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Diverse transformations of sulfur in seabird-affected sediments revealed by microbial and stable isotope analyses*

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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 ($\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$) in sediments were analyzed to reveal the biogeochemical transformations of sulfur in a seabird-affected lake Y2 and a seabird-free 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 $\delta^{34}S_{SO_4}$ 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. The 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 $\delta^{34}S_{SO_4}$ (35.2‰) and $\delta^{34}S_{CRS}$ (2.5‰) indicate the strongest sulfate reduction in 28-cm layer. High abundance of *Pseudomonas* indicates active remineralization of organic sulfur in 3–5-cm layer in YO. The medium $\delta^{34}S_{SO_4}$ and considerable abundance of SOB and sulfate-reducing bacteria (SRB) indicate concurrence of sulfur oxidation and sulfate reduction in other layers in YO. Therefore, a high level of organic matter input from penguin populations supported the diverse microbial community and transformations of sulfur in aquatic ecosystems in Antarctica.

Keyword: sulfur and oxygen isotope; dissimilatory sulfate reduction; sulfur oxidation; sulfate-reducing bacteria; Antarctica

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., 2020b). For example, sulfur biogeochemistry is coupled closely with the remineralization of organic matter, acidification of water bodies, recycling of nutrients and control of trace metal bioavailability in aquatic environments (Pester et al., 2012; Sheng et al., 2013; Liu et al., 2017; Jørgensen et al., 2019; Chen et al., 2020a).

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., 2020b). 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 III et al., 2003). 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) (Purcell et al., 2014; Jørgensen et al., 2019).

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The composition of sulfur isotopes is a useful tool to trace sulfur biogeochemical processes in aquatic environments, since different isotope fractionations were observed during these transformations (Sela-Adler et al., 2016; Jørgensen et al., 2019). The remineralization of organic sulfur could result in sulfur isotope fractionation effects of 10‰–30‰ (Norman et al., 2002; Shawar et al., 2018). Dissimilatory sulfate reduction (DSR) in sediments driven by anaerobic microorganisms is the main process imparting sulfur isotope fractionation with a variation between 0 and 70‰ (Canfield, 2001). 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 under the condition of considerable oxidants (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 aquatic ecosystems are 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 and Madigan, 2006; Saxton et al., 2016). Sulfur and oxygen isotope ratios of sulfate in lake waters from Deception Island, Antarctic Peninsula suggest mixing of sulfate from atmospheric deposition and from oxidation of local sulfide minerals (Kim et al., 2017, 2021).

In Polar Regions, as well as in the globe, seabirds transport and accumulate 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., 2009; Emslie et al., 2014). Nine bio-elements in the ornithogenic sediments from Y2 Lake, one of our study sites, have been identified and used to reconstruct the penguin population change in the past 3 000 years at Ardley Island, West Antarctic Peninsula (Sun et al., 2000). High level of organic matter and nutrients including nitrogen, phosphorus, and sulfur in the ornithogenic waste products provide abundant nutrients that are often promoting 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 various sulfur species in a seabird-affected sediment core Y2 and a seabird-free sediment core YO from Ardley Island and Fildes Peninsula, discussed the indicated sulfate reduction in those sediments and concluded the main forms of organic sulfur for Y2 and organic sulfur and sulfate for YO (Chen et al., 2020b). The specific biogeochemical transformations of sulfur in Y2 and YO, however, remain unclear since sulfur species provides rare indication on micro-geochemical processes. Therefore, in the present study, we analyze the compositions of sulfur and oxygen isotope for sulfate/sulfides and the microbial community in sediments to study and reveal the diverse transformations of sulfur in the seabird-affected aquatic ecosystem.

