# Diverse Biotransformation of Sulfur in Antarctic Lake Sediments

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# Diverse biotransformation of sulfur in Antarctic lake sediments

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## Abstract

Microbial communities, sulfur isotope of sulfides ($\delta^{34}S_{AVS}$ and $\delta^{34}S_{CRS}$) and sulfur and oxygen isotopes of sulfate ($\delta^{34}S_{SO4}$ and $\delta^{18}O_{SO4}$) in sediments were analyzed to study the biotransformation of sulfur in a penguin-affected lake Y2 and a pristine YO from Fildes Peninsula, Antarctic Peninsula. The microbial communities in Y2 were mainly associated with penguin activities, while those in YO were limited by nutrients. The much enriched $\delta^{34}S_{SO4}$ recorded at depth of 30, 41 and 52 cm in Y2 indicates very strong sulfate reduction therein. The sulfur-degrading bacteria *Pseudomonas* in 0–23 cm of Y2 was 3.5 times as abundant as that of sulfur oxidizing bacteria (SOB), indicating remarkable remineralization of organic sulfur. While abundant SOB and $^{34}S$-depleted sulfate indicate considerable sulfur oxidation in 34–56 cm layer in Y2. In YO sediments, the highest abundance of *Desulfotalea* and the most enriched $\delta^{34}S_{SO4}$ (35.2‰) and $\delta^{34}S_{CRS}$ (2.5‰) indicate strongest sulfate reduction in 28 cm layer. High abundance of *Pseudomonas* indicates active remineralization of organic sulfur in 3–5 cm layer in YO. While the medium $\delta^{34}S_{SO4}$ and considerable abundance of SOB and SRB indicate concurrence of sulfur oxidation and sulfate reduction in other layers in YO. Our results show that high level of organic matter inputs from penguin populations support the diverse microbial community and biotransformation of sulfur in freshwater ecosystems in Antarctica.

**Keywords**

Microbial community · Sulfur and oxygen isotope · Dissimilatory sulfate reduction · Sulfur oxidation · Sulfate-reducing bacteria

**Declarations**

Not applicable

**Conflicts of interest**

The authors declare no conflict of interests.

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**Authors' contributions**

**Shen Lili:** Data curation, Investigation, Writing-Original draft preparation, Formal analysis. **Huang Tao:** Conceptualization, Investigation, Writing-Reviewing and Editing, Resources, Funding acquisition, Supervision. **Chen Yuanqing:** Investigation, Formal analysis. **Chu Zhuding:** Formal analysis. **Xie Zhouqing:** Formal analysis.

## 1 Introduction

Sulfur is one of the important nutrient elements, and its biogeochemical transformation and recycling play an important role in maintaining the function of aquatic ecosystems (Orem et al. 2015; Poulin et al. 2017; Chen et al. 2020a). For example, sulfur biogeochemistry is coupled closely with the remineralization of organic matter, acidification of water bodies, formation of biogenic pyrite and control of trace metal bioavailability in aquatic environment (Pester et al. 2012; Sheng et al. 2013; Van de Velde et al. 2017; Liu et al. 2017; Jørgensen et al. 2019; Chen et al. 2020b).

A variety of sulfur species occur in aquatic sediments, including sulfide, elemental sulfur, sulfate and organic sulfur compounds. Transformations among these species are controlled by the microbial activity, redox condition, organic matter, and pH (Norman et al. 2002; Sánchez-Andrea et al. 2014; Chen et al. 2020a). Organic sulfur compounds can be degraded to sulfate by sulfur-degrading bacteria (Couture et al. 2016). Sulfate-reducing bacteria (SRB) reduce sulfate to sulfide under anoxic and anaerobic conditions (Luther et al. 2003; Werne et al. 2008; Raven et al. 2018; Fakhraee et al. 2019); Correspondingly, some sulfides could also be oxidized to sulfate and intermediate species of elemental sulfur, thiosulfate, sulfite and pyrite by chemical oxidant and/or sulfur oxidizing bacteria (SOB) (Jørgensen et al. 2019).

The composition of sulfur isotopes is a useful tool to trace sulfur biogeochemical processes in sediments, since different isotope fractionations were observed during these transformations (Jørgensen et al. 2019). The remineralization of organic sulfur could result in sulfur isotope fractionation effects on the order of 10‰–30‰ (Norman et al. 2002; Amrani, 2014; Shawar et al. 2018). Dissimilatory sulfate reduction (DSR) in sediments drove by anaerobic microorganisms is the main process imparting sulfur isotope fractionation (Canfield 2001). The magnitudes of sulfur isotopic fractionation produced by DSR vary between 0 and 70‰ (Canfield and Thamdrup 1994; Canfield 2001; Wortmann et al. 2001; Sim et al. 2011; Halevy et al. 2012; Kunzmann et al.

2017). While the assimilatory sulfate reduction produces very small sulfur isotope fractionation of 1–3‰ (Sela-Adler et al. 2016). Most of the sulfides are ultimately reoxidized back to sulfate with only negligible fractionations (Zerkle et al. 2009; Balci et al. 2012).

The oxygen isotope compositions of sulfate ($\delta^{18}O$) in sediments could also provide information on sulfur biogeochemistry. The intermediate sulfur species generated from the DSR inherit enriched $\delta^{18}O$ in the residual sulfate (Brunner and Bernasconi 2005). During the oxidation of sulfides, the formed sulfate inherits the depleted $^{18}O$ from water in the cytoplasm (Poser et al. 2014). Dual stable sulfur and oxygen isotopes of sulfate in natural environments therefore have been used increasingly to study the net rate and pathway of DSR (Antler et al. 2013; Feng et al. 2016).

Studies of sulfur geochemistry in Antarctic freshwater ecosystems are very limited. The sulfur isotope value of sulfate in the bottom water of the Ace Lake in Vestfold Hills, East Antarctica was as high as 67‰ and associated with sulfate reduction by microorganisms (Burton and Barker 1979). Subsequent studies analyzed the microbial composition and metabolic function in waters of the adjacent Organic Lake, discussed the coupling relationship between carbon and sulfur transformations, and indicated how the microbial communities adapt to the specific Antarctic environment (Ng et al. 2010; Yau et al. 2013). The microbial compositions of SRB and SOB in the sediments of Subglacial Lake Whillans in Antarctica were also determined to study the sulfur transformations (Purcell et al. 2014). In the extremely dry and cold McMurdo valleys, geochemical and molecular microbial community analyses were performed to investigate the anaerobic oxidation of methane and associated sulfate reduction in Lake Fryxell (Karr et al. 2005; Sattley et al. 2006; Saxton et al. 2016).

In Polar area, as well as globally, seabirds transport and focus large amounts of nutrients and pollutants in the form of guano from ocean to lacustrine ecosystems (Sun et al. 2000, 2013; Blais et al. 2005; Michelutti et al. 2008; Emslie et al. 2014). In the ground-breaking study of Sun et al. (2000), nine bio-elements in the

ornithogenic sediments from Y2 lake were identified and used to reconstruct the penguin population change in the past 3000 years at Ardley Island, West Antarctic Peninsula. High level of organic matter and nutrients including nitrogen, phosphorus and sulfur in the ornithogenic waste products provide abundant nutrients that are often promoting to the growth of microorganisms (Li et al. 2006). The bacterial richness and diversity in Y2 lake is strongly associated with historical penguin activity (Zhu et al. 2015). In our previous study, we analyzed the sulfur species and its vertical distribution in a penguin-affected sediment core Y2 and a pristine sediment core YO from Ardley Island and Fildes Peninsula and discussed the indicated reduction and transformation of sulfate in those sediments (Chen et al. 2020a). The specific microbial and geochemical transformations of sulfur, however, remain unclear. Therefore in this study, based on the sulfur species from Chen et al. (2020a), we analyze the compositions of sulfur and oxygen isotope for sulfate/sulfides and the microbial community in these distinct lake sediments to exposit the microbial and geochemical transformations of sulfur.

## 2 Materials and methods

### 2.1 Study area and sample collection

Ardley Island is a special ecological reserve designated by the Scientific Committee on Antarctic Research (SCAR), which is connected to Fildes Peninsula with a sandbar. The island covers an area of about 2 $km^2$ with a flat and stable terrain, where lichens and mosses populated therein. Ardley Island is famous for the breeding colonies of penguin populations, with those occupied the eastern part of the island nowadays and the western part in the past (Roberts et al. 2017; Yang et al. 2019). During the summer breeding period, abundant penguin guano washes into nearby lakes and ponds and leaves distinct ornithogenic signatures in the sediments (Sun et al. 2000). In contrast, the lakes and ponds on Fildes Peninsula, including our study site YO, are relatively pristine and not affected by penguin activity (Chu et al. 2019). The YO lake covers an area of about 9,000 $m^2$ and locates about 200 m from the nearest coastline of the Great Wall Bay. Sediment cores Y2 (60 cm) and YO

(30 cm) were collected in austral summer 2012/2013 during the 29th Chinese Antarctic Expedition. In laboratory, Y2 and YO were sectioned at 1 cm intervals and obtained 60 and 30 subsamples, respectively. The penguin-affected Y2 core discharges a strong and unpleasant smell of guano, especially in the bottom layer; while the pristine YO core was characterized by a dominance of greyish deposits.

