Supplementary Material

Metagenomics Sequencing and Binning Reveal the Diversity, Assembly and Synergistic Variation of Microbial Sulphur Cycling Genes under Corpse Decay

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16S rRNA sequencing

PCR amplicons were sequenced to obtain 2 × 250 bp paired-end sequences by the Illumina Miseq system (Illumina, USA). The raw data was handled with the QIIME pipeline [1]. By 16S high-throughput sequencing, we obtained 3,163,291 high-quality bacterial readings (range from 7,547 to 339,343, and the average count of reading was 79,082) from 40 samples. Bioinformatics analysis methods were the same with our foregone study [2].

Metagenomic analysis and annotation

Totally, 50.9 million reads, namely 7.42 GB high-quality sequence was retrieved from per sample. Then the sulphur functional annotations of unigenes were annotated via the databases SCycDB (https://github.com/Qichao 1984/SCycDB) [3] through DIAMOND v0.7.12. The sulphur cycle pathway was set up by KEGG PATHWAY data base (https://www.kegg.jp/kegg/pathway.html) [4]. Unigene abundance was calculated by TPM (Transcripts Per Kilobase of exon model per Million mapped reads) abundance as follows [5]:

$$G_k = \frac{r_k}{L_k} * \frac{1}{\sum_{i=1}^{n}\frac{r_i}{L_i}} * 10^6 \tag{S1}$$

r_k: the count of reads aligned to the k_{th} exon; L_k: length of the k_{th} exon; $\sum_{i=1}^{n}\frac{r_i}{L_i}$: after normalization of all exons according to length, sum the values. The value n means the count of exons.

Binning analysis

The clean data obtained from the standard quality control process of metagenomic method was directly used for subsequent binning analysis. The clean data was assembled by optional assembly software Megahit [6], and contig sequence was obtained. The assembly result was evaluated based on Quast software, and the data of R1 and R2 of all samples were merged respectively before assembly [7]. Then the assembled contigs and cleandata from the 40 samples were binned based on three methods: maxbin2, metabat2 and CONCOCT [8, 9]. Only contigs met both of the following criteria were retained: (1) Bin completion ≥ 50%; (2) Bin contamination ≤ 10%. Based on the quantification of Salmon [10], the average abundance of every sacffold and bin was evaluated. The retained metagenome assembled genomes (MAGs) were used for the sequencing annotation. Using Taxator-tk [11], the contigs were aligned against the NCBI_nt and NCBI-tax database, and the contigs had no affiliation to any known taxa were defined as "Unknown". The gene sequence and corresponding annotation information of each bins were obtained through annotation of PROKKA [12]. The visualization of genome map was achieved through GSDS2.0 (http://gsds.gao-lab.org/index.php) [13].

Statistical analysis

The differences between different groups of alpha diversity of microbes and sulphur cycling genes were compared by *Mann-Whitney U* test [14] through SPSS 26.0 (SPSS Inc., Chicago, IL, USA). Based on Bray-Curtis and Jaccard distance matrices, beta diversity was calculated and generated using graphical visualization by principal co-ordinates analysis (PCoA) [15]. The Bray-Curtis distance matrix reflects species abundance, while Jaccard distance matrix represents the information of existence of species [16, 17]. To probe into the relationship between the TPM abundance of sulphur cycling genes and temperature, linear fitting was used. The main drawing software was Origin 2018 (Originlab, Northampton, USA). With the help of R version 4.1.3, we completed the following contents of calculation and draft: permutational multivariate analysis of variance (PERMANOVA) [18], multiple regression matrix (MRM) [19], heatmap and correlation heatmap, by the packages 'vegan', 'ecodist' 'heatmap' and 'pheatmap', respectively. To constitute the network and calculated the topological parameters (including average path length, diameter, average degree, density, modularity and average clustering coefficient), Gephi 0.9.2 was utilized [20]. All sulphur cycling genes were included into the analysis of network, and only the co-occurrence relationships with significance were showed ($|r| > 0.5$, $P < 0.05$).

Table S1. PERMANOVA of sulphur cycling microbes based on Bray-Curtis and Jaccard distance matrices. *P < 0.05. Abbreviations: T, control groups; E, experimental groups.

PERMANOVA	Bray-Curtis			Jaccard		
	F	R^2	P	F	R^2	P
T23 vs T26	3.565	0.373	**0.036***	6.009	0.500	**0.036***
T26 vs T29	0.929	0.134	0.507	14.558	0.708	**0.034***
T29 vs T32	3.310	0.356	0.059	12.099	0.668	**0.033***
T32 vs T35	7.400	0.552	**0.031***	7.251	0.547	**0.031***
E23 vs E26	0.532	0.081	0.891	0.850	0.124	0.576
E26 vs E29	1.836	0.234	0.065	0.887	0.129	0.585
E29 vs E32	1.250	0.172	0.257	1.974	0.248	0.145
E32 vs E35	4.189	0.411	0.059	3.029	0.336	0.081
T23 vs E23	70.412	0.921	**0.032***	170.678	0.966	**0.032***
T26 vs E26	21.190	0.779	**0.033***	100.552	0.944	**0.033***
T29 vs E29	30.779	0.837	**0.028***	65.112	0.916	**0.028***
T32 vs E32	14.029	0.700	**0.032***	28.129	0.824	**0.032***
T35 vs E35	25.323	0.808	**0.026***	19.907	0.768	**0.026***

Table S2. PERMANOVA of sulphur cycling genes based on Bray-Curtis and Jaccard distance matrices. *P < 0.05. Abbreviations: T, control groups; E, experimental groups.

