Reviewer#1's first comment, January 2

The authors aimed to review on direct and indirect contributions of sulfate to methane oxidation in upland soils. As far as I'm concerned, influence of sulfate on methane oxidation is an important topic in biogeochemistry, but the current manuscript failed to make a clear and concise presentation. Here I list my major concerns as follows.

We sincerely appreciate Reviewer#1's comments, which we believe are insightful and constructive. Below are the specific suggestions provided by Reviewer #1, along with our responses and the corresponding revisions we have made.

1) Methane oxidation or aerobic methane oxidation?

The manuscript is entitled '*Contribution of sulfate to methane oxidation...*', but throughout the manuscript the authors seemed to talk only about aerobic methane oxidation. Note that both aerobic and anaerobic methane oxidation exist in upland soils. If the authors just want to review on aerobic methane oxidation processes, please change the title into '*...Contribution of sulfate to aerobic methane oxidation...*'. If not, previous studies regarding anaerobic methane oxidation should also be reviewed in the manuscript.

Although many studies use the term "CH_4 oxidation" without specifying "aerobic," we recognize the importance of clarity in our manuscript. Therefore, we have revised all instances of "CH_4 oxidation" to "aerobic CH_4 oxidation" to ensure consistency and accuracy.

While SO_4^{2-} has been shown to promote aerobic CH_4 oxidation in a limited number of studies, its effect on aerobic CH_4 oxidation remains unclear and has not yet been definitively established. Therefore, our review focuses specifically on aerobic CH_4 oxidation in upland soils, as this area requires further clarification and synthesis of existing literature. To better reflect the focus of our study, we have revised the title to "*Contribution of Sulfate to Aerobic Methane Oxidation in Upland Soils: A Mini-Review.*" This change ensures that the scope of our review is clearly communicated

to readers.

We greatly appreciate your clarification on the distinction between aerobic and anaerobic CH_4 oxidation and the need to align the title with the study's focus. These insights have helped us improve the clarity and accuracy of our manuscript.

2) Direct effects?

The authors stated that '*This review provides a comprehensive summary of the direct and potential indirect impacts of SO_4^{2-} on CH_4 oxidation*' (L. 82 - 84), but I cannot see how sulfate directly influences methane oxidation in the manuscript. It seems that the authors tried to figure out the direct effect in the third part of the manuscript (L. 186 – 250), but they only concluded that '*due to the scarcity of studies investigating the direct effect of SO_4^{2-} on CH_4 oxidation, no definitive conclusion regarding its impact could be drawn*' (L. 245 - 247).

Before discussing direct impacts of sulfate on methane oxidation, the authors should first answer whether sulfate could directly influence methane oxidation. Unfortunately, the authors didn't even try to demonstrate existence of direct influence of sulfate on methane oxidation, but they made a conclusion that they provided '*a comprehensive summary of the direct and potential indirect impacts*'. Therefore, I think the conclusion regarding direct impacts should be modified throughout the manuscript.

From my perspective, direct effects of sulfate on methane oxidation is associated with coupling of anaerobic methane oxidation to sulfate reduction. Under anoxic conditions, numerous studies have demonstrated that sulfate could be the electron acceptor for methane oxidation, which can be considered a direct effect of sulfate on methane oxidation. However, the authors didn't review on aerobic methane oxidation in the current manuscript.

We acknowledge that certain phrasing in our article may have led to some misunderstanding. Following the referees' suggestions, we have reorganized the article, adjusting our approach to describe the findings by focusing on macroscopic

manifestations (i.e., the effects of SO_4^{2-} on aerobic CH_4 oxidation rates as reported in the literature) and microscopic mechanisms (i.e., the potential pathways through which SO_4^{2-} influences aerobic CH_4 oxidation). We have also revised lines 82-84 and lines 245-247 accordingly, as recommended by the reviewer.