### 2.2 Sample pretreatment

Sub-sediments of Y2 and YO analyzed in the present study were parallel samples to those reported in Chen et al (2020a). Before chemical analyses and DNA extraction, one part of each subsample was stored at -20°C, and the other part was centrifugalized to extract the pore water prior to freeze-dried and powdered. The final powder was passed through a 200-mesh sieve, placed in a drying apparatus and then prepared to barite for stable sulfur and oxygen isotope analyses.

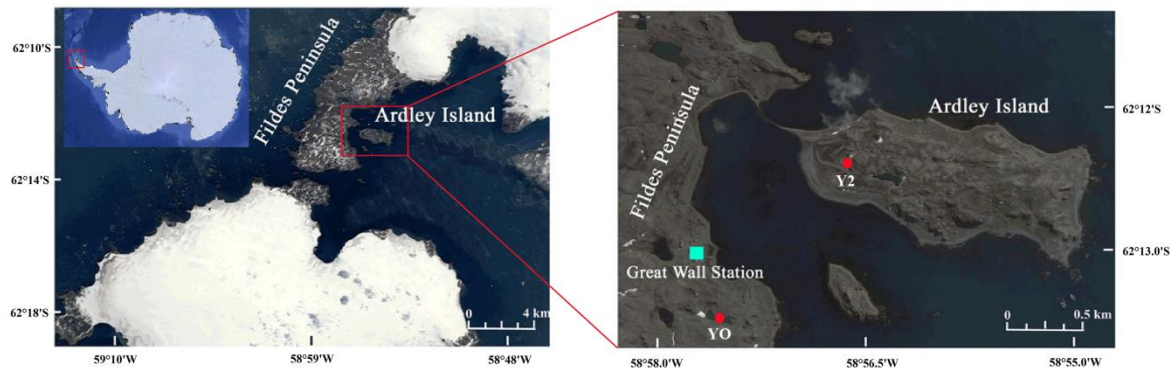



**Fig.1** Study area and sampling sites on Fildes Peninsula and Ardley Island

### 2.3 Analyses of microbial community compositions

**Sediment DNA extraction:** DNA was extracted from the sediments by SDS high salt method (Zhou et al. 1996). Sediment samples of 0.5 g were mixed with 600 μL of DNA extraction buffer (1 mol/L Tris-HCl, 0.5 mol/L EDTA, 0.5 mol/L phosphate, 3 mol/L NaCl, 1% CTAB） and 50 μL of proteinase K (20 mg/mL) in Oakridge tubes by horizontal shaking at 225rpm for 30 min at 37°C. After the shaking treatment, 600 μL 20% SDS was added, and the samples were incubated in a 65°C water bath for 30 min. The supernatants were

129 collected after centrifugation at 8000 rpm for 10 min at room temperature and transferred into 2 mL centrifuge
130 tubes. Supernatants from extractions were combined and mixed with an equal volume of
131 Phenol-chloroform-isoamyl alcohol (25:24:1). The aqueous phase was recovered by centrifugation and
132 precipitated with PEG-NaCl at 4°C for 2h. The pellet of crude nucleic acids was obtained by centrifugation at
133 14,000 rpm for 10 min at 4°C, washed with 75% ethanol, add 100 μL Tris-EDTA buffer and store at -20°C.

134 **PCR amplification and 16S rDNA sequencing:** The V3-V4 region of the bacterial 16S rRNA gene was
135 amplified using the 341F (5'-CCTACGGGNGGCWGCAG-3') /805R (5'-GACTACHVGGGTATCTAATCC-3')
136 primers. PCR reaction was done for each sample under the following conditions: 98°C for 30s; 35 cycles of
137 denaturation at 98°C for 10 s, annealing at 54°C or 52°C for 30s, and extension at 72°C for 45s; followed by a
138 final extension at 72°C for 10 min. The PCR products were collected and purified using the Agarose Gel DNA
139 purification kit (TaKaRa Bio Inc., Shiga, Japan), and then sequencing was conducted using the Illumina MiSeq
140 PE300 Sequencer (Illumina, Inc., CA, USA) at LC-Bio Technologies (Hangzhou, P. R. China) Co., Ltd.

141 **Data processing:** Paired-end reads were merged using the FLASH program. Chimeric sequences were
142 filtered using Vsearch software (v2.3.4). Sequences with ⩾97% similarity were assigned to the same
143 operational taxonomic units (OTUs) using Vsearch. Representative sequences were selected for each OTU, and
144 taxonomic data were then assigned to each representative sequence using the Ribosomal Database Project
145 (RDP) classifier. OTU abundance information was normalized using the sequence number of the sample with
146 the fewest sequences as a standard.

147 **2.4 Sulfur and oxygen isotope analyses**

148 Geochemical analysis was not performed for pore water due to the low content. The sulfates in Y2 and YO
149 sediments were extracted by solution of $NaH_2PO_4$ (pH=6, 0.016 mol/L), collected through centrifugation and
150 filtration, purified by dissolution and reprecipitation in a chelating solution of DTPA (Bao 2006), and prepared

151 to barite by adding saturated $BaCl_2$ solution. The collected $BaSO_4$ was washed repeatedly by Milli-Q water and
152 then heated in an oven at 105℃. The dried $BaSO_4$ was powdered and placed into a 2-mL centrifuge tube in
153 drying apparatus.

154 Since the main sulfides in Y2 and YO sediments are AVS and CRS, respectively (Chen et al. 2020a), and
155 the precipitation of sulfides is associated with only negligible fractionations (Böttcher et al. 1998). The
156 precipitation of AVS and CRS in Y2 and YO for stable sulfur isotope analyses was prepared according to
157 Habicht and Canfield (1997). The detail steps were as follows: AVS in Y2 and CRS in YO were extracted into
158 ZnS; the ZnS was rinsed sequentially by NaOH (2 mol/L, 15 mL) and weakly alkaline water (pH=8.0, 15 mL);
159 then the ZnS was converted to $Ag_2S$ by adding $AgNO_3$ solution (0.1 mol/L, 10 mL); finally, the precipitated
160 $Ag_2S$ was separated by centrifugation, washed twice by 15 mL distilled water, dried in an oven at 105℃ and
161 placed in a 2 mL centrifuge tube in a drying dish.

162 Stable sulfur and oxygen isotope analyses of barite and sulfides were performed in the State Key
163 Laboratory of Ore Deposit Geochemistry. Weighed samples of barite as well as $V_2O_5$ (1:3) and $Ag_2S$,
164 respectively, were compacted into tin cups for sulfur isotope analysis; while the weighed $BaSO_4$ was compacted
165 into silver cups for oxygen isotope analysis. Stable sulfur and oxygen isotope ratios were determined by isotope
166 ratio mass spectrometer (Thermo Fisher Delta V Advantage) coupled to an elemental analyzer (Flash 2000 for
167 sulfur and TC/EA for oxygen). The instrument precision was ±0.2‰ for $\delta^{34}S$ and 0.30‰ for $\delta^{18}O$. IAEA-SO-5
168 ($\delta^{34}S$, 0.5‰), IAEA-SO-6 (-34.1‰) and NBS-127 ($\delta^{34}S$, 20.3‰) were used as the standard samples for sulfur
169 isotope analysis of barite and IAEA S1 (−0.3‰), IAEA S2 (+22.6‰) and IAEA S3 (−32.5‰) for sulfur isotope
170 analysis of sulfides. NBS-127 ($\delta^{18}O$, 8.59‰) was used as the standard sample for the oxygen isotope analysis.
171 Stable isotope results were presented in $\delta$ (‰) and expressed relative to the VCDT for $\delta^{34}S$ and VSMOW for
172 $\delta^{18}O$ according to the equation of $\delta$ (‰) = $[(R_{sample} - R_{standard})/R_{standard}] * 1000$, where $\delta$ (‰) represents the $\delta^{34}S$

or $\delta^{18}O$, $R_{sample}$ is the isotopic ratio of the sample, and $R_{standard}$ the isotopic ratio of VCDT and VSMOW.

