

Flourishing chemosynthetic life at the greatest depths of hadal trenches

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

Version 0:

Reviewer comments:

Referee #1

(Remarks to the Author)

This manuscript offers an exciting glimpse of a host of newly discovered methane seeps at hadal depths. Although hadal vents have been described previously, the great depths probed here and flourishing communities revealed extend known habitat and depth (and biogeographic) distributions for a great many species. The authors could dwell on this more.

This represents a significant advance in the realm of discovery and exploration. While the contribution of this work is clear, there are many ways that this paper could be strengthened to meet the rigorous requirements of Nature.

The title refers to ...reveal huge methane reservoirs. While they are probably correct there is no mention of the nature or size of the reservoir feeding the seeps, therefore I suggest revising the title.

Line 38 The authors should probably mention the observation of likely chemosynthetic mats associated with serpentinization at the bottom of the Mariana Trench cited by Hand et al. 2020 <https://doi.org/10.1016/j.dsr.2020.103238>
These are deeper than the current paper, although not associated with specific animal communities.

Line 60 Please give the depth capability of the sub – is full ocean depth = 10,980 m?

Line 77 – What are the previous investigations in these trenches at 9500 m depth?

Terminology – The authors refer to tube-dwelling polychaetes (e.g., line 139, 143) when referring to non-siboglinids – however technically siboglinids ARE tube-dwelling polychaetes so this is very confusing. Also the authors refer to siboglinid tubeworms when referring to frenulates, but most uses of this term refer to the larger vestimentiferans. To avoid confusion I suggest the authors use the scientific terms (frenulate siboglinids) or family names (Ampharetidae) when referring to polychaetes. Similarly the use of common names throughout the paper (gastropods, bivalves, anemones, sea cucumbers, spoon worms) mixed with species/genus names is not appropriate. If no higher resolution ID is available use the scientific name for the order, class or family.

Please clarify if all IDs were based on morphological analyses or if genetic sequencing was carried out on any of the species collected. No genetic sequence information is given in the methods so the assumption is this is absent.

Line 120 – Note should be taken to how similar the clambered observations are to those made by Suess (et al. 1998) further east in the Aleutian Trench at similar depths.

Please give water depths when referring to the different site names since this information actually appears only in the supplementary Table I think.

Line 163 please define dDSMOW at first use.

Line 184. Note that many seeps have very diffuse flow and do not emit gas bubbles. Please see the literature on this.

Implications – this section makes claims without supporting data.

Line 225. This sentence refers to high abundances but these are only given in a supplementary Table – primarily as

densities of siboglinids. There are no methods describing how abundances were assessed, or if quantitative sampling took place. Given the statement that these surpass prior estimates for trenches it would be appropriate to provide the density data, methods for assessment, and previous trench data to which the densities are being compared.

Line 228 The authors also refer to richness of hadal biodiversity – but no diversity data/metrics are given. Similarly line 232 refers to hadal trenches as overlooked biodiversity hotspots. We are not told how diverse the communities are (only a few taxa are mentioned) and seeps have notoriously low alpha diversity. Are any of the observed species likely new? If so this could help support the claim of rich hadal biodiversity. It is important not to confuse biodiversity with productivity.

Line 240-241. The authors refer to sea anemones, spoon worms, sea cucumbers and amphipods as background animals – but how sure are they that this is true? While I agree the Scotoplanes are likely to occur on and off the seeps – have they looked for evidence of chemosymbionts in any of the taxa? Anemones have been shown to have sulfide oxidizing symbionts in other chemosynthetic ecosystems. It is possible that many of the species observed here are seep specialist, even if heterotrophic. Also please use scientific names for the taxa mentioned.

Do the authors speculate how much hadal methane is overlooked in the global carbon models? Does adiabatic heating in trenches influence hydrate stability and possible dissociation?

In summary, this is a valuable contribution providing the best support yet of previous conjectures that chemosynthetic communities should be common in trenches.

Lisa Levin

Referee #2

(Remarks to the Author)

This is an exciting study that documents the widespread occurrence of methane seeps and associated benthic ecosystems at hadal depths in deep-sea subduction trenches, essentially around the entire northern Pacific from the Kuril Islands to Alaska. This study will change the dominant perspective on hadal trenches: from the outermost frontier of marine life where dwindling input from the productive surface ocean imposes severe restrictions on density and diversity of benthic life, to a thriving hydrocarbon-driven geo-ecosystem where methane of sedimentary origin fuels dense and diverse assemblages of (previously unseen) seep fauna. As the authors note in their introduction, some previous studies have glimpsed hadal seep systems and taken same samples, but this survey makes it clear that hadal seep ecosystems are consistently wide-ranging and harbor diversified invertebrate communities. Such seep sites might even constitute a defining feature of subduction trenches, just as hydrothermal vents are a defining feature of mid-ocean spreading centers. In brief, this is a major discovery (enabled by newly developed deep-sea submersibles that are capable of accessing hadal depths) that should certainly be reported in *Nature*.

That said, the authors should consider changing the title of their study and place the emphasis on the seep ecosystem, not on the size of the hadal methane reserves. The chemosynthetic seep ecosystems are nicely documented by direct observation and consistent with geochemistry data, but the “huge methane reservoirs” would require more documentation. There is certainly abundant methane that fuels the hadal seep ecosystems, and it is very likely that these methane reserves are substantial and take the form of methane hydrates. However, the data do not explicitly quantify the size of these methane reservoirs; their “huge” size remains a working hypothesis at present, and the methane hydrates are not observed directly (apart from an unsatisfactory photo in Suppl. Figure 4). It would be safer (and closer to the truth) to emphasize the consistency of observing methane seeps and detecting microbially produced methane in sediments along thousands of miles in hadal trenches around the Northern Pacific.

I have some suggestions how to further improve this study, not in the sense of altering the general conclusions in the widespread hadal seeps, but for enlarging the underlying data. At present, geochemical and physical metadata provide the absolute minimum for documenting methane seeps: methane stable isotopes, sulfide and sulfate porewater, some mineral collection. Since porewater samples and sediment cores exist, it would be possible to add NH₄ or Total nitrogen, DOC (typically elevated at seeps), and salinity (a signature of salt exclusion due to methane hydrate formation). Also, are there any subbottom seismic profiles or multichannel seismic profiles that identify whiteout zones due to methane flux, (examples in Lapham et al. 2008, *Geochem. Geophys. Geosyst.*, 9, Q06001, doi:10.1029/2008GC001944; Teske et al. 2019 *Scientific Reports* 9:13847; <https://doi.org/10.1038/s41598-019-50200-5>) or Bottom simulating reflectors that typically indicate the presence of solid hydrate layers? Also, are there any thermal data for bottom water and fluids? These questions do not mean that I doubt the existing data; however, this publication would become the benchmark report on hadal trench-associated seep ecosystems, and there should be some effort to document this ecosystem as comprehensively as possible, beyond what is absolutely necessary.

