

Episodic records of deep microbial methanogenesis throughout the Caledonian mountain range exhumation

Corresponding Author: Dr Femke van Dam

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Dear Editor and Authors,

Here are my comments regarding the manuscript titled “Episodic record of deep microbial methanogenesis throughout the Caledonian mountain range exhumation” by van Dam and co-authors.

The manuscript reports on the authors’ investigation of methanogenic microbial activity deep within the crust, using mineralized fracture samples from a deep rock core in the Swedish Caledonides. The authors have discovered calcite from these fractures with extremely positive $\delta^{13}\text{C}$ values, suggesting protracted methane formation within a closed system and precipitation of calcite from the residual dissolved inorganic carbon. Similarly, but only at certain depths, the authors report high $\delta^{34}\text{S}$ values in pyrite, suggesting closed-system sulfate reduction. These analyses are supplemented by geothermometry, carried out using clumped isotope techniques, and U-Pb dating. This data shows that the temperature of formation is consistent with microbial activity (contra thermogenic formation) and that methanogenic activity occurred multiple times over the last several hundred million years during tectonic events. They also report data on samples from fluids and gases that do not indicate microbial methanogenic activity, underlining that such activity may be greatest during tectonic events when fractures form more readily.

I find the authors’ data to be thorough and of high quality, and their conclusions both interesting and robust. Specifically, linking metabolic activity to punctuated tectonic events is elegant and provides greater insight into how the deep biosphere operates. I therefore recommend publication of this manuscript with only minor revisions.

Below, I add my comments on specific lines in the manuscript:

Line 37–39: Revise the sentence starting with “This ancient...” to clarify that the metabolic process is an analogue of a metabolism that may have been important for early Earth conditions.

Line 82–84: Rephrase the opening sentence to first clearly state what the samples were (fractures? mineralized fractures?) and then present the components explored (calcite, fluid, gas). I had to re-read this sentence several times to understand it.

Fig. 2: These images should be labeled to highlight mineral phases and important observations presented in the text.

Line 121 [whole section]: It may be worth spelling out exactly what type of clumped isotope measurement is being made, even if it is obvious to experts.

Line 168–170: Rephrase the sentence starting with “The stable...” as it currently reads as if the stable isotopes were “40 to 2081 m,” which is non-sensical.

Fig. 7: Consider dividing the legend into reference data (with a header titled “Reference Data”) and the new data presented in the paper. This would make the figure easier to interpret at a glance.

Fig. 7: The horizontal arrow suggests that only the oxygen isotopic composition is changing during water–rock interaction, whereas the text describes mechanisms that change both hydrogen and oxygen isotopic compositions. If possible, this could be remedied.

Line 185 [whole section]: The section discusses one type of methanogenesis (hydrogenotrophic). If there is a reason only this mechanism is discussed, it should be made clear. It may also be interesting to discuss how AOM interacts with methanogenesis. Since this paragraph presents a key result, it would be helpful to have a strong concluding sentence stating exactly what is being claimed. Are you arguing that all these metabolisms were occurring?

Fig. 8: Are the fields in the figure interpreted or derived from other work? If derived from other work, it should be cited in the figure caption.

Line 346: The text reads that the work has a number of implications but does not explicitly spell them out. Please clarify what these implications are.

Kind regards
Ausitn Jarl Boyd

Reviewer #2

(Remarks to the Author)

General Comments

Review of "Episodic records of deep microbial methanogenesis throughout the Caledonian mountain range exhumation"
This manuscript combines detailed carbonate analyses (e.g., in-situ C-O isotopes, clumped isotopes) from a deep scientific borehole in central Sweden with multiple lines of evidence (including CH₄ C-H isotopes and groundwater H-O isotopes) to link deep methanogenesis with regional crustal exhumation and mountain erosion. This approach is highly innovative. The heavy $\delta^{13}\text{C}$ values of the calcite provide exclusive and compelling evidence for microbial methanogenesis, offering excellent geological constraints on deep subsurface microbial activities. The manuscript integrates various analytical techniques, presents robust datasets, and provides a reasonable interpretation of the results. Overall, I like the story and support the publication of this manuscript. Yet, I feel several issues need to be addressed to sharpen the core arguments. I hope the following suggestions will be helpful for the revision.