## 3 Results

### 3.1 Compositions of microbial community

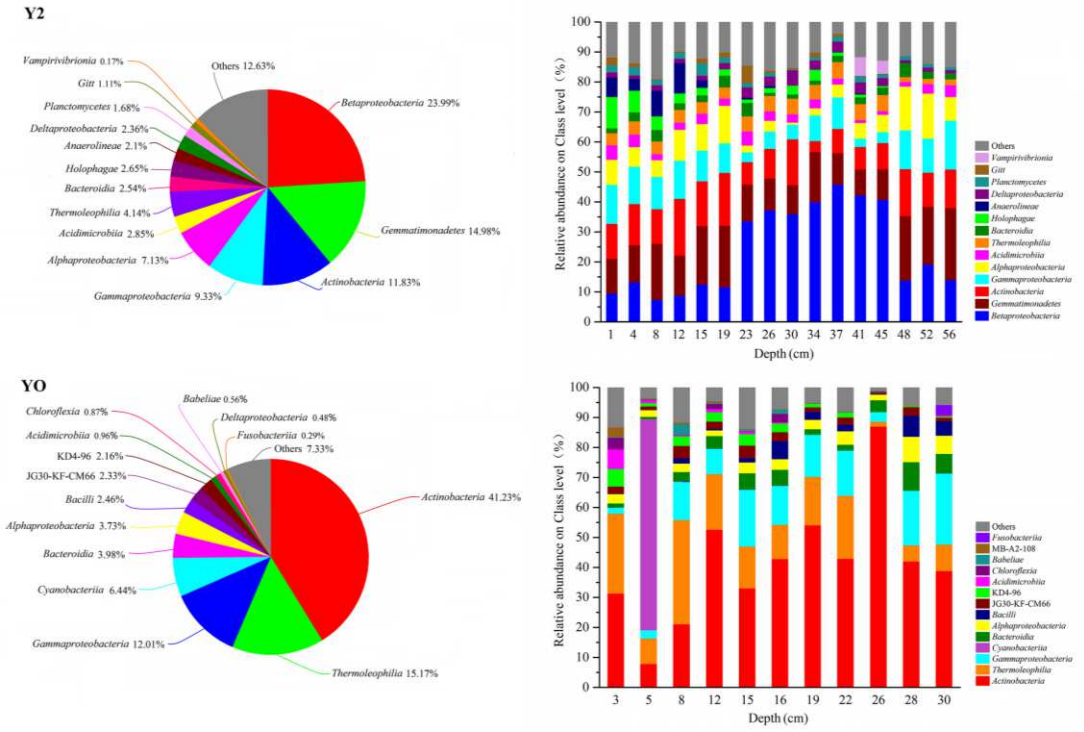



**Fig.2** Relative abundance of microorganisms at the class level in Y2 and YO sediments.

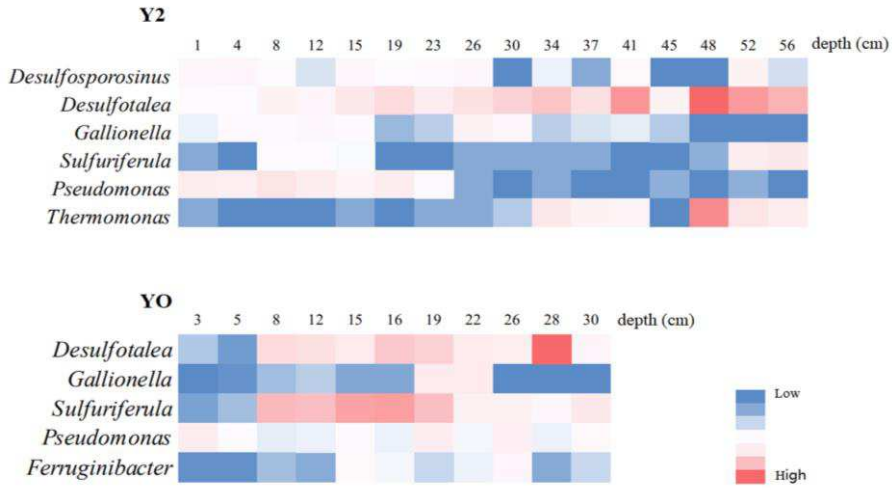



**Fig.3** Heatmap diagram of the sulfur cycle-related microbial genus in Y2 and YO sediments.

1,045,366 and 811,921 high-quality microbial sequences were obtained from Y2 and YO sediments, with a range of 43,123–81,825 and 39,185–93,981 sequences per sample. The compositions of microbial community in Y2 and YO sediments were plotted in Fig.2. At the class level, the dominant microbial groups in Y2 sediments were *Betaproteobacteria* (23.99%), *Gemmatimonadetes* (14.98%), *Actinobacteria* (11.83%), *Gammaproteobacteria* (9.33%) and *Alphaproteobacteria* (7.13%). The average abundance of each of other groups was less than 5%. The dominant bacteria in YO sediments were *Actinobacteria* (41.23%), *Thermoleophilia* (15.17%) and *Gammaproteobacteria* (12.01%).

The vertical distribution of microorganisms in Y2 and YO was plotted in Fig.2 at class level. In Y2 sediment profile, bacteria of *Gammaproteobacteria*, *Gemmatimonadetes*, *Actinobacteria* and *Alphaproteobacteria* show higher relative abundance in 0–19 cm and 48–56 cm and lower in 23–45 cm; while *Betaproteobacteria* and *Deltaproteobacteria* show an opposite vertical trend with those above. The dominant bacteria of *Actinobacteria*, *Thermoleophilia* and *Gammaproteobacteria* in YO show high relative abundance in the vertical profile except for the layer of 5 cm, where the relative abundance of *Cyanobacteriia* is as high as 70.34%.

Heatmap diagram of microbial communities at genus level in Y2 and YO sediments was plotted in Fig.3. In Y2 sediments, *Desulfotalea* was the dominant SRB genus (0.37%–13.40%), with the highest abundance at depth of 48 cm; the abundance of *Pseudomonas* in the section of 0–23 cm was higher than that of 23–56 cm; while the abundance of *Thermomonas* was very low in the up 30 cm layer in contrast to those high in 34–56 cm. Other sulfur cycle-related microbial groups in Y2 included *Desulfosporosinus* (0.48%), *Gallionella* (0.40%) and *Sulfuriferula* (0.36%). In YO sediments, *Sulfuriferula* (0.30%–6.19%) and *Desulfotalea* (0.22%–8.84%) were the primary groups of SOB and SRB, and high abundance of them was observed in 16 cm and 28 cm, respectively. Other sulfur cycle-related microbial groups in YO include *Pseudomonas* (1.63%), *Gallionella*

(0.64%) and *Ferruginibacter* (0.90%).

### 3.2 Compositions of sulfur and oxygen isotope for sulfate and sulfides

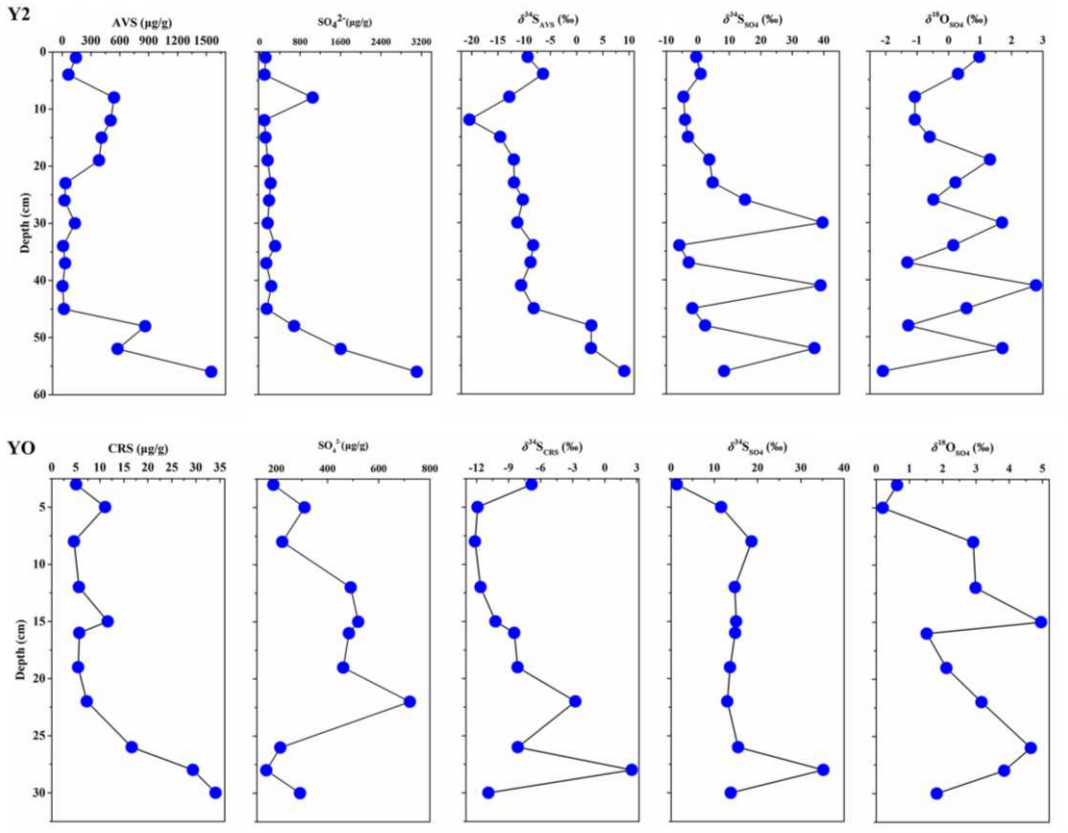



**Fig.4** Vertical distribution of sulfur species of AVS, CRS and sulfate (data from Chen et al., 2020a) and the corresponding $\delta^{34}S_{AVS}$, $\delta^{34}S_{CRS}$, $\delta^{34}S_{SO4}$ and $\delta^{18}O_{SO4}$ in Y2 and YO sediments.