PERMANOVA	Bray-Curtis			Jaccard		
	F	R^2	P	F	R^2	P
T23 vs T26	2.164	0.265	0.086	0.663	0.100	0.608
T26 vs T29	0.285	0.045	0.641	0.871	0.127	0.468
T29 vs T32	2.531	0.297	0.120	0.843	0.123	0.584
T32 vs T35	9.288	0.608	**0.031***	5.622	0.484	0.055
E23 vs E26	0.864	0.126	0.570	0.213	0.034	0.944
E26 vs E29	1.433	0.193	0.086	0.259	0.041	0.944
E29 vs E32	0.939	0.135	0.437	1.471	0.197	0.386
E32 vs E35	1.699	0.221	0.059	0.956	0.137	0.316
T23 vs E23	108.839	0.948	**0.032***	27.358	0.820	**0.032***
T26 vs E26	9.312	0.608	0.061	20.596	0.774	**0.033***
T29 vs E29	13.639	0.694	**0.028***	12.115	0.669	**0.028***
T32 vs E32	2.961	0.330	0.083	28.284	0.825	**0.032***
T35 vs E35	29.233	0.830	**0.026***	2.343	0.281	0.050

Table S3. PERMANOVA of sulphur cycling genes and the influential factors based on Bray-Curtis and Jaccard distance matrices. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Abbreviations: NO_3-N, nitrate nitrogen; NH_4-N, ammonium nitrogen; TC, total carbon, TN, total nitrogen; IP, inorganic phosphorus; OP, organic phosphor; TS, total sulphur.

PERMANOVA	Bray-Curtis			Jaccard		
	R^2	F	P	R^2	F	P
Corpse	0.439	54.939	**0.001*****	0.471	48.017	**0.001*****
Temperature	0.222	27.836	**0.001*****	0.090	9.152	**0.001*****
NH_4-N	0.035	4.361	**0.046***	0.013	1.300	0.236
NO_3-N	0.001	0.095	0.821	0.022	2.221	0.101
TC	0.005	0.677	0.465	0.017	1.686	0.159
TN	0.007	0.926	0.328	0.024	2.453	0.074
IP	0.020	2.447	0.131	0.010	0.977	0.398
OP	0.005	0.569	0.479	0.043	4.354	**0.011***
TS	0.021	2.602	0.101	0.001	0.082	0.952
CON	0.245	23.535	**0.001*****	0.218	15.620	**0.001*****
TDS	0.007	0.702	0.447	0.017	1.217	0.294
SALT	0.000	0.021	0.971	0.032	2.311	0.073
PH	0.017	1.645	0.205	0.040	2.882	**0.044***
ORP	0.075	7.176	**0.015***	0.121	8.674	**0.001*****
DO	0.312	29.896	**0.001*****	0.111	7.937	**0.001*****
Alpha diversity	0.026	3.265	0.083	0.024	2.395	0.081
PCoA1	0.001	0.185	0.707	0.017	1.759	0.159
PCoA2	0.003	0.349	0.570	0.005	0.489	0.687
Number of bacteria	0.001	0.128	0.791	0.007	0.655	0.561

Table S4. MRM of sulphur cycling genes. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Abbreviations: NO_3-N, nitrate nitrogen; NH_4-N, ammonium nitrogen; TC, total carbon, TN, total nitrogen; IP, inorganic phosphorus; OP, organic phosphor; TS, total sulphur; CON, conductivity; TDS, total dissolved solids; SALT, salinity; ORP, oxidation-reduction potential; DO, dissolved oxygen.

MRM	R^2	*F*	*P*
Temperature	0.011	8.321	**0.033***
Corpse	0.104	90.630	**0.001*****
NO_3-N	0.005	3.989	0.352
NH_4-N	0.000	0.062	0.892
TN	0.007	5.231	0.108
TS	0.011	9.041	0.063
IP	0.002	1.479	0.588
OP	0.004	2.844	0.460
TC	0.004	3.492	0.371
CON	0.011	8.377	**0.042***
TDS	0.006	4.724	0.130
SALT	0.009	6.714	0.061
pH	0.001	1.103	0.386
ORP	0.013	10.159	**0.022***
DO	0.285	310.087	**0.001*****
PCoA1	0.342	404.107	**0.001*****
PCoA2	0.001	0.877	0.579
Number of bacteria	0.001	0.665	0.714
Observed species	0.353	424.985	**0.001*****

Table S5. MRM of sulphur cycling genes. *P <0.05, **P <0.01, ***P <0.001.

MRM	R^2	*F*	*P*
Temperature	0.013	10.561	**0.023***
Corpse	0.110	95.955	**0.001*****
Environment factor	0.010	8.148	**0.039***
Microbe	0.348	414.749	**0.001*****

Table S6. The network topology parameters of sulphur cycling genes.

