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Major contribution of sulfide-derived sulfur to the benthic food web in a large freshwater lake

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Abstract

In freshwater systems, contributions of chemosynthetic products by sulfur-oxidizing bacteria in sediments as nutritional resources in benthic food webs remain unclear, even though chemosynthetic products might be an important nutritional resource for benthic food webs in deep-sea hydrothermal vents and shallow marine systems. To study geochemical aspects of this trophic pathway, we sampled sediment cores and benthic animals at two sites (90 and 50 m water depths) in the largest freshwater (mesotrophic) lake in Japan: Lake Biwa. Stable carbon, nitrogen, and sulfur isotopes of the sediments and animals were measured to elucidate the sulfur nutritional resources for the benthic food web precisely by calculating the contributions of the incorporation of sulfide-derived sulfur to the biomass and of the biogeochemical sulfur cycle supporting the sulfur nutritional resource. The recovered sediment cores showed increases in ³⁴S-depleted sulfide at 5 cm sediment depth and showed low sulfide concentration with high $\delta^{34}\text{S}$ in deeper layers, suggesting an association of microbial activities with sulfate reduction and sulfide oxidation in the sediments. The sulfur-oxidizing bacteria may contribute to benthic animal biomass. Calculations based on the biomass, sulfur content, and contribution to sulfide-derived sulfur of each animal comprising the benthic food web revealed that 58%–67% of the total biomass sulfur in the benthic food web of Lake Biwa is occupied by sulfide-derived sulfur. Such a large contribution implies that the chemosynthetic products of sulfur-oxidizing bacteria are important nutritional resources supporting benthic food webs in the lake ecosystems, at least in terms of sulfur. The results present a new trophic pathway for sulfur that has been overlooked in lake ecosystems with low-sulfate concentrations.

KEYWORDS

chemosynthesis, Lake Biwa, microbial sulfate reduction, organic matter decomposition, stable isotopes

1 | INTRODUCTION

In freshwater lakes, biogeochemical processes in sediments, including the decomposition of organic matter, nutrient regeneration, and

microbial primary production, are important to elucidate the function and structure of an entire benthic lake ecosystem. As one example, carbon that is well known to originate from methane produced by methanogens in anoxic sediment is incorporated into benthic

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food webs via methane-oxidizing bacteria (MOB) (Grey, 2016; Jones & Grey, 2011). That fact suggests MOB as a valuable diet resource for the benthic food web, as are photoautotrophic product particles sinking from the epilimnion. Along with methane, oxygen, nitrate, iron, manganese, and sulfate can be electron acceptors for organic matter decomposition (i.e., energy production) along with the “thermodynamic ladder” or “redox tower” (Bethke et al., 2011; Champ et al., 1979; Froelich et al., 1979). Subsequently, re-oxidation (i.e., chemoautotrophic primary production, which does not generally include methane oxidation) of reduced compounds, such as ammonium and hydrogen sulfide generated from the decomposition, occurs in benthic biogeochemical processes (Bethke et al., 2011; Champ et al., 1979; Froelich et al., 1979). Consequently, bacterial primary productive processes other than methane oxidation may contribute to benthic food webs.

Sulfur (S) compounds play a pivotal role in various biological, chemical, and physical processes at the redox boundary (Wu et al., 2019). The biogeochemical cycle and behavior of S in anoxic sediments have been well studied in marine environments (e.g., Jørgensen et al., 2019) with vigorous microbial sulfate reduction (MSR) by sulfate-reducing bacteria (SRB) in high sulfate concentrations in seawater (28 mmolL⁻¹; Lowenstein et al., 2001). MSR is the terminal step among microbial metabolism associated with anaerobic organic matter decomposition. Its importance in marine ecosystems has long been recognized (Canfield & Des Marais, 1991; Jørgensen, 1982; Jørgensen et al., 2019; Martens & Berner, 1974). Aside from MSR, the degradation of organic sulfur (putrefaction) has also been recognized as an important S cycling process in especially low-sulfate environments (Fakhraee et al., 2017; King & Klug, 1982). Moreover, the chemoautotrophic primary production of sulfur-oxidizing bacteria (SOB), which oxidize and assimilate reduced S species, can also be important in an S cycle (Figure 1). Although hydrogen sulfide is generally toxic, its toxicity can be reduced by combination with iron. Hydrogen sulfide in sediment and the amorphous iron sulfides formed by this reaction are collectively designated as acid volatile sulfide (AVS) (Berner, 1964; Rickard & Morse, 2005). The SOB biomass fueled by AVS is an important diet resource for

benthic animals in some environments, such as cold seeps and hydrothermal vents (Onishi et al., 2020; Onishi, Yamanaka, et al., 2018; Yamanaka et al., 2015). Despite the occurrence of sulfide by MSR and putrefaction even in freshwater environments with low-sulfate concentrations (typically 0–2.5 mmolL⁻¹, mostly <1 mmolL⁻¹; Fry, 1986; Jones et al., 1982; Zak et al., 2021), effect of the SOB on benthic food webs remains unclear because of inadequate interest and research methods.

No direct evidence has been reported to date of sulfide-originated S derived from SOB (hereinafter sulfide-S) incorporation into benthic food webs in freshwater environments. No report of the relevant literature describes benthic animals that are reliant on the SOB biomass as their diet resource. Several possible reasons exist for why the incorporation of sulfide-S into benthic food webs in freshwater environments has not been considered. First, as the benthic food web carbon source, methane-derived carbon has been studied more thoroughly in freshwater ecosystems than SOB-derived carbon because methane-derived carbon is much more conspicuous and detectable. Many ecological studies of freshwater lakes use stable carbon isotopes rather than S isotopes to evaluate diet resources and to construct the trophic structure of benthic ecosystems (Agasild et al., 2014; Bunn & Boon, 1993; Jones et al., 1999, 2008; Kiyashko et al., 2001), where biogenic methane can be readily distinguished with its low carbon isotope ratio (–110 to –50‰; e.g., Deines & Grey, 2006; Taipale et al., 2007; Whiticar, 1999). The use of S isotope has therefore not been pursued intensively. Secondly, few studies have examined details of the biogeochemical S cycle in lacustrine settings (e.g., Jørgensen, 1990; Wu et al., 2019) because of its low-sulfate concentration compared with marine sulfate concentrations, which makes the S isotope measurement difficult. Because sulfate available for SRB is small, MSR is regarded as less important in freshwater sediments where methanogenesis occurs preferentially (Capone & Kiene, 1988; Holmer & Storkholm, 2001).

However, some intermittent evidence exists for the importance of SOB as primary productive processes supporting benthic food webs in anoxic freshwater sediments. Santoro et al. (2013) reported that chemoautotrophic primary production, including SOB, is equivalent to up to 80% of heterotrophic bacterial production by measuring dark carbon fixation rate in sediments taken from 11 freshwater lakes in Sweden and Brazil. Moreover, Jin et al. (2017) demonstrated that chemoautotrophic SOB account for up to 70% of total bacteria in anoxic sediments and that they drive the S cycle in the sediments of the freshwater Daechung Reservoir (Korea). Moreover, Grey and Deines (2005) found that Chironomid larvae in Wyresdale Park Lake and Esthwaite Water (UK) might be rather reliant on sulfide for their primary S resource, which implies that the chironomids feed on SOB that assimilated the sulfide. Sanders Jr. et al. (2011) found that in Lake Huron (USA), bacterial mats (including chemosynthetic SOB mat; Biddanda et al., 2009, 2023) flourished by nutrient-rich groundwater venting from the lake bottom supported a benthic fauna. Evaluating the S resource of benthic

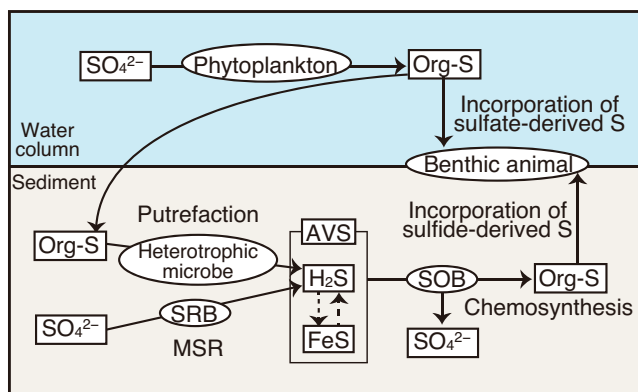


FIGURE 1 Biogeochemical sulfur cycles and the contributions of benthic animals on and in sediments in aquatic systems. Processes indicated by solid arrows have been well known.

animals quantitatively necessitates measuring the S isotope ratios of sulfide and sulfate, as well as animal tissues. However, no report has ever described a study of the S resource for benthic animals in freshwater. The contribution of sulfide-S as a S resource for benthic animals remains unclear (Figure 1) because of a gap in the interests of ecologists and geochemists.