Major Concerns

Given that the central theme of this manuscript revolves around deep methanogenesis during the exhumation of the Caledonian orogeny, I argue that the precipitation temperatures of the carbonates should be another core focus of the paper, alongside the heavy $\delta^{13}\text{C}$ signatures and geochronology. The authors utilize these calcite precipitation temperatures to back-calculate paleodepths and further estimate the amount of denudation. However, the current discussion on this aspect is not sufficiently prominent. For instance, the authors offer only a brief speculation regarding why the youngest calcite occurs at a depth of 1270 m, even though some implications stated in the abstract might rely on this specific observation. Conversely, the sections discussing groundwater sources, modern microbial communities, and the stable isotopes of methane gas occupy a substantial portion of the text. Because these particular results do not strongly support the primary theme or the conclusions of the paper, they seem to distract from the core focus. I suggest reducing the length of these sections to make the central theme more clear and coherent. Furthermore, the authors might consider adding a schematic diagram to conceptually illustrate the linkage between mountain exhumation and deep episodic methanogenesis.

Specific Comments

1. Abstract: The U-Pb dating result of 605 ± 27 Ma for the 986 m sample is missing in the abstract. It should be included to ensure chronological completeness.
2. Figure 3: Please explain why pyrite $\delta^{34}\text{S}$ data were only obtained/presented for three specific depths/intervals.
3. Line 197 (and throughout): The authors append standard notations (e.g., V-PDB, V-CDT) to some permil symbols ‰ but omit them in other places. Please ensure strict consistency throughout the text and figures.
4. Line 202: Since the $\delta^{13}\text{C}$ values of methane in the borehole are around -30 ‰, which overlaps with the isotopic signatures of organic carbon mentioned by the authors, is it possible that these lower $\delta^{13}\text{C}$ values are derived from the anaerobic oxidation of methane (AOM)?
5. Line 218: Large intra-crystal $\delta^{34}\text{S}$ variations in a single pyrite grain are a diagnostic feature of Rayleigh fractionation. Did all analyzed pyrite grains from this specific layer exhibit such wide-ranging variations? Additionally, could the authors briefly explain why the pyrite $\delta^{34}\text{S}$ signatures vary so drastically across different depths? Note that choosing modern groundwater sulfate as the baseline $\delta^{34}\text{S}$ of sulfate for calculating the fractionation factor has inherent limitations, as the pyrite could be ancient.
6. Line 315: I am a bit concerned about the paleodepth estimation. If we follow the authors' calculation scheme, the ambient surface temperature at that time would be close to 0 °C. Does this align with the authors' geological expectations or paleoclimate records for that period?
7. Lines 314-316: The authors only discussed the data from 42.7 °C and 52 °C. Does the temperature of 69.6 ± 14 °C at 128 m yield a consistent denudation estimate? Can all three shallow clumped isotope temperatures be explained by the same paleogeothermal gradient?
8. Line 336: Do the other stable or clumped isotope signatures of the youngest calcite sample at 1270 m show any anomalies? Do these observations corroborate the late-stage glaciation-induced fracture reactivation hypothesis proposed by the authors?

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Reviewer #1 (Remarks to the Author):

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I find the authors’ data to be thorough and of high quality, and their conclusions both interesting and robust. Specifically, linking metabolic activity to punctuated tectonic events is elegant and provides greater insight into how the deep biosphere operates. I therefore recommend publication of this manuscript with only minor revisions.

Thank you for the positive review. The line-by-line comments are addressed point by point below and changes are highlighted in yellow in the revised manuscript word document.