	Node counts	Edge counts	Average degree	Average clustering coefficient	Average path length	Diameter	Density	Modularity
Control group	29	130	8.966	0.722	1.781	4	0.320	0.227
Experimental group	22	36	3.273	0.707	1.658	3	0.156	0.104

Table S7. The sulphur cycling genes in assimilatory sulphate reduction pathway.

Pathways	Gene	Annotation	Detected
Assimilatory sulphate reduction	*cysC*	Adenylylsulphate kinase	Yes
	cysND	Sulphate adenylyltransferase	No
	cysH	Phosphoadenosine phosphosulphate reductase	Yes
	cysIJ	Sulphite reductase	Yes
	cysNC	Bifunctional enzyme CysN/CysC	No
	cysQ	3′(2′), 5′-bisphosphate nucleotidase	Yes
	nrnA	Bifunctional oligoribonuclease and PAP phosphatase	No
	sat	Sulphate adenylyltransferase	Yes
	sir	Sulphite reductase (ferredoxin)	Yes

Table S8. The sulphur cycling genes in dissimilatory sulphur reduction and oxidation pathway.

Pathways	Gene	Annotation	Detected
Dissimilatory sulphur reduction and oxidation	*aprAB*	Adenylylsulphate reductase	No
	dsrAB	Dissimilatory sulphite reductase	No
	dsrC	Dissimilatory sulphite reductase related protein	No
	dsrDNT	Protein DsrD DsrN DsrT	No
	dsrEFH	Sulfurtransferase	No
	dsrL	NADPH: acceptor oxidorductase DsrL	No
	dsrMKJOP	Membrane-Bound DsrMKJOP complex	No
	qmoABC	Quinone-modifying oxidoreductase	No
	rdsr	Reverse dissimilatory sulphite reductase	No
	sat	Sulphate adenylyltransferase	No

Table S9. The sulphur cycling genes in sulphur reduction pathway.

Pathways	Gene	Annotation	Detected
Sulphur reduction	*asrABC*	Anaerobic sulphite reductase	Yes
	fsr	Sulphite reductase (coenzyme F420)	No
	hydABDG	Sulphhydrogenase	No
	mccA	Dissimilatory sulphite reductase	No
	otr	Octaheme tetrathionate reductase Otr	No
	psrABC	Polysulphide reductase	No
	rdlA	Putative rhodanese-like protein	No
	shyABCD	Sulphhydrogenase 2	No
	sreABC	Sulphur reductase	No
	sudAB	Sulphide dehydrogenase	No
	ttrABC	Tetrathionate reductase	Yes

Table S10. The sulphur cycling genes in sox system pathway.

Pathways	Gene	Annotation	Detected
sox system	*soxAX*	l-cysteine *S*-thiosulphotransferase	No
	soxB	*S*-sulfosulfanyl-l-cysteine sulphohydrolase	No
	soxC	Sulphite dehydrogenase	Yes
	soxD	Sulphite dehydrogenase	Yes
	soxYZ	Sulphur-oxidizing protein SoxYZ	No

Table S11. The sulphur cycling genes in sulphur oxidation pathway.

Pathways	Gene	Annotation	Detected
Sulphur oxidation	*doxAD*	Thiosulphate dehydrogenase [quinone]	No
	fccA	Sulphide dehydrogenase	Yes
	glpE	Thiosulphate sulfurtransferase	No
	soeABC	Sulphite dehydrogenase	No
	sorAB	Sulphite cytochrome c oxidoreductase	No
	sqr	Sulphide:quinone oxidoreductase	No
	sseA	Thiosulphate sulfurtransferase	No
	tsdAB	Thiosulphate dehydrogenase	Yes

Table S12. The sulphur cycling genes in sulphur disproportionation pathway.

Pathways	Gene	Annotation	Detected
Sulphur disproportionation	*phsABC*	Thiosulphate reductase	No
	tetH	Tetrathionate hydrolase TetH	No
	sor	Sulphur oxygenase/reductase	No

Table S13. The sulphur cycling genes in organic sulphur transformation pathway.

Pathways	Gene	Annotation	Detected
Organic sulphur transformation	*acuI*	Acrylyl-CoA reductase AcuI	No
	acuNK	Acrylyl-CoA transferase and hydratase	No
	betAB	Betaine biosynthesis protein	Yes
	betC	Choline-sulphatase	No
	comABCDE	Coenzyme M biosynthesis protein	Yes
	dddAC	3-Hydroxypropionate dehydrogenase	No
	dddDKLPQWY	Dimethylsulphonioproprionate lyase	No
	dddT	Betaine/carnitine/choline transporter	No
	ddhABC	Dimethylsulphide dehydrogenase	No
	dmdABCD	Dimethylsulphoniopropionate demethylation protein	No
	dmoA	Dimethyl-sulphide monooxygenase	No
	dmsABC	Anaerobic dimethyl sulphoxide reductase	Yes
	dsyB	DsyB	No
	gdh	Glutamate dehydrogenase (NADP+)	No
	hpsN	Sulphopropanediol 3-dehydrogenase	No
	hpsOP	*R* or *S*-dihydroxypropanesulphonate−2-dehydrogenase	No
	iseJ	Isethionate dehydrogenase	No
	isfD	Sulphoacetaldehyde reductase	No
	mddA	Methanethiol *S*-methyltransferase	No
	mdh	Malate dehydrogenase	No
	mtsAB	Methylthiol:coenzyme M methyltransferase	No
	prpE	Propionate–CoA ligase	No
	pta	Phosphate acetyltransferase	Yes
	sfnG	Dimethylsulphone monooxygenase	No
	slcCD	Sulpholactate dehydrogenase	Yes
	sqdBDX	Sulpholipid sulphoquinovosyl diacylglycerol biosynthesis protein	No
	tauXY	Taurine dehydrogenase	No
	tmm	Trimethylamine monooxygenase	No
	toa	Taurine:2-oxoglutarate transaminase	No
	tpa	Taurine-pyruvate aminotransferase	No
	yihQ	Sulphoquinovosidase	No