Stable isotope compositions of biophile elements have been used widely for elucidating the diet resources of animals in aquatic ecosystems (e.g., Wada, 2009). Carbon and nitrogen isotopic ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) are useful for ascertaining the trophic position of taxa and the trophic structure of communities (DeNiro & Epstein, 1981; Minagawa & Wada, 1984). However, S isotopic compositions ($\delta^{34}\text{S}$) are used to trace diet resources, especially primary S resources, because of the slight isotopic fractionation during assimilation, similar to that of carbon (Onishi et al., 2020; Onishi, Yamanaka, et al., 2018). The three potential food resources of sinking particles (i.e., photoautotrophic product), MOB, and SOB in benthic food webs can potentially be distinguished isotopically. Although the $\delta^{34}\text{S}$ values of phytoplankton and MOB are identical to those of the lake water sulfate used (Fry et al., 1983), the $\delta^{34}\text{S}$ values of SOB are clearly different from those of phytoplankton and MOB because of their different resources and isotope fractionations (Yamanaka et al., 2015). The $\delta^{34}\text{S}$ value of SOB reflects that of the sulfide used (Fry et al., 1983). Isotope fractionation factors during the sulfide assimilation have been reported as -5.2 to $+2\%$ (Kaplan & Raftar, 1958; Poster et al., 2014). The $\delta^{34}\text{S}$ value of sulfide generated by SRB is often negative (Kaplan & Rittenberg, 1964; Sim et al., 2011). Therefore, the relative contributions of three organic matter resources to the benthic animals can be evaluated with $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$, where S isotope ratios are particularly powerful for identifying organisms feeding on SOB.

Using S isotopes, this study was conducted to clarify the contribution of SOB as a diet resource for benthic animals in and on sediments of Lake Biwa during the stratification period. In the lake, the water is fully circulated during winter (January–February), whereas the dissolved oxygen (DO) concentration decreases in the stagnant bottom water during the stratification period (March–November). High sulfide concentrations (up to $20\ \mu\text{mol-Sg}^{-1}$ -wet sediment; Maeda & Kawai, 1988; Mitamura et al., 2015; Murphy et al., 2001) in sediment and the thick bacterial mat of the SOB *Thioploca* have been observed (Kojima et al., 2003; Nishino et al., 1998). The biomass was homogeneous at an average $10^6\ \mu\text{m-trichome cm}^{-3}$ -sediment in the deepest area (Furuta et al., 2020; Mitamura et al., 2015; Nishino et al., 1998). We hypothesized that MSR and then chemoautotrophic primary production by SOB occur in the sediment, and that the SOB biomass can be a valuable diet resource for the benthic food web. Sulfur isotope ratio measurements of sulfide and benthic animals in the sediments from two sampling points with different DO concentrations quantitatively revealed that the SRB produce some of the sulfide that SOB use to produce organic matter chemosynthetically. The measurements also revealed that benthic animals indirectly assimilated sulfide-S by feeding on the biomass of SOB.

2 | MATERIALS AND METHODS

2.1 | Study areas

Located in Shiga Prefecture, the largest freshwater lake in central Japan, Lake Biwa, covers $672\ \text{km}^2$. Lake Biwa has two basins: a shallow southern basin (mean depth = 4 m) and a deep northern basin (mean depth = 44 m). The lake's maximum depth is 103.6 m. Lake Biwa is a warm monomictic lake (Fukushima et al., 2019). The northern basin exhibits stratification during spring–autumn and vertical circulation in winter. The vertical circulation extends to the lake bottom during January–February. A seasonal thermocline appears during the stratification period and disappears during the circulation period. During the stratification period, the DO concentration in lake bottom water at the deepest area decreases to a few milligram per liter, although the DO concentration in the lake bottom water exceeds $10\ \text{mg L}^{-1}$ during the circulation period (Yamada et al., 2021). Vertical circulation has been observed every winter since 1979. However, incomplete circulation was observed for the first time in 2019 (Yamada et al., 2021).

For this study, sampling was carried out at two locations in the northern basin (Figure 2a). Station 1 (St. 1) at 90.8 m depth and Station 2 (St. 2) at 49.1 m depth were established with the aim of investigating the response of the microbial sulfur cycle to the intensity of anoxic conditions. Observation and sampling were performed with the R/V *Hasu* belonging to the Center for Ecological Research, Kyoto University on September 27, 2021. The water temperature and DO concentration at the time of sampling are measured using a CTD profiler with a fast response DO sensor (Rinko profiler ASTD 102; JFE Advantech Co. Ltd, Japan) (Figure 2b). At both sites, clear thermoclines were observed at depths of ca. 20 m. A sharp decline in DO concentrations was observed at the thermocline, and then, a more gradual decline was observed approximately 10–20 m above the lake floor at both sites. The DO concentrations decreased with water depth from $7.9\ \text{mg L}^{-1}$ in the epilimnion to 0.5 and $5.8\ \text{mg L}^{-1}$, respectively, just above the lake bottom in St. 1 and 2, respectively.

2.2 | Sampling procedure and sample preparation

Sediment cores were sampled using a push core (50 cm long, 11 cm diameter; HR type core sampler; Rigosha Co. Ltd, Japan) at St. 1 and 2. The gravity push cores were dropped with a line from the ship. Both of the collected sediment cores were ca. 30 cm long. St. 1 core contained white and filamentous materials in the sediment on the top. The sediment cores were cut into 3–5-cm sections from the top on deck. The subsamples were refrigerated and brought back to the onshore laboratory. To minimize core oxidation as much as possible, the sediment subsamples were collected from the center of the core of each section and brought back sealed.

In the laboratory, around 10 g of the subsample sediments were used for AVS extraction on the sampling day, according to the procedures described by Onishi, Shimamura, et al. (2018), Onishi,

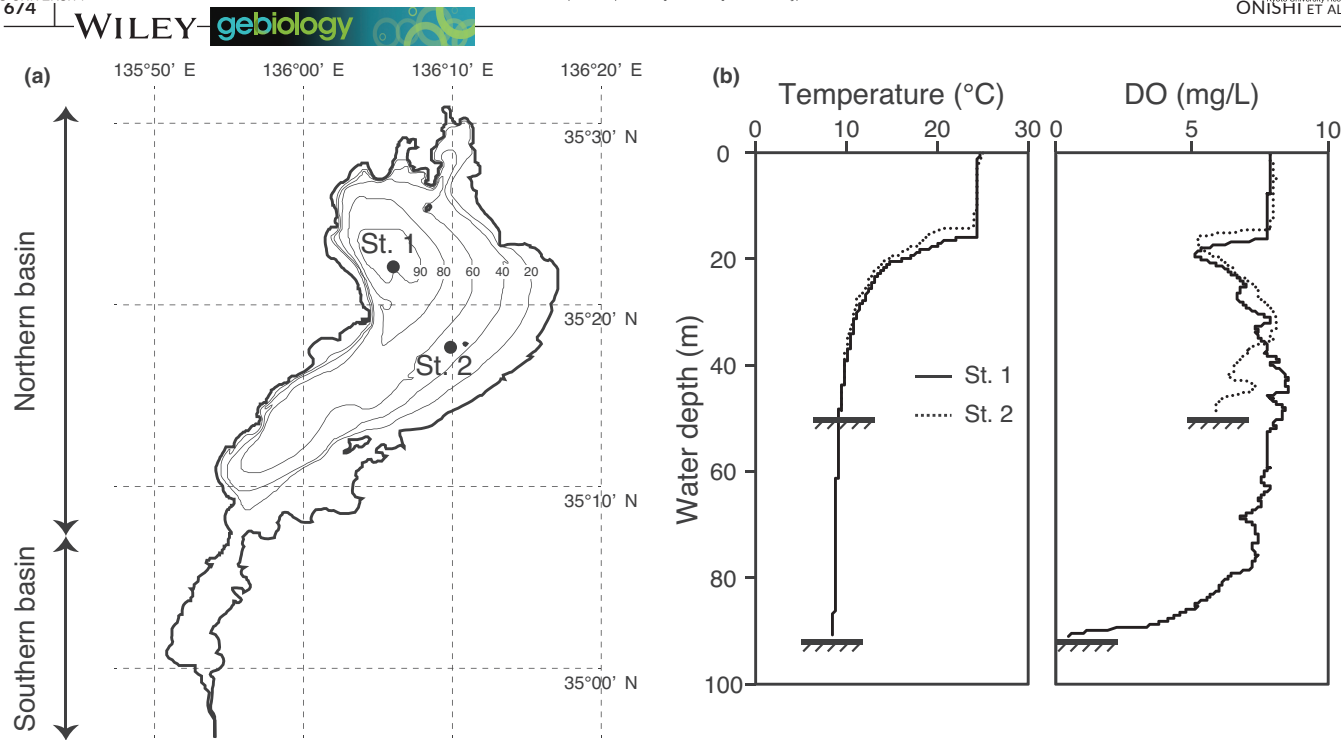


FIGURE 2 (a) Map of the two sampling points for the study in Lake Biwa. (b) Vertical profiles of the water temperature and the dissolved oxygen (DO) concentration of each sampling point. Solid and dashed lines respectively represent data from St. 1 and 2.