Below, I add my comments on specific lines in the manuscript:

Line 37–39: Revise the sentence starting with “This ancient...” to clarify that the metabolic process is an analogue of a metabolism that may have been important for early Earth conditions.

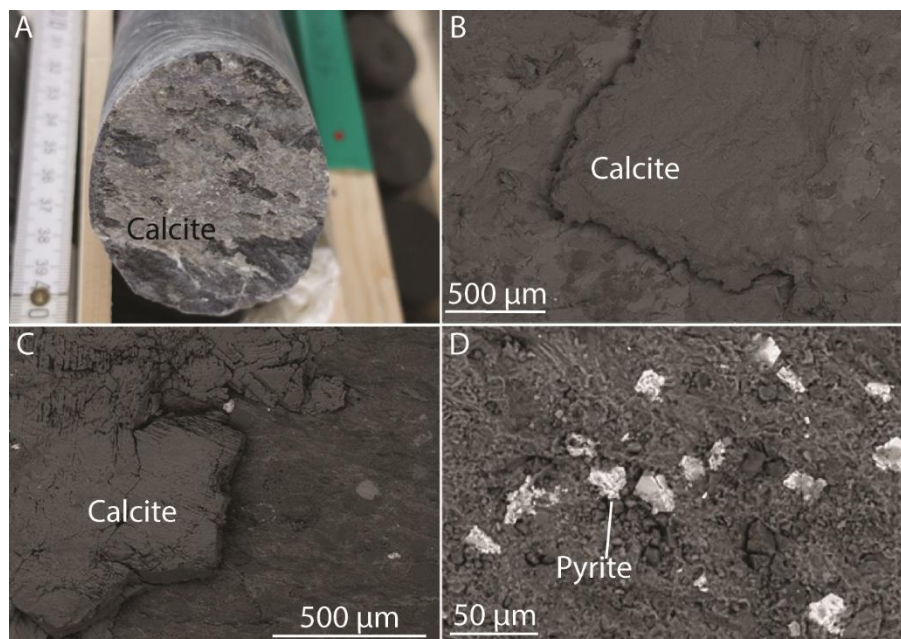
The sentence meant to refer to ‘analogues of early Earth’ and thus has been changed to “*This ancient metabolic process likely played a crucial role in shaping the early Earth’s atmosphere and climate (Sauterey et al., 2020), making it a key component for understanding early Earth history and planetary bodies analogous to early Earth. (Glass & Hörst, 2025).*” Lines 36-38

Line 82–84: Rephrase the opening sentence to first clearly state what the samples were (fractures? mineralized fractures?) and then present the components explored (calcite, fluid, gas). I had to re-read this sentence several times to understand it.

The sentence has been made clearer and was changed to: “*The drill core was samples for calcite- and/or pyrite-bearing open fractures from depths corresponding to those sampled for water and gas analysis. This includes the major conductive fracture (zone) of the thrust sheets at 975 m, that corresponds to the depth interval investigated for microbiological analyses (Westmeijer et al., 2024).*” Lines 81-85

Fig. 2: These images should be labeled to highlight mineral phases and important observations presented in the text.

The images have been labeled with mineral phases (Fig. 2, line 88).



Line 121 [whole section]: It may be worth spelling out exactly what type of clumped isotope measurement is being made, even if it is obvious to experts.

To clarify, the section has been made more clear by adding “*the carbonate clumped isotope composition derived using laser spectroscopy was only acquired from calcite at shallow depth*”. Lines 119-120

Line 168–170: Rephrase the sentence starting with “The stable...” as it currently reads as if the stable isotopes were “40 to 2081 m,” which is non-sensical.