We have reviewed the potential direct effect of SO_4^{2-} on aerobic CH_4 oxidation, with studies suggesting that SO_4^{2-} may promote aerobic CH_4 oxidation, with an observed range of 0-42% (L. 75). In the revised version, we have updated this range to 0-42% (L. 26 and L.75), and the corresponding references are provided in Table 1. Section 3 (L. 187-250) provides a detailed review of the potential direct impact of SO_4^{2-} on aerobic CH_4 oxidation. However, we did not sufficiently emphasize the conclusion that SO_4^{2-} affects aerobic CH_4 oxidation. In the revised version, we will explicitly state that SO_4^{2-} may influence aerobic CH_4 oxidation before discussing its effects. The statement 'due to the scarcity of studies investigating the direct effect of SO_4^{2-} on aerobic CH_4 oxidation, no definitive conclusion regarding its impact could be drawn' (L. 245-247) reflects our view that there is currently insufficient research to fully understand the direct influence of SO_4^{2-} on aerobic CH_4 oxidation. While we did not explicitly highlight the potential promoting effect of SO_4^{2-} in the original manuscript, existing studies suggest that such an effect may exist. We will revise the manuscript to clarify and acknowledge the potential positive influence of SO_4^{2-} on aerobic CH_4 oxidation.

Our review exclusively focuses on the impact of SO_4^{2-} on aerobic CH_4 oxidation because our primary concern is with upland surface soils, which are important sites for aerobic CH_4 oxidation and can absorb CH_4 from the atmosphere, thereby reducing atmospheric CH_4 concentrations. Although anaerobic CH_4 oxidation does occur in upland soils, it is limited to deeper soil layers, and the amount of CH_4 oxidized is relatively small. We believe that, even though SO_4^{2-} acts as an electron acceptor for anaerobic CH_4 oxidation, it is situated far below the aerobic CH_4 oxidation layer and, given the minimal amount of CH_4 oxidized in this context, its contribution to overall CH_4 absorption is likely negligible. Furthermore, most studies on the effect of SO_4^{2-} on anaerobic CH_4 oxidation have been conducted in wetlands, marine environments,

and paddy fields—anaerobic environments—while little research has been conducted on anaerobic CH_4 oxidation in upland soils. Therefore, we did not review the effect of SO_4^{2-} on anaerobic CH_4 oxidation, nor did we include treat its contribution as part of the effect of dryland soil SO_4^{2-} on CH_4 oxidation.

3) Concerns regarding the major conclusion

From table 1, the authors drew the conclusion that '*the enhancement of SO_4^{2-} on CH_4 oxidation is prominent in numerous studies*' (L. 74) and that '*SO_4^{2-} facilitates CH_4 oxidation within a range of 3-42%*' (L. 75). However, table 1 only contains results from 5 study sites, and no effect of sulfate addition was observed in 2 out of the 5 sites. In the 5 sites, effects of sulfate addition are 0%, 0%, 3%, 25% and 42%, respectively, and no statistical analysis has been conducted to support the statement that sulfate facilitate methane oxidation by 3-42%. Therefore, I think this conclusion is greatly undermined because of insufficient literature and lack of effective statistics. Additionally, title of table 1 is '*Promotion effect of sulfate on methane oxidation in diverse biome soils*', but I don't think 5 forests could be called "*diverse biome*".

We included studies from five forests because the effect of SO_4^{2-} on aerobic CH_4 oxidation has only been reported in these specific studies. We will revise the title of Table 1 to 'Promotion effect of SO_4^{2-} on aerobic CH_4 oxidation in diverse upland soils.' Based on the available literature, we hypothesize that SO_4^{2-} may influence aerobic CH_4 oxidation; however, research on this topic remains limited. To date, only these five studies conducted in different forests have explicitly examined the impact of SO_4^{2-} on aerobic CH_4 oxidation. Therefore, our analysis is based on these studies.

We acknowledge that drawing the conclusion 'SO_4^{2-} promotes aerobic CH_4 oxidation' based solely on these five studies may not be sufficiently robust. To address this limitation, we have summarized the potential microscopic mechanisms

through which SO_4^{2-} may influence aerobic CH_4 oxidation in the article. Our goal is to explore potential connections between SO_4^{2-} and aerobic CH_4 oxidation in soils, thereby supporting the hypothesis that SO_4^{2-} can influence this process.

4) Concerns regarding manuscript structure

I also have some concerns regarding structure of the manuscript. First, the authors spent 2 pages introducing the methane oxidation processes and associated microbes (Pages 4 - 5), which are not much associated with the main topic of the manuscript. These contents are not reviewed in the following parts, and I suggest the authors to shorten these contents. Second, Lines 174 – 182 in section 2.3 repeated contents in the introduction (Lines 60 - 68). Similar sentences also emerged in the implication section (Lines 355 - 360). Third, contents in section 3 are disorganized, and I suggest the authors to reorganize these contents (e. g. by adding a topic sentence to the beginning of each paragraph of this section). Forth, presentation of table 1 (L. 75 - 78) should be in the result section (e.g. section 3), instead of the introduction.