Yamanaka, et al. (2018). The AVS in the sediment was extracted as H_2S by addition of 47% sulfuric acid to the sample. The evolved H_2S was introduced into a 0.5 M cadmium acetate aqueous solution with a N_2 stream to give precipitates of cadmium sulfide (CdS). The CdS precipitate was oxidized to sulfate using a few drops of ca. 30% hydrogen peroxide solution, and it was converted into $BaSO_4$ precipitate by the addition of $BaCl_2$ solution.

Porewaters were extracted from about one-quarter of the subsample sediments, according to the squeezing procedure described by Nakaseama et al. (2008). For total organic carbon (TOC), total nitrogen (TN), and total sulfur (TS) analyses, the squeezed cakes were frozen at $-80^\circ C$ until analysis. A part of each subsample was used for water content measurements. Sample preparations were done immediately after recovery of the samples in the laboratory.

For suspended particulate organic matter (POM) analyses, lake water samples were collected with a Niskin bottle (5 L volume; Rigosha Co. Ltd, Japan) at St. 1 and 2. The POM in the lake water samples was subsampled on deck using a glass fiber filter (pore size = $0.5 \mu m$; GC-5; Advantec) that had been heated at ca. $450^\circ C$, 2 h before use. Filtrates were used for sulfate recovery. The filters and filtrates were preserved in a $-80^\circ C$ freezer until analyses were done.

Benthic animals were sampled from the sediment surface (approx. 5 cm sediment depth) using an Ekman–Birge grab ($15 cm \times 15 cm$; Rigosha Co. Ltd, Japan). Animals in the sediment were screened with $500 \mu m$ Surber net. Collections were made four times at each station. In the laboratory, organisms were identified based on their morphology (Ohtaka & Nishino, 1995, 1999) by microscopy. Then they were counted. The organisms were frozen and preserved at $-80^\circ C$ for analyses. The animal samples were freeze-dried. The

biomass of each species ($mg m^{-2}$) was calculated as the average of four samplings.

2.3 | Analytical methods

For TOC and TN measurements, the sediment samples were lyophilized. Then 1 N HCl solution was added to remove carbonate, before finally being dried in vacuo using NaOH pellets as a desiccant. For TS measurement, the dried sediments were washed several times with distilled water to remove sulfate. Then, they were converted into sulfate by heating with a hot hydrogen peroxide solution (Yamanaka et al., 1999). The resulting sulfate was recovered as $BaSO_4$. The resulting $BaSO_4$ precipitates derived from AVS and TS and dried sediment fractions were weighed precisely. Then, the AVS and TS concentrations were calculated ($\mu mol-Sg^{-1}$ -dry sediment). The analytical error, which is based on duplicate measurement of same samples, associated with the overall process was $<5\%$. Concentrations of organic sulfur (Org-S) were calculated by subtracting AVS from TS.

The POM samples were lyophilized. Then, 1 N HCl solution was added to remove carbonate before finally being dried in vacuo using NaOH pellets as a desiccant. Sulfate concentrations in lake water and pore water samples were measured using an ion chromatograph (ICS-1600; Dionex Corp., USA) with greater than 5% accuracy. In addition, the sulfate in lake water was recovered as precipitated $BaSO_4$ (Onishi, Shimamura, et al., 2018; Onishi, Yamanaka, et al., 2018; Yamanaka et al., 1999). The water content of the sediment was found by drying the fresh subsamples (48 h at $105^\circ C$).

For the carbon and nitrogen isotope measurements, the animal samples were treated with 1N HCl, followed by chloroform and methanol mixed solvent (3:1 by volume), to remove carbonates and lipids. They were then lyophilized and pulverized. For S isotope measurements, the animal samples were dialyzed repeatedly using 1M LiCl solution to remove excess lake water sulfate. For S isotope measurements, the animal species were composited: approximately 50 individuals. Each composited sample was measured and averaged. $\delta^{34}\text{S}$ values of POM samples were not measured for this study. However, the $\delta^{34}\text{S}$ value of the POM can be regarded as similar to the $\delta^{34}\text{S}_{\text{sulfate}}$ value because of limited isotope fractionation during sulfate assimilation by phytoplankton (Karube et al., 2012).

The TOC and TN concentrations in the sediments and all carbon and nitrogen isotopic ratios of sediments, POM, and animals were measured using EA/IRMS (Delta V Plus; Thermo Fisher Scientific Inc., Germany). All S isotopic ratios and S contents of BaSO_4 precipitates and animals were measured using an EA/IRMS (IsoPrime EA; GV Instruments Ltd., UK). All isotopic values are expressed using δ notation in per mil deviation (‰) from international reference materials (VPDB for $\delta^{13}\text{C}$, atmospheric N_2 for $\delta^{15}\text{N}$, and CDT for $\delta^{34}\text{S}$). Analyses were done within a month after arrival at the onshore laboratory. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ values were corrected using working reference materials CERKU-02, CERKU-03, and CERKU-05 (Tayasu et al., 2011) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and MSS-2 and MSS-3 (Ueda & Sakai, 1983) for $\delta^{34}\text{S}$. Analytical errors, which are based on measurements of standard materials, associated with the overall processes of these determinations were <5% for quantitative analyses and ± 0.2 , ± 0.3 , and $\pm 0.3\%$, respectively, for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$. The $\delta^{34}\text{S}$ values of Org-S ($\delta^{34}\text{S}_{\text{Org-S}}$) were calculated by considering TS as composed of two components: AVS and Org-S as

$$\delta^{34}\text{S}_{\text{Org-S}} = \frac{[\text{TS}] \times \delta^{34}\text{S}_{\text{TS}} - [\text{AVS}] \times \delta^{34}\text{S}_{\text{AVS}}}{[\text{TS}] - [\text{AVS}]} \quad (1)$$

where [TS] and [AVS], respectively, represent the concentrations of TS and AVS in sediment ($\mu\text{mol-S g}^{-1}$ -dry sediment). Also, $\delta^{34}\text{S}$ represents the $\delta^{34}\text{S}$ values of each S species represented by subscripts.

2.4 | Estimation of sulfide-S contribution

To estimate the contribution of sulfide-S to animals, a simple two-endmember (assuming SOB and POM) isotope mixing model was used (Phillips & Gregg, 2001). This model was often applied to estimate the contribution of methane-derived carbon in a freshwater lake (e.g., Tsuchiya et al., 2020). We modified the model to estimate the contribution of sulfide-S. Because the S isotope discrimination between animal and diet resource is negligible (Fry et al., 1983), the isotope mixing model can be represented simply as shown below:

$$f(\%) = \frac{\delta^{34}\text{S}_{\text{animal}} - \delta^{34}\text{S}_{\text{POM}}}{\delta^{34}\text{S}_{\text{SOB}} - \delta^{34}\text{S}_{\text{POM}}} \times 100 \quad (2)$$

In that equation, $\delta^{34}\text{S}_{\text{animal}}$ was measured as the $\delta^{34}\text{S}$ value of macroinvertebrates, f represents the relative contributions of SOB to the animal S, and $\delta^{34}\text{S}_{\text{SOB}}$ and $\delta^{34}\text{S}_{\text{POM}}$, respectively, denote the $\delta^{34}\text{S}$ values of SOB and POM. Also, $\delta^{34}\text{S}_{\text{SOB}}$ can be calculated using the $\delta^{34}\text{S}$ value of AVS ($\delta^{34}\text{S}_{\text{AVS}}$) and fractionation factor ($\epsilon_{\text{oxidation}}$) during sulfide oxidation by SOB. Because phytoplankton use sulfate as their S resource and because the S isotope fractionation can be assumed to be small (<1‰ in Lake Biwa; Karube et al., 2012), $\delta^{34}\text{S}_{\text{POM}}$ should be similar to $\delta^{34}\text{S}$ values of lake water sulfate ($\delta^{34}\text{S}_{\text{sulfate}}$). Consequently, Equation 2 can be rewritten as presented below:

$$f(\%) = \frac{\delta^{34}\text{S}_{\text{animal}} - \delta^{34}\text{S}_{\text{sulfate}}}{(\delta^{34}\text{S}_{\text{AVS}} + \epsilon_{\text{oxidation}}) - \delta^{34}\text{S}_{\text{sulfate}}} \times 100 \quad (3)$$

For $\delta^{34}\text{S}_{\text{AVS}}$, we used the measured minimal values in the surface sediment (3–8 cm sediment depth) of Lake Biwa in this study (−3.8 and +0.3‰, respectively, at St. 1 and 2; see Results). For $\delta^{34}\text{S}_{\text{sulfate}}$, we used measured values in the epilimnion water of Lake Biwa (+1.1‰; see Results). Lastly, a value of −4‰ was assumed for $\epsilon_{\text{oxidation}}$ (kinetic sulfur isotope fractionation factor during sulfide oxidation), based on the AVS concentration and the $\delta^{34}\text{S}_{\text{AVS}}$ value in the sediment cores (Supporting Information 1: Appendix S1 and Figure 5 in Discussion).