This sentence has been changed to “*The stable isotope composition of water was analyzed over the depth interval 40-2081 m depth in the borehole (Supplementary Table 1), with $\delta^{18}O_{H_2O}$ values ranging from -14.6 to -12.9 ‰_{V-SMOW} and $\delta^2H_{H_2O}$ values ranging from -89.3 to -83.7 ‰_{V-SMOW} (Fig. 7 and Supplementary Table 2).*” Lines 167-170

Fig. 7: Consider dividing the legend into reference data (with a header titled “Reference Data”) and the new data presented in the paper. This would make the figure easier to interpret at a glance.

Thank you for this suggestion, this change has been made to Figure 7.

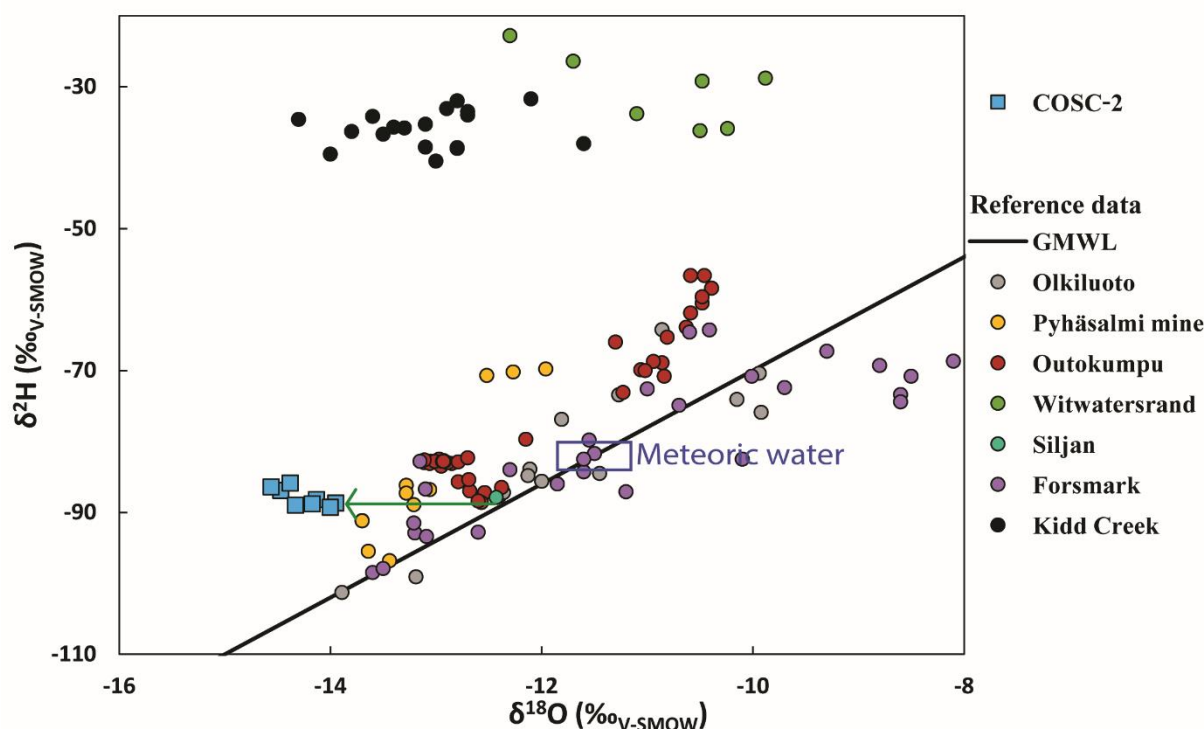


Fig. 7: The horizontal arrow suggests that only the oxygen isotopic composition is changing during water–rock interaction, whereas the text describes mechanisms that change both hydrogen and oxygen isotopic compositions. If possible, this could be remedied.

Following this and Reviewer 2's suggestions, the section on the text describing mechanisms that change hydrogen and oxygen isotopic composition has been shortened and clarified to only include relevant information. The main mechanism is that oxygen is depleted due to rock-water interactions.