The vertical distributions of AVS, CRS and sulfate and the corresponding $\delta^{34}S_{AVS}$, $\delta^{34}S_{CRS}$, $\delta^{34}S_{SO4}$ and $\delta^{18}O_{SO4}$ in Y2 and YO sediments were plotted in Fig.4. The $\delta^{34}S_{SO4}$ values in the 0–23 cm layer in Y2 sediments ranged between -4.5‰ and 4.7‰; While those below 30 cm showed a fluctuated trend, with large enrichment in 30, 41 and 52 cm, and much depletion in 34–37 and 45–48 cm layers. The $\delta^{34}S_{AVS}$ in Y2 sediments depleting from 5 cm to 12 cm and enriching between 12 cm and 56 cm, with much enriched values in 48–56 cm layer. The $\delta^{18}O_{SO4}$ values in Y2 sediments ranged from -2.09‰ to 2.78‰ with a fluctuation. In YO sediments, almost of the $\delta^{34}S_{SO4}$ ranged narrowly between 11.6‰ and 18.6‰, except the depleted values in 3–5 cm and the most

enriched in 28 cm. The $\delta^{34}S_{CRS}$ in YO sediments enriching from 5 cm to 22 cm; the most enriched value in 28 cm was similar to that of $\delta^{34}S_{SO4}$. $\delta^{18}O_{SO4}$ values in YO sediments ranged from 0.20‰ to 3.96‰ with a fluctuation.

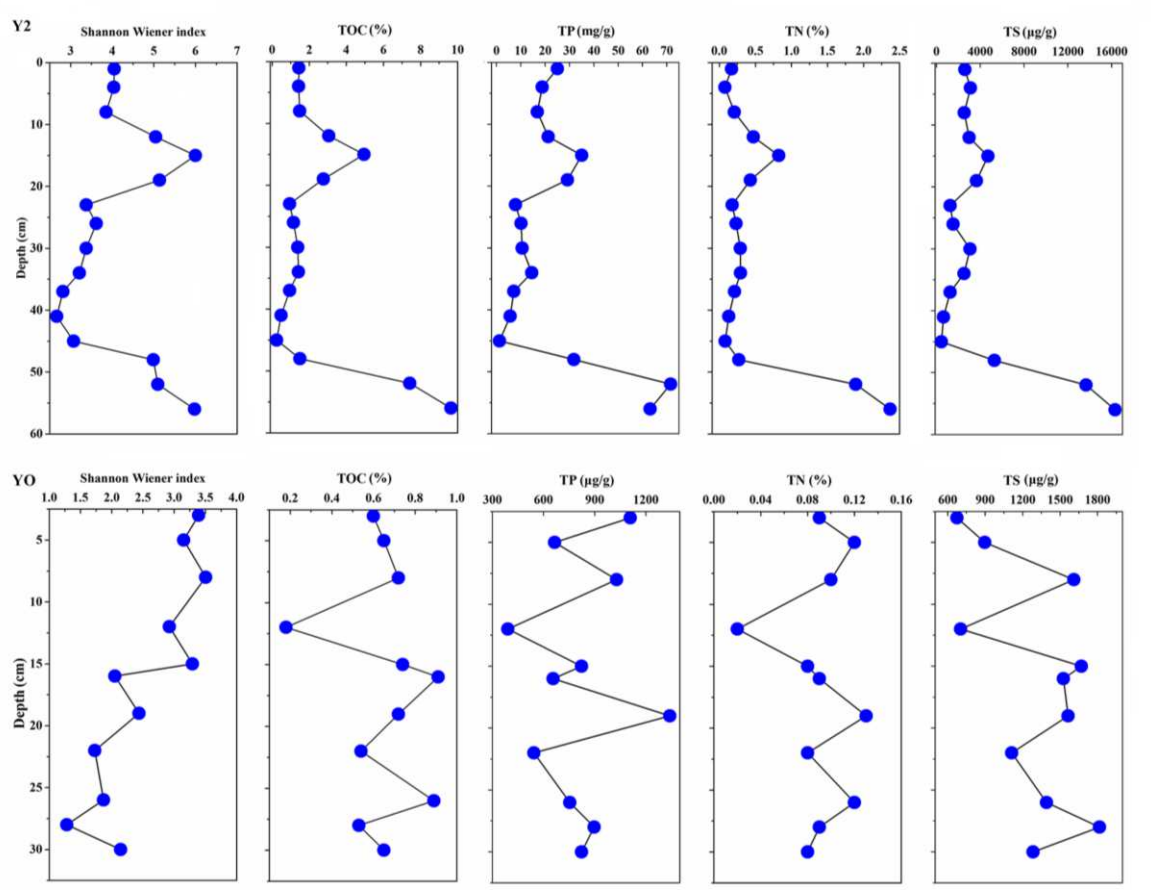
## 4 Discussion

### 4.1 Vertical distribution of microbial diversity

**Table 1** Correlations between Shannon–Wiener index and physicochemical indicators in Y2 and YO sediments.

| Physicochemical indicators | Shannon–Wiener index | |
|---|---|---|
| | Y2 | YO |
| TOC | 0.791** | -0.095 |
| TN | 0.678** | -0.081 |
| TP | 0.808** | 0.189 |
| TS | 0.704** | -0.371 |

**correlation is significant at the 0.01 level (2-tailed)




225

226 **Fig.5** Vertical distribution of Shannon–Wiener index and the reported TOC, TP, TN and TS in Y2 and YO

227 sediments (Chen et al. 2020a).

228 Shannon–Wiener index was calculated in this study to evaluate the microbial diversity in Y2 and YO

229 sediments. Seabird-derived nutrients such as phosphorus, nitrogen, sulfur as well as associated increases in

230 organic matter content in Y2 sediments have been used to reconstruct penguin population dynamics over the

231 past 3000 years (Sun et al. 2000). Significant and positive correlations between the Shannon–Wiener index and

232 TOC, TP, TN and TS in Y2 sediments (table 1), as well as their consistent vertical distributions (Fig.6)

233 indicated that the microbial communities at the class level were associated with penguin population changes in

234 the past, similar to those reported in phylum level in Zhu et al. (2015). While the nutrients in YO sediments

235 were very low in contrast to those in Y2, and the microbial diversity showed a decreasing trend from the top

236 down.

237 The sulfur cycle-related bacterial genus plotted in Fig.3 includes sulfate-reducing bacteria (SRB),

238 sulfur-oxidizing bacteria (SOB) and the sulfur-degrading bacteria. SRB is a diverse group of anaerobic
239 microorganisms which use sulfate as terminal electron acceptor to obtain energy through catabolism, electron
240 transfer and oxidation of organic matter (Hao et al. 1996; Mußmann et al. 2005; Bhattarai et al. 2018), and
241 reduce sulfate to sulfide and/or elemental sulfur. Therefore, high organic matter in sediments promotes the
242 growth of SRB community (Brodersen et al. 2019). Two SRB of *Desulfosporosinus* and *Desulfotalea* were
243 observed in Y2 sediments while only *Desulfotalea* in YO; *Desulfosporosinus* inhabited in acid sediments (Sen
244 and Johnson 1999), while *Desulfotalea* was determined primarily from marine sediments in cold region (Rabus
245 et al. 2004). High abundance of SRB has been reported in subsurface sediments (Leloup et al. 2005; Finke et al.
246 2007). While in Y2 sediments, the abundance of *Desulfotalea* in deep layer of 48 cm was the highest, with 3.6
247 times as abundant as those in subsurface layer (8–19 cm). This is likely due to the high level of organic matter
248 inputs from penguin guano and strictly anaerobic conditions therein which promote the growth of SRB
249 community. The abundance of *Desulfotalea* was low in 3–5 cm layer in YO sediments (Fig.3), corresponding to
250 the low level of organic matter and nutrients. The organic matter in sediments is the electron donor for sulfate
251 during the DSR; it correlated positively with the sulfate reduction rate (Taketani et al. 2010). The highest
252 abundance of *Desulfotalea* in 28 cm indicated strongest sulfate reduction in YO sediments, coincides with that
253 reported by RIS/$SO_4^{2-}$ in Chen et al. (2020a).