Table S14. The sulphur cycling genes in linkages between inorganic and organic sulphur transformation pathway.

Pathways	Gene	Annotation	Detected
Linkages between inorganic and organic sulphur transformation	*cuyA*	l-cysteate sulpho-lyase	No
	cysEKMO	Cysteine biosynthesis protein	Yes
	hdrABCDE	Heterodisulphide reductase	No
	mccB	Cystathionine gamma-lyase/homocysteine desulphydrase	Yes
	metABCXYZ	l-Cystathionine biosynthesis protein	Yes
	msmAB	Methanesulphonate monooxygenase	No
	mtoX	Methanethiol oxidase	No
	ssuDE	Alkanesulphonate monooxygenase	No
	suyAB	(2*R*)-sulpholactate sulpho-lyase	No
	tauD	Taurine dioxygenase	No
	tbuBC	Toluene−3-monooxygenase	No
	tmoCF	Toluene−4-monooxygenase	No
	touCF	Toluene *o*-xylene monooxygenase	No
	xsc	Sulphoacetaldehyde acetyltransferase	No

Table S15. The sulphur cycling genes in other pathway.

Pathways	Gene	Annotation	Detected
Others	*cuyZ*	Sulphite exporter	No
	cysAPUWZ	Sulphate/thiosulphate transporter	Yes
	hpsKLM	Dihydroxypropanesulphonate transporter	No
	iseKLM	Isethionate TRAP transporter	No
	sbp	Sulphate-binding protein	Yes
	sgpABC	Sulphur globule protein	Yes
	soxL	Sulphur transferase, periplasm	No
	ssuABC	Sulphonate transport system	Yes
	sulP	Sulphate permease	Yes
	tauABC	Taurine transport system	Yes
	tauE	Sulphite/organosulphonate exporter	No
	tauZ	Membrane protein TauZ	No
	tusA	Sulphur carrier protein TusA	No
	tusBCDE	tRNA 2-thiouridine synthesizing protein	No

Table S16. The MAGs, taxonomy and the corresponding functions.

Bin	Taxonomy	Functional contigs	Function
bin138	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; Azospirillum	k141_1486403_length_20968	ID=GKMFNEBJ_02733;eC_number=2.3.3.15; gene=xsc; product=Sulfoacetaldehyde acetyltransferase
		k141_1486403_length_20968	ID=GKMFNEBJ_02737;eC_number=1.8.5.5; gene=phsA; product=Thiosulfate reductase molybdopterin-containing subunit PhsA
		k141_1486403_length_20968	ID=GKMFNEBJ_02740;eC_number=1.2.1.81; gene=sauS; product=Sulfoacetaldehyde dehydrogenase (acylating)
		k141_162616_length_18321	ID=GKMFNEBJ_03204;eC_number=1.8.7.1; gene=sir; product=Sulfite reductase [ferredoxin]
		k141_162616_length_18321	ID=GKMFNEBJ_03208;eC_number=1.8.4.8; gene=cysH; product=putative phosphoadenosine phosphosulfate reductase
		k141_162616_length_18321	ID=GKMFNEBJ_03209;eC_number=2.7.7.4; gene=cysD; product=Sulfate adenylyltransferase subunit 2
		k141_162616_length_18321	ID=GKMFNEBJ_03211;gene=ssuA_1; product=Putative aliphatic sulfonates-binding protein
		k141_162616_length_18321	ID=GKMFNEBJ_03212;gene=ssuC_4; product=Putative aliphatic sulfonates transport permease protein SsuC
bin141	Bacteria; Proteobacteria; Alphaproteobacteria	k141_1443419_length_31861	ID=HDGPBJIB_02456;eC_number=2.7.7.4; gene=cysD; product=Sulfate adenylyltransferase subunit 2
		k141_1443419_length_31861	ID=HDGPBJIB_02457;eC_number=1.8.4.8; gene=cysH; product=putative phosphoadenosine phosphosulfate reductase
		k141_1443419_length_31861	ID=HDGPBJIB_02459;eC_number=1.8.7.1; gene=sir; product=Sulfite reductase [ferredoxin]
		k141_1443419_length_31861	ID=HDGPBJIB_02462;eC_number=1.8.5; gene=msrP_2;product=Protein-methionine-sulfoxide reductase catalytic subunit MsrP
		k141_2885297_length_29662	ID=HDGPBJIB_02663;gene=sbp; product=Sulfate-binding protein
		k141_2885297_length_29662	ID=HDGPBJIB_02664;gene=cysT; product=Sulfate transport system permease protein CysT
		k141_2885297_length_29662	ID=HDGPBJIB_02665;gene=cysW; product=Sulfate transport system permease protein CysW
		k141_2885297_length_29662	ID=HDGPBJIB_02666;eC_number=3.6.3.25; gene=cysA_3;product=Sulfate/thiosulfate import ATP-binding protein CysA
bin153	Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; Azospirillum; Azospirillum thiophilum	k141_331820_length_18177	ID=BDPCBGIO_02031;eC_number=1.8.7.1; gene=sir; product=Sulfite reductase [ferredoxin]
		k141_331820_length_18177	ID=BDPCBGIO_02034;eC_number=1.8.4.8; gene=cysH; product=putative phosphoadenosine phosphosulfate reductase
		k141_331820_length_18177	ID=BDPCBGIO_02035;eC_number=2.7.7.4; gene=cysD; product=Sulfate adenylyltransferase subunit 2
		k141_3615490_length_17077	ID=BDPCBGIO_02237; gene=sbp; product=Sulfate-binding protein
		k141_3615490_length_17077	ID=BDPCBGIO_02238; gene=cysT; product=Sulfate transport system permease protein CysT
		k141_3615490_length_17077	ID=BDPCBGIO_02239;gene=cysW_1; product=Sulfate transport system permease protein CysW
		k141_3615490_length_17077	ID=BDPCBGIO_02240;eC_number=3.6.3.25; gene=cysA_1;product=Sulfate/thiosulfate import ATP-binding protein CysA
		k141_2095138_length_14031	ID=BDPCBGIO_02923;eC_number=3.6.3.-; gene=ssuB_2;product=Aliphatic sulfonates import ATP-binding protein SsuB
		k141_2095138_length_14031	ID=BDPCBGIO_02933; gene=fccA; product=Cytochrome subunit of sulfide dehydrogenase
		k141_2095138_length_14031	ID=BDPCBGIO_02934;eC_number=1.8.2.3; gene=fccB; product=Sulfide dehydrogenase [flavocytochrome c] flavoprotein chain
		k141_2095138_length_14031	ID=BDPCBGIO_02937;eC_number=2.5.1.47; gene=cysK1_2; product=O-acetylserine sulfhydrylase

