

Response to Reviewer #1

General comments.

Berg et al. present an interesting and mostly original study on sulfur transformations and sulfur isotope partitioning into different inorganic and organic sulfur fractions in a previously well studied Swiss lake. The isotope geochemical study includes microbiological informations, which is nice, but of secondary importance, since it represents the present situation and not necessarily for the 'paleo'-ones that are responsible for the formation of benthic geochemical signatures. Lago Cadagno is well known for its unusual element cycling, including the development of euxinic conditions due to a groundwater derived carbonate-buffered substantial sulfate supply derived from gypsum and dolomite dissolution in the catchment area.

I suggest to change the title and remove 'active', since no rates measurements of the current sulfur transition have been carried out and are only deduced from sediment solid phase parameters. What is missing is a competent discussion of the oxidative part of the sulfur cycle and relevant associated sulfur cycling.

It is true that rates have not been measured, though steep gradients of redox-active compounds along with clear isotopic signatures in these pools are telling evidence of microbial activity in surface sediments and at the sediment-aquifer transition. We have removed 'active' from the title. We have also added a discussion of sulfur oxidation, especially as a contribution to light sulfate isotopes in deep, glacial sediments to the extent possible in the absence of *soxB* gene identities. We have also cited the 16S dataset of Berg et al (2022) in which some sulfide oxidizers were recovered.

The presentation of data, for instance in the supplementary data, file has to be substantially improved. The labels at X axes of TIS, TOC etc. are missing, the label at the $\delta^{34}\text{S}$ profiles from 1991 are not of publishable format/quality. In general, the basic geochemical data should be presented in the main text.

The supplementary figures have now been imported into the main manuscript (Figure 2). We have ensured that all the axes are correctly labeled and are logically presented in terms of dissolved versus solid phase compounds. We apologize for the poor quality of the 1991 data, which was stored on an original floppy disk. The data has now been retrieved and overlain on the 2019 dataset revealing a high level of consistencies. The surface and deep sediments are now presented in separate figures for better visualization (axes scales).

Detailed comments:

- I suggest to add the fundamental PhD thesis of Losher (1989) on S cycling in Lago di Cadagno into the introduction and reference list.

The PhD thesis of Losher (1989) has now been cited in the introduction.

-L33: ...catchment groundwater... instead of 'aquifer groundwater'

This was changed from 'aquifer water' to 'groundwater'. Analyses were made on water collected directly from the subsurface springs.

-L33: Is the $\delta^{34}\text{S}$ of lake water column sulfate really constant?

According to Canfield et al. (2010) water column sulfate signatures change the most at the redoxcline, but are relatively constant within the deep, anoxic water which is in contact with the sediment. We have now specified 'compared to sulfate in the lake monimolimnion (+24 ‰)'.

-L34: d34S values for dissolved sulfate in the deduced steep gradient are not measured, but only deduced. Therefore, this statement on apparent isotope enrichment factors is a substantial oversimplification.

The d34S values for dissolved sulfate were in fact measured, but from pooled samples. This simply means that there is a lower depth resolution. The deduction to be made is that the measured d34S of groundwater sulfate seeping into the lake is the same as the sulfate seeping into the deep sediment, but that is not what is referred to here.

-L35: Due to the lack of pore water data, it stays questionable if the term 'closed system' really describes the boundary conditions

In nature it is rather improbable that any system is 100% open or closed and we could only estimate where Lake Cadagno lies on a spectrum. Due to the lack of available porewater data for the deep sediments, we have cautiously rephrased this statement and text in the discussion. The line has been rephrased as follows: 'The isotopic offsets between pools of humic acid-sulfur, acid-volatile sulfur (AVS) and chromium-reducible sulfur (CRS) in both surface and deep sediments suggest differential timing of formation, with sulfide oxidation to sulfur/polysulfides playing an integral role in organic matter sulfurization.' Now lines (31-34).

-L53: I am sure that there are really more basic studies from the 90s of the last century that should be cited here: e.g., Jorgensen, 1990, Thamdrup et al. etc.

These citations are from marine sediments which have historically been greatly studied compared to freshwater sediments. We specifically want to distinguish studies of sulfur cycling in freshwater environments. We have added the study of Hansel et al 2015.

-L57/58 ...cultures and marine sediments... References should include: Kaplan & Rittenberg (1964), Wortmann et al. (Geology, 2001); Rudnicki et al., (GCA, 2001)

These three citations have been added.

-L66: add reference: Jorgensen et al. (GCA, 2004)

We thank the reviewer for pointing out this interesting reference which has been added to this line.

-L68: This is not a new result of Bryant et al., but has already been found out in the 80s of the last century and the 90s. You should refer to the original studies.