We appreciate the reviewer's valuable comments and have made the following revisions to address the issues raised:

1) We have reduced the discussion of the aerobic CH_4 oxidation process and the related content on microorganisms to focus more on the main topic of the manuscript.

2) We have addressed the repetition between Lines 174–182 in Section 2.3 and Lines 60–68 in the Introduction by either removing or integrating the overlapping content to improve the overall coherence of the article.

3) To enhance the clarity and organization of Section 3, we have added topic sentences at the beginning of each subsection, ensuring a more logical flow of ideas.

4) To better align with the manuscript's structure, we have moved the results presented in Lines 75–78 from the Introduction to the Results section.

Reviewer#1's second comment, January 13

Hi Lihua, I'm glad to see your willingness to address my comments. Your responses are

overall satisfactory, while I think you should take note of the following two problems.

First, you may misunderstand my Point 2. Before addressing this point, you should first distinguish gross effect, direct effect and indirect effect. For example, the '3-42%' effect in your manuscript is the gross effect (not direct effect) of sulfate on methane oxidation, and the gross effect can then be partitioned into direct and indirect effects. In your manuscript, you said that 'This review provides a comprehensive summary of the direct and potential indirect impacts of SO42- on CH4 oxidation'. What I expect from this statement is that you might have categorized the gross effect into different aspects of direct and indirect effects, but you actually didn't.

We understand your perspective, and it appears that the misunderstanding arose from our imprecise wording. We have now revised the terms 'direct effect' and 'indirect effect' to 'macroscopic manifestations' and 'microscopic pathways,' of SO_4^{2-} 's impact on aerobic CH_4 oxidation respectively. Our focus is on arid or semi-arid grassland ecosystems, where we have summarized the influence of SO_4^{2-} on the rate of aerobic CH_4 oxidation. The macroscopic manifestations refer to the impact of SO_4^{2-} on the overall rate of aerobic CH_4 oxidation, without considering any underlying microscopic mechanisms. It simply examines how SO_4^{2-} affects the CH_4 oxidation rate under aerobic conditions. The microscopic pathways, on the other hand, describe how SO_4^{2-}, after entering the soil, alters the soil's physical and chemical properties, methanotrophs, and other factors, thereby influencing aerobic CH_4 oxidation.

Second, the conclusion of 3-42% promotion is inaccurate without effective statistical tests. Additionally, the effect size in the 5 studies should be 0%, 0%, 3%, 25% and 42%, but why did you ignore the two sites of no effect when drawing the conclusion of 3-42% promotion?

Regarding your second point, we acknowledge that research on the impact of SO_4^{2-}

on aerobic CH_4 oxidation remains limited. Due to the small number of available studies, it is currently not feasible to perform robust statistical analyses to draw definitive conclusions about the effect of SO_4^{2-} on aerobic CH_4 oxidation. Therefore, our analysis is based on the five studies cited. We recognize that we inadvertently omitted the two studies showing no significant effect of SO_4^{2-} on CH_4 oxidation. To more accurately reflect the findings of the existing literature, we will revise the statement 'SO_4^{2-} facilitates CH_4 oxidation within a range of 3-42%' (L. 26 and L. 75) to SO_4^{2-} facilitates CH_4 oxidation within a range of 0-42%.

Reference

[1] P.L.E. Bodelier, (2011). Interactions between nitrogenous fertilizers and methane cycling in wetland and upland soils, Current Opinion in Environmental Sustainability 3(5) 379-388. https//doi.org/10.1016/j.cosust.2011.06.002.

[2] J.H. Guo, H.L. Feng, C.H. Peng, H. Chen, X. Xu, X.H. Ma, L. Li, D. Kneeshaw, H.H. Ruan, H.Q. Yang, W.F. Wang, (2023). Global climate change increases terrestrial soil CH_4 emissions, Global Biogeochemical Cycles 37(1) e2021GB007255. https//doi.org/10.1029/2021gb007255.

[3] A. Boetius, K. Ravenschlag, C. Schubert, D. Rickert, F. Widdel, A. Gieseke, R. Amann, B. Jorgensen, U. Witte, O. Pfannkuche, (2000). A marine microbial consortium apparently mediating anaerobic oxidation of methane, Nature 407(6804) 623-626. https//doi.org/10.1038/35036572.