2.5 | Statistical analysis

The isotopic values of animal samples were examined using Mann-Whitney U -tests to evaluate the significance ($p < .05$) of the observed differences.

3 | RESULTS

3.1 | Chemical and isotopic compositions of lake water, sediment, and pore water samples

Sulfate concentrations in the lake bottom water from St. 1 and 2 were, respectively, 65.6 and 67.7 $\mu\text{mol L}^{-1}$. Also, $\delta^{34}\text{S}_{\text{sulfate}}$ were +1.2‰ and +1.0‰, respectively, for St. 1 and 2. These values show good agreement with values obtained from earlier studies (Karube et al., 2012). Because the concentrations and $\delta^{34}\text{S}_{\text{sulfate}}$ values were almost identical between sites and because the Lake Biwa water shows uniformity with respect to sulfate (Nakano et al., 2005), these average values (66.7 $\mu\text{mol L}^{-1}$ and +1.1‰) were regarded as those of the lake water.

The AVS concentrations in the sediments (Figure 3a) were highly variable within cores. They were 3.6–35.7 $\mu\text{mol-S g}^{-1}$ -dry sediment

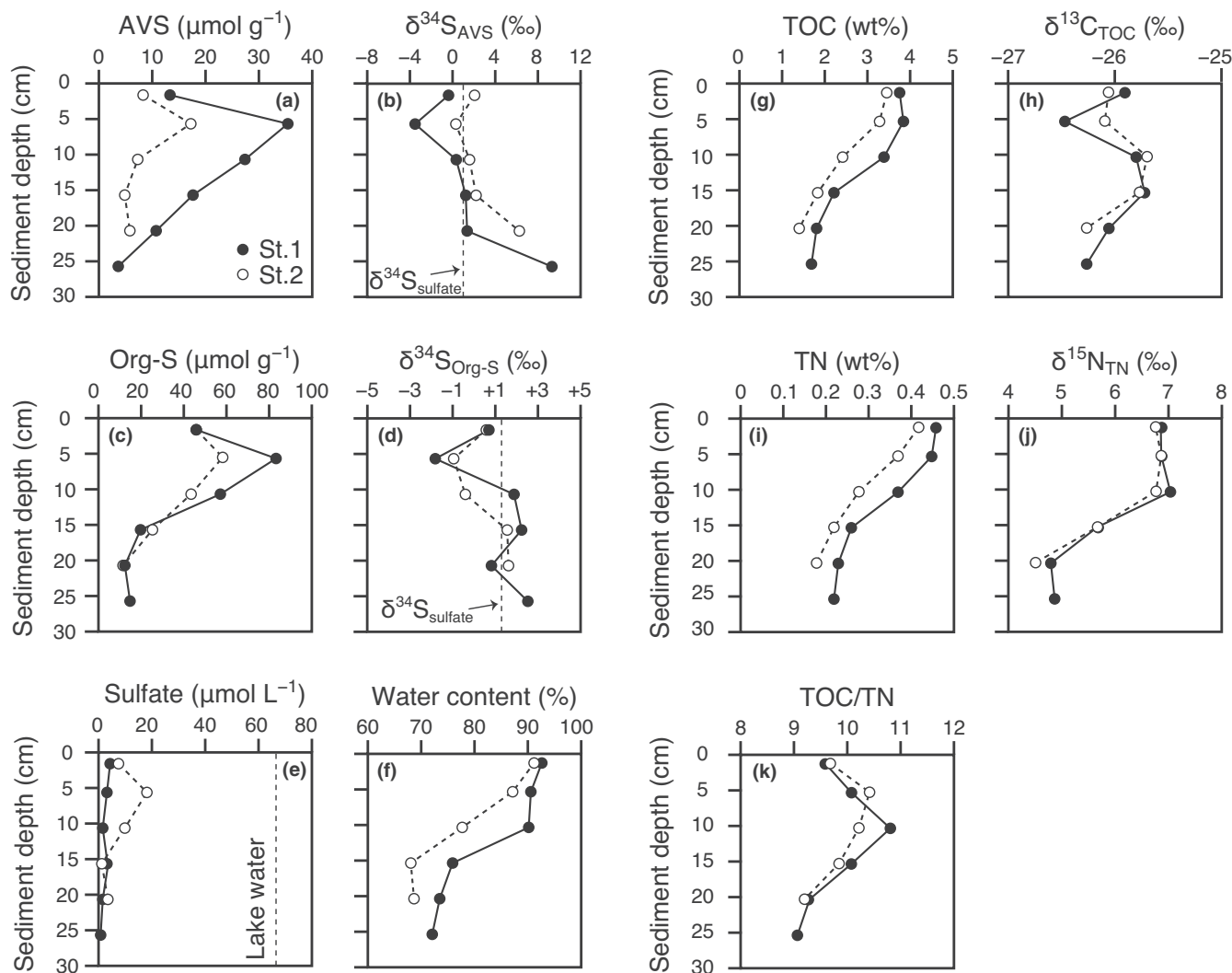


FIGURE 3 Vertical profiles of the chemical and isotopic compositions and water contents of sediment and pore water samples. Black and white symbols with solid and dashed lines, respectively, represent data from core samples obtained from St. 1 and 2.

in the St. 1 core and $4.9\text{--}17.4\ \mu\text{mol-Sg}^{-1}$ -dry sediment in the St. 2 core (Table A1). A peak in the AVS profile was observed in the layer at 3–8 cm below the lake bottom in both cores. $\delta^{34}\text{S}_{\text{AVS}}$ (Figure 3b) showed measured values of -3.8 to $+9.4\text{‰}$ in the St. 1 core and from $+0.3$ to $+6.4\text{‰}$ in the St. 2 core. In each core, the minimum $\delta^{34}\text{S}_{\text{AVS}}$ value was observed in the layer at 3–8 cm below the lake bottom where the AVS concentration was maximum (Figure 3b). These values were lower than the $\delta^{34}\text{S}_{\text{sulfate}}$ value ($+1.1\text{‰}$). However, the maximum $\delta^{34}\text{S}_{\text{AVS}}$ value was at the deepest layers in both cores. These values were higher than the $\delta^{34}\text{S}_{\text{sulfate}}$ value. The Org-S concentrations (Figure 3c) also were highly variable within the St. 1 core: $12.9\text{--}84.3\ \mu\text{mol-Sg}^{-1}$ -dry sediment. A peak in the Org-S profile was observed in the layer 3–8 cm below the lake bottom in both cores. Values of $\delta^{34}\text{S}_{\text{Org-S}}$ (Figure 3d) ranged from -1.8 to $+2.6\text{‰}$ in the St. 1 core and from -1.0 to $+1.7\text{‰}$ in the St. 2 core. In each core, the minimum $\delta^{34}\text{S}_{\text{Org-S}}$ value was observed in the layer 3–8 cm below the lake bottom, where the Org-S concentration was maximum. These values were lower than the $\delta^{34}\text{S}_{\text{sulfate}}$ value ($+1.1\text{‰}$),

whereas the $\delta^{34}\text{S}_{\text{Org-S}}$ values at deeper layers (>8 cm) were similar to the $\delta^{34}\text{S}_{\text{sulfate}}$ value.

Sulfate concentration in porewaters decreased with depth (Figure 3e). The sulfate concentration in the pore waters (<0.1 to $3.9\ \mu\text{molL}^{-1}$ in St. 1 core, <0.1 to $17.7\ \mu\text{molL}^{-1}$ in St. 2 core) were much lower than in the lake water ($66.7\ \mu\text{molL}^{-1}$) although it was possible that some of sulfide in the sediments might have been oxidized because of exposure to the air during the processing of the samples. The water contents of the sediments (Figure 3f) were higher at shallower layers (<13 cm) in both cores. The pore waters were sampled by core squeezing. At this time, if SOB (*Thioploca*) was present, this could rupture their cells, causing an increase in pore-water nitrate, and potentially sulfate. However, we could not detect nitrate in the pore waters with colorimetric analysis (detection limit is $<0.1\ \mu\text{molL}^{-1}$). Thus, even if *Thioploca* cells were involved in the squeezed sediments, the effect was below our detection limits.