We have reworded this sentence as follows (L. 80-84): While the $\delta^{34}\text{S}$ -composition of pyrite has been widely used to interpret global changes in the Earth's sulfur cycle or microbial metabolic pathways, an earlier body of work (e.g., Schwarcz and Burnie, 1973; Goldhaber and Kaplan, 1975, 1980; Maynard, 1980) showed that $\delta^{34}\text{S}$ -isotopic variations in marine sediments are largely controlled by local physical factors, such as sedimentation rate, along with the supply of Fe and OM.' A reference to Bryant et al. was moved to the later in the text.

-L71: cite for closed system behaviour: Hartmann & Nielsen (1969 Geol. Rundschau, or 2012, Isot. Env. Health Stud.)

We have added the citation of (Hartmann and Nielsen, 2012) because this version is more accessible to anglophone readers.

-L73: This statement is questionable. Whereas AVS may reflect system behaviour, pyrite often reflects dominantly the conditions provided by essentially open system conditions

It is true that pyrite often reflects the compositions reflecting open conditions, especially in Fe-limited systems, but the large spread in $\delta^{34}\text{S}$ of sedimentary pyrites and gradients measured with pyrite grains (e.g. (Och and Shields-Zhou, 2012)) clearly shows that pyrite can also record sulfate reduction under closed system conditions.

-L78: Add Putschew et al. (OGC, 1996) and something from the 90s of the NIOZ group and more recently Amrani et al

This finding has been published in many papers and it is difficult to cite exhaustively but we have added two of the earlier articles of Putschew et al (1996) and Damste et al. (1998).

-L80: 'substantially' instead of 'significantly'?

Changed as suggested.

-L361: add Jorgensen et al. (2004, GCA)

This citation was not included because in this paper, unique conditions of extremely rapid sulfate reduction rates in an open system with iron trapping lead to the formation of very heavy CRS, which is the conditions in Cadagno where CRS becomes rather light compared to sulfate.

-L364: ...disproportionation of sulfur intermediates... (Canfield & Thamdrup, 1994; Cypionka et al., 1998 FEMS)...

Question: Is a repeated disproportionation cycling of sulfur intermediates really a plausible process in sediments? Canfield & Thamdrup (1994) proposed the model for an euxinic water column, the substantial role for benthic anaerobic sulfur cycling in competition to reductive processes is still to be shown

The discussion of disproportionation has been removed as it is quite unlikely, as mentioned in the original manuscript, requiring the removal of heavy sulfate from the system. We still mention the detection of bacteria belonging to clades of known disproportionators in line 351 and 389 out of general interest to the reader.

-L370: Which evidence of S^0 accumulation? What would be the role of S^0 reduction for isotope discrimination?

We have updated the figures to better highlight peaks in elemental sulfur at the surface and deep sediments. It is also mentioned that by reaction with elemental sulfur to form polysulfide (and rapid isotope exchange), the isotopic signature of sulfate reduction can be transferred to polysulfide reaction products (Amrani and Aizenshtat, 2004).

-L453: Is Fichtel et al. a suitable reference? The study belongs to a completely different type of system.

In the Conclusions we aim to demonstrate that inverse geochemical gradients and S-isotope overprinting can occur across a wide variety of environments, as Lake Cadagno is quite a unique lake. We have therefore changed the sentence to encompass a full range of terrestrial to deep subsurface environments as follows: In other environments, the reduction of sulfate diffusing from terrestrial

aquifers (Porowski et al., 2019), submarine groundwater discharge sites (McAllister et al., 2015) and even deep crustal aquifers (Engelen et al., 2008), could essentially result in overprinting of new $\delta^{34}\text{S}$ signatures on buried sediments.

-L455. Why referring to the ocean crust as aquifer, instead of much shallower submarine or limnic groundwater discharge sites(?)

We have added references for sulfur cycling influenced by terrestrial and submarine aquifers (L. 765-766).

-L474: I do not understand this statement. Why should membership in an editorial board lead for an academic trained person lead to a competition of interests?

This statement was included upon specific request by the journal editor.

-L479: Didn't the co-authors also contributed to revision and editing of the ms? Who provided funding?

We have added that All co-authors contributed to revision and editing of this manuscript.

-Fig.2 Presentation should be improved: No combination of dissolved and solid phase species in one each plot; spread of the X axis for S isotope results

Plots in Figure 2 have been separated into surface sediments and deep sediments (as Figure 2 and Figure 3) for better scaling. Solid and dissolved species have also been separated. Supplementary data has now been incorporated into Figures 2 and 3. Ratios are presented as a separate Figure 4.