(Continued)

Bin	Taxonomy	Functional contigs	Function
bin159	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales	k141_186678_length_153681	ID=FJIPPPMI_01371;eC_number=1.8.4.-; gene=yghU_1; product=Disulfide-bond oxidoreductase YghU
		k141_186678_length_153681	ID=FJIPPPMI_01372;eC_number=1.8.5.3; gene=dmsA_1; product=Dimethyl sulfoxide reductase DmsA
		k141_186678_length_153681	ID=FJIPPPMI_01374;eC_number=3.6.3.25; gene=cysA_3; product=Sulfate/thiosulfate import ATP-binding protein CysA
		k141_186678_length_153681	ID=FJIPPPMI_01375;gene=cysW; product=Sulfate transport system permease protein CysW
bin213	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales	k141_5168135_length_40468	ID=PCBNDNOG_01886;gene=sbp_1; product=Sulfate-binding protein
		k141_5168135_length_40468	ID=PCBNDNOG_01893;gene=sbp_2; product=Sulfate-binding protein
		k141_5168135_length_40468	ID=PCBNDNOG_01894;gene=cysT; product=Sulfate transport system permease protein CysT
		k141_5168135_length_40468	ID=PCBNDNOG_01895;gene=cysW; product=Sulfate transport system permease protein CysW
		k141_5168135_length_40468	ID=PCBNDNOG_01896;eC_number=3.6.3.25; gene=cysA; product=Sulfate/thiosulfate import ATP-binding protein CysA
		k141_4445261_length_23863	ID=PCBNDNOG_02981;eC_number=2.7.7.4; gene=cysD; product=Sulfate adenylyltransferase subunit 2
		k141_4445261_length_23863	ID=PCBNDNOG_02982;eC_number=1.8.4.10; gene=cysH; product=Thioredoxin-dependent 5'-adenylylsulfate reductase
		k141_4445261_length_23863	ID=PCBNDNOG_02984;eC_number=1.8.7.1; gene=sir; product=Sulfite reductase [ferredoxin]
bin249	Bacteria; Proteobacteria; Gammaproteobacteria;Pseudomonadales; Pseudomonadaceae; Pseudomonas	k141_1454792_length_10891	ID=JDAMOIHB_04599;gene=sbp_1; product=Sulfate-binding protein
		k141_1454792_length_10891	ID=JDAMOIHB_04600;gene=cysT; product=Sulfate transport system permease protein CysT
		k141_1454792_length_10891	ID=JDAMOIHB_04601;gene=cysW_5; product=Sulfate transport system permease protein CysW
		k141_1454792_length_10891	ID=JDAMOIHB_04602;eC_number=3.6.3.25;gene=cysA_2; product=Sulfate/thiosulfate import ATP-binding protein CysA

