

Dear editor, dear reviewer,

We thank you for your thoughtful comments and the constructive suggestions which will be helpful to further improve the manuscript. Attached you can find our comments to those points that require a response as well as the suggested changes for the revised manuscript in bold print. We hope that we successfully addressed each point raised. References used in the author comments are listed at the end of the document.

All the best

Franziska Koebisch

Anonymous Referee #2

The manuscript "Sulfate deprivation triggers high methane production in a disturbed and rewetted coastal peatland" by Koebisch et al. is very interesting and informative. The effect of dyking and freshwater rewetting on S and C transformation of a peatland has been studied using various analysis methods. The authors studied four spots with different solid S and sulfate concentrations and discussed the sulfate reduction and CH₄ production and consumption among these sites. They conclude that a replenishment of marine water to dyked wetlands would reduce methane emissions.

Abstract:

Lines 30-32, the word of "suppression" is not accurate since results showed "high contents of labile iron minerals and dissolved ferrous iron at depth of spot 2, coincided with a high abundance of *Thermodesulfovibrionaceae* at concurrently minor occurrence of *Delta-proteobacteria*. (402-404)".

Lines 34-36: Is that useful to re-exposure of dyked wetlands to natural coastal dynamics since high amounts of sulfate did not interfere with high methane emissions on ecosystem scale?

Author's response

- **Indeed, we think that dissimilatory sulfate reduction at spot 2 is inhibited because we suggest *Thermodesulfovibrionaceae* present at this spot to utilize mainly ferric iron as electron acceptor (Fortney et al., 2016). This is indicated by the long-term persistence of the brackish sulfate reservoir at concurrently high contents of labile iron minerals and dissolved ferrous iron. This interpretation is stated in detail in line 394f. of the manuscript**
- **We admit that the used formulations in line 34-36 may be confusing for the reader.**

Suggested change in the manuscript

- **We will replace 'suppression' in line 30-32 with 'inhibition'**
- **We will rephrase the sentences in line 34-36 accordingly. In particular, we will emphasize that the local sulfate remnants did not interfere with high methane emissions on ecosystem scale as sulfate was spatially confined to depths below the methane production zone. In contrast, the re-exposure to coastal dynamics would replenish the brackish sulfate reservoir within the entire wetland and is therefore likely to lower methane emissions on ecosystem scale.**

Introduction

Lines 46-47: "dissimilatory sulfate reduction of dissolved organic matter (DSR)" changed to "dissimilatory sulfate reduction (DSR) of dissolved organic matter".

Line 63: Wrong left parenthesis. {Wen & Unger, 2018).

Line 75: Lack of "."

Author's response

- **Text mistakes will be corrected accordingly**

Material and Methods

Lines 105-107: Please check the parenthesis and should not be set in italic.

Line 159: Please define the abbreviation of DOC. It seems that the results of stable carbon isotope ratio of DOC was not presented in the result or discussion.

Line 160: Ertl and Spitzky (2004), not list in the Reference. Please check the manuscript completely.

Lines 168, 285, 308: Böttcher et al. (2007), not list in the Reference. Please check the manuscript completely.

Line 185: Please clarify DNA or RNA? Since only DNA has been extracted from samples, hence, I suggest to delete RNA.

Line 191: Please define the abbreviation of AVS. e.g. (acid volatile sulfur).

Lines 196-199: What's the meaning of "reactive iron"? How to detect the dissolved Fe and the valence of Fe (Fe(II) or (Fe(III))? Line 200 indicated the extracted iron fraction consists of iron(III) oxyhydroxides and iron(II) monosulfides, was the dissolved Fe(II) also existed (Heronet al., 1994. Speciation of Fe(II) and Fe(III) in contaminated aquifer sediments using chemical extraction techniques. Environmental Science & Technology, 28(9), 1698-705.)? The reference of Canfield, 1989 was not list, please check.

Line 200: Stookey (1970), not list in the Reference.

Line 223: "Sequence raw reads" changed to "Raw sequence reads".

Lines 229-230: Clarify the number of the sequence. 18,500 or 18,500? 12,500 or 12,500?

Author's response

- **Reactive iron is considered here as the sum of those iron fractions that still may react with dissolved sulfide. The applied analytical extraction scheme extracts the sum of remaining iron(III) oxyhydroxides and acid volatile sulfide (AVS, essentially FeS) as well as a very minor contribution from dissolved Fe²⁺ in the pore water (Canfield, 1989). No further quantification of different di- and trivalent iron was carried out.**

Suggested change in the manuscript

- **The meaning of 'reactive iron' will be specified in the method section and the reference Canfield 1989 will be added in the reference list**
- **Text mistakes will be corrected accordingly, references will be checked, abbreviations will be defined**

Results

Lines 240-241: Lack of ")". Please check the manuscript completely.

Lines 271-272: What's the detailed detection method of organic-bond S and where is the data of organic-bond S? Is it in the Figure 4 or TABLE A1? The note in the Figure 4 indicated that the solid residual S fraction is suggested to present primarily organic-bond S. However, the content (0.2 to 1.6%dwt) in spot 1 (lines 271-272) seems inconsistent with Figure 4b (far less than 1%dwt). What's the relationship between TABLE A1 and Figure 4 and 5^{ijj}§

Lines 291, 322: Similar question to the line 271-272. Table A1 seems to address the organic-bond S content (0.2 to 1.6 %dwt). What's the difference between orgS in Table A1 and solid residual S in Figure 4b? Please clarify.

Lines 298-299 and 392-393: It seems inconsistent to Figure 4a. In the figure, the 1 mM sulfate concentration was appeared at 60cmbsf of spot 3, however, the relative abundance of Chloroflexi was missed at 60cmbsf of spot 3. Please clarify.