Seep invertebrates were identified based on the species and genus level, using video imagery or by shipboard examination of recovered specimens. In first approximation, this is probably acceptable, since this is not a taxonomy paper; the point of this study is to demonstrate generally the existence of seep communities and seep species in deep-sea trenches. However, the authors should also note that molecular identification using taxonomically informative key genes would be required to check species identity. The sampling area (from near Japan to Alaska) is huge and provides more than enough scope for biogeographical separation and speciation.

Finally, the manuscript needs some careful editing to make sure that the word choice, figures and figure legends are as

precise as possible:

Line 165: “methyl-type fermentation” is not the term that a microbiologist or biochemist would use. Methyl groups are the carbon substrate for specific types of methanogenic archaea, and their metabolism would be called methylotrophic or methyl-based methanogenesis (Bueno de Mesquita et al. 2023. *Microbiology and Molecular Biology reviews*, vol 87, 10.1128/mmr.00024-22). This process is actually widespread in surficial marine sediments where it competes successfully with sulfate reduction (Zhuang et al. 2018. *Limnol. Oceanogr.* 63:2080-2092; <https://doi.org/10.1002/lno.10925>).

Lines 240, 243: “background animals” should be replaced by “heterotrophic benthic fauna”

line 525: it should be “... microbial and thermogenic methanogenesis.” At present, all known methanogenic microbes are members of the archaea, not bacteria.

The a) and b) labels in Figure 3 are missing. In the methane hydrate stability diagram (3b), the attempt to insert dashed lines (indicating T-P lines for different geothermal gradients) and explanatory text into the upper right corner of this plot lead to overcrowding. I suggest to draw a separate diagram for the supplementals, with appropriate supplemental discussion. At present, the manuscript text relating to Fig. 3b (lines 179-191) does not even mention the T-P lines and the extent of methane hydrate stability at greater sediment depths.

A separate plot for illustrating the potential depth extent of methane hydrate stability would also allow to remove the ill-fitting label “sediment depth” on the right side of Fig 3b. Since the pressure on the left side of this plot shows hydrostatic pressure, depth is implied as water depth.

Figure 4. There are no “black hollow arrows” (line 541) in this figure. I see thin black arrows (without any filling) indicating fault formation in the subducted plate, and larger arrows (filled in white or in local background color) indicating plate movement and organic matter input.

Also, I am wondering about the “striped zone” (line 544) indicating seeps. The diagrams 4a and 4b appear to show methane flares (generally conflated with bubble streams) emanating into the water column, but the manuscript does not provide evidence for flares. It is more likely that methane is gradually seeping and diffusing from the sediment into the overlying water, and appropriate symbols should be chosen to represent this process.

The interpretation of Supplementary Figure 4 as visual evidence for methane hydrates is not very convincing. The arrow points at something that could just as well be grey sediment that is transitioning into black sediment; on top of this is a light brown and beige-colored crust with small specks of black that could be anything. Toning down the interpretation of this figure does not mean denying the existence of hydrates; they should be there, but their unequivocal recovery would most likely require a more determined coring effort (long piston cores instead of short submersible push cores). Revisiting these seeps should provide ample opportunity to try coring.

Referee #3

(Remarks to the Author)

A.Summary of the key results:

This study documents the deepest known methane seep ever observed at 9533m, expanding the known depth range of these ecosystems. It provides a detailed characterization of seep-associated communities and environmental conditions at hadal depths, contributing to our understanding of deep-sea chemosynthetic habitats.

B.Originality and significance: if not novel, please include reference:

This study is novel, as no previous methane seep has been documented at this depth. It provides crucial insights into the biogeography and ecological limits of seep ecosystems, challenging previous assumptions about the depth constraints of chemosynthetic communities. While primarily descriptive, this study offers a detailed characterization of the seep environment and associated fauna, establishing a valuable foundation for future research. Beyond its ecological significance, this discovery has implications for deep-sea methane reservoirs and their role in global biogeochemical cycling. Investigating the fate of methane at hadal depths could enhance our understanding of carbon sequestration, microbial methane consumption, and the broader contribution of deep-sea processes to global methane fluxes. Future studies could build on these findings by examining functional processes, trophic interactions, and microbial mediation of methane dynamics in these extreme environments

C.Data & methodology: validity of approach, quality of data, quality of presentation:

The methodology appears robust, utilizing comprehensive visual surveys, in situ measurements, and sample analyses to characterize the seep habitat and its associated fauna. The data are well presented, with clear figures and tables that effectively convey key findings. No major concerns regarding the validity of the approach or data quality.

D.Appropriate use of statistics and treatment of uncertainties:

The study is primarily observational and descriptive, therefore statistics were not used. However, if quantitative comparisons (e.g., community composition across depths) are suggested to be included, addressing variability in sampling effort between depth zones could be valuable.

E.Conclusions: robustness, validity, reliability:

The conclusions are well-supported by the data and effectively highlight the ecological significance of hadal seeps. The

study provides compelling evidence for the presence of chemosynthetic communities at extreme depths and discusses the implications for deep-sea biogeography and energy flux.

F.Suggested improvements: experiments, data for possible revision:

The manuscript does not have fundamental flaws that would prohibit publication, but there are areas that could be strengthened. The study is primarily descriptive, and while the discovery of a methane seep at hadal depths is certainly novel and significant, the manuscript could benefit from a more in-depth analysis of functional processes. Additionally, while estimated densities of fauna are provided, further quantitative analyses could enhance ecological interpretations.

Some other small suggestions:

1. Taxonomic correction – The manuscript mislabels the genus *Lamellisabella* as *Lamellesabelia*. This should be corrected to ensure accurate taxonomic representation.
2. Clarity and Conciseness – Some sections are quite dense. Breaking up long paragraphs and streamlining descriptions could improve readability.
3. Clarify sample numbers - While the manuscript provides a thorough description of the sampling techniques and analytical methods, it does not explicitly state how many push cores or gas samples were collected in each area. Clarifying the sample numbers for each site would improve transparency and allow for a better assessment of the dataset's robustness.
4. Site imagery – The manuscript describes the "Dead Valley" site but does not include any images of this area. Given that it is discussed in the text, adding an image or figure showing this site would enhance the reader's understanding and provide visual context for its features

G.References: appropriate credit to previous work? - Yes

H.Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions:

The abstract and introduction clearly present the study's novelty, objectives, and key findings, making it accessible to a broad audience. The conclusions effectively summarize the study's significance. Minor refinements to the introduction could strengthen the contextual link between shallower seeps and the newly discovered hadal seep.