This section has now been changed to: *“The source of the groundwater and potential long-term water-rock interactions affect the isotope composition of water. The $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ deviation to the left of the global meteoric water line (Fig. 7) is typical for deep brines in crystalline basement (e.g., Fritz & Frape, 1982). Comparing the $\delta^2\text{H}_{\text{H}_2\text{O}}$ and $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ results to other deep groundwaters, the composition was similar to the groundwater at Pyhäsalmi mine at depths between 2000-2400 m below surface level and the $\delta^2\text{H}_{\text{H}_2\text{O}}$ values were similar to the groundwater at the Outokumpu borehole down to 1300 m (Kietäväinen et al., 2013). Silicate hydrolysis and formation of clay minerals result in ^{18}O -depletion and ^2H -enrichment (Fritz & Frape, 1982) and precipitation of calcite and zeolite may deplete the liquid phase in ^{18}O (Karlsson & Clayton, 1990; Kloppmann et al., 2002; Warr et al., 2021). These mechanisms could explain the observed depleted $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ values, suggesting long-term water rock interaction in the studied groundwater samples.”*

Line 185 [whole section]: The section discusses one type of methanogenesis (hydrogenotrophic). If there is a reason only this mechanism is discussed, it should be made clear. It may also be interesting to discuss how AOM interacts with methanogenesis. Since this paragraph presents a key result, it would be helpful to have a strong concluding sentence stating exactly what is being claimed. Are you arguing that all these metabolisms were occurring?

This section has been edited, and acetoclastic methanogenesis is discussed as well in lines 192-198: *“Acetoclastic methanogenesis can lead to enriched calcites as well. In this pathway, acetate that is produced from organic matter degradation is becoming ^{13}C -enriched due to continuous fermentation (Dimitrakopoulos & Muehlenbachs, 1987; Franks et al., 2001). The ^{13}C -enriched acetate is subsequently utilized by methanogens, producing ^{13}C -enriched CO_2 alongside CH_4 , resulting in a ^{13}C rich inorganic carbon pool. The significantly ^{13}C -enriched calcite crystals in this study of up to 28.9*

%_{ov-PDB} (Figs. 3, 7) thus support formation following microbial methane production, but the pathway is elusive”

The concluding sentence was changed: “Taken together, the mineral-infilling record provided several lines of support for ancient methanogenesis as well as microbial sulfate reduction in the fracture system.” Lines 229-230

Fig. 8: Are the fields in the figure interpreted or derived from other work? If derived from other work, it should be cited in the figure caption.

A citation has been added to the figure caption.

Line 346: The text reads that the work has a number of implications but does not explicitly spell them out. Please clarify what these implications are.

This sentence was added: “Geological processes such as denudation and fault reactivation could be essential for facilitating deep subsurface methanogenesis.” Lines 345-346

Reviewer #2 (Remarks to the Author):

General Comments

Review of "Episodic records of deep microbial methanogenesis throughout the Caledonian mountain range exhumation"

This manuscript combines detailed carbonate analyses (e.g., in-situ C-O isotopes, clumped isotopes) from a deep scientific borehole in central Sweden with multiple lines of evidence (including CH₄ C-H isotopes and groundwater H-O isotopes) to link deep methanogenesis with regional crustal exhumation and mountain erosion. This approach is highly innovative. The heavy $\delta^{13}\text{C}$ values of the calcite provide exclusive and compelling evidence for microbial methanogenesis, offering excellent geological constraints on deep subsurface microbial activities. The manuscript integrates various analytical techniques, presents robust datasets, and provides a reasonable interpretation of the results. Overall, I like the story and support the publication of this manuscript. Yet, I feel several issues need to be addressed to sharpen the core arguments. I hope the following suggestions will be helpful for the revision.

Thank you for your review, the suggestions were very helpful in improving the manuscript. Below, all revisions are addressed, and changes in the revised manuscript are highlighted in green.