Lines 331-332: How to understand the role of DIC in methane production and consumption?

Line 340: Whiticar (1986), not list in the Reference. Please check.

Author's response

- In our study we assume that the solid residual S fraction corresponds primarily to organic-bond S. Unfortunately, we have made a mistake in the residual S fraction values in table A1. The mistake will be corrected. However, this will not affect the main patterns and results of our work.
- We are sorry that the depth indications for spot 3 in Fig. 4d are shifted. The text is correct as the 40% relative abundance of Chloroflexi of the class Dehalococcoidetes represented the dominating bacterial group at the 1 mM sulfate concentration at 60 cm depth of spot 3.
- The formation of methane is a highly fractionating process and results in CO₂ that is considerably enriched in ¹³C compared to the starting organic material (~-27‰ in this study) (Whiticar et al. 1986). The isotopic composition of DIC-C can therefore be used as indicator for methane formation in relation to non-fractionating pathways (organic matter fermentation, sulfate reduction, e. g. Corbett et al. 2013). Dissolved methane is highly volatile and especially at high methane production rates, the indicative value of methane concentration profiles can be easily impaired by erratic ebullitive release. Therefore, in this study we decided to use the $\delta^{13}\text{C}$ values of DIC as indicator for methane production. In concert, with the microbial structure, these can provide a comprehensive picture on methane cycling in our study site.

Suggested change in the manuscript

- the residual S fraction values in table A1 will be corrected and then correspond to the organic bond S values in Fig. 4. The text will be checked thoroughly for mistakes.
- The depth indications for Fig. 4d spot 3 will be corrected.
- Text mistakes will be corrected accordingly, references will be checked.
- We will add a note on the indicative value of $\delta^{13}\text{C}$ -DIC for methanogenesis in the text.
- Text mistakes will be corrected accordingly, references will be checked

Discussion

Line 394: "dsrAB" should be italicized.

Line 439: Please define the abbreviation of AOM-SR.

Lines 447-448: In the Figure 7, the obvious increase of $\delta^{13}\text{C-CH}_4$ and $\delta\text{D-CH}_4$ ratios were appeared at 50 cmsf of spot 1, however, the relative abundance of methanotrophy was missed at 50 cmsf, hence, how to understand this sentence.

Lines 452-453: Why high sulfate concentration did not interfere with methane production?

Author's response

- Likewise with Fig 4d, depth indications for Fig. 7d are accidentally shifted. The 48% abundance of ANME-2d ranges down to a depth of 50 cmbsf. Accordingly, the observed patterns in $\delta\text{D-CH}_4$ and $\delta^{13}\text{C-CH}_4$ are consistent with the microbial community structure.
- The remnants of the brackish sulfate reservoir were spatially separated from the zones of methane production: As a result of intense dissimilatory sulfate reduction, residual sulfate persisted only at depth of spot 2 (and in lower amounts at depth of spot 3). Methane production in wetlands is usually confined to the upper peat horizons, and also in our study methane was mainly produced in the upper 30-40 cm. Therefore, we assume that the residual sulfate does not significantly affect methane production. This is stated in line 452-459: *As intense methane production was confined to the upper pore water layers in the entire peatland, it did not interfere with high sulfate concentrations locally preserved as legacy of former brackish impact in the bottom.*

Suggested change in the manuscript

- Text mistakes will be corrected accordingly, abbreviations will be defined
- The depth indications for Fig. 7d will be corrected.

Table A1: TS concentrations were inconsistent with dry weight ratios of TS. e.g. why the value of TS percentage is not 0 while TS concentration is 0. What is the meaning of orgS? Ionic valence should be added e.g. Cl-, Br-, Na+.

Author's response

- TS in mmol corresponds to the total dissolved S fraction whilst TS in dry weight ratios corresponds to the solid dissolved S fraction. Anyway, we admit that it would be better to use different abbreviations to avoid confusion.
- Organic S is the residual non-specified solid S fraction.

Suggested change in the manuscript

- We will implement different abbreviations for solid and dissolved total S
- As stated above, we will correct the values of orgS so that they are consistent with the quantities represented in Fig 4
- Ionic valences will be added

Reference

The format of the references should be carefully checked. Non-English language appeared e.g. lines 504-506, 523-524, 602-603. No journal e.g. lines 507-508, 566. Capitalize the first word of journal or not e.g. lines 548, 568, 573, 577. Other format errors in lines e.g. 523-524, 537-538, 546, 585, 624, 628.

Suggested change in the manuscript

- The reference list will be carefully checked

Figures

Figure 2: Lines 645-646, please rewrite this sentence.

Figure 4: Please unify the formats with Figure 7. e.g. mmol l-1 in Figure 4 while mM in Figure 7. e.g. the name of y-coordinate.

Suggested change in the manuscript

- **Figure captions and axis labels will be corrected accordingly**

Author's response reference list

Corbett, J. E., Tfaily, M. M., Burdige, D. J., Cooper, W. T., Glaser, P. H., & Chanton, J. P. (2013). Partitioning pathways of CO₂ production in peatlands with stable carbon isotopes. *Biogeochemistry*, 114(1-3), 327-340.

Fortney, N. W., He, S., Converse, B. J., Beard, B. L., Johnson, C. M., Boyd, E. S., & Roden, E. E. (2016). Microbial Fe (III) oxide reduction potential in Chocolate Pots hot spring, Yellowstone National Park. *Geobiology*, 14(3), 255-275.

Whiticar, M. J., Faber, E., & Schoell, M. (1986). Biogenic methane formation in marine and freshwater environments: CO₂ reduction vs. acetate fermentation—isotope evidence. *Geochimica et Cosmochimica Acta*, 50(5), 693-709.