[4] W. La, X. Han, C.Q. Liu, H. Ding, M. Liu, F. Sun, S. Li, Y. Lang, (2022). Sulfate concentrations affect sulfate reduction pathways and methane consumption in coastal wetlands, Water Res. 217 118441. https//doi.org/10.1016/j.watres.2022.118441.

[5] L.C. Fan, D. Schneider, M.A. Dippold, A. Poehlein, W.C. Wu, H. Gui, T.D. Ge, J.S. Wu, V. Thiel, Y. Kuzyakov, M. Dorodnikov, (2021). Active metabolic pathways of anaerobic methane oxidation in paddy soils, Soil Biol. Biochem. 156 108215. https//doi.org/10.1016/j.soilbio.2021.108215.

Reviewer#2's comment, January 25

The authors aimed to review the recent literature regarding the impact of sulfate on aerobic methane oxidation in soils. Indeed, this is in interesting biogeochemical topic, given that in the past the focus was on the role of SO_4^{2-} on anaerobic methane oxidation. I am in agreement with the first reviewer that the current manuscript failed to present the state of knowledge in a clear way and although this is a mini-review, it lacks depth. At the current state, for me, the aim of this manuscript is to show how little research has been done on the influence of sulfate on aerobic methane oxidation and not to summarize the research to date into an overall picture (review) and to point out specific starting points for further studies (syntheses). If I have understood correctly, the authors are currently working on another manuscript including experiments to observe the effects of SO_4^{2-} on aerobic methane oxidation. By shortening the text considerably and formulating it more precisely, it would be ideally suited as an introduction and part of the discussion for such a manuscript.

We sincerely appreciate Reviewer #2's insightful comments, which have helped us recognize the need for a more focused and cohesive synthesis of the existing literature. In the initial version, we may have overemphasized the scarcity of research on the effects of SO_4^{2-} on aerobic CH_4 oxidation rather than provided a comprehensive synthesis of the available studies. Based on your suggestion, we will restructure the manuscript to provide a clearer synthesis of the existing literature. Specifically, we will organize the findings into thematic sections, highlight key insights, and identify gaps for future research, ensuring a more balanced and comprehensive overview. We also agree with your suggestion to shorten and refine the text, making it more concise and focused. This will give the manuscript a strong foundation for future experimental work, particularly in the introduction and discussion sections.

In the following, I have outlined my major points for each section and provided more detailed feedback in the comments of the attached document.

We have carefully reviewed the detailed feedback provided in the attached document and have addressed each point in our revisions. Below, we address each of Reviewer #2's specific suggestions in detail, outlining the revisions we have made to improve the manuscript.

1) The introduction would benefit from enhanced clarity, with the relocation of sections from section 4 having the potential to significantly improve its quality. However, the text does not provide a compelling rationale for why the impact of SO_4^{2-} on aerobic methane oxidation could be of interest. Furthermore, some of the references utilized (marked in the PDF) do not correspond to the original works cited in the text, and some references are employed incorrectly.

We consider Section 4 as an independent segment aimed at identifying the microscopic pathways through which SO_4^{2-} influences aerobic CH_4 oxidation by reviewing the literature, serving as a supplement to the five studies listed in Table 1. We have reorganized Section 4 to enhance clarity and coherence in response to your suggestion. Additionally, we have added a new section in the revised manuscript to provide a compelling rationale for why the impact of SO_4^{2-} on aerobic CH_4 oxidation warrants attention, highlighting its ecological and biogeochemical significance. These revisions aim to address your concerns and improve the overall quality of the manuscript.

Regarding the cited literature, we have carefully reviewed the references and corrected the discrepancies and inaccuracies identified in your comments. We have replaced incorrect references with the original works and ensured that all citations are used appropriately to support our arguments. We greatly appreciate the detailed feedback in the PDF, which has helped us identify areas for improvement. We have carefully addressed your suggestions in the revised manuscript and reorganized the content accordingly.