254 SOB is a group of microorganisms which oxidize the sulfide, elemental sulfur, thiosulfite and sulfite to
255 sulfate or intermediates. In Y2 sediments, SOB of *Gallionella*, *Sulfuriferula* and *Thermomonas* was observed,
256 and the abundance of *Gallionella* and *Sulfuriferula* was very low; *Thermomonas* is a strict anaerobic bacteria
257 that could drives denitrification coupling with the oxidation of reduced inorganic sulfur compounds (He et al.
258 2017; Yavuz et al. 2007; Ucar et al. 2020). *Gallionella*, *Sulfuriferula* and *Ferruginibacter* were observed in YO
259 sediments. High abundance of *Sulfuriferula* as well as *Ferruginibacter* increased rapidly below 8 cm in YO

indicates that they are anaerobic SOB, which could oxidize sulfide to sulfate under anaerobic and/or anoxic conditions.

*Pseudomonas* is one of the sulfur-degrading bacteria that could produce sulfatase and degrade the sulfuric acid ester to sulfate (Wallner et al. 2004; Hagelueken et al. 2006). High abundance of *Pseudomonas* indicated active remineralization of organic sulfur compounds in 0–23 cm layer of Y2 sediments. The abundance of *Pseudomonas* was the highest in surface layer (3–5 cm) and the third abundant in other layer indicated the strong and moderate degradation of organic sulfur compounds in YO sediments.

### 4.2 Remineralization of organic sulfur compounds

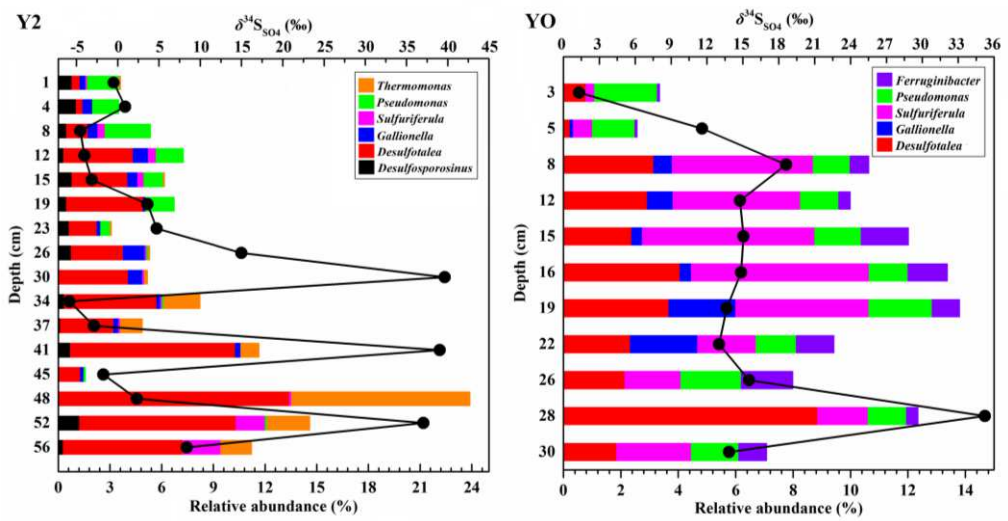
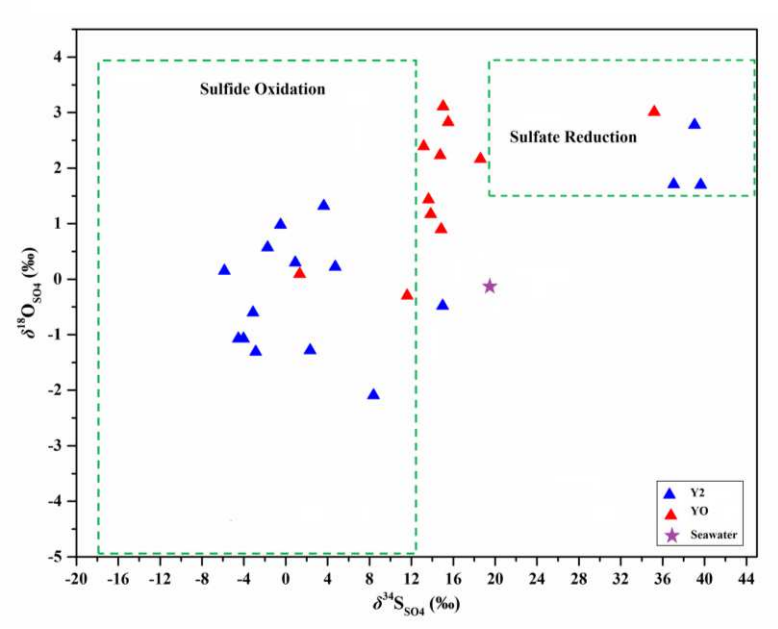



**Fig.6** Relative abundance of sulfur cycle-related bacteria (Histogram) and $\delta^{34}S_{SO4}$ (Dot-line) in Y2 and YO sediments.

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272 **Fig.7** Bivariate plot of $\delta^{34}S_{SO4}$ and $\delta^{18}O_{SO4}$ values for sulfate from sediments (Triangles) in this study and
273 worldwide seawater (Star) (data from Krouse and Mayer 2000).

274 Total sulfur in Y2 sediments originated primarily from inputs of penguin guano with a main form of
275 organic sulfur compounds (Chen et al. 2020a), which would degrade to sulfate by microbial activity. This
276 degradation, also known as remineralization of organic sulfur compounds, would produce ~10‰ sulfur isotope
277 fractionation (Norman et al. 2002). Organic sulfur compounds are formed through the assimilatory sulfate
278 reduction and sulfurization of organic matter (Werne et al. 2008; Fakhraee et al. 2017; Raven et al. 2018;
279 Rosenberg et al. 2018), and the $\delta^{34}S$ of them inherits those of their precursor, sulfate and sulfide (Aizenshtat et
280 al. 2004). Therefore, the $\delta^{34}S$ of sulfate degraded from organic sulfur compounds ranges between those of
281 sulfate and sulfide.

282 Very high abundance of *Pseudomonas* indicates strong remineralization of organic sulfur compounds in
283 the layer of 0–23 cm in Y2 sediments (Fig.6), consistent with those reported in Chen et al. (2020a). Although
284 the high abundance of *Desulfotalea* (SRB) observed in the section of 12–19 cm in Y2 indicated remarkable
285 sulfate reduction, the sulfur isotopic depletion of -4.5‰–4.7‰ for sulfate suggested that the intensity of

remineralization of organic sulfur compounds was much higher than that of sulfate reduction in this section. While the very low level of *Pseudomonas* in the layer below 23 cm in Y2 suggested weak degradation of organic sulfur compounds. The organic sulfur-degrading bacteria *Pseudomonas* observed from the top down indicates remineralization of organic sulfur compounds in every layer in YO, with high intensity in 3–5 cm and considerable in other layer.

### 4.3 Sulfate reduction and sulfur oxidation

Dissimilatory sulfate reduction by SRB is a key step of the sulfur biogeochemical transformations (Jørgensen and Parkes 2010; Orem et al. 2015; Wasmund et al. 2017), which would led to a large enrichment in $\delta^{34}S$ and $\delta^{18}O$ for the residual sulfate and depletion in $\delta^{34}S_{AVS}$ and $\delta^{34}S_{CRS}$ for the reduced products (Wehrmann et al. 2017; Riedinger et al. 2017). While the sulfur isotope fractionation for oxidation of sulfides was negligible, and thus the formed sulfate inherits the depleted sulfur isotopic ratio of sulfides (Zerkle et al. 2009; Balci et al. 2012; Jørgensen et al. 2019). The observed large enrichment in $\delta^{34}S_{SO4}$ and $\delta^{18}O_{SO4}$ and high abundance of SRB in Y2 sediments in the 30, 41 and 52 cm layer indicated a strong sulfate reduction therein (Fig.7). This indication, however, is inconsistent with the strong sulfate reduction in 12–19 cm layer as indicated by high proportion of RIS/$SO_4^{2-}$ in Chen et al. (2020a). This inconsistency is likely due to that the sulfur and oxygen isotope compositions of sulfate were affected simultaneously by sulfate reduction, sulfur oxidation and remineralization of organic sulfur compounds, because sulfate reduction results large enrichment in $\delta^{34}S_{SO4}$ and $\delta^{18}O_{SO4}$ while sulfur oxidation and remineralization of organic sulfur compounds would deplete $\delta^{34}S_{SO4}$ and $\delta^{18}O_{SO4}$ in system. Similar high abundance of SRB in 12–19 cm and 30 cm in Y2 sediments (Fig.6) indicated strong sulfate reduction therein, while high abundance of SOB and sulfur-degrading bacteria *Pseudomonas* in 12–19 cm layer indicated concurrent strong sulfur oxidation and remineralization of organic sulfur compounds and consequently deplete the $\delta^{34}S_{SO4}$ in system. High abundance of SRB and *Thermomonas*

(SOB) observed in 34–37, 48 and 56 cm in Y2 sediments (Fig.6) indicated concurrent sulfate reduction and sulfur oxidation, and thus result depletion of $\delta^{34}S_{SO4}$ and $\delta^{18}O_{SO4}$ therein (Fig.7). In addition, the comparative $\delta^{34}S_{AVS}$ and $\delta^{34}S_{SO4}$ in 56 cm in Y2 may also be due to the high rate of sulfate reduction in the bottom layer with a very high level of sulfate. It was reported that high concentration of substrate promotes faster sulfate reduction and results smaller fractionation effects (Habicht and Canfield 1997; Habicht et al. 2005; Canfield et al. 2010).