Version 1:

Reviewer comments:

Referee #1

(Remarks to the Author)

Review of 502133_1

This ms is improved with response to reviewers. However, I do not recommend publication until the authors have addressed the issues below. I am concerned that the biology seems like an afterthought in its mode of presentation.

TITLE: I strongly suggest that unless you focus more on diversity (and prove these are really diverse communities) that you take the word 'Diverse' out of the title. Nothing in the list of species given suggests high diversity. You could say "Flourishing communities...". Diverse means that there are either a lot of species present and/or that they individuals are evenly distributed among many species. You deleted 'biodiversity' hotspots in the text – this also needs removal from the title.

Please list background species where you refer to them.

Line 13 – Suggest you say siboglinid Polychaeta

Line 43 - suggest seep animal communities, microbes are organisms and form communities

Line 69... No part of the ocean is 11,000 m. So I'm not sure this makes sense. (10,980 is the deepest?). Maybe say capable of reaching the deepest part of the ocean at nearly 11,000 m)

Line 97 and 99 and 101 – PLEASE do not call the frenulates tubeworms

Line 263-4 High biomass and density (compared to what?) Non seep settings? Other seeps? Your densities are comparable to reports from other seep sites.

This sentence is not correct. Biomass is measured as weight (e.g., g or kg). Numbers of bivalves is not biomass. The biomass at most cold seep sites in this study far exceeds previous Japan Trench records^{3,4}, where only tens to hundreds of chemosynthetic bivalves were noted.

I would remove discussion of biomass unless you weighed samples and calculated wt/unit area.

Why are the siboglinid and bivalve density data presented in implications section. These need to be in the results section where the fauna is described. Are these values maximum values? Where are the data deposited?

In the implications - are there actually density data for the Japan Trench? Other trenches to compare?

Line 268 Bivalves misspelled.

Line 290 – remove ‘sea’

Line 614 – Where are the COI data deposited? Found this material in supplemental – probably needs to be in the paper.

Line 598 to 602. The very brief video methods listed are insufficient to describe how density assessments were done. The type of information needed would include:

*What was analyzed – replicate frame grabs from each site? Or 1 minute video segments? Or something else?

*No error bars are given. Are these counts based on single images? They list the maximum so presumably there were areas counted with fewer?

*How was area in these images determined? – the lasers alone do not provide enough information. Or did you just do counts between the laser points?

Please refer to Table S1 in the main text as the source of density information – ideally in the results.

Supplementary Table 2 Why is *Lamellisabella* not listed here.? It seems you updated the main text but not the Supplemental table.

Lisa Levin

Referee #2

(Remarks to the Author)

My comments and suggestions have been taken up constructively; the manuscript is now much improved, and contains more geochemical metadata that provide further context for hadal seep sediment and the habitat for these seep communities. The new version also draws a clear distinction towards factors that remain unknown, such as the size of hadal methane reservoirs.

Some details require attention:

Line 155: This should read “During dive FDZ 278...”

The new title is odd. The central point of this manuscript is not that these chemosynthetic communities are located at the greatest depth of hadal seeps. You want to emphasize that these hadal seeps and their chemosynthetic communities are located at the greatest depth of hadal trenches.

Line 165 & 166: “Fields of siboglinids and clusters of bivalves within the KAT were usually adjacent, but spatially separated”. This makes no sense, if they are spatially separated (meaning, there is a gap in between) they cannot be adjacent. You probably want to say that these fields were located next to each other, but did not overlap (meaning, there was no zone where these organisms coexisted).

Line 238 and 239: include not only the permille fractionation, but also the isotopic reference standards

Line 271-272: When you say that “methane is present in the form of dissolved fluid (liquid)” this could be misunderstood as gas in liquid form, such as liquid nitrogen (which requires very cold temperatures). You want to say that methane occurs in dissolved form, in aqueous solution in porewater.

Line 298: Better: “A focused coring and drilling campaign is needed...” (a few push cores do not count as a focused campaign)

Line 356: bivalves, not biovalves

Line 386: Omit “sea”, therefore: “such as Actiniaria...”

Line 389: better: “is demonstrated and quantified by follow-up research...”

Line 447: better: “It remains unknown whether the current findings can be extrapolated...”

Line 447: Please note the ODP drilling has recovered methane hydrates not just on the continental margin, but in trench sediments in the Peru Trench at > 5000m depth (Shipboard Scientific Party, 2003. Site 1230. In D'Hondt, S.L., Jørgensen, B.B., Miller, D.J., et al., Proc. ODP, Init. Repts., 201: College Station, TX (Ocean Drilling Program), 1–107.

doi:10.2973/odp.proc.ir.201.111.2003)

Referee #3

(Remarks to the Author)

I'm satisfied with the revisions made in this version of the manuscript. The authors have addressed the major concerns raised in the initial round of review, and the paper now presents a well-structured, compelling, and enjoyable account of hadal methane seeps and associated communities. It reads clearly and concisely, and represents an exciting contribution to deep-sea research. In my opinion, it is ready for publication pending a few very minor editorial adjustments and some general finalizing of the text, such as:

- Line 54 – correct “research”
- Line 56 – remove “a” before “predominantly”
- Lines 102–105 – for clarity, consider adding commas after each faunal group or placing the taxonomic names in parentheses

- Line 111 – correct to “siboglinids”
- Line 116 – add a comma after “polychaetes”
- Line 162 – add a comma after “siboglinids”
- Line 163 – consider changing “east to” to “east of”
- Line 182 – the sentence could be refined
- Lines 200–205 – this sentence is a bit dense. Consider splitting it into two
- Line 268 – correct the spelling of “bivalves”
- Line 274 – suggest rewording as “the distribution ranges observed”
- Line 310 – remove the comma after “unknown”

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Response to the Editor and Referees

Referees' comments:

Referee #1 (Remarks to the Author):

This manuscript offers an exciting glimpse of a host of newly discovered methane seeps at hadal depths. Although hadal vents have been described previously, the great depths probed here and flourishing communities revealed extend known habitat and depth (and biogeographic) distributions for a great many species. The authors could dwell on this more.

Reply:

We extend our heartfelt appreciation for Prof. Lisa Levin's insightful comments. We have dwelled on these points more within the Implications section.

