.8.7.1
Sulfide
0.04-0.02
0.05-0.04
0.06-0.05
0.07-0.06
0.08-0.07
0.09-0.08
0.23-0.09
0.25-0.23
0.30-0.25
Relative abundance %