Major Concerns

Given that the central theme of this manuscript revolves around deep methanogenesis during the exhumation of the Caledonian orogeny, I argue that the precipitation temperatures of the carbonates should be another core focus of the paper, alongside the heavy $\delta^{13}\text{C}$ signatures and geochronology. The authors utilize these calcite precipitation temperatures to back-calculate paleodepths and further estimate the amount of denudation. However, the current discussion on this aspect is not sufficiently prominent. For instance, the authors offer only a brief speculation regarding why the youngest calcite occurs at a depth of 1270 m, even though some implications stated in the abstract might rely on this specific observation.

The clumped isotope measurements are quite few, as the amount of material needed for this type of analysis was not sufficient for each sample. The three clumped measurements that were obtained were used at the full of its potential, by estimating the paleodepth at the time of precipitation. The discussion on paleodepths has been altered to include all three measurements now, see lines 306-308.

“The temperature of 69.6 ± 14 °C at 128 m would then correspond to a paleodepth of 2.6 km, although its age is unresolved ($124 \pm 20/378 \pm 65$ Ma), therefore a denudation estimate is uncertain.”

The youngest calcite at 1270, is an observation that denotes the heterogeneity of water infiltration in the fracture system. This reiterates the observation that different flow paths have been active at different times. Another interesting factor is that this sample occurs close to the Caledonian decollement. This might be of interest as well, as this is a zone of weakness that has been reactivated so late. These discussion points and an extended discussion of this sample have been added to the lines 329-336: “A possible explanation could be that the Late Cenozoic Ice Age beginning around 2.6 Ma with ice sheets loading and unloading the crust may have induced minor fracturing events and fluid circulation, potentially linking the microbial activity linked to late-stage fracture reactivation. Although no evidence of glacial meltwater penetrating to this depth was not recorded in the calcite mineral, the sample occurs directly below the Caledonian décollement, which may be a zone of weakness susceptible to reactivated after a long period of stability. This young calcite age at this depth therefore suggests that water infiltration within the fracture network has been heterogeneous through time, with different flow paths becoming active at different times.”

Conversely, the sections discussing groundwater sources, modern microbial communities, and the stable isotopes of methane gas occupy a substantial portion of the text. Because these particular results do not strongly support the primary theme or the conclusions of the paper, they seem to distract from the core focus. I suggest reducing the length of these sections to make the central theme more clear and coherent.

The section on the modern groundwater isotopes has been shortened (lines 237-246), according to this and clarified as well as according to reviewer 1's suggestions (see above). The section on gas composition and discussion on $O_2 + Ar$ has been moved to Supplementary Material Text 2 (referenced in manuscript in line 153).

Furthermore, the authors might consider adding a schematic diagram to conceptually illustrate the linkage between mountain exhumation and deep episodic methanogenesis.

Thank you for your suggestion. It is a good idea, but as the mountain range feature erosion and uplift histories that are much more dynamic and complicated than basin scale models, it will be too speculative to try to tie each sample to different events of the mountain range architecture.

Specific Comments

1. Abstract: The U-Pb dating result of 605 ± 27 Ma for the 986 m sample is missing in the abstract. It should be included to ensure chronological completeness.

This date was excluded since this calcite sample did not show any indication of microbial methanogenesis. To adhere to the abstract word count, only the dates of calcite with microbial methanogenesis signatures were included.

2. Figure 3: Please explain why pyrite $\delta^{34}S$ data were only obtained/presented for three specific depths/intervals.

Pyrite was less common than calcite on the fracture surfaces. The presented pyrite data reflects the depths at which pyrite was observed on fracture surfaces and subsequently sampled. This is clarified now in lines 85-86 “and pyrite was more rare (e.g. cubic in Fig 2d). In total, three samples carried pyrite and eleven carried calcite.”

3. Line 197 (and throughout): The authors append standard notations (e.g., V-PDB, V-CDT) to some

permil symbols ‰ but omit them in other places. Please ensure strict consistency throughout the text and figures.