This represents a significant advance in the realm of discovery and exploration. While the contribution of this work is clear, there are many ways that this paper could be strengthened to meet the rigorous requirements of Nature. The title refers to ...reveal huge methane reservoirs. While they are probably correct there is no mention of the nature or size of the reservoir feeding the seeps, therefore I suggest revising the title.

Reply:

We fully agree with this valuable and constructive suggestion. We have accordingly softened the language concerning the nature and scale of the hadal methane reservoirs throughout the manuscript. Additionally, we have revised the title to "Diverse Chemosynthetic Communities at the Greatest Depths of Hadal Seeps," as recommended by the referees and the editor.

Line 38 The authors should probably mention the observation of likely chemosynthetic mats associated with serpentinization at the bottom of the Mariana Trench cited by Hand et al.

2020 <https://doi.org/10.1016/j.dsr.2020.103238>. These are deeper than the current paper, although not associated with specific animal communities.

Reply:

Many thanks for this suggestion. We have mentioned this study in the revised manuscript as follows: Additionally, likely chemosynthetic mats were observed at a depth of 10,677 m at the bottom of the Mariana Trench; however, they are not associated with any seep communities.

Line 60 Please give the depth capability of the sub – is full ocean depth = 10,980 m?

Reply:

Full ocean depth = 11,000 m. Change has been made to the manuscript.

Line 77 – What are the previous investigations in these trenches at 9500 m depth?

Reply:

We have added a sentence to introduce the previous investigations in these trenches.

Terminology – The authors refer to tube-dwelling polychaetes (e.g., line 139, 143) when referring to non-siboglinids – however technically siboglinids ARE tube-dwelling polychaetes so this is very confusing. Also the authors refer to siboglinid tubeworms when referring to frenulates, but most uses of this term refer to the larger vestimentiferans. To avoid confusion I suggest the authors use the scientific terms (frenulate siboglinids) or family names (Ampharetidae) when referring to polylchaetes. Similarly the use of common names throughout the paper (gastropods, bivalves, anemones, sea cucumbers, spoon worms) mixed with species/genus names is not appropriate. If no higher resolution ID is available use the scientific name for the order, class or family.

Reply:

We agree with the referee's comment. Changes have been made to the manuscript.

Please clarify if all IDs were based on morphological analyses or if genetic sequencing was carried out on any of the species collected. No genetic sequence information is given in the methods so the assumption is this is absent.

Reply:

Most of our identification works are based on morphological analyses and confirmed by experts in respective fields. We are grateful to the referee for this remark and agree with it. For this reason, we do not provide species names for most taxa, for which all necessary data and sequences have not yet been obtained. The exceptions are two typical chemosynthetic frenulate siboglinids (FD Z271 and FDZ 294 in Supplementary Figure 1) and all the four chemosynthetic bivalves (Supplementary Figure 3). We provided their *coxI* gene sequences as extra evidences (Supplementary Table 2). However, the two frenulates and the bivalve FDZ 298 could not be assigned to known species because of the lack of closely relative gene references.

Line 120 – Note should be taken to how similar the clambed observations are to those made by Suess (et al. 1998) further east in the Aleutian Trench at similar depths.

Reply:

We agree with the referee's comment. Changes have been made to the manuscript.

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Reply:

We agree with the referee's constructive comment and sorry for this confusion. In this study, we only use photographs and video observations to obtain the density data (please find the method for density in the section of Method- Processing of benthic fauna and seafloor video footage). Because no quantitative sampling took place during the cruise, we cannot provide abundance data. To state this clearer, we have changed “abundances” to “density”. We have further compared biomass and density data with that in Japan Trench.

Line 228 The authors also refer to richness of hadal biodiversity – but no diversity data/metrics are given. Similarly line 232 refers to hadal trenches as overlooked biodiversity hotspots. We are not told how diverse the communities are (only a few taxa are mentioned) and seeps have notoriously low alpha diversity. Are any of the observed species likely new? If so this could help support the claim of rich hadal biodiversity. It is important not to confuse biodiversity with productivity.

Reply:

We concur with the referee's insightful comment. While certain specimens we observed and collected may represent new species, we require additional time for their taxonomic identification. To avoid an overestimation of the unappreciated biodiversity, we have deleted two sentences from the Implications section.

Line 240-241. The authors refer to sea anemones, spoon worms, sea cucumbers and amphipods as background animals – but how sure are they that this is true? While I agree the Scotoplanes are likely to occur on and off the seeps – have they looked for evidence of chemosymbionts in any of the taxa? Anemones have been shown to have sulfide oxidizing symbionts in other chemosynthetic ecosystems. It is possible that many of the species observed here are seep specialist, even if heterotrophic. Also please use scientific names for the taxa mentioned.

Reply:

We agree with the referee's remark that there are specialized species among the listed taxa. However, in the mentioned sentences we mean species common to areas outside the seeps (e.g., the holothurian *Elpidia hansenii*, the echiuran *Vitjazema ultraabyssalis*, the free-moving

polychaetes *Macellicephaloides*, etc.). These taxa have been well studied previously, and the identification of the specimens has been confirmed by experts.

Do the authors speculate how much hadal methane is overlooked in the global carbon models?

Reply:

We are currently unable to quantify the magnitude of hadal methane that remains unaccounted for in global carbon models. This is due to the current absence of the required subbottom seismic profiles or multichannel seismic data, which are essential for determining the amount of methane stored within these trenches.

Does adiabatic heating in trenches influence hydrate stability and possible dissociation?

Reply:

Based on CTD (conductivity, temperature, and depth) data from submersible, the seawater within the study area demonstrates an adiabatic heating value of roughly 0.15°C. The coldest temperature recorded was 1.51°C at a depth of 4,350 meters. At the deepest site, where a cold seep ecosystem was identified at 9,533 meters, the seawater temperature was approximately 2.25°C, accompanied by a pressure of 98 MPa. These temperature and pressure conditions fall within the stability zone for methane hydrates. Consequently, as long as the methane concentration in the sediment (pore water) remains above the solubility threshold for hydrates, the adiabatic heating effect of the seawater will not disrupt hydrate stability or trigger hydrate dissociation.

In summary, this is a valuable contribution providing the best support yet of previous conjectures that chemosynthetic communities should be common in trenches.