The TOC and TN concentrations in both cores were higher in shallower layers (<8 cm); they decreased downward. The $\delta^{13}\text{C}_{\text{TOC}}$

values were almost constant throughout the core (−26.9 to −25.4‰ and −26.5 to −25.4‰ for St. 1 and 2, respectively), whereas the $\delta^{15}\text{N}_{\text{TN}}$ values were constant at +6.8 to +7.1‰ in shallower layers (<13 cm) and then decreasing in deeper layers (>13 cm) down to ca. +5‰. The TOC/TN atomic ratios (Figure 3k) were fairly uniform throughout the cores: 9.1–10.8.

3.2 | Biomass and isotopic compositions of benthic species

At St. 1, three benthic animal species were found (Table 1). The biomass of the oligochaete *Tubifex tubifex* was the greatest (8.5 mg m^{−2}) among these species. The next greatest biomass was that of another oligochaete, *Branchiura sowerbyi* (5.1 mg m^{−2}). These oligochaetes accounted for more than 90% of the total biomass, but the biomass of Chironomids was small (0.3 mg m^{−2}). At St. 2, six benthic animal species were found (Table 1). The biomass of Chironomids was the greatest (14.8 mg m^{−2}) among these species, accounting for 44.1% of the total biomass in St. 2. The biomass of oligochaetes was large (5.8 mg m^{−2} for *T. tubifex* and 7.4 mg m^{−2} for *B. sowerbyi*), accounting for 40.0% of the total biomass. The biomass of *Jesogammarus annandalei* (5.1 mg m^{−2} and 15.4% of total biomass) was also large in St. 2. The biomasses of a species of bivalves (0.2 mg m^{−2} and 0.7% of total biomass) and *Kamaka biwae* (0.1 mg m^{−2} and 0.3% of total biomass) were small.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the POM samples were $-27.0 \pm 0.4\%$ and $+6.5 \pm 0.3\%$ (mean \pm standard deviation; Figure 4). Oligochaetes (*T. tubifex* and *B. sowerbyi*) in St. 1 had similar $\delta^{13}\text{C}$ values (−27.2‰: −27.9 to −26.5‰ and −26.1‰, mean: range, respectively) to those of POM (Mann–Whitney U test; $p = .257$), although their $\delta^{15}\text{N}$ values (+10.3‰: +9.5 to +10.8‰ and +12.3‰, respectively) were 3.8 and 5.8‰ higher, respectively, than that of the POM ($p < .05$) (Table 1; Figure 4). The $\delta^{34}\text{S}$ values of these taxa (−1.0‰: −1.1 to −0.9‰ and −7.3‰, respectively) yielded f -values (the estimated relative contribution of sulfide-S to animal S based on these $\delta^{34}\text{S}$ values; Equation 3), respectively, of 24.4 and 97.7%. Chironomid

larvae in St. 1 had low $\delta^{13}\text{C}$ (−39.9‰) and $\delta^{15}\text{N}$ (+6.1‰) values. The $\delta^{34}\text{S}$ values of the Chironomid larvae were not measured because of their small sample size. The $\delta^{13}\text{C}$ values in all taxa, except for Chironomid larvae, in St. 2 (−26.6‰: −27.3 to −25.8‰ for *B. sowerbyi* to −21.9‰ for *J. annandalei*) were slightly higher than those of the POM ($p < .01$), whereas their $\delta^{15}\text{N}$ values (+8.9‰: +7.9 to +9.5‰ for *B. sowerbyi* to +11.6‰ for *K. biwae*) were 2.4 to 5.1‰ higher than that of the POM ($p < .01$). The Chironomid larvae in St. 2 had low $\delta^{13}\text{C}$ (−35.6‰: −43.1‰ to −25.8‰) and similar $\delta^{15}\text{N}$ (+6.5‰: +3.8 to +11.2‰) values compared with those of the POM ($p < .05$ and $p = .133$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively). The $\delta^{34}\text{S}$ values of *T. tubifex* (−1.0‰: −2.1 to 0.0‰), *B. sowerbyi* (−4.4‰), and Chironomid larvae (−2.6‰: −2.6 to −2.5‰) were lower than that of the sulfate ($p < .05$), leading to high f -values (46.7, 100, and 82.2%, respectively), whereas the $\delta^{34}\text{S}$ values of *J. annandalei* (+2.6‰: +1.9 to +3.4‰) were similar to that of the sulfate ($p = .17$), with an f -value of <0%. Sulfur contents in bulk tissues were similar among all taxa (0.93 wt% for *B. sowerbyi* at St. 1 to 0.46 wt% for Chironomid at St. 2; Table 1).

4 | DISCUSSION

Vertical profiles of $\delta^{13}\text{C}_{\text{TOC}}$ and $\delta^{15}\text{N}_{\text{TN}}$ values and TOC/TN atomic ratio are fairly consistent between and within the cores (Figure 3), suggesting that the source of sedimentary organic matter on the bottom of Lake Biwa is similar, at water depths of 50 m or more. The $\delta^{13}\text{C}_{\text{TOC}}$ and $\delta^{15}\text{N}_{\text{TN}}$ values in the sedimentary layer at <13 cm below the lake bottom in both cores were similar to those of POM in this study (−27.0 \pm 0.4‰ and +6.5 \pm 0.3‰, respectively). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the POM are consistent with those of phytoplankton in the epilimnion of Lake Biwa (ca. −26‰ and ca. +6‰, respectively; Okuda et al., 2020), suggesting that the measured values of the POM samples can be regarded as those of phytoplankton. The correspondence of isotopic ratios between the surface sediment and POM indicates that the sedimentary organic matter consists mainly of phytodetritus such as phytoplankton. The TOC and TN concentrations are higher near the sediment surface, implying

TABLE 1 Analytical results of biomass, isotopic compositions, and S contents of animal samples.

Station	Fauna	Class	Biomass (mg m ^{−2})	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{34}\text{S}$ (‰)	S content (wt%)	f^a (%)
1	<i>T. tubifex</i>	Oligochaeta	8.5	−27.2 \pm 0.5	+10.3 \pm 0.5	−1.0 \pm 0.2	0.67 \pm 0.04	24
	<i>B. sowerbyi</i>	Oligochaeta	5.1	−26.1	+12.3	−7.3	0.93	98
	Chironomid	Insecta	0.3	−39.9	+6.1	n.d.	n.d.	−
2	Chironomid	Insecta	14.8	−35.6 \pm 6.4	+6.5 \pm 2.8	−2.6 \pm 0.1	0.46 \pm 0.01	77
	<i>B. sowerbyi</i>	Oligochaeta	7.4	−26.6 \pm 0.6	+10.1 \pm 0.1	−4.4	0.69	114
	<i>T. tubifex</i>	Oligochaeta	5.8	−26.2 \pm 1.2	+8.9 \pm 0.6	−1.0 \pm 1.5	0.61 \pm 0.10	47
	<i>J. annandalei</i>	Malacostraca	5.1	−21.9	+11.1	+2.6 \pm 1.1	0.51 \pm 0.01	−30
	Bivalves	Bivalvia	0.2	−24.7 \pm 1.7	+9.1 \pm 0.1	n.d.	n.d.	−
	<i>K. biwae</i>	Malacostraca	0.1	−25.0	+11.6	n.d.	n.d.	−

Abbreviation: n.d., not determined.

^a f = relative contribution of sulfide-S to benthic animal S.