2 MATERIAL AND METHOD

2.1 Study area and sample collection

Ardley Island is a special ecological reserve designated by the Scientific Committee on Antarctic Research, which is connected to Fildes Peninsula with a sandbar (Fig.1). 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 (Yang et al., 2019). During the summer breeding period, abundant penguin guano is washed into nearby lakes and ponds and leaves distinct ornithogenic signatures

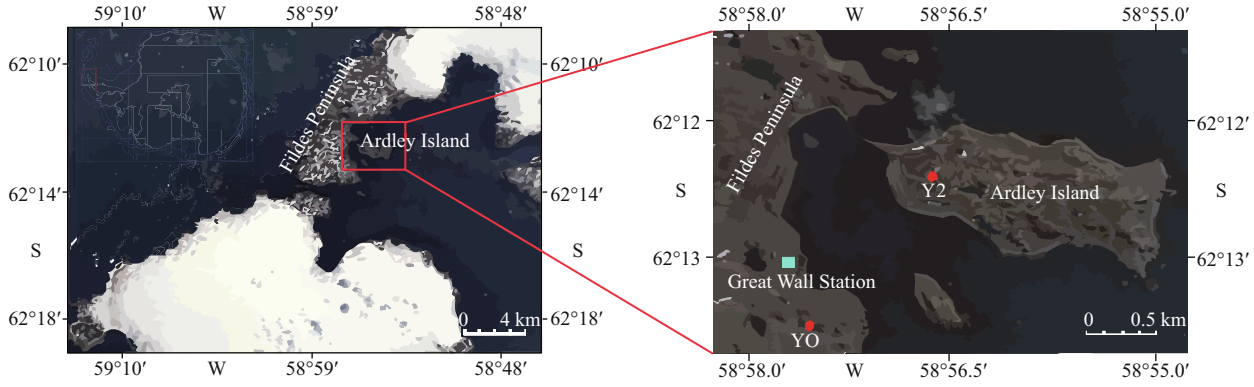

Fig.1 Study area and sampling sites on Fildes Peninsula and Ardley Island (based on Chen et al., 2020b)

in the sediments. In contrast, the lakes and ponds on Fildes Peninsula, including our study site YO, are seabird-free and not affected by penguin activity (Chu et al., 2019). The YO lake covers an area of about 9 000 m^2 and located 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; and the seabird-free 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. (2020b). 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.

2.3 Analyses of microbial community compositions

2.3.1 Sediment DNA extraction

DNA was extracted from the sediments by the sodium dodecyl sulfate (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 225 r/min for 30 min at 37 ℃. After the shaking treatment, 600-μL 20% SDS was added, and the samples were incubated in a 65-℃ water bath for 30 min. The supernatants were collected after centrifugation at 8 000 r/min for 10 min at room temperature and transferred into 2-mL centrifuge tubes. Supernatants from extractions were combined and mixed with an equal volume of Phenol-chloroform-isoamyl alcohol (25∶24∶1). The aqueous phase was recovered by centrifugation and precipitated with PEG-NaCl at 4 ℃ for 2 h. The pellet of crude nucleic acids was obtained by centrifugation at 14 000 r/min for 10 min at 4 ℃, washed with 75% ethanol, add 100-μL Tris-EDTA buffer, and store at -20 ℃.

2.3.2 PCR amplification and 16S rDNA sequencing

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

2.3.3 Data processing

Paired-end reads were merged using the FLASH program. Chimeric sequences were filtered using Vsearch software (v2.3.4). Sequences with ≥97% similarity were assigned to the same operational taxonomic units (OTUs) using Vsearch. Representative sequences were selected for each OTU, and taxonomic data were then assigned to each representative sequence using the Ribosomal

Database Project (RDP Release 11.5) classifier. OTU abundance information was normalized using the sequence number of the sample with the fewest sequences as a standard. The microbial communities at the class level and the sulfur-cycle related microbial genus in Y2 and YO sediments were obtained by using the above method.

2.4 Sulfur and oxygen isotope analyses

Geochemical analysis was not performed for pore water due to the low content. The sulfates in Y2 and YO sediments were extracted by solution of NaH_2PO_4 (pH=6, 0.016 mol/L), collected through centrifugation and filtration, purified by dissolution and re-precipitation in a chelating solution of diethylene triamine penta acetic acid (DTPA) (Bao, 2006), and to precipitated as barite by adding saturated $BaCl_2$ solution. The collected $BaSO_4$ was washed repeatedly by Milli-Q water and then heated in an oven at 105 ℃. The dried $BaSO_4$ was powdered and placed into a 2-mL centrifuge tube in drying apparatus.