Lisa Levin

Referee #2 (Remarks to the Author):

This is an exciting study that documents the widespread occurrence of methane seeps and associated benthic ecosystems at hadal depths in deep-sea subduction trenches, essentially around the entire northern Pacific from the Kuril Islands to Alaska. This study will change the dominant perspective on hadal trenches: from the outermost frontier of marine life where dwindling input from the productive surface ocean imposes severe restrictions on density and diversity of benthic life, to a thriving hydrocarbon-driven geo-ecosystem where methane of

sedimentary origin fuels dense and diverse assemblages of (previously unseen) seep fauna. As the authors note in their introduction, some previous studies have glimpsed hadal seep systems and taken same samples, but this survey makes it clear that hadal seep ecosystems are consistently wide-ranging and harbor diversified invertebrate communities. Such seep sites might even constitute a defining feature of subduction trenches, just as hydrothermal vents are a defining feature of mid-ocean spreading centers. In brief, this is a major discovery (enabled by newly developed deep-sea submersibles that are capable of accessing hadal depths) that should certainly be reported in Nature.

Reply:

We are greatly appreciative of the referee's comments.

That said, the authors should consider changing the title of their study and place the emphasis on the seep ecosystem, not on the size of the hadal methane reserves. The chemosynthetic seep ecosystems are nicely documented by direct observation and consistent with geochemistry data, but the "huge methane reservoirs" would require more documentation. There is certainly abundant methane that fuels the hadal seep ecosystems, and it is very likely that these methane reserves are substantial and take the form of methane hydrates. However, the data do not explicitly quantify the size of these methane reservoirs; their "huge" size remains a working hypothesis at present, and the methane hydrates are not observed directly (apart from an unsatisfactory photo in Suppl. Figure 4). It would be safer (and closer to the truth) to emphasize the consistency of observing methane seeps and detecting microbially produced methane in sediments along thousands of miles in hadal trenches around the Northern Pacific.

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I have some suggestions how to further improve this study, not in the sense of altering the general conclusions in the widespread hadal seeps, but for enlarging the underlying data. At present, geochemical and physical metadata provide the absolute minimum for documenting methane seeps: methane stable isotopes, sulfide and sulfate porewater, some mineral collection. Since porewater samples and sediment cores exist, it would be possible to add NH₄ or Total nitrogen, DOC (typically elevated at seeps), and salinity (a signature of salt exclusion due to methane hydrate formation).

Reply:

We agree with the referee's constructive suggestions. We have added the supplementary Figure 4, which shows the profiles of CH₄, NH₄, DOC, DIC, $\delta^{13}\text{C}$ -DIC, SO₄²⁻, H₂S and salinity in the two sediment pushcores. These data showed typical distribution patterns associated with cold seeps. Changes have been made to the manuscript as follows:

The vertical profiles of methane, ammonia (NH₄⁺), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), $\delta^{13}\text{C}$ -DIC, sulfate (SO₄²⁻), H₂S and salinity in two pushcores (Figure S4) showed typical distribution patterns associated with cold seep environments, indicating the processes of anaerobic methane oxidation coupled with sulphate reduction^{5,29,30}, organic matter diagenesis^{5,30} and hydrate decomposition³¹.

Also, are there any subbottom seismic profiles or multichannel seismic profiles that identify whiteout zones due to methane flux, (examples in Lapham et al. 2008, *Geochem. Geophys. Geosyst.*, 9, Q06001, doi:10.1029/2008GC001944; Teske et al. 2019 *Scientific Reports* 9:13847; <https://doi.org/10.1038/s41598-019-50200-5>) or Bottom simulating reflectors that typically indicate the presence of solid hydrate layers? Also, are there any thermal data for bottom water and fluids? These questions do not mean that I doubt the existing data; however, this publication would become the benchmark report on hadal trench-associated seep ecosystems, and there should be some effort to document this ecosystem as comprehensively as possible, beyond what is absolutely necessary.

Reply:

We are grateful for this insightful suggestion. However, it is regrettable that no sub-bottom seismic or multichannel seismic profiles are available to identify whiteout zones or bottom simulating reflectors, as the expedition's focus was on dive operations and no seismic equipment was aboard the vessel. To date, there is also a lack of thermal data for bottom water and fluids. We acknowledge the importance of these data for a deeper understanding of fluid migration in the deep and for assessing the size of methane reservoirs beneath the surface. We believe that these studies can be conducted during future geophysical investigation expeditions, which will aid in our enhanced comprehension of these hadal ecosystems.

Seep invertebrates were identified based on the species and genus level, using video imagery or by shipboard examination of recovered specimens. In first approximation, this is probably acceptable, since this is not a taxonomy paper; the point of this study is to demonstrate generally the existence of seep communities and seep species in deep-sea trenches. However, the authors should also note that molecular identification using taxonomically informative key genes would be required to check species identity. The sampling area (from near Japan to Alaska) is huge and provides more than enough scope for biogeographical separation and speciation.

Reply:

Most of our identification works are based on morphological analyses and confirmed by experts in respective fields. We are grateful to the referee for this remark and agree with it. For this reason, we do not provide species names for most taxa, for which all necessary data and sequences have not yet been obtained. The exceptions are two typical chemosynthetic frenulate siboglinids (FD Z271 and FDZ 294 in Supplementary Figure 1) and all the four chemosynthetic bivalves (Supplementary Figure 3). We provided their *coxI* gene sequences as extra evidences (Supplementary Table 2). However, the two frenulates and the bivalve FDZ 298 could not be assigned to known species because of the lack of closely relative gene references.

Finally, the manuscript needs some careful editing to make sure that the word choice, figures and figure legends are as precise as possible:

Line 165: “methyl-type fermentation” is not the term that a microbiologist or biochemist would use. Methyl groups are the carbon substrate for specific types of methanogenic archaea, and their metabolism would be called methylotrophic or methyl-based methanogenesis (Bueno de Mesquita et al. 2023. Microbiology and Molecular Biology reviews, vol 87, 10.1128/membr.00024-22). This process is actually widespread in surficial marine sediments where it competes successfully with sulfate reduction (Zhuang et al. 2018. Limnol. Oceanogr. 63:2080-2092; <https://doi.org/10.1002/lno.10925>).

Reply:

Thanks for this valuable suggestion. We have changed “methyl-type fermentation” to “methyl-based methanogenesis” throughout the manuscript and have cited the reference (Bueno de Mesquita et al. 2023) in the manuscript.

Lines 240, 243: “background animals” should be replaced by “heterotrophic benthic fauna”

Reply:

Thanks for this suggestion. We have done it.

line 525: it should be “... microbial and thermogenic methanogenesis.” At present, all known methanogenic microbes are members of the archaea, not bacteria.