Thank you for noticing this, the text has been made consistent, with style ‰_{V-PDB} as the standard notation.

4. Line 202: Since the $\delta^{13}\text{C}$ values of methane in the borehole are around -30 ‰, which overlaps with the isotopic signatures of organic carbon mentioned by the authors, is it possible that these lower $\delta^{13}\text{C}$ values are derived from the anaerobic oxidation of methane (AOM)?

Yes, this is an interesting notion and AOM could have affected the $\delta^{13}\text{C}$ values. However, it is not certain the original methane produced in this borehole would have a more depleted $\delta^{13}\text{C}$ signature, or whether -30 ‰ is the original signature, there is very little evidence of methanotrophs in the microbial analysis (lines 283-285), we did not invoke AOM as an explanation for the methane isotopic signature, as the methane signature also overlaps in thermogenic field. This has been added in line 287:

“Therefore, the genomic evidence provides limited evidence for current microbial methanogenesis or methanotrophy occurring.”

5. Line 218: Large intra-crystal $\delta^{34}\text{S}$ variations in a single pyrite grain are a diagnostic feature of Rayleigh fractionation. Did all analyzed pyrite grains from this specific layer exhibit such wide-ranging variations? Additionally, could the authors briefly explain why the pyrite $\delta^{34}\text{S}$ signatures vary so drastically across different depths? Note that choosing modern groundwater sulfate as the baseline $\delta^{34}\text{S}$ of sulfate for calculating the fractionation factor has inherent limitations, as the pyrite could be ancient.

Other pyrite grains at this depth had intra-crystal variability of 10 ‰ and 3 ‰. At the other two depths intra-crystal variability was <1 ‰. Variations across the different depths would indicate different formation processes, hydrothermal formation or microbial sulfate reduction would alter the pyrite sulfur signature. The pyrite is likely ancient and $\delta^{34}\text{S}$ values are modern, which has its limitations and assumes stable $\delta^{34}\text{S}$ values. Changes have been made in lines 212-219:

“The dissolved sulfate in the modern groundwater at 975 m depth has a $\delta^{34}\text{S}_{\text{sulfate}}$ of 12.4 ‰_{V-CDT} (Westmeijer et al., 2024). Though the $\delta^{34}\text{S}_{\text{sulfate}}$ of the groundwater at the time of pyrite precipitation is unknown, assuming a similar isotopic composition of the dissolved sulfate at the time of precipitation of the pyrite, the enrichment factor $\epsilon^{34}\text{S}$ ($\epsilon^{34}\text{S} = \delta^{34}\text{S}_{\text{sulfate}} - \delta^{34}\text{S}_{\text{pyrite}}$) was between 9.4 to 16.2 ‰_{V-CDT}. However, $\epsilon^{34}\text{S}$ overlaps with thermochemical sulfate reduction values and as fractionation factors for microbial sulfate reduction are typically above 20 ‰_{V-CDT} (Machel et al., 1995), it is not sole evidence for microbial sulfate reduction. Instead, this pyrite could have been hydrothermal pyrite, formed under high temperature”

6. Line 315: I am a bit concerned about the paleodepth estimation. If we follow the authors' calculation scheme, the ambient surface temperature at that time would be close to 0 °C. Does this align with the authors' geological expectations or paleoclimate records for that period?