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

Stable sulfur and oxygen isotope analyses of barite and sulfides were performed in the State Key Laboratory of Ore Deposit Geochemistry. Weighed samples of barite as well as V_2O_5 (1∶3) and Ag_2S, respectively, were compacted into tin cups for sulfur isotope analysis and the weighed $BaSO_4$ was compacted into silver cups for oxygen isotope analysis. Stable sulfur and oxygen isotope ratios were determined by isotope ratio mass spectrometer (Thermo Fisher Delta V Advantage) coupled to an elemental analyzer (Flash 2000 for sulfur and thermal conversion eElemental analyzer (TC/EA) for oxygen). The instrument precision was ±0.2‰ for $\delta^{34}S$ and 0.30‰ for $\delta^{18}O$. IAEA-SO-5 ($\delta^{34}S$, 0.5‰), IAEA-SO-6 (-34.1‰), and NBS-127 ($\delta^{34}S$, 20.3‰) were used as the standard samples for sulfur isotope analysis of barite and IAEA S1 (-0.3‰), IAEA S2 (+22.6‰) and IAEA S3 (-32.5‰) for sulfur isotope analysis of sulfides. NBS-127 ($\delta^{18}O$, 8.59‰) was used as the standard sample for the oxygen isotope analysis. Stable isotope results were presented in δ (‰) and expressed relative to the vienna canyon diablo troilite (VCDT) for $\delta^{34}S$ and VSMOW for $\delta^{18}O$ according to the equation of $\delta\ (‰)=[(R_{sample}-R_{standard})/R_{standard}]\times 1\,000$, where δ (‰) 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 vienna standard mean ocean water (VSMOW).

3 RESULT

3.1 Compositions of microbial community

Amounts of 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. 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%.

Relative abundance of the sulfur-cycle related microbial communities at genus level in Y2 and YO sediments were 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