2) It is imperative to briefly outline the pathway through which methane is oxidized by

methanotrophs, highlighting the genera responsible for this process. However, the focus should be on the enzymes that may be influenced by sulfate, either directly or indirectly, through changes in pH. Additionally, it would be intriguing to examine the effects of sulfate on changes in the methanotrophic community or their abundance. If these aspects are not addressed, it would be advisable to combine and significantly shorten these sections. The topic of high and low affinity methanotrophy is not addressed at all, despite the fact that this distinction is very important because high affinity methanotrophs mainly oxidize atmospheric methane, whereas low affinity methanotrophs focus on the methane produced in the soil. If the authors want to argue that sulfate addition increases the atmospheric soil sink, this is of utmost importance, especially as some of the indirect effects may only target one of the two groups. For instance, the inhibition of methanogenesis would primarily affect the oxidation of methane by low-affinity methanotrophs, while the atmospheric sink may remain unaltered.

Based on your suggestion, we have added a brief overview of the aerobic CH_4 oxidation pathway in the revised manuscript, highlighting the key genera involved and providing a classification. We have also elaborated on the role of CH_4 monooxygenase (MMO) and how SO_4^{2-} influences its activity through changes in Cu availability and NH_4^+ adsorption. Additionally, we have included a detailed discussion on high-affinity and low-affinity methanotrophs, emphasizing their distinct roles and characteristics. Regarding the statement that "sulfate addition increases the atmospheric soil CH_4 sink," we have removed this claim from the manuscript due to the lack of specific literature investigating the effects of sulfate on low-affinity and high-affinity methanotrophs. This revision ensures that our conclusions are fully supported by available evidence.

In addition, the methanotroph types are nowadays only defined by phylogeny and not as mentioned by their metabolic pathways, membranes etc.

In response to your suggestion, we have revised the classification of methanotrophs in the manuscript to align with current phylogenetic definitions, replacing the outdated classification based on metabolic pathways and membrane structures. This update ensures that our discussion reflects the latest scientific understanding of methanotroph diversity and evolution. We greatly appreciate your clarification on the modern classification of methanotrophs based on phylogeny. This insight has helped us align our manuscript with current scientific standards. This revision not only improves the accuracy of our classification but also strengthens the overall coherence of our manuscript by adopting a more scientifically rigorous approach.

Most of section 2.3 can be moved to the introduction and the rest should be discussed in sections 3 or 4.

Based on your suggestion, we have relocated the majority of Section 2.3 to the Introduction section, including the role of sulfate deposition and its impact on the CH_4 cycle. The remaining content has been integrated into Sections 3 and 4 to enhance the logical coherence of the manuscript.

3) I find the titles of section 3 and 4 misleading, as most of the processes described in section 3 are indirect effects of SO_4^{2-} on methane oxidation Moreover, a substantial portion of the argumentation appears to suggest that the primary effect of the sulfate is to mitigate the inhibitory effects of the ammonium. However, it is subsequently presented as that sulfate increases methane oxidation rates. This should be clarified.

We sincerely thank Reviewer #2 for their insightful and constructive comments, which have significantly helped us improve the manuscript. In response to your suggestion, we have revised the titles of Section 3 and Section 4 to better reflect their content. Section 3 is now titled 'Macroscopic Manifestations of SO_4^{2-} on Aerobic CH_4 Oxidation,' while Section 4 is titled 'Microscopic Pathways of SO_4^{2-} on Aerobic

CH_4 Oxidation.' These changes aim to eliminate any potential confusion and provide a clearer structure for the manuscript.

Additionally, we have clarified the distinction between the primary effect of SO_4^{2-} in mitigating ammonium inhibition and its role in directly enhancing aerobic CH_4 oxidation rates. Specifically, we now emphasize that SO_4^{2-} indirectly promotes aerobic CH_4 oxidation by reducing ammonium availability through enhanced adsorption rather than directly increasing oxidation rates. The content related to ammonium ions has been moved to Section 4, where it is presented as part of the microscopic pathways.

4) This section provides a description of the various soil properties that may be influenced by SO_4^{2-}, yet the processes by which these properties are influenced are only described in rudimentary terms and often unstructured. The impact of SO_4^{2-} is often speculated. Consequently, this section lacks in depth and needs to be reworked. Furthermore, the sections 5 and 6 should be reworked and combined.

Based on your suggestion, we have reorganized this section to provide a more detailed and structured discussion on how SO_4^{2-} influences soil properties and related substances, thereby affecting the process of aerobic CH_4 oxidation. We have also integrated additional references to support our arguments and removed most of the speculative content. Additionally, we have reworked and combined Sections 5 and 6 to eliminate redundancy and provide a more focused discussion on the broader implications of SO_4^{2-}'s effects on soil properties and aerobic CH_4 oxidation.