In YO sediments, the large enriched $\delta^{34}S_{SO4}$, $\delta^{34}S_{CRS}$ and highest abundance of *Desulfotalea* in 28 cm consistent with the highest ratio of RIS/$SO_4^{2-}$ reported in Chen et al. (2020a) and indicated strongest sulfate reduction. While the medium $\delta^{34}S_{SO4}$ value of 11.6‰–18.6‰ and high abundance of SRB and SOB in 8–26 cm and 30 cm indicated concurrence of sulfate reduction and sulfur oxidation.

## 5 Conclusions

The compositions of microbial community and sulfur and oxygen isotope of sulfate and sulfides in ornithogenic sediments Y2 and pristine sediments YO from Antarctic were analyzed in order to unravel the microbial and geochemical transformations of sulfur in these two distinct lake systems. The microbial communities in Y2 sediments were associated with elevated sediment organic matter content and track the nutrient-rich inputs from past penguin activities. Diverse sulfur cycle-related bacteria were observed in both of Y2 and YO sediments, with the main SRB of *Desulfotalea* and the sulfur degrading-bacteria *Pseudomonas*; while the main SOB of *Thermomonas* was observed in Y2 in contrast to that of *Sulfuriferula* in YO. The much enriched sulfur isotope ratios of sulfate in Y2 (30, 41 and 52 cm) and YO (28 cm) indicated very strong sulfate reduction therein. High abundance of *Pseudomonas* indicated remarkable remineralization of organic sulfur in Y2 (0–23 cm) and YO (3–5 cm). While the depleted and medium sulfur isotope values of sulfate and the considerable abundance of SOB and SRB in other layers in Y2 and YO indicated concurrence of sulfur

oxidation and sulfate reduction. This study indicates that microbial community and stable sulfur isotope analysis in ornithogenic sediments could provide potential tracking of penguin activities around freshwater systems in Antatctica.

## Data availability

All data used in this manuscript are present in the manuscript.

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# Figures

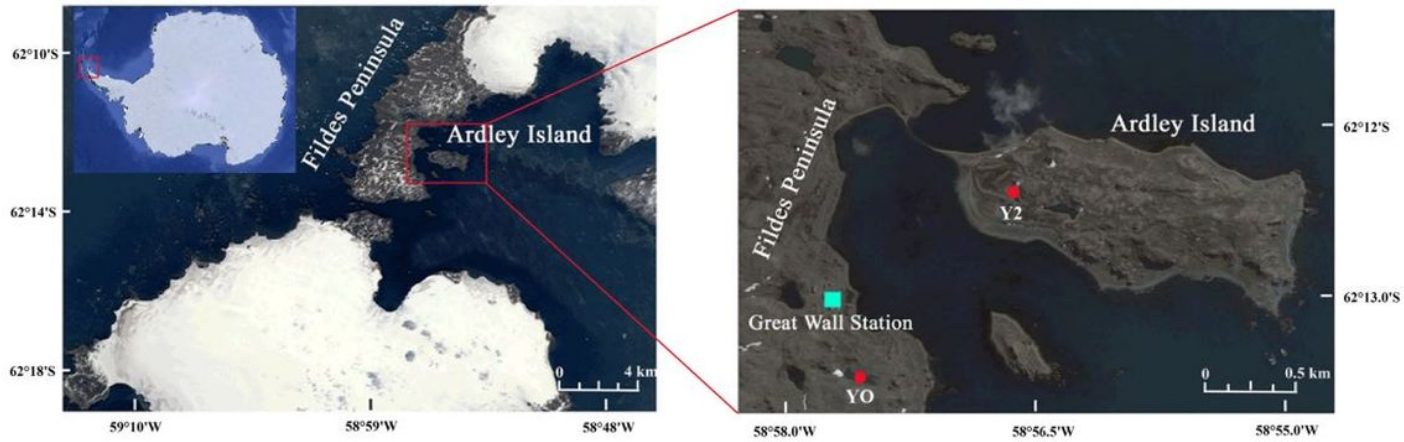
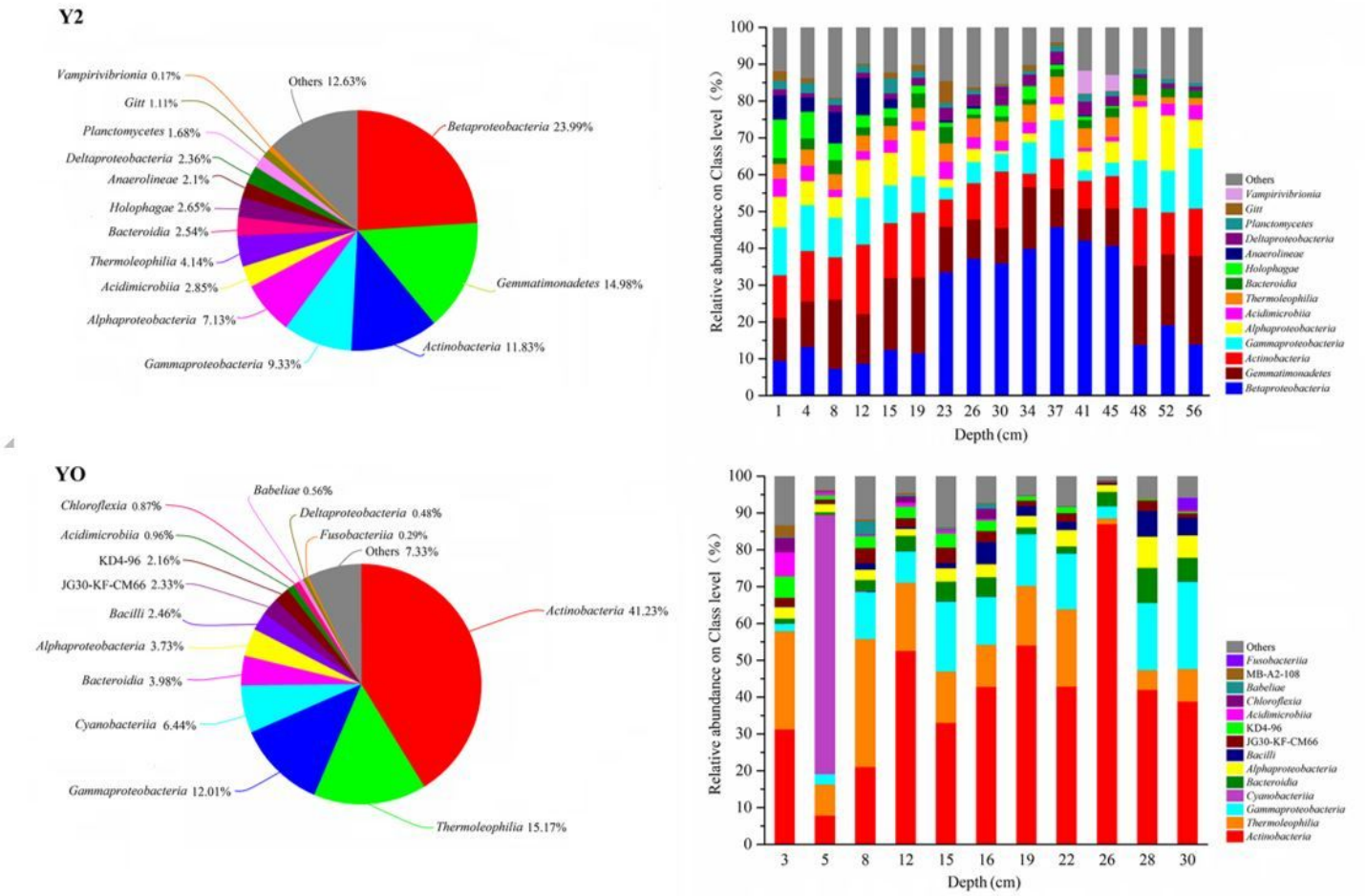