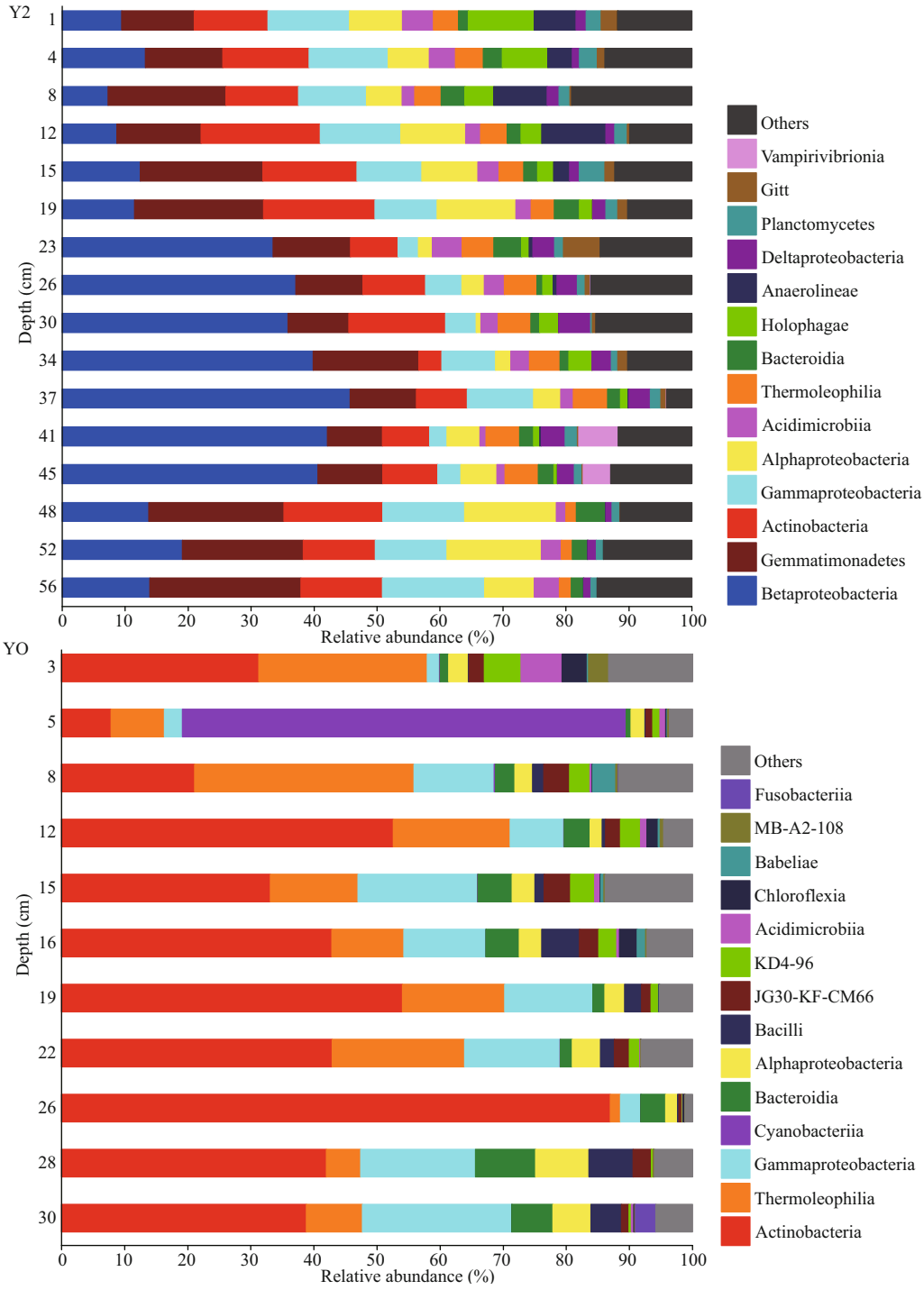

Fig.2 Relative abundance of microorganisms at the class level in Y2 and YO sediments

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

The vertical distributions of AVS, CRS, and

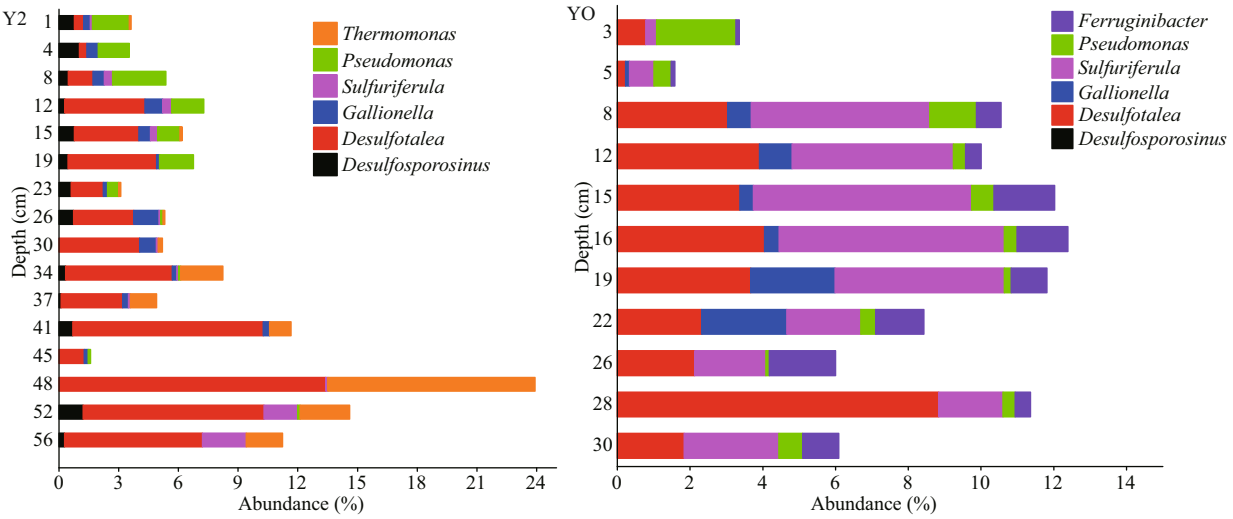

Fig.3 Relative abundance of the sulfur cycle-related microbial genus in Y2 and YO sediments