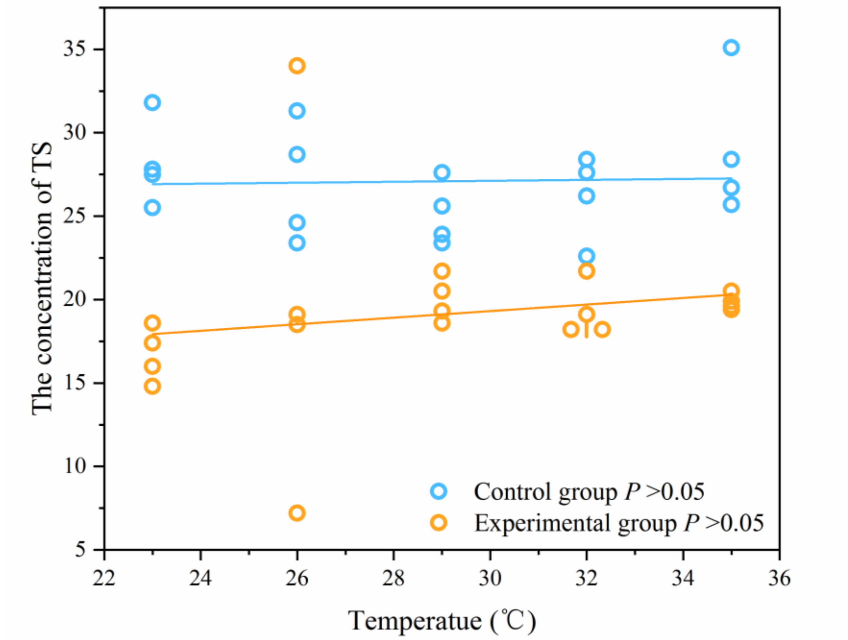

Figure S1. Line chart revealed the change trend of sulphur concentration in all water samples. Abbreviations: TS, total sulphur.

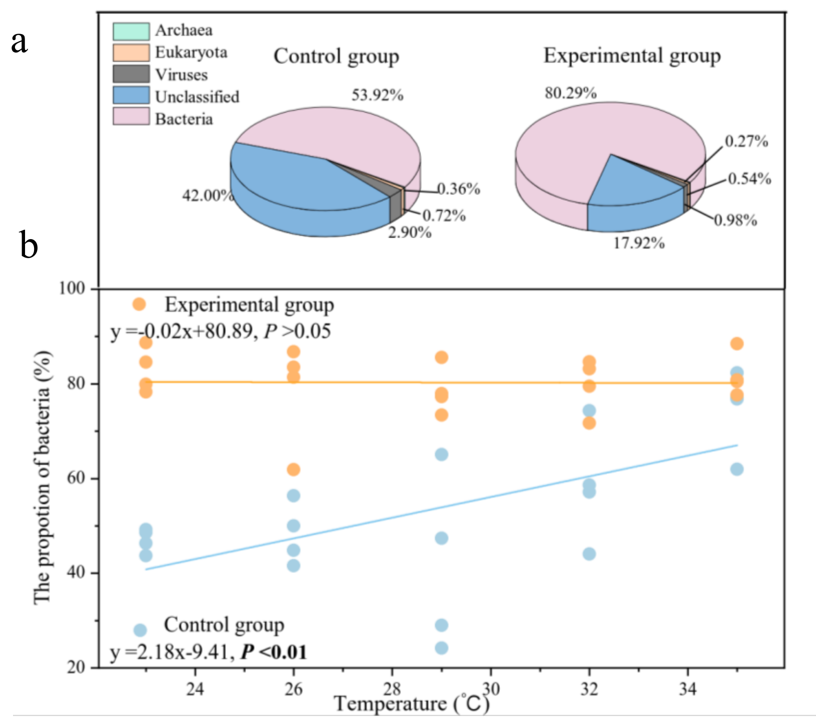

Figure S2. The pie chart (a) showed the composition of sulphur cycling microorganisms in control and experimental groups at the domain level. The linear fitting (b) showed the correlation between temperature and the proportion of bacteria.

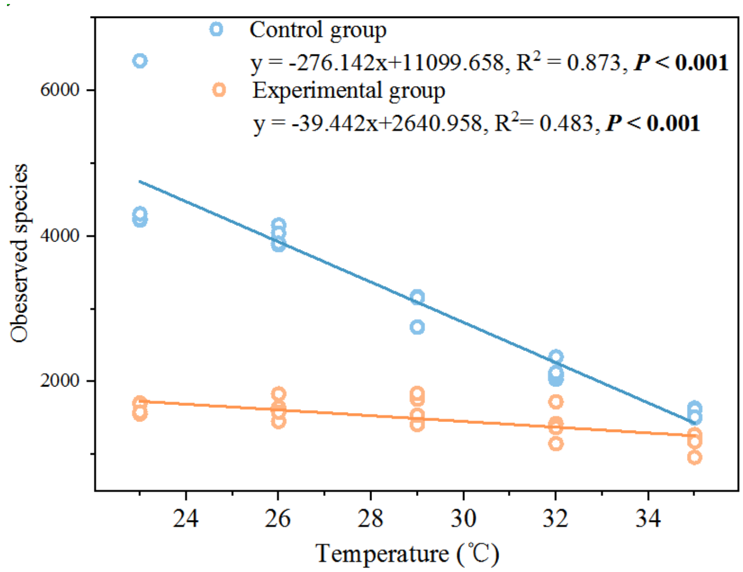

Figure S3. The linear fitting showed the correlation between temperature and alpha diversity (observed species) of sulphur cycling microbial communities.