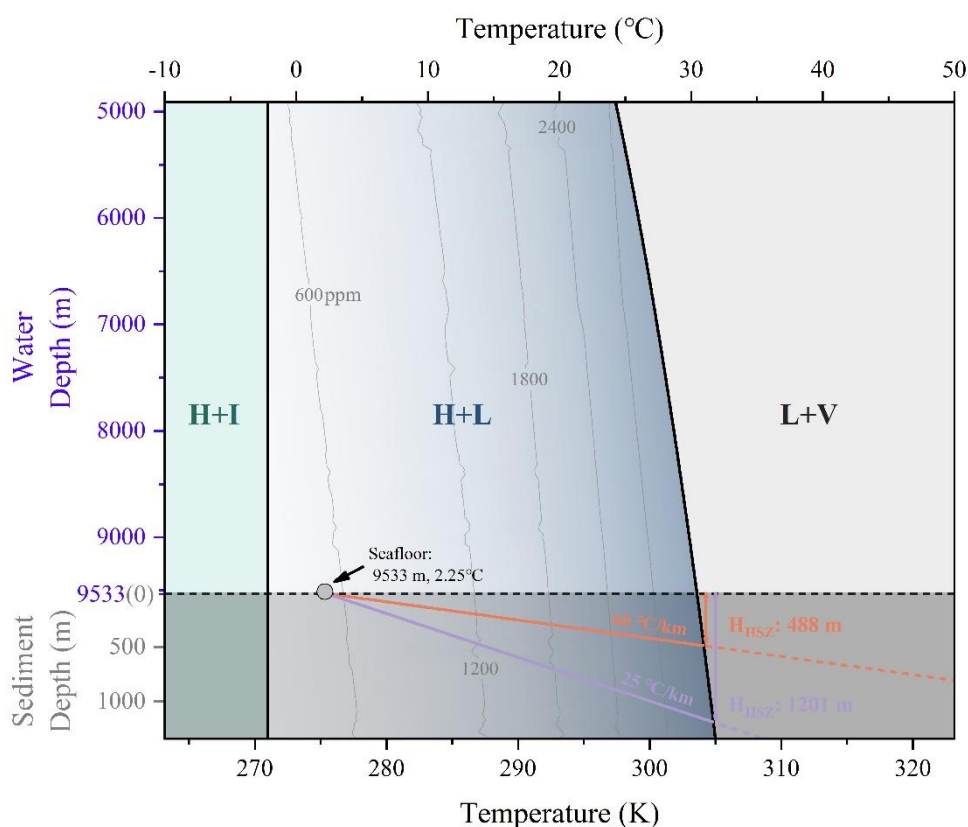
Reply:

Thanks for this valuable comments. It has been done.

The a) and b) labels in Figure 3 are missing. In the methane hydrate stability diagram (3b), the attempt to insert dashed lines (indicating T-P lines for different geothermal gradients) and explanatory text into the upper right corner of this plot lead to overcrowding. I suggest to draw a separate diagram for the supplementals, with appropriate supplemental discussion. At present, the manuscript text relating to Fig. 3b (lines 179-191) does not even mention the T-P lines and the extent of methane hydrate stability at greater sediment depths. A separate plot for illustrating the potential depth extent of methane hydrate stability would also allow to remove the ill-fitting label “sediment depth” on the right side of Fig 3b. Since the pressure on the left side of this plot shows hydrostatic pressure, depth is implied as water depth.

Reply:

Many thanks for this constructive comment. According to the referee’s suggestion, we have revised Figure 3b and have also added a separate diagram (supplementary Fig.6) for the supplemental with appropriate discussion.



Supplementary Figure 6 A phase diagram illustrating hydrate stability conditions in the sediments of the investigated hadal zone. The horizontal black dashed line denotes the 9,533-meter water depth, corresponding to 98.0 MPa of hydrostatic pressure (assuming an average water density of 1.045 g/cm³), above which lies the unconsolidated sub-seafloor sediments. The gray circle denotes the seafloor temperature-pressure conditions at 9,533 meters, with a temperature of 2.25°C and a pressure of 98.0 MPa. The purple and red lines represent the minimum and maximum

geothermal gradients (ranging from 25 to 60°C/km) within the unconsolidated sediments, respectively. The point of intersection between these geothermal gradients and HLV delineates the vertical limits of the hydrate stability zone (HSZ) within the sediment.

Figure 4. There are no “black hollow arrows” (line 541) in this figure. I see thin black arrows (without any filling) indicating fault formation in the subducted plate, and larger arrows (filled in white or in local background color) indicating plate movement and organic matter input.

Reply:

We have modified the figure in which we use light green arrows indicating organic matter input

Also, I am wondering about the “striped zone” (line 544) indicating seeps. The diagrams 4a and 4b appear to show methane flares (generally conflated with bubble streams) emanating into the water column, but the manuscript does not provide evidence for flares. It is more likely that methane is gradually seeping and diffusing from the sediment into the overlying water, and appropriate symbols should be chosen to represent this process.

Reply:

We apologize for the confusion. In the previous version, we utilized flare-like symbols to denote the seep community (tubeworms), which has seemingly caused some misunderstanding. We have now updated the symbols for the seep community and have also included this change in the legend for clarity.

The interpretation of Supplementary Figure 4 as visual evidence for methane hydrates is not very convincing. The arrow points at something that could just as well be grey sediment that is transitioning into black sediment; on top of this is a light brown and beige-colored crust with small specks of black that could be anything. Toning down the interpretation of this figure does not mean denying the existence of hydrates; they should be there, but their unequivocal recovery would most likely require a more determined coring effort (long piston cores instead of short submersible push cores). Revisiting these seeps should provide ample opportunity to try coring.

Reply:

We highly appreciated the referee’s constructive comments. We have toning down the statement of potential existence of gas hydrate and also have deleted Supplementary Figure 4. A sentence has been added at the end of this paragraph as follows: “Calculation of the hydrate

stability zone (Fig.S6) suggests the potential presence of hydrates at these profound depths in sedimentary layers. A more focused coring and drilling campaign is needed to revisit these seeps in order to validate this hypothesis”.

Referee #3 (Remarks to the Author):

A. Summary of the key results:

This study documents the deepest known methane seep ever observed at 9533m, expanding the known depth range of these ecosystems. It provides a detailed characterization of seep-associated communities and environmental conditions at hadal depths, contributing to our understanding of deep-sea chemosynthetic habitats.

Reply:

We are greatly appreciative of the referee's comments.