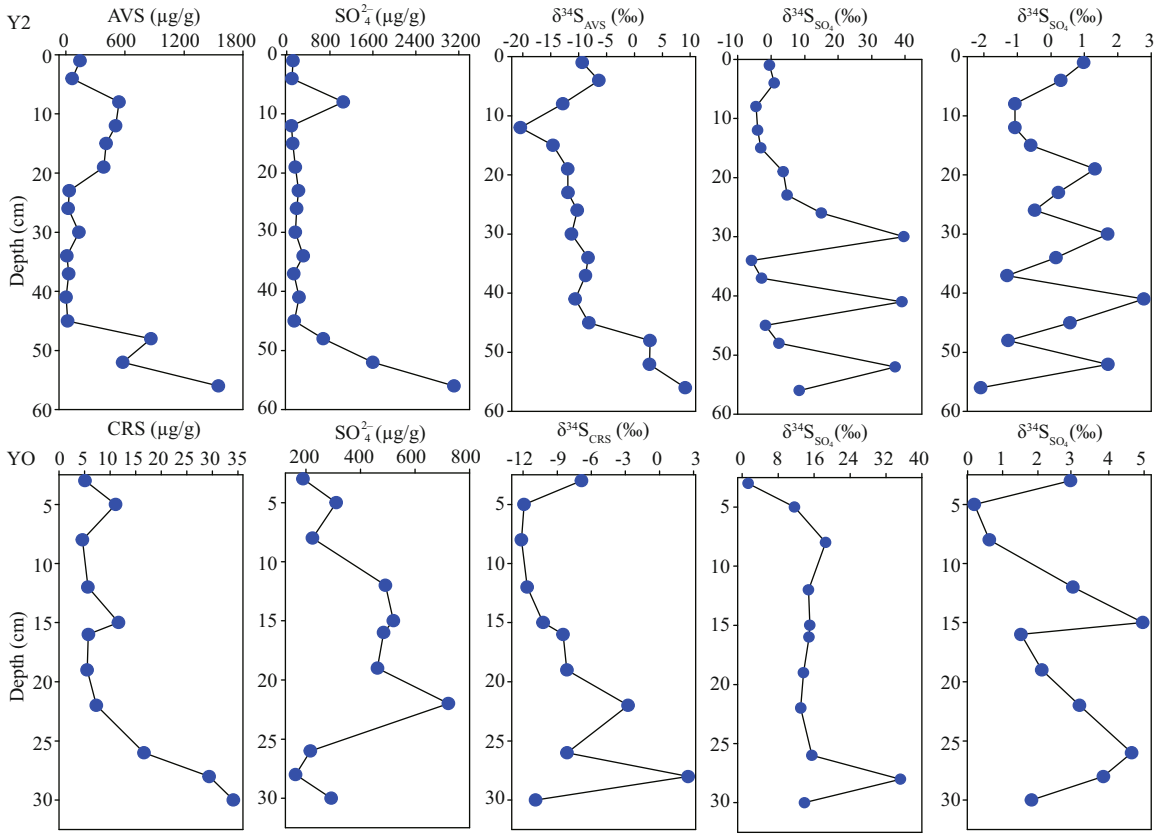

Fig.4 Vertical distribution of sulfur species of AVS, CRS, and sulfate (data from Chen et al., 2020b) and the corresponding $\delta^{34}S_{AVS}$, $\delta^{34}S_{CRS}$, $\delta^{34}S_{SO_4}$, and $\delta^{18}O_{SO_4}$ in Y2 and YO sediments

sulfate and the corresponding $\delta^{34}S_{AVS}$, $\delta^{34}S_{CRS}$, $\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$ in Y2 and YO sediments were plotted in Fig.4. The $\delta^{34}S_{SO_4}$ 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 to 12 cm and enriching between 12 and 56 cm, with much enriched values in 48–56-cm layer. The $\delta^{18}O_{SO_4}$ values in Y2 sediments ranged from -2.09‰ to 2.78‰ with a fluctuation. In YO sediments, almost of the $\delta^{34}S_{SO_4}$ 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