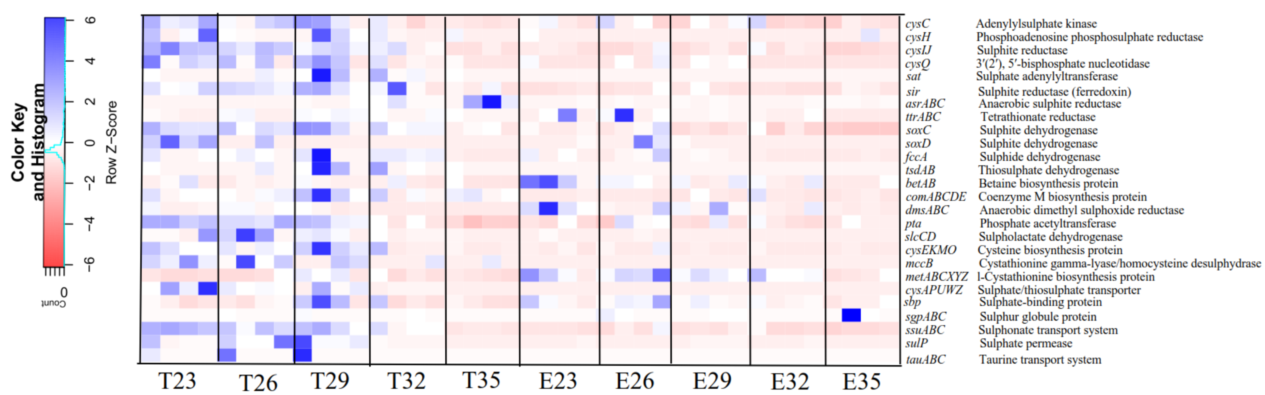

Figure S4. The heatmap of sulphur cycling genes detected in this research in all samples. The functions of these sulphur cycling genes were listed after the gene name. Abbreviations: T, control groups; E, experimental groups.

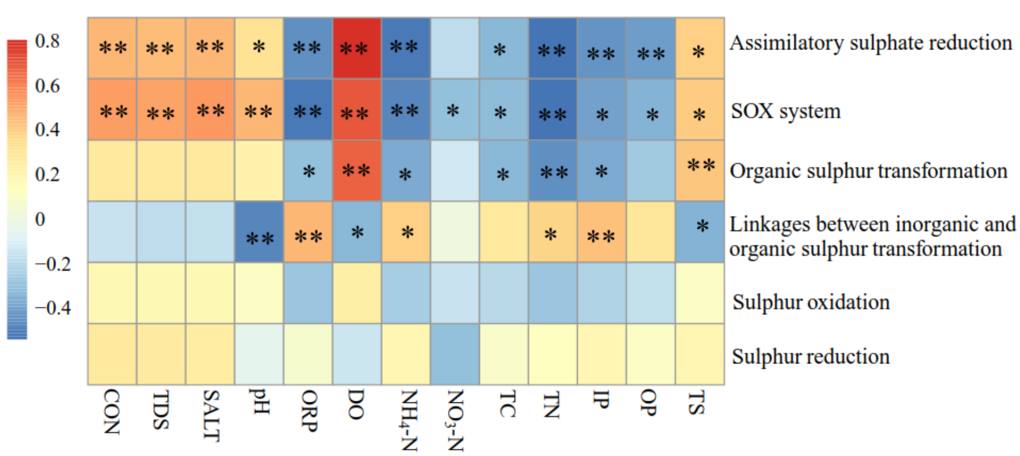

Figure S5. The heatmap of correlation between the sulphur cycling pathways and physicochemical properties. Abbreviations: NO_3-N, nitrate nitrogen; NH_4-N, ammonium nitrogen; TC, total carbon, TN, total nitrogen; IP, inorganic phosphorus; OP, organic phosphor; TS, total sulphur; CON, conductivity; TDS, total dissolved solids; SALT, salinity; ORP, oxidation-reduction potential; DO, dissolved oxygen. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