B. Originality and significance: if not novel, please include reference:

This study is novel, as no previous methane seep has been documented at this depth. It provides crucial insights into the biogeography and ecological limits of seep ecosystems, challenging previous assumptions about the depth constraints of chemosynthetic communities. While primarily descriptive, this study offers a detailed characterization of the seep environment and associated fauna, establishing a valuable foundation for future research. Beyond its ecological significance, this discovery has implications for deep-sea methane reservoirs and their role in global biogeochemical cycling. Investigating the fate of methane at hadal depths could enhance our understanding of carbon sequestration, microbial methane consumption, and the broader contribution of deep-sea processes to global methane fluxes. Future studies could build on these findings by examining functional processes, trophic interactions, and microbial mediation of methane dynamics in these extreme environments

Reply:

We are greatly appreciative of the referee's comments.

C. Data & methodology: validity of approach, quality of data, quality of presentation:

The methodology appears robust, utilizing comprehensive visual surveys, in situ measurements, and sample analyses to characterize the seep habitat and its associated fauna. The data are well presented, with clear figures and tables that effectively convey key findings. No major concerns regarding the validity of the approach or data quality.

Reply:

We are greatly appreciative of the referee's comments.

D. Appropriate use of statistics and treatment of uncertainties:

The study is primarily observational and descriptive, therefore statistics were not used. However, if quantitative comparisons (e.g., community composition across depths) are suggested to be included, addressing variability in sampling effort between depth zones could be valuable.

Reply:

We are thankful to the referee for this valuable comment and concur with the suggestion that further quantitative analysis of the data is essential for ecological interpretation. We hold that such an analysis should be the focal point of comprehensive studies, which would necessitate detailed data on the fauna's composition and trophic interactions. Without such robust data, the analysis would be limited to a superficial level. The pursuit of these studies is among our future objectives. However, their successful completion is contingent upon extensive, long-term research. This includes the discovery of numerous species new to science and the requirement for a broad spectrum of identification methods to accurately classify these taxa.

E. Conclusions: robustness, validity, reliability:

The conclusions are well-supported by the data and effectively highlight the ecological significance of hadal seeps. The study provides compelling evidence for the presence of chemosynthetic communities at extreme depths and discusses the implications for deep-sea biogeography and energy flux.

Reply:

We are greatly appreciative of the referee's comment.

F. Suggested improvements: experiments, data for possible revision:

The manuscript does not have fundamental flaws that would prohibit publication, but there are areas that could be strengthened. The study is primarily descriptive, and while the discovery of a methane seep at hadal depths is certainly novel and significant, the manuscript could benefit from a more in-depth analysis of functional processes. Additionally, while estimated densities of fauna are provided, further quantitative analyses could enhance ecological interpretations.

Reply:

We sincerely appreciate the referee's insightful comments. Nevertheless, due to the limitations of our current timeframe and the lack of quantitative sampling, we are unable to proceed with a more in-depth analysis of functional processes and quantitative assessments at this juncture. These aspects will, however, be our primary focus in our subsequent research endeavors.

Some other small suggestions:

1. Taxonomic correction – The manuscript mislabels the genus *Lamellisabella* as *Lamellesabelia*. This should be corrected to ensure accurate taxonomic representation.

Reply:

Thanks for this suggestion. We have updated this in the text.

2. Clarity and Conciseness – Some sections are quite dense. Breaking up long paragraphs and streamlining descriptions could improve readability.

Reply:

Thanks for this suggestion. It has been done.

3. Clarify sample numbers - While the manuscript provides a thorough description of the sampling techniques and analytical methods, it does not explicitly state how many push cores or gas samples were collected in each area. Clarifying the sample numbers for each site would improve transparency and allow for a better assessment of the dataset's robustness.

Reply:

We highly appreciate this suggestion. We have incorporated a description of the sample number in the "Methods" section, specifically under "Gas and Porewater Sampling," as follows:

During each dive, 6 to 12 sediment pushcores were collected utilizing the manipulators of the submersible. Upon recovery, these pushcores were immediately transported to the ship's cold room, which is kept at around 4° C, to facilitate subsequent processing. Among them, 1 to 2 pushcores were allocated for gas concentration analysis onboard, with only subsamples exhibiting high methane concentrations being prepared for further carbon and deuterium isotope analysis. Additionally, 1 to 2 pushcores were employed to extract porewater samples for geochemical analysis.

4. Site imagery – The manuscript describes the "Dead Valley" site but does not include any images of this area. Given that it is discussed in the text, adding an image or figure showing this site would enhance the reader's understanding and provide visual context for its features

Reply:

Thanks for this suggestion. We have added a figure as supplementary Figure 1 which shows Dead Valley, as well as other two seep sites Clam Valley and Turtle Egg that were also listed in the supplementary Table1.

G. References: appropriate credit to previous work? - Yes

H. Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions:

The abstract and introduction clearly present the study's novelty, objectives, and key findings, making it accessible to a broad audience. The conclusions effectively summarize the study's significance. Minor refinements to the introduction could strengthen the contextual link between shallower seeps and the newly discovered hadal seep.

Reply:

We highly appreciated the referee's constructive comments. Minor refinements to the introduction have been made.

Response to the Editor and Referees

We thank the editor and three referees for their insightful and valuable feedback. We've revised the manuscript in response, addressing all referee comments, particularly those from Referee #1 on textual matters. We've also streamlined the paper to meet the Guide to Authors' guidelines, ensuring all editorial and Reporting Summary issues are resolved. The detailed response to each referee's points is provided below.

Referees' comments:

Referee #1 (Remarks to the Author):

Review of 502133_1

This ms is improved with response to reviewers. However, I do not recommend publication until the authors have addressed the issues below. I am concerned that the biology seems like an afterthought in its mode of presentation.

TITLE: I strongly suggest that unless you focus more on diversity (and prove these are really diverse communities) that you take the word 'Diverse' out of the title. Nothing in the list of species given suggests high diversity. You could say "Flourishing communities...". Diverse means that there are either a lot of species present and/or that they individuals are evenly distributed among many species. You deleted 'biodiversity' hotspots in the text – this also needs removal from the title.

Response:

We sincerely appreciate this insightful comment. In response, we have changed the title to "Flourishing chemosynthetic life at the greatest depths of hadal trenches", as recommended by Referees #1 and #2.

Line 13 – Suggest you say siboglinid Polychaeta

Response:

Have done.

Line 43 - suggest seep animal communities, microbes are organisms and form communities

Response:

Have done.

Line 69... No part of the ocean is 11,000 m. So I'm not sure this makes sense. (10,980 is the deepest?). Maybe say capable of reaching the deepest part of the ocean at nearly 11,000 m)

Response:

Have done.

Line 97 and 99 and 101 – PLEASE do not call the frenulates tubeworms

Response:

Have done.