Response to Reviewer #2

Berg and colleagues present a depth profile of sulfur geochemistry and target gene abundance for a ~10-meter-long core from Lake Cadagno sediments (Switzerland). This well-studied lake has moderately low concentrations of sulfate in deep porewater and experiences an influx of groundwater from below, making it a valuable intermediate case study between the ocean and very-low-sulfate lakes (e.g., Lake Superior). This dataset is likely to be of broad interest in the field. Before publication, however, there are several discussion topics that need additional attention, especially related to the interpretation of the organic S and C results and the deeper S-isotope trends. Detailed comments below are in manuscript order.

Line 111 – Please provide the essential details about your sampling procedure here so the manuscript is complete on its own without reference to Berg 2022. The reader needs to be able to quickly understand e.g. that these are piston cores, at what coordinates and elevation, without an internet connection.

All sampling procedures for data presented in this manuscript have now been described in the Materials and Methods. This also applies to the 1991 data. XRF data presented in Figure 1 are cited (Berg et al. 2022) but procedures are not described as this data is not used in further detail. Manganese was removed from these plots because it is not discussed in the manuscript.

Line 230 – I was looking for a profile of TOC to make sense of the C:S ratio profiles before I found it in the Supplement. The x-axis for TOC concentrations appears to be missing on figure S1. Consider moving this to the main text, it's very useful for thinking about the ratio profiles.

The concentration profiles have been moved to main Figures 2 and 3, and ratio profiles have been separated as Figure 4. The supplementary figures have been replaced with all the data in numerical form for easier access (Table S1).

Fig. 2 – I realize that the x-axis in D is the full range of values observed, but it is not feasible to glean information about the relationships among CRS, HAS, and AVS in the main core at this scale. Please provide an additional zoomed-in scale or some other approach to make it possible to resolve $\delta^{34}\text{S}$ differences of a few permil. Similarly, the TC/TS range goes to 40 when the data max is 12 – it would help to adjust this so the data are easier to see.

In order to better visualize variations in compounds downcore, we have separated the figure into two figures (Fig. 2: uppermost sediments (0-50 cm; obtained with a gravity corer); Fig. 3: deep sediments (0-1000 cm; obtained with a percussion coring system). We have also incorporated some of the main data (TOC) from the supplementary information, and created a separate figure for TC/TS ratios. We have also added the data in numerical form in the supplementary materials (Table S1).

Line 222–230 – I found it challenging to make sense of the humic acid sulfur data referenced only to total or total organic C. Values for humic acid carbon would be extremely helpful if they exist. Do we know at least roughly what proportion of TOC was extracted as HA? Either way, a discussion is warranted about the relationship between HA extracts and total or residual (protokerogen-like) OC. How should the reader think about statistics like TOC:HAS when the ratios of C:S in HA, the ratio of C:S in non-soluble OC, and the relative abundances of those pools are all potentially changing? Additional discussion on this topic would also help translate this data to comparison sites, many of which report OC and OS from protokerogen or lipids rather than HAs. Exchanges of sulfur between HA, DOM, and this pool should likely also be considered.

Unfortunately, we did not do a targeted quantification of HA carbon as all of the extract was used up to measure sulfur content and isotopes. However, we could calculate that on average $2.6 \pm 3.2\%$ of total organic carbon was extracted as HA, which was added to the text (L 451). Nonetheless, the addition of the TOC profile to the main text should reveal that changes in TOC:HAS ratios are most susceptible to large fluctuations in TOC content. There is a weak correlation between TOC and TOC:HAS ($r^2 = 0.31$) that becomes very strong ($r^2 = 0.81$) when lacustrine layers are excluded and this is now discussed in the text (L 444).

A huge number of organic sulfur pools were not analysed, but due to time constraints and analytical capacities, a selection was made to analyze humic acid sulfur as representative of one organic sulfur pool expected to form in recent sediments and that could be comparable to several previously published studies from marine and lacustrine environments (namely (Urban et al., 1999). Due to the lack of supporting data, we have refrained from speculation and suggested that further studies would be needed to elucidate how these pools are changing relative to each other (L. 469).

Line 236 / Fig S2 – The comparison with 1991 data is potentially intriguing but insufficiently explained to be useful. A description of the sampling type and associated concentration data would make this much more valuable, perhaps as a Supplement section. Otherwise this data is undersupported. (Why were they able to get sulfate data when the current study was not? Different sampling volumes I presume?) Can the new sulfate data be overlain on the 1991 data for a more direct comparison?

We thank the reviewer for pointing this out. The porewater data from 1991 are now overlain in the main figures and reveal a surprising consistency with 2019 data. We also added detailed sampling methods from 1991 to the main text. Indeed, a different sampling method was used and also analyses were more targeted (sulfur species only) while we had to divide our samples for additional porewater analyses of dissolved Fe, Mn, major anions and cations, and DIC, leaving much less porewater for sulfate isotopes in layers where sulfate is depleted. This was most problematic in the deep glacial sediments which contained much less water and only one sample could be measured by pooling the porewater.