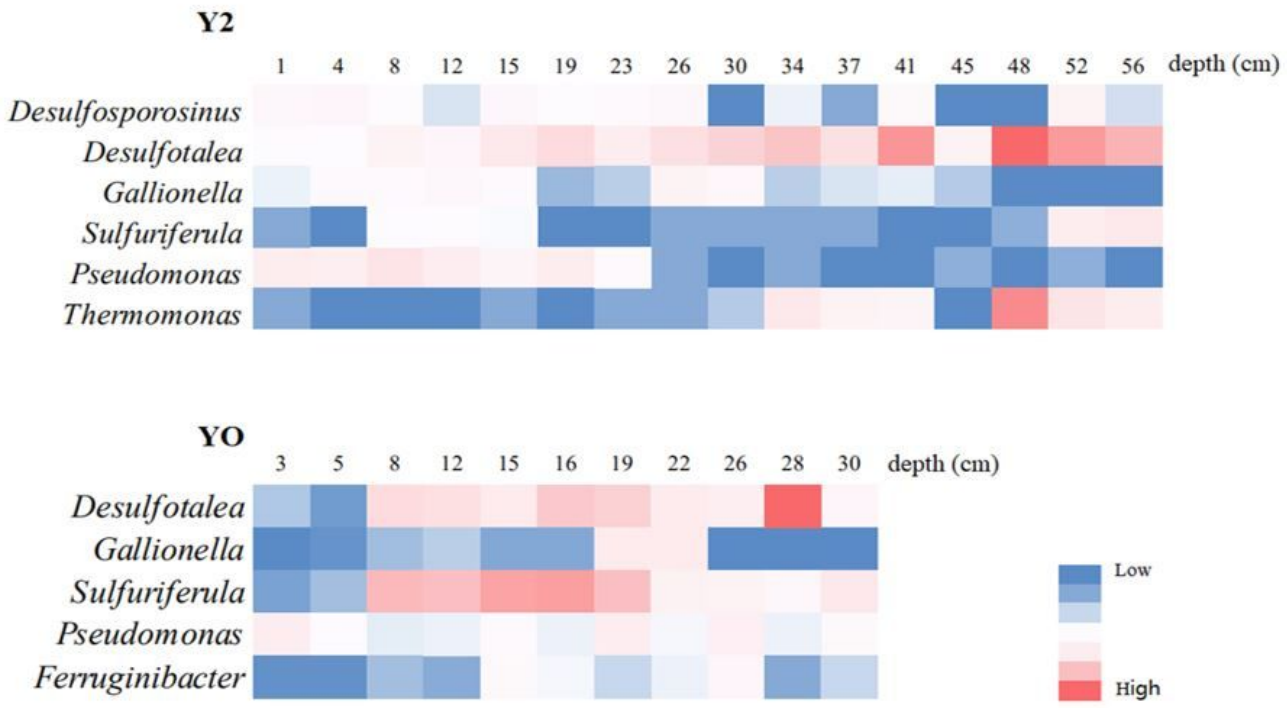

**Figure 1**

Study area and sampling sites on Fildes Peninsula and Ardley Island Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.



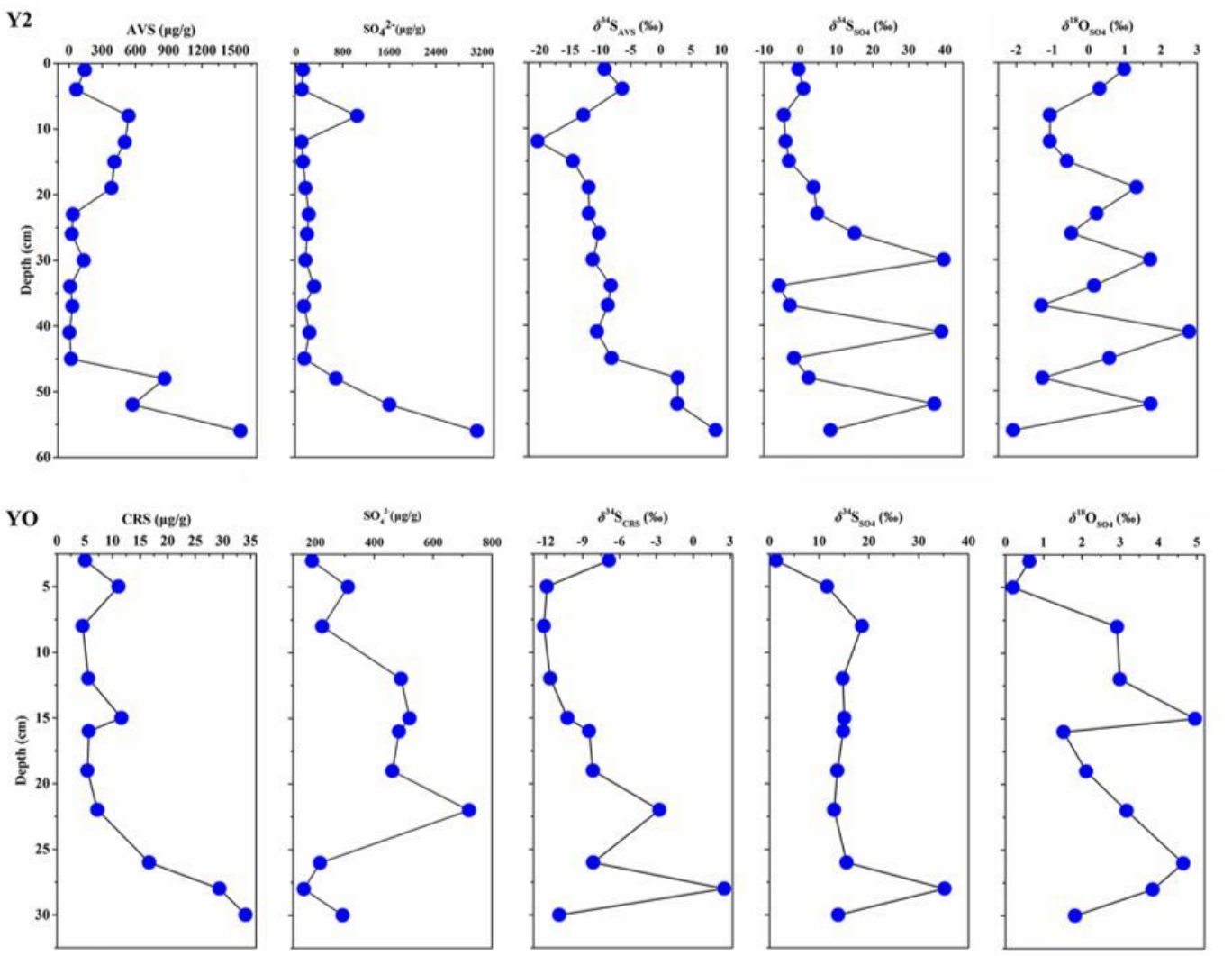

## Figure 2

Relative abundance of microorganisms at the class level in Y2 and YO sediments.



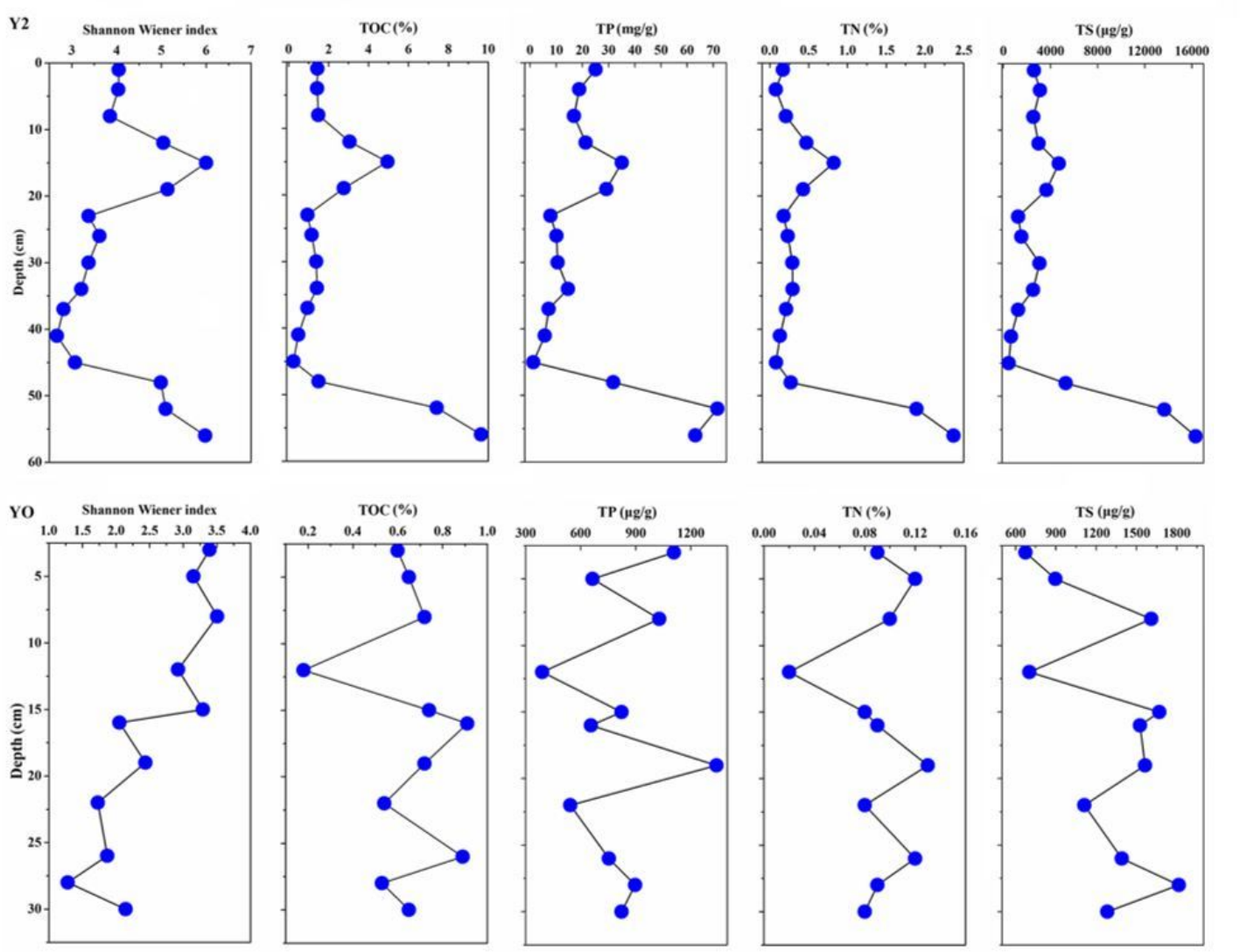

## Figure 3

Heatmap diagram of the sulfur cycle-related microbial genus in Y2 and YO sediments.