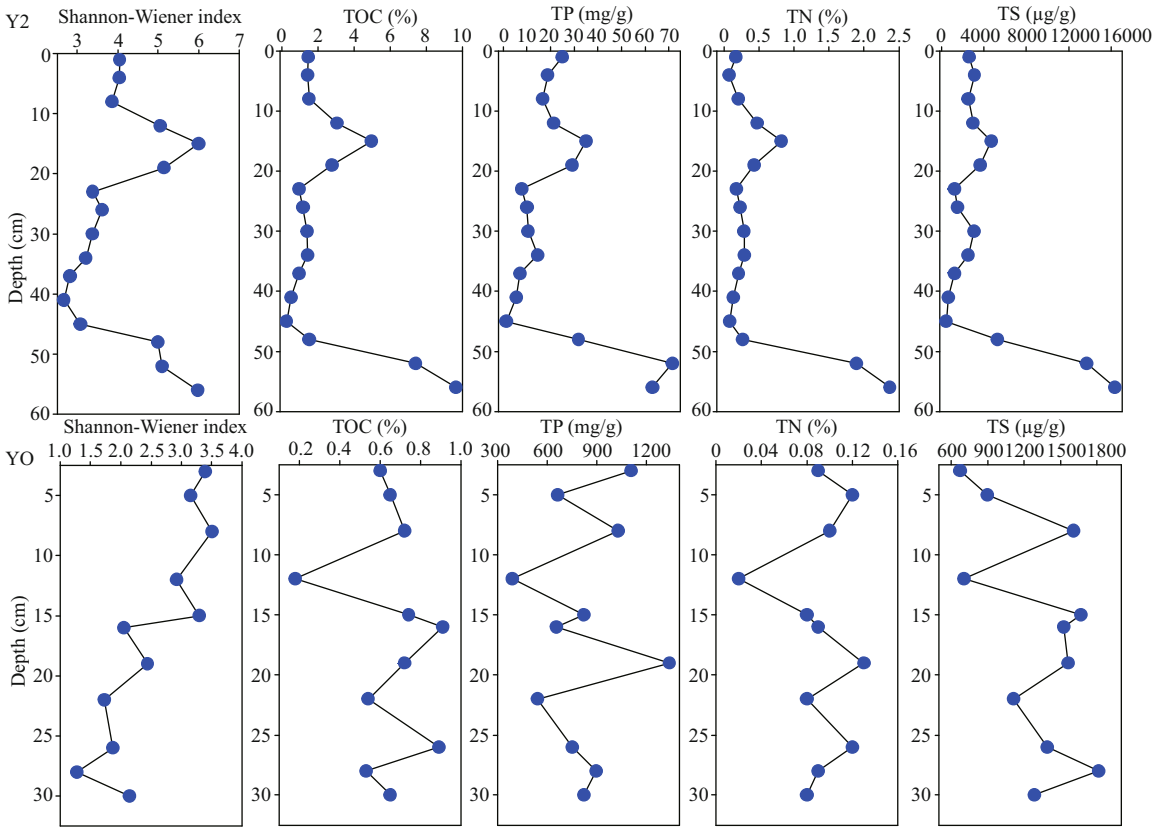

Fig.5 Vertical distribution of Shannon-Wiener index and the reported TOC, TP, TN, and TS in Y2 and YO sediments (Chen et al., 2020b)

Table 1 Correlations between Shannon-Wiener index and physicochemical indices in Y2 and YO sediments

Physicochemical indices	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).

was similar to that of $\delta^{34}S_{SO_4}$. The $\delta^{18}O_{SO_4}$ values in YO sediments ranged from 0.20‰ to 3.96‰ with a fluctuation.

4 DISCUSSION

4.1 Association between microbial diversity and penguin activity

Shannon-Wiener index was calculated in this study to evaluate the microbial diversity in Y2 and YO sediments. Seabird-derived nutrients such as phosphorus, nitrogen, and sulfur, as well as associated increases in organic matter content in Y2 sediments have been used to reconstruct penguin population dynamics over the past 3 000 years (Sun et al., 2000). Significant and positive correlations between the Shannon-Wiener index and total organic carbon (TOC), total phosphorus (TP), total nitrogen (TN), and total sulfur (TS) in Y2 sediments (Table 1), as well as their consistent vertical distributions (Fig.5) indicated that the microbial communities at the class level were associated tightly with penguin population changes in the past, similar to those reported in phylum level in Zhu et al. (2015). The nutrients in seabird-free YO sediments were very low in contrast to those in Y2, and the microbial diversity showed a decreasing trend from the top down (Fig.5).