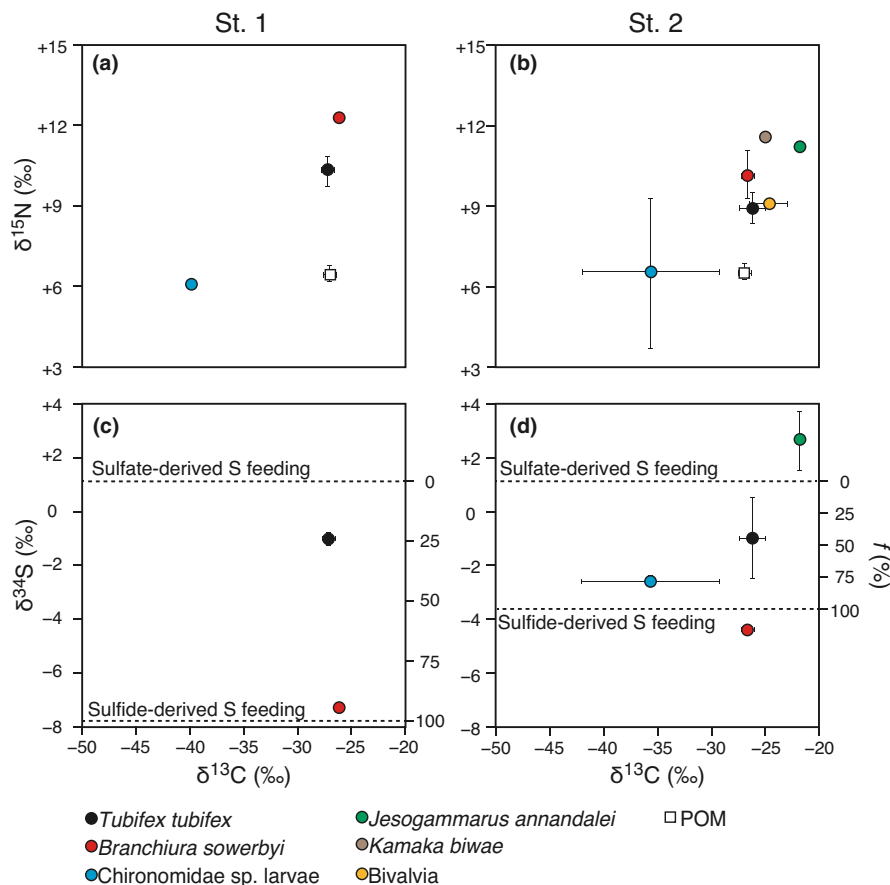


FIGURE 4 Plots of carbon versus nitrogen and carbon versus sulfur isotopic compositions and f -value (relative contribution of sulfide-S) to their primary sulfur source of animal samples obtained from St. 1 (a and c) and St. 2 (b and d). The second y-axis was calculated by Equation 3 based on their $\delta^{34}\text{S}$ values measured.

that microbes degrade the sedimentary organic matter over time. These profiles are consistent with the results of an earlier study using sediment cores from 85 m water depth in Lake Biwa (Ogawa et al., 2001).

Similarly to the TOC and TN concentrations, the vertical profile of Org-S concentrations (Figure 3c) also showed a tendency to be lower in the deeper layers and higher toward the top layer. This trend might be explained by an microbial decomposition of Org-S (putrefaction), as explained above. Indeed, the $\delta^{34}\text{S}_{\text{Org-S}}$ values are similar to the $\delta^{34}\text{S}_{\text{sulfate}}$ values through the cores, indicating that most Org-S in the layers is derived from phytodetritus.

In contrast to the vertical profiles of concentrations and isotope ratios of TOC and Org-S, the AVS concentrations and $\delta^{34}\text{S}_{\text{AVS}}$ values in sediments varied vertically within and between the cores. The trend is readily apparent for St. 1 core, where lake water DO concentrations were lower than those from St. 2. The profiles are characterized by an increase in AVS with low $\delta^{34}\text{S}_{\text{AVS}}$ values (-3.8‰ and +0.3‰ in St. 1 and 2 respectively; Figure 3b) in the layer at 3–8 cm below the lake bottom in both sediment cores. Based on the $\delta^{34}\text{S}_{\text{AVS}}$ values relative to lake water sulfate (Figure 3b), we discern different biogeochemical zones: a sulfide production zone in the layer at 3–8 cm below the lake bottom and a sulfide consumption zone in the layer at >8 cm below the lake bottom. The discussion hereinafter addresses the use of $\delta^{34}\text{S}_{\text{AVS}}$ in sediment as a proxy of microbial processes that control the cycling of sulfur within these zones.

4.1 | Vertical profiles of concentrations and $\delta^{34}\text{S}$ values of AVS and Org-S

4.1.1 | Sulfide production zone: surface layer in the cores

The highest AVS concentrations and lowest $\delta^{34}\text{S}_{\text{AVS}}$ values were found in the layer 3–8 cm below the lake bottom in each core. The peak depth of the AVS concentrations in both cores correspond to the depth with the highest rate of MSR in Lake Biwa as mentioned by Koizumi et al. (2003), suggesting that sediments in the layer at 3–8 cm below the lake bottom at the same site to this study are the most active depth of sulfide production. Occurrence of MSR activity in the sediments is also suggested by the much lower concentration of sulfate in the porewaters at the sediment depth (up to $17.7 \mu\text{mol L}^{-1}$; Figure 3e) compared with lake water ($66.7 \mu\text{mol L}^{-1}$). However, in addition to MSR, putrefaction of Org-S (e.g., methionine and cysteine) might also be the other important process yielding AVS in freshwater lake sediments (Fry, 1986; Jones et al., 1982; King & Klug, 1982). We have therefore estimated the contributions of these two processes (MSR and putrefaction) to sulfide production in sediments under several assumptions.

Our estimates revealed that assuming the sediments as a completely closed system with respect to S does not adequately explain the observed profiles of the concentration and isotope ratio of AVS (Supporting Information 2: Appendix S1). Assuming a closed system

for S, sulfate consumption per gram of wet sediment (0.058 and 0.043 $\mu\text{mol-Sg}^{-1}$ -wet sediment, respectively, at St. 1 and 2), which is calculated from the difference in sulfate concentrations between the pore water and lake water, can only explain <5% of the sulfide production per gram of wet sediment (3.4 and 2.2 $\mu\text{mol-Sg}^{-1}$ -wet sediment in St. 1 and 2, respectively; Supporting Information 2: Appendix S1). Furthermore, because most of the sulfate in the pore water is consumed at this time, the $\delta^{34}\text{S}_{\text{AVS}}$ values of sulfide produced by the MSR is expected to be equivalent to the $\delta^{34}\text{S}$ values of original sulfate (i.e., $\delta^{34}\text{S}_{\text{sulfate}}$) when sulfate is the sole substrate for MSR. Therefore, the MSR in the closed sediment cannot yield lower observed $\delta^{34}\text{S}_{\text{AVS}}$ values than those of $\delta^{34}\text{S}_{\text{sulfate}}$. However, it is expected that $\delta^{34}\text{S}$ values of sulfide produced by putrefaction are almost equal to $\delta^{34}\text{S}$ values of the organic matter being decomposed because of the small isotope fractionation ($\epsilon_{\text{putrefaction}} = \text{ca. } 0\text{‰}$; Fry, 1986). Most of the sedimentary organic matter in Lake Biwa is particles of phytoplankton sinking from the epilimnion. Because the $\delta^{34}\text{S}$ value of organic matter derived from phytoplankton is similar to that of lake water sulfate (Karube et al., 2012), the putrefaction-derived sulfide can be expected to be equivalent to $\delta^{34}\text{S}_{\text{sulfate}}$. Therefore, putrefaction is also inadequate to explain the observed low $\delta^{34}\text{S}_{\text{AVS}}$. It is therefore more appropriate to regard the sediments as a semi-closed system, controlled by sulfate diffusion, rather than completely closed system associated with the sulfate.

Our estimation revealed that assuming the sediments as a completely open system with respect to sulfate yield can show the contribution of MSR to sulfide production in the sediment (Supporting Information 3: Appendix S1). The estimation yields the contribution of MSR (the A values in the Supporting Information 3: Appendix S1) of 28% and 5% in St. 1 and 2, respectively (Table 2). Nriagu (1968) observed that about 45% of sulfide in Lake Mendota (USA) originated from putrefaction based on the difference between observed Org-S concentrations in sediment and S content of organic matter supplied to the sediment. Experiments conducted by Jones and Simon (1980) revealed, based on production of sulfide and loss of sulfate, that 40%–50% of the sulfide production in the lake sediments of Blelham Tarn (UK) originate from MSR. Comparing values from these earlier studies and this study, it might be reasonable for our estimated value that the contribution of MSR is lower than that of putrefaction if water temperature effects on MSR and putrefaction (Nedwell & Floodgate, 1972) are taken into account. It is noteworthy that this estimate includes uncertainty because the sediments are actually

semi-closed systems rather than open systems, and because we ignore sulfide consumption that would have occurred simultaneously in the sediment. In practice, AVS consumption is also regarded as occurring simultaneously with AVS production, so the contribution of MSR to sulfide production (the A value) estimated for this study is the minimum contribution.

4.1.2 | Sulfide consumption zone: deeper layer in the cores

The $\delta^{34}\text{S}_{\text{AVS}}$ values in deeper layers of the cores in this study were found to be higher than $\delta^{34}\text{S}_{\text{sulfate}}$ values in lake water, suggesting that active AVS consumption occurs in the deeper sediment. The higher $\delta^{34}\text{S}_{\text{AVS}}$ value than $\delta^{34}\text{S}_{\text{sulfate}}$ implies negative isotope fractionation involved with AVS consumption because sulfate reduction rate at deeper layers (>14 cm) in Lake Biwa sediments is extremely low (Koizumi et al., 2003). Therefore, the profiles of AVS concentrations and $\delta^{34}\text{S}_{\text{AVS}}$ values in this study suggest that AVS generated in sediments in the layer at 3–8 cm below the lake bottom are consumed with progress of burial to deeper layers. This trend has been observed for previous studies of mesotrophic Lake Biwa (Maeda & Kawai, 1988; Mitamura et al., 2015; Murphy et al., 2001) and earlier studies of eutrophic Lake Mendota (Nriagu, 1968), oligotrophic freshwater lakes of the Adirondack Mountains (Fry, 1986), and oligotrophic to mesotrophic Lake Constance (Bak & Pfennig, 1991).