Line 241 – is the support for this opening sentence the 1991 pocket dataset? Please tell us more about it! If this $\delta^{34}\text{S}$ sulfide trend is directly comparable, can it be included in Figure 2 so that it can be seen next to the AVS etc?

The 1991 porewater data were overlain in the main figures to be directly comparable and some text was added to the results comparing H_2S , AVS and other reduced sulfur pools (L. 260-267).

Line 370 – I don't see abundant SO at the deep redox transition in Fig 2A, please clarify.

We have now separated the data from surface and deep sediments to better visualize concentrations using different x-axis scales. The peak in elemental sulfur at 772 cm is now visible.

Line 383 – The statement that HAS increases over the top 10 cm (without mention of its immediate decrease below) is a bit misleading. Please provide some explanation for the peak of HAS abundance between 5–15 cm that can account for both sides of the profile.

We have now added a discussion of the decrease at 10-15cm (L. 436-441) which, although difficult to explain based on the persistence of HA in sediments, could account for heavier CRS values measured at those depths.

Line 411 – 413 – The description of organic sulfur sources is a bit tangled. Are you referring to the possibility of sulfate reducers using organic sulfate esters as a sulfate source in extremely low-S lakes?

(Fakharee / Phillips should be cited if so). If sulfate esters are used for MSR, they could find their way into any product of reactions with sulfate, theoretically including pyrite as well as OS, and would not necessarily be associated with specific OS functional groups. To understand the OS pool, the much larger effect is expected to be the incorporation of biogenic OS materials, either from PP or potentially also from secondary production by sediment microbes (Anderson and Pratt 1995 and many others).

We thank the reviewer for pointing this out. After re-reading the literature thoroughly, the vast majority of publications on humic sulfur point to an enriched ^{34}S - polysulfide source of S in HA formed by sulfide re-oxidation. We agree with the reviewer that different sulfur sources would ultimately be distributed into all the reduced sulfur pools and have rewritten the text to emphasize the timing of formation and involvement of an oxidation step in generating relatively heavy HAS.

The discussion in 416-418 seems disconnected from the observations of HAS concentration in Figure 2. How do that data support (or not support) an argument about timing of HAS formation and stability, when peak HAS concentrations mostly experience loss at shallow depths? How might biogenic OS be related to this?

We have removed these lines and focused the timing of formation as the control on HAS isotope signatures. Adjusting the scales in Figures 2 and 3 now reveal that HAS is persistent throughout the sediment column. HAS is less abundant in terrestrial than lacustrine sediments and it therefore may appear that there is a 'loss' below 10cm (now discussed in L.436-441). However, HAS reappears at concentrations up to 4 $\mu\text{mol/g}$ sediment which is comparable to surface sediments.

Line 438 – I was confused by the statement that “closed-system sulfate reduction is leading to similar $\delta^{34}\text{S}$ fractionations as in surface sediments.” Closed-system processes don't affect fractionation factors, but instead they affect the expression of those fractionations in the environment. It is not clear how closed-system distillation is creating consistent $\delta^{34}\text{S}$ distributions at depths with very different levels of system openness.

We have deleted this line to avoid confusion.

The argument for this closed-system control is, if I understand properly, the trend in CRS $\delta^{34}\text{S}$ values between 600 and 800 cm depth above the groundwater source. This hypothesis needs to be explained in much greater detail to understand the mechanism being invoked and how other previous datasets might support it. Humic acid and AVS $\delta^{34}\text{S}$ values also tend light in that zone – how is that related? (and sulfate is light... why are these values not light just because they are offset from light sulfate?) Some simple calculations to compare offsets among the sulfur pools would be great. Alternative explanations for the CRS $\delta^{34}\text{S}$ trend in the deep zone should be considered.

We have now mentioned the offsets between sulfate and reduced sulfur pools: $\epsilon_{\text{sulfate-HAS}} = 14\text{-}32$ per mil, $\epsilon_{\text{sulfate-AVS}} = 9\text{-}45$ per mil, $\epsilon_{\text{sulfate-CRS}} = 36\text{-}53$ per mil. The discussion has been reworded to state that the main control on light sulfide isotopes could be light sulfate originating from reoxidation of buried sulfides with a contribution of very slow sulfate reduction supplied by sulfate diffusing from groundwater. Since no system is a 100% open or closed system, we have refrained from labelling it as one or the other but highlighted the ongoing dominant processes governing S isotopes in the sediments.