4.2 Change of sulfur cycle-related bacterial genus

The sulfur cycle-related bacterial genus plotted in Fig.3 includes SRB, SOB, and the sulfur-degrading bacteria. Sulfate-reducing bacteria are a diverse group of anaerobic microorganisms that use sulfate as terminal electron acceptor to obtain energy through catabolism, electron transfer, and oxidation of organic matter (Mußmann et al., 2005; Bhattarai et al., 2018), and reduce sulfate to sulfide and/or elemental sulfur. Therefore, high organic matter in sediments

promotes the growth of SRB community (Brodersen et al., 2019). Two SRB of *Desulfosporosinus* and *Desulfotalea* were observed in Y2 sediments but only *Desulfotalea* in YO. *Desulfosporosinus* inhabited in acid sediments (Sen and Johnson, 1999), while *Desulfotalea* was maninly found from marine sediments in cold regions (Rabus et al., 2004). High abundance of SRB has been reported in subsurface sediments (Leloup et al., 2005; Finke et al., 2007). In Y2 sediments, the abundance of *Desulfotalea* in deep layer of 48 cm was the highest, being 3.6 times as abundant as that in subsurface layer (8–19 cm). This is likely due to the high level of organic matter inputs from penguin guano and strictly anaerobic conditions therein, which promote the growth of SRB community. The abundance of *Desulfotalea* was low in 3–5-cm layer in YO sediments (Fig.3), corresponding to the low level of organic matter and nutrients. The organic matter in sediments is the electron donor for sulfate during the DSR; it correlated positively with the sulfate reduction rate (Taketani et al., 2010). The highest abundance of *Desulfotalea* in 28 cm indicated strongest sulfate reduction in YO sediments, which coincides with the results concluded by the ratios of reduced inorganic sulfur and sulfate (RIS/SO_4^{2-}) in Chen et al. (2020b).

Sulfur-oxidizing bacteria are a group of microorganisms who oxidize the sulfide, elemental sulfur, thiosulfate, and sulfite to sulfate or intermediates. In Y2 sediments, SOB of *Gallionella*, *Sulfuriferula*, and *Thermomonas* was observed, and the abundance of *Gallionella* and *Sulfuriferula* was very low; *Thermomonas* is a strict anaerobic bacteria that could drive denitrification coupling with the oxidation of reduced inorganic sulfur compounds (He et al., 2017; Ucar et al., 2020). *Gallionella*, *Sulfuriferula*, and *Ferruginibacter* were observed in YO sediments. High abundance of *Sulfuriferula* as well as *Ferruginibacter* increased rapidly below 8 cm in YO indicates that they are anaerobic SOB and can use nitrate and ferric iron as electron acceptors and oxidize sulfide to sulfate under anaerobic and/or anoxic conditions (Sugio et al., 1985; Mahmood et al., 2009).

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.

4.3 Remineralization of organic sulfur compounds

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

Very high abundance of *Pseudomonas* indicates strong remineralization of organic sulfur compounds in the layer of 0–23 cm in Y2 sediments (Fig.3), consistent with those indicated by sulfur species in Chen et al. (2020b). Although the high abundance of *Desulfotalea* (SRB) observed in the section of 12–19 cm in Y2 indicated remarkable 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. 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.

4.4 Concurrence of sulfate reduction and sulfur oxidation

Dissimilatory sulfate reduction by SRB is a key step of the sulfur biogeochemical transformations in aquatic ecosystems (Orem et al., 2015; Wasmund and Mußmann, 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 (Antler et al., 2013). As the sulfur isotope fractionation for oxidation of sulfides was negligible,