To calculate the contributions of sulfide-S to animal S resources, we roughly ascertained the S isotope fractionation factor during sulfide oxidation ($\epsilon_{\text{oxidation}}$). Linear correlation between AVS concentrations and the corresponding $\delta^{34}\text{S}_{\text{AVS}}$ values of all the data (Supporting Information 1: Appendix S1 and Figure 5) suggest that the $\epsilon_{\text{oxidation}}$ is estimated as $-4.0 \pm 0.8\text{‰}$ (mean \pm standard error). The estimated $\epsilon_{\text{oxidation}}$ values are within the range obtained from a laboratory culture study of SOB (-5.2 to $+2\text{‰}$; Balci et al., 2007; Fry et al., 1984; Hubert et al., 2009; Kaplan & Rafter, 1958; Poster et al., 2014; Thurston et al., 2010; Toran & Harris, 1989). As this study did not observe the microbial communities associated with sulfide oxidation, it is not unclear what type of SOB actually drive the sulfide oxidation. However, particularly good agreement of our estimated $\epsilon_{\text{oxidation}}$ value (-4.0‰) can be found with the fractionation factor (-4.3‰) obtained from freshwater nitrate-reducing sulfide-oxidizers (Poster et al., 2014). In

TABLE 2 Contributions of MSR- and putrefaction-derived sulfide to total sulfide in freshwater sediment.

Lake	Water temp. of lake bottom at sampling time	Proportion to total sulfide production		References
		MSR	Putrefaction	
Lake Biwa (St. 1)	<10°C	28%	72%	This study
Lake Biwa (St. 2)	<10°C	5%	95%	This study
Lake Mendota	ca. 25°C (summer)		ca. 45%	Nriagu (1968)
Blelham Turn	10–12°C	40%–50%		Jones and Simon (1980)

Lake Biwa, a dense bacterial mat of the sulfide-oxidizer, *Thioploca* sp., is widely distributed in and on sediments in the profundal zone at water depth >50 m (up to 37 m-trichome cm⁻³-sediment and 0.09 m-trichome cm⁻³-sediment in the layer at 0–2 cm and 11–14 cm below the lake bottom at an area with 90 m water depth; Furuta et al., 2020; Mitamura et al., 2015; Nishino et al., 1998). They can oxidize sulfide with nitrate accumulated in their cells (Fossing et al., 1995; Kojima et al., 2015). The genus *Thioploca*, of gliding filamentous bacteria, includes species that can oxidize sulfides in deeper sediments (>8 cm) by gliding through the sheath (Jørgensen & Gallardo, 1999). Therefore, the almost identical $\epsilon_{\text{oxidation}}$ values to those observed from this study and that determined from the culture experiment (Poster et al., 2014) suggest that the vertical profiles of AVS concentrations and $\delta^{34}\text{S}_{\text{AVS}}$ values in Lake Biwa sediments are explainable by the sulfide oxidation by SOB such as *Thioploca* sp.

The AVS concentrations were lower in the deeper layers than in the surface layers, suggesting AVS consumption at the deeper layers. Although the AVS consumption process is not clear, sulfide consumption by SOB was assumed for this study as explained above. Consumed AVS should be incorporated into Org-S as SOB biomass (Mizota & Maki, 1999) or it should be eventually returned to pore water as sulfate. However, in the present study, the sulfate concentrations in the pore water were low even in the deeper sediments (Figure 3e). Therefore, sulfate produced by the oxidation might be leaking out of the system. The upward movement of pore water might be causing the sulfate generated at depth to migrate to shallower areas. Indeed, sulfate concentration in the layer at 3–8 cm below the lake bottom at St. 2 were slightly higher than those at other depths (Figure 3e). Because MSR is active at this depth, the sulfate concentration may be due to the migration of sulfate produced by sulfide oxidation at deeper layers. The migrated sulfate from deeper is likely to be used for MSR in the shallower layers, and be eventually leaked out the system.

4.2 | Contributions of sulfide-S to S nutrient resources for benthic animals

According to the $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ plots (Figure 5), all animals except Chironomids had similar $\delta^{13}\text{C}$ values and ca. +2 to +6‰ higher $\delta^{15}\text{N}$ values than those of POM, suggesting that they directly or indirectly rely mainly on phytodetritus from epilimnion as their diet resource. However, the $\delta^{13}\text{C}$ values of Chironomids in both St. 1 and 2 are sufficiently lower than POM. Such low $\delta^{13}\text{C}$ values of Chironomids might be attributed to contribution of MOB production. In fact, MOB are widely known as a diet resource for benthic animals, especially Chironomids, in lake sediments (Grey, 2016; Jones & Grey, 2011). These results, indicating that the oligochaetes and Chironomids in Lake Biwa rely on phytodetritus and/or MOB, correspond with results reported from an earlier study (Kiyashko et al., 2001).

In contrast to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, $\delta^{34}\text{S}$ values vary depending on the animal's primary S resources and therefore represent its dependence on SOB. Our evaluation of sulfide-S contribution to S resource for benthic animals (the *f*-value in Equation 3) revealed that the *f*-values of *Branchiura sowerbyi* and *Tubifex tubifex* at St. 1 were, respectively, 98% and 24%. The *f*-values suggest that phytodetritus was not the unique S resource for *B. sowerbyi* and *T. tubifex* sampled at St. 1. The high contribution of sulfide to *B. sowerbyi* suggests that they acquire sulfur nutrients primarily from chemosynthetic organic matter, whereas *T. tubifex* might rely on both chemosynthetic organic matter and phytodetritus in the sediment for their S resource. Of the animals collected from St. 1, they accounted for >90% of the biomass at St. 1. Consequently, chemosynthetic organic matter plays an important role in the nutrition of the benthic food web at St. 1, although $\delta^{34}\text{S}$ values for Chironomids have not been measured. In St. 2, the *f*-value of *B. sowerbyi*, *T. tubifex*, and Chironomids were 47%–114%. As their S resources, they might rely completely or partly on both chemosynthetic organic matter of both types in the sediment. In contrast, the *f*-value of *J. annandalei* (–30%) could not be calculated. However, its negative value suggests that they feed primarily on phytodetritus without any dependence on chemosynthetic products. Consequently, chemosynthetic organic matter plays an important role in the nutrition of the benthic food web, although the sulfide contributions vary among animal species.

The different sulfide-S contributions of different animal species observed for this study are likely to reflect the feeding behavior of the animals. The two oligochaetes that had the highest biomass at St. 1 possess high hypoxia tolerance (Volpers & Neumann, 2005). For example, the tubifex worm can survive for 16 days under anoxia (Chapman et al., 1982). Furthermore, their high hypoxia tolerance allows them to bury their heads in the organic-rich anoxic sediment (up to 20 cm and 10 cm for *B. sowerbyi* and *T. tubifex*, respectively; Matisoff et al., 1999) and feed on organic detritus and bacteria in the sediment (Rodriguez et al., 2001). Thus, they may be able to feed on chemosynthetic organic matter in the sediment. Although it is well known that chironomids in freshwater feed on methane-oxidizing bacteria in sediments (Agasild et al., 2014; Bunn & Boon, 1993; Jones et al., 1999, 2008; Kiyashko et al., 2001), their reliance on chemosynthetic organic matter has not been well established. However, their bacterial feeding ability may assist their reliance on chemosynthetic organic matter. On the contrary, the *J. annandalei* identified in St. 2 inhabits the sediment surface during the day and vertically migrates to 15–20 m water depth at night for feeding on the plentiful copepod and branchiopod species found in the epilimnion (Trevorrow & Tanaka, 1997). Therefore, they are likely to have more reliance on the photosynthetic products than other benthic animals.

Our quantitative data on the dependence on sulfide-S in each fauna showed that the sulfide-S is also a fairly important nutritional resource for the entire benthic food web. We calculated the proportion of sulfide-S to total body-S in the communities from the

mean biomass, S contents, and dependence on sulfide-S in dominant fauna. Chironomids at St. 1 and bivalves and *K. biwae* at St. 2 were excluded from the calculations because their biomasses accounted for a fairly small proportion of the total biomass (Table 1). The calculations revealed that 58% and 67% of the total body-S in St. 1 and 2 communities, respectively, were derived from sulfide in the sediments. Therefore, the chemosynthetic products by SOB are an

important nutritional resource for the entire benthic food web in the Lake Biwa, whereas MSR and putrefaction are also important as an energy resource to drive chemosynthesis.