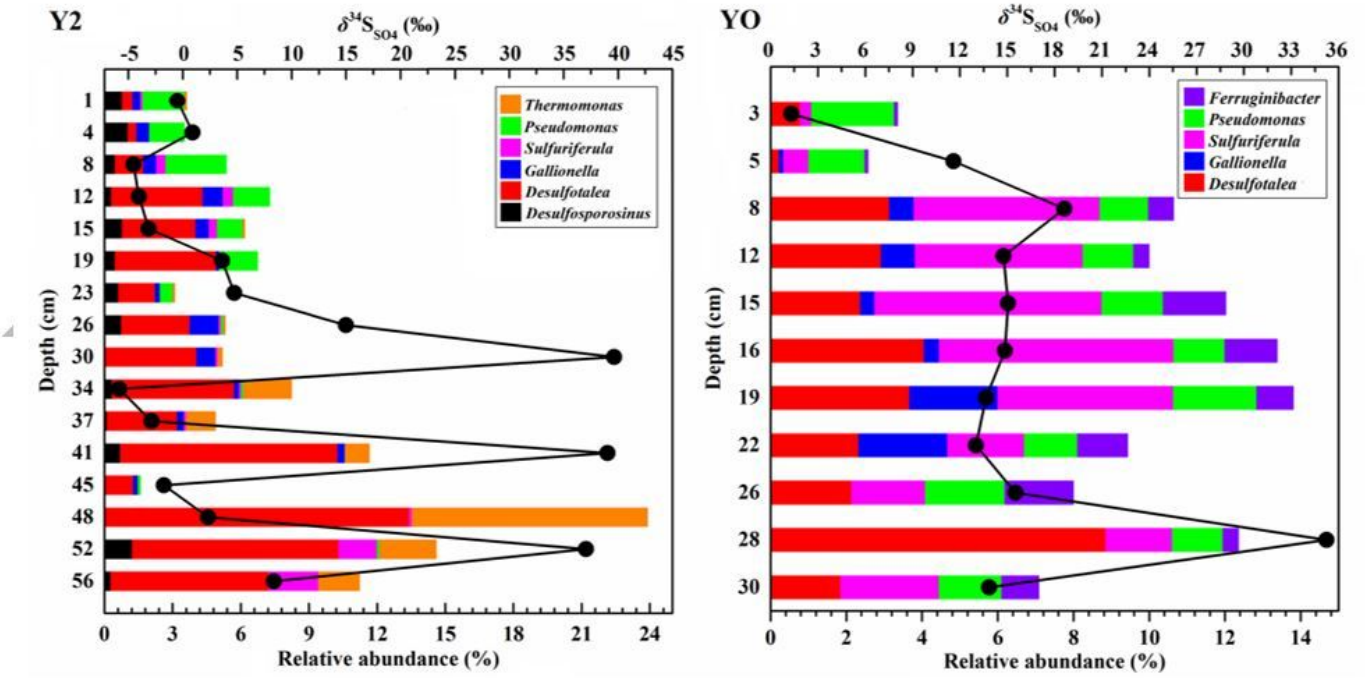

## Figure 4

Vertical distribution of sulfur species of AVS, CRS and sulfate (data from Chen et al., 2020a) and the corresponding δ34SAVS, δ34SCRS, δ34SSO4 and δ18OSO4 in Y2 and YO sediments.




## Figure 5

Vertical distribution of Shannon–Wiener index and the reported TOC, TP, TN and TS in Y2 and YO sediments (Chen et al. 2020a).




## Figure 6

Relative abundance of sulfur cycle-related bacteria (Histogram) and δ34SSO4 (Dot-line) in Y2 and YO sediments.

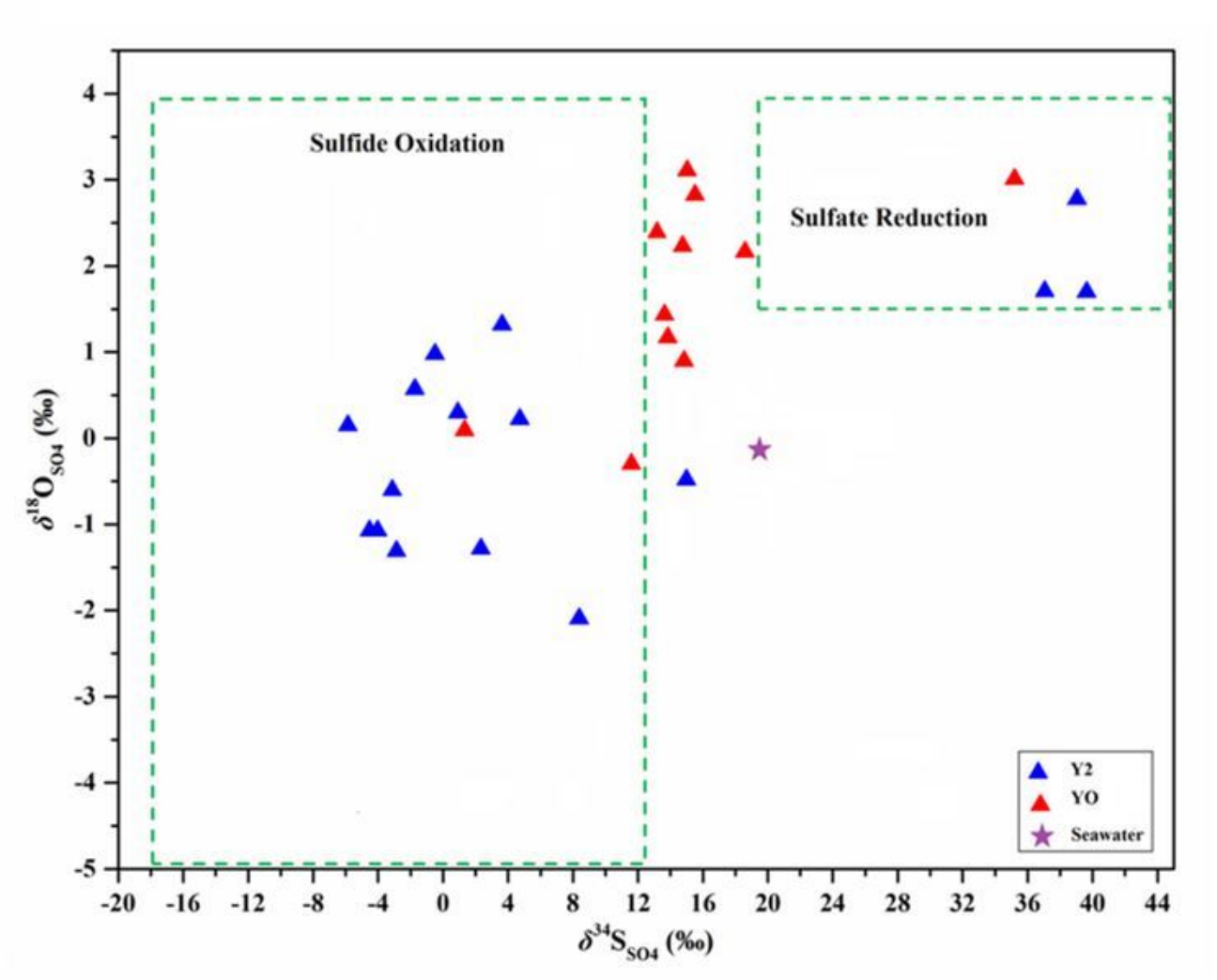



**Figure 7**

Bivariate plot of δ34SSO4 and δ18OSO4 values for sulfate from sediments (Triangles) in this study and worldwide seawater (Star) (data from Krouse and Mayer 2000).