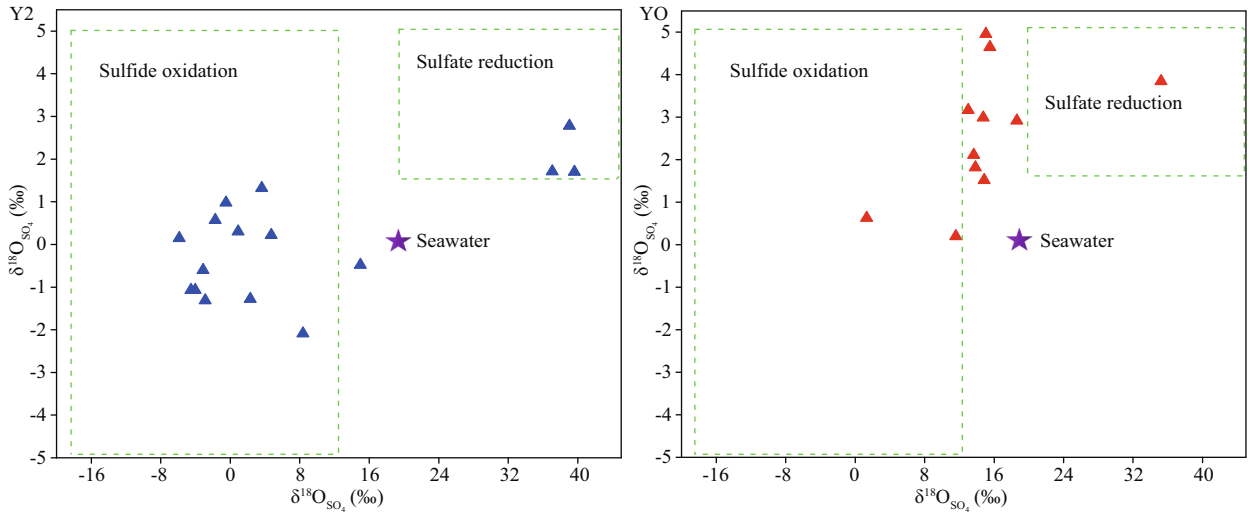

Fig.6 Bivariate plots of $\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$ for sulfate in sediments in this study and the worldwide seawater (data from Tostevin et al., 2014)

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 $\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$ (Fig.4) and high abundance of SRB (Fig.3) in Y2 sediments in the 30-, 41-, and 52-cm layer indicated a strong sulfate reduction therein (Fig.6). This indication, however, is inconsistent with the strong sulfate reduction in 12–19-cm layer as suggested by high proportion of RIS/SO_4^{2-} in Chen et al. (2020b). This inconsistency is likely because 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 in large enrichment in $\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$, whereas sulfur oxidation and remineralization of organic sulfur compounds would deplete $\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$ in system. Similar high abundance of SRB in 12–19 cm and 30 cm in Y2 sediments (Fig.3) indicated strong sulfate reduction there, 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 $\delta^{34}S_{SO_4}$ in system. High abundance of SRB and *Thermomonas* (SOB) observed in 34–37, 48, and 56 cm in Y2 sediments (Fig.3) indicated concurrent sulfate reduction and sulfur oxidation, and thus resulted in depletion of $\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$ (Fig.6). In addition, the comparative $\delta^{34}S_{AVS}$ and $\delta^{34}S_{SO_4}$ 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 $\delta^{34}S_{SO_4}$, $\delta^{34}S_{CRS}$ and highest abundance of *Desulfotalea* in 28 cm consistent with the highest ratio of RIS/SO_4^{2-} reported in Chen et al. (2020b) and indicated strongest sulfate reduction. The medium $\delta^{34}S_{SO_4}$ 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.

Compared to those of YO, the sulfur isotope compositions of Y2 varied widely due to much stronger sulfate reduction activity (Chen et al., 2020b; this study). Other potential mechanism such as the local terrestrial inputs from sulfide oxidation may be responsible for some of the observed $\delta^{34}S_{SO_4}$, since the depleted $\delta^{34}S$ of volcanic rocks and sulfides were reported in King George Island (Kim et al., 2017, 2021). This process, however, may not be a meaningful contribution to the observed $\delta^{34}S_{SO_4}$ in the present study, because the sulfur inputs from terrestrial runoff was negligible in comparison with the guano depositions in Y2. The consistent associations between sulfur cycle-related bacteria and sulfur isotope compositions in both Y2 and YO indicated that internal bio-transformations of sulfur species were the main trigger for the change of sulfur isotope compositions.

5 CONCLUSION

The microbial communities in Y2 sediments were associated with elevated sediment organic matter

content and track the nutrient-rich inputs from past penguin activities. Stable sulfur and oxygen isotope ratios of sulfate and the sulfur cycle-related bacteria indicated remarkable and diverse transformations of sulfur in the seabird-affected sediments. Our study indicates that microbial community and stable sulfur isotope analysis in ornithogenic sediments could provide potential tracking of penguin activities around freshwater systems in Antarctica.

6 DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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