This is a good point, as Cretaceous climates would be much warmer than current temperatures. The paleodepth calculation has been adjusted, taking into account the surface temperature at that time according to (Sellwood & Valdes, 2006), which on average would be roughly 15 °C (model estimate for Late Cretaceous). Note that the paleodepth is a rough estimate, based on a paleogeothermal which is also an average estimate of the area. The original text:

“Assuming a paleo-geothermal gradient of 20 °C km⁻¹ (Huigen & Andriessen, 2004; Lorenz et al., 2022), this would suggest approximately 2.5 km of denudation since the Early Jurassic and is supported by similar results of 42.7 ± 6 °C at 109 ± 17 Ma, suggesting a denudation of 2 km since the

Late Cretaceous. Indications for ancient methanogenesis go as deep as 1907 m in ^{13}C -rich calcite dated to 100 ± 22 Ma, corresponding to a paleodepth of approximately 4 km depth given the aforementioned apparent denudation of at least 2 km since the Late Cretaceous, which is among the deepest ^{13}C -rich secondary calcite fracture-fills ever recorded (Liu et al., 2024). ”

The revised text (lines 304-312):

“Assuming a paleo-geothermal gradient of $20\text{ }^{\circ}\text{C km}^{-1}$ (Huigen & Andriessen, 2004; Lorenz et al., 2022), and an average surface temperature estimate of 15°C (Sellwood & Valdes, 2006), this would suggest approximately 1.7 km of denudation since the Early Jurassic and is supported by similar results of $42.7 \pm 6\text{ }^{\circ}\text{C}$ at 109 ± 17 Ma, suggesting a denudation of 1.3 km since the Late Cretaceous. The temperature of $69.6 \pm 14\text{ }^{\circ}\text{C}$ at 128 m would then correspond to a paleodepth of 2.6 km, although its age is unresolved ($124 \pm 20/378 \pm 65$ Ma), therefore a denudation estimate is uncertain.

Indications for ancient methanogenesis go as deep as 1907 m in ^{13}C -rich calcite dated to 100 ± 22 Ma, corresponding to a paleodepth of approximately <3 km depth given the aforementioned apparent denudation of at least 1.3 km since the Late Cretaceous, which is among the deepest ^{13}C -rich secondary calcite fracture-fills ever recorded (Liu et al., 2024). ”

7. Lines 314-316: The authors only discussed the data from $42.7\text{ }^{\circ}\text{C}$ and $52\text{ }^{\circ}\text{C}$. Does the temperature of $69.6 \pm 14\text{ }^{\circ}\text{C}$ at 128 m yield a consistent denudation estimate? Can all three shallow clumped isotope temperatures be explained by the same paleogeothermal gradient?

As the paleogeothermal gradient is an estimate that would apply to the whole of this area, on average, yes. However, sample 128 m yielded an age of 124 ± 20 Ma on the outer zone of the calcite mineral, whereas the inner zones appeared to be older but did not provide a robust isochron (378 ± 65 Ma). For this reason, a denudation estimate was not made, as the age was not certain for this particular mineral grain. This point of discussion has been added to lines 304-312 (see above).

8. Line 336: Do the other stable or clumped isotope signatures of the youngest calcite sample at 1270 m show any anomalies? Do these observations corroborate the late-stage glaciation-induced fracture reactivation hypothesis proposed by the authors?

No clumped isotope measurement was made on this sample, due to insufficient amount of material. The stable isotopes of this sample are consistent with microbial methanogenesis. The proposed mechanism of late-stage glaciation-induced fracture reactivation could promote occurrence of microbial methanogenesis at these depths, by creating space and allowing inflow. However, evidence of glacial meltwater penetrating to these depths is not found in the calcite mineral, as the oxygen isotope values are relatively enriched. This has been revised in the manuscript, the text has been changed from:

“A possible explanation could be that the Late Cenozoic Ice Age beginning around 2.6 Ma with ice sheets loading and unloading the crust induced minor fracturing events and microbial activity linked to late-stage fracture reactivation. ”

To:

“A possible explanation could be that the Late Cenozoic Ice Age beginning around 2.6 Ma with ice sheets loading and unloading the crust may have induced minor fracturing events and fluid circulation, potentially linking the microbial activity linked to late-stage fracture reactivation. Although no evidence of glacial meltwater penetrating to this depth was not recorded in the calcite mineral, ... ” (Lines 329-332)