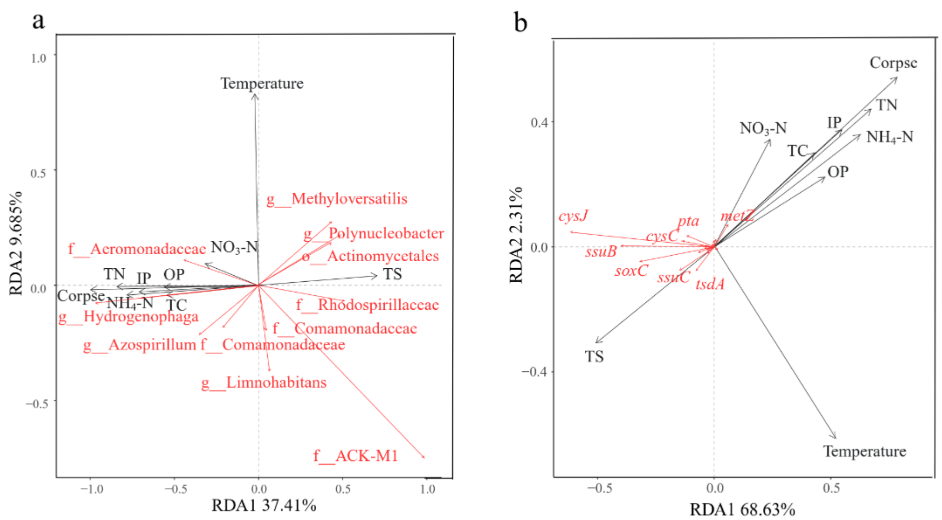

Figure S6. The RDA of sulphur cycling microbes (a) and genes (b). Abbreviations: NO_3-N, nitrate nitrogen; NH_4-N, ammonium nitrogen; TC, total carbon, TN, total nitrogen; IP, inorganic phosphorus; OP, organic phosphor; TS, total sulphur

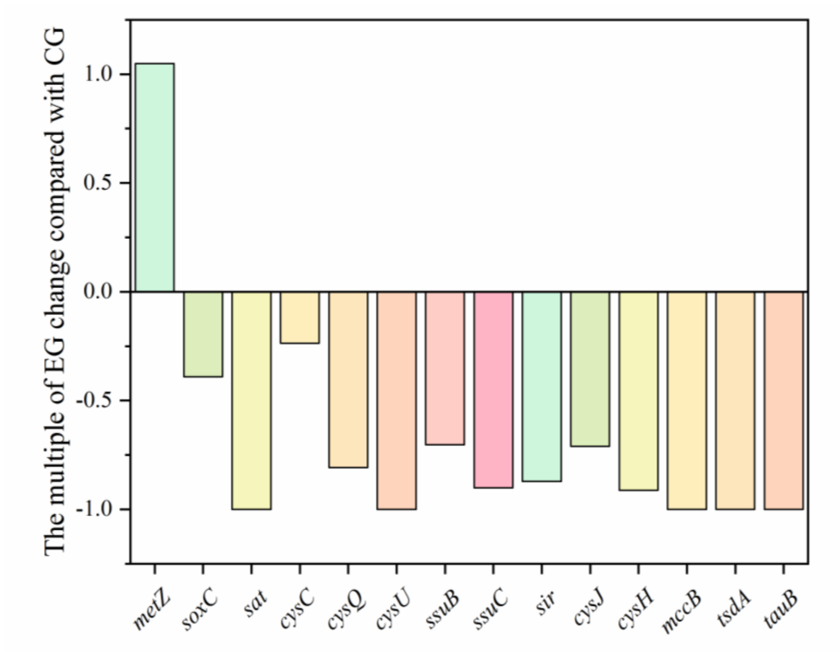

Figure S7. The multiple of the sulphur cycling genes in EG compared with CG in Fig. 7. Only the genes with significant differences were included. The calculation formula was as follows: average TPM abundance of experimental groups/ average TPM abundance of control groups -1. Abbreviations: CG, control group; EG, experimental group.

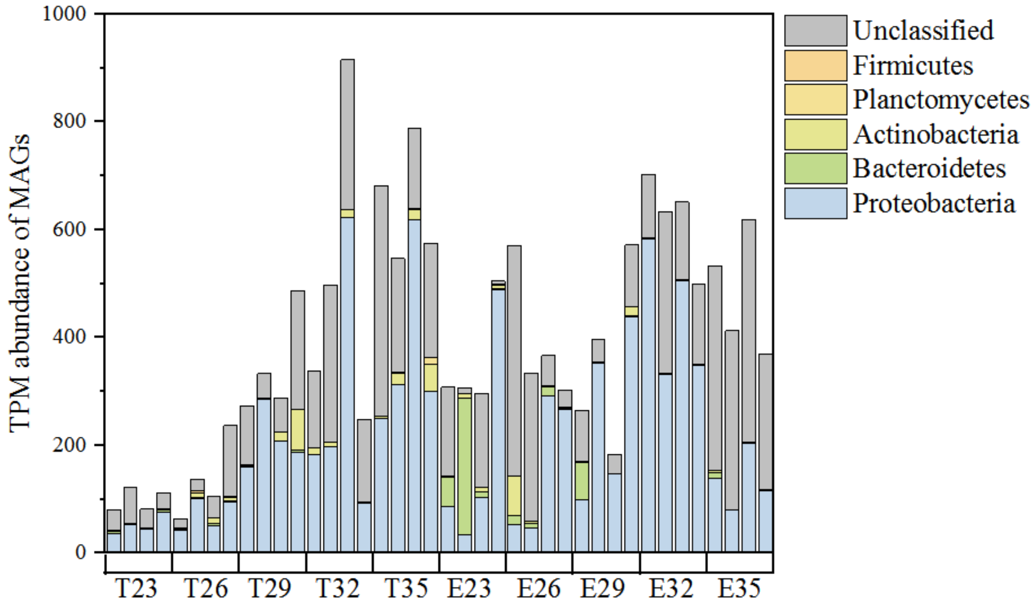

Figure S8. The TPM abundance of the phyla of MAGs in CG and EG. Abbreviations: CG, control group; EG, experimental group.

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