5 | CONCLUSIONS

For this study investigating the relation between microbial S cycle and the S resources of benthic food web, sediment cores collected at two sites with different DO concentrations during the stratification period of Lake Biwa. Results show that at more anoxic St. 1, the microbial S cycle is actively operating within the sediments. It has a strong influence on the S resources of benthic food web at the site. Organic carbon and nitrogen deposited from the epilimnion decompose monotonically in the anoxic sediment and are lost out from the system as CO₂ and N₂/N₂O (Reddy & DeLaune, 2008). In contrast, S from the water column enters the sediment as Org-S of sinking particles and sulfate in the lake water and reduces to sulfide by putrefaction and MSR at 5 cm sediment depth (Figure 6). However, loss of the sulfide from the system is difficult because it is recycled in situ by SOB as technically elemental sulfur (Fike et al., 2015). The S recycling provides Org-S that is also available to benthic animals. For that reason, unlike carbon and nitrogen, recycling of S in sediments serves an important function as a nutrient supply for benthic food webs. Biogeochemical redox reactions of S in sediments drive this S recycling. The redox reactions ensure a nutrient S resource for benthic animals, making less abundant S efficiently available throughout the lake. These findings suggest that sulfide oxidation by the putatively nitrate-reducing sulfur-oxidizer *Thioploca* in Lake Biwa sediments might provide a sulfur nutritional resource for benthic animals. Therefore, observations targeting nitrogen in sediments are

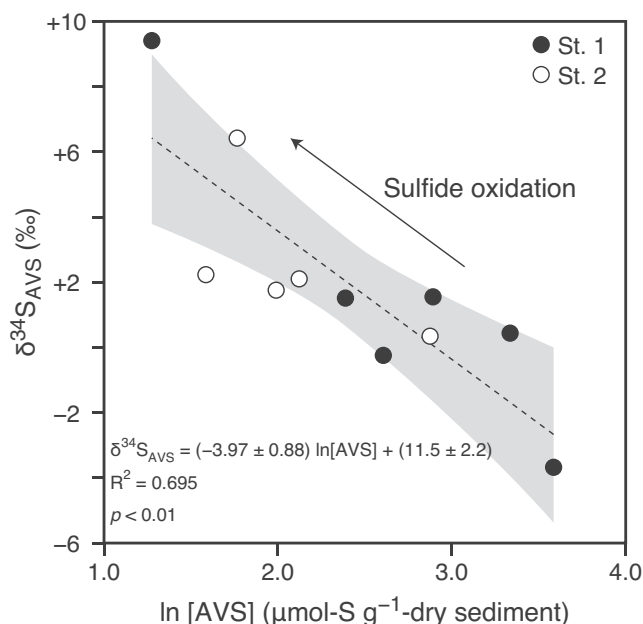


FIGURE 5 Acid volatile sulfide (AVS) concentrations ([AVS]) versus the corresponding isotopic ratios ($\delta^{34}\text{S}_{\text{AVS}}$) from sediment samples taken from St. 1 and 2. The dashed line ($\delta^{34}\text{S}_{\text{AVS}} = (-4.0 \pm 0.84) \ln[\text{AVS}] + (11.7 \pm 2.1)$; $R^2 = 0.721$) is the regression line for all data in the plot.

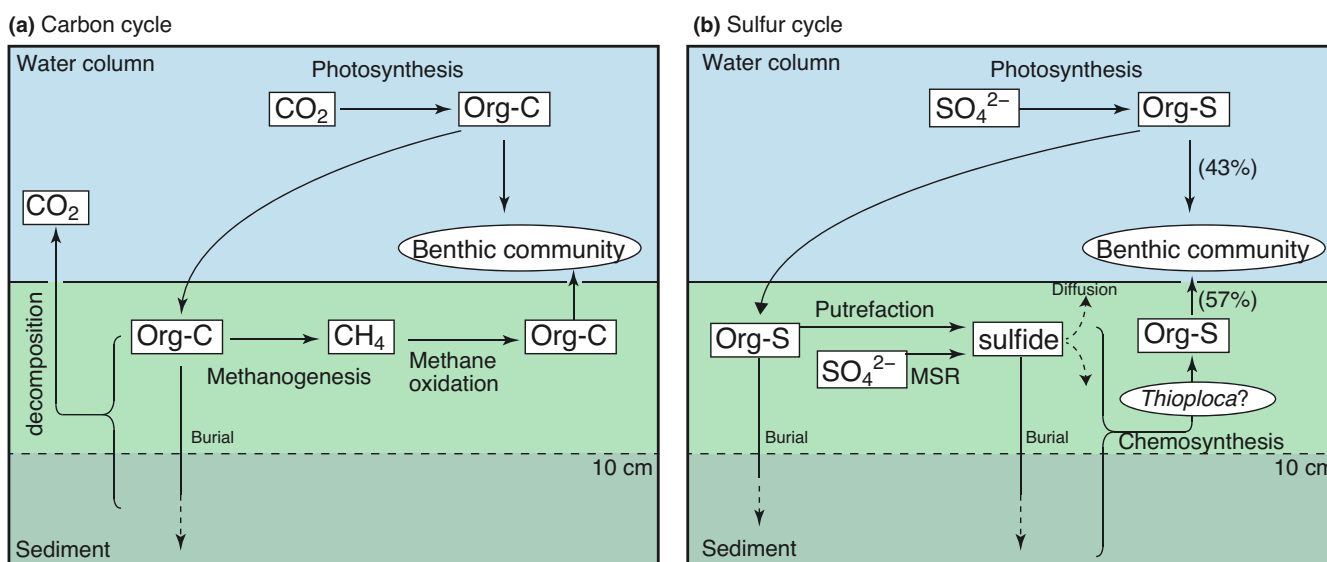


FIGURE 6 Schematic diagrams showing (a) carbon and (b) sulfur cycles in Lake Biwa sediment in this study. The carbon cycle (a) revealed in this study corresponds with those found in earlier studies. The percentages in the sulfur cycle (b) represent the contributions of the respective S resources to total body-S in the St. 1 community.

expected to lead to a more detailed assessment of the role of microbial activity in sediments on benthic fauna.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding authors (YO) upon reasonable request.

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SUPPORTING INFORMATION

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APPENDIX

TABLE A.1 Analytical results of chemical and isotopic compositions in sediment and porewater and water content.

Sediment depth (cm)	AVS ($\mu\text{mol-S g}^{-1}$ dry sediment)	$\delta^{34}\text{S}_{\text{AVS}}$ (‰)	Org-S ($\mu\text{mol-S g}^{-1}$ dry sediment)	$\delta^{34}\text{S}_{\text{Org-S}}$ (‰)	Sulfate ($\mu\text{mol L}^{-1}$)	Water content (%)	TOC (wt%)	$\delta^{13}\text{C}_{\text{TOC}}$ (‰)	TN (wt%)	$\delta^{15}\text{N}_{\text{TN}}$ (‰)	TOC/TN (atomic ratio)	Org-S/TOC (atomic ratio)
St. 1												
0-3	13.4	-0.3	46.6	+0.6	3.9	93.1	3.8	-25.8	0.46	+6.9	9.6	0.015
3-8	35.7	-3.8	84.3	-1.8	2.5	90.5	3.9	-26.9	0.45	+6.9	10.1	0.026
8-13	27.6	+0.4	57.8	+1.9	<0.1	90.4	3.4	-25.6	0.37	+7.1	10.8	0.020
13-18	17.7	+1.5	20.3	+2.2	2.4	75.7	2.3	-25.4	0.26	+5.7	10.1	0.011
18-23	10.8	+1.5	12.9	+0.8	1.9	73.4	1.8	-26.1	0.23	+4.8	9.3	0.008
23-28	3.6	+9.4	15.2	+2.6	<0.1	72.4	1.7	-26.5	0.22	+4.9	9.1	0.011
St. 2												
0-3	8.3	+2.4	46.9	+0.6	6.9	91.6	3.5	-26.1	0.42	+6.8	9.7	0.016
3-8	17.4	+0.3	59.1	-1.0	17.7	87.5	3.3	-26.2	0.37	+6.9	10.4	0.022
8-13	7.3	+1.9	44.0	-0.4	9.3	78.0	2.5	-25.4	0.28	+6.8	10.2	0.022
13-18	4.9	+2.5	26.1	+1.6	<0.1	68.2	1.9	-25.5	0.22	+5.7	9.9	0.017
18-23	5.9	+6.4	12.4	+1.7	2.6	68.9	1.4	-26.5	0.18	+4.5	9.2	0.011