Line 263-4 High biomass and density (compared to what?) Non seep settings? Other seeps? Your densities are comparable to reports from other seep sites. This sentence is not correct. Biomass is measured as weight (e.g., g or kg). Numbers of bivalves is not biomass. The biomass at most cold seep sites in this study far exceeds previous Japan Trench records^{3,4}, where only tens to hundreds of chemosynthetic bivalves were noted. I would remove discussion of biomass unless you weighed samples and calculated wt/unit area.

Response:

Thanks for reviewer's valuable suggestions. We have removed the discussion of biomass in this section according to the referee's suggestion.

Why are the siboglinid and bivalve density data presented in implications section. These need to be in the results section where the fauna is described. Are these values maximum values? Where are the data deposited?

Response:

Thanks for reviewer's valuable suggestions. We have moved the siboglinid and bivalve density data to the results section (please see the last paragraph in *Chemosynthetic community distribution & diversity* section). These values are maximum values and data have been deposited in the Supplementary Table 1.

In the implications - are there actually density data for the Japan Trench? Other trenches to compare?

Response:

Density data were not reported previously for the Japan Trench. In the last version, we roughly calculated the density of bivalves in Japan Trench based on single image from reference 3, which shows bivalve aggregates associated with the seep. This may not be sufficiently rigorous. To clarify, we have removed the relevant sentence to prevent confusion. Hadal seep animal communities were only previously discovered in Japan Trench, so there are no other trenches to compare.

Line 268 Bivalves misspelled.

Response:

We have corrected it.

Line 290 – remove 'sea'

Response:

We have done it.

Line 614 – Where are the COI data deposited? Found this material in supplemental – probably needs to be in the paper.

Response:

The data reported in this paper have been deposited in the GenBase in National Genomics Data Center, Beijing Institute of Genomics, Chinese Academy of Sciences/China National Center for Bioinformatics, under accession number C_AA106802.1 to C_AA106807.1 that is publicly accessible at <https://ngdc.cncb.ac.cn/genbase>.

Line 598 to 602. The very brief video methods listed are insufficient to describe how density

assessments were done. The type of information needed would include: What was analyzed – replicate frame grabs from each site? Or 1 minute video segments? Or something else?

Response:

Thank you for this constructive comment. We agree that the original description of the video-based density assessment was too brief. We have now revised the Methods Section to include the requested details. The revised text is listed as follows:

For each dive, 3~10 representative screenshots showing the densest cold seep communities were selected from the video footage. The area of each image was estimated using the submersible's laser scale, which projects two parallel laser points 10 cm apart onto the seafloor. This provided a reliable spatial reference for calculating the seafloor area captured in each image. A standardized quadrat (e.g. 50×50 cm) was drawn near the laser dots by using this laser scale as a reference in the image. The calculated area was then converted into square meters for standardized density calculations. Animals visible within each quadrat were manually counted, and faunal density was expressed as the number of individuals per square meter (individual·m⁻²). For each dive, density values from the selected images were used to calculate the mean faunal density, and the standard deviation was computed to quantify the variability among replicate images.

No error bars are given. Are these counts based on single images? They list the maximum so presumably there were areas counted with fewer?

Response:

Thank you for your thoughtful question. The density counts were not based on single images but on 3~10 representative screenshots extracted from video footage of the densest seep communities observed in each dive. The area within each image was estimated using scale information provided by the submersible's laser scale, which projects a known distance of 10 cm onto the seafloor. For each dive, density values from the selected images were used to calculate the mean faunal density, and the standard deviation was computed to quantify the variability among replicate images. The standard deviations of the density estimates have now been included in Supplementary Table 1. We have updated the manuscript accordingly.

How was area in these images determined? – the lasers alone do not provide enough information. Or did you just do counts between the laser points?

Response:

Thanks for your question. The area is determined by using the laser scale which provide a fixed scale reference (10 cm apart). We did not simply count organisms between the laser dots, instead, a standardised quadrat (e.g. 50×50 cm) was drawn near the laser dots by using this laser scale in the image. Organisms visible within each image were manually counted, and faunal density was expressed as the number of individuals per square meter (individual·m⁻²).

Please refer to Table S1 in the main text as the source of density information – ideally in the results.

Response:

We have done it.

Supplementary Table 2 Why is *Lamellisabella* not listed here.? It seems you updated the main text but not the Supplemental table.

Response:

Lamellisabella is identified based on morphological analyses and we have not obtained the molecular data for it. In this study, most of our identification works are based on morphological analyses and confirmed by experts in respective fields. The exceptions are two typical frenalate siboglinids (FD Z271 and FDZ 294 in Supplementary Figure 1) and four chemosynthetic bivalves (Supplementary Figure 3), whose *coxI* gene sequences are provided as extra evidences in Supplementary Table 2.

Referee #2 (Remarks to the Author):

My comments and suggestions have been taken up constructively; the manuscript is now much improved, and contains more geochemical metadata that provide further context for hadal seep sediment and the habitat for these seep communities. The new version also draws a clear distinction towards factors that remain unknown, such as the size of hadal methane reservoirs.

Response:

Thanks for the referee's comments.

Some details require attention:

Line 155: This should read "During dive FDZ 278..."

Response:

We have done it.

The new title is odd. The central point of this manuscript is not that these chemosynthetic communities are located at the greatest depth of hadal seeps. You want to emphasize that these hadal seeps and their chemosynthetic communities are located at the greatest depth of hadal trenches.

Response:

Many thanks for this suggestion. We have changed the title to "Flourishing chemosynthetic life at the greatest depths of hadal trenches"

Line 165 & 166: "Fields of siboglinids and clusters of bivalves within the KAT were usually adjacent, but spatially separated ". This makes no sense, if they are spatially separated (meaning, there is a gap in between) they cannot be adjacent. You probably want to say that these fields were located next to each other, but did not overlap (meaning, there was no zone where these organisms coexisted).

Response:

We have revised it according to the reviewer's suggestion.

Line 238 and 239: include not only the permille fractionation, but also the isotopic reference standards

Response:

We have done it.

Line 271-272: When you say that "methane is present in the form of dissolved fluid (liquid)" this could be misunderstood as gas in liquid form, such as liquid nitrogen (which requires very cold

temperatures). You want to say that methane occurs in dissolved form, in aqueous solution in porewater.

Response:

We have revised it according to the reviewer's suggestion.

Line 298: Better: "A focused coring and drilling campaign is needed..." (a few push cores do not count as a focused campaign)

Response:

We have revised it according to the reviewer's suggestion.

Line 356: bivalves, not biovalves

Response:

We have revised it according to the reviewer's suggestion.

Line 386: Omit "sea", therefore: "such as Actiniaria..."

Response:

We have done it.

Line 389: better: "is demonstrated and quantified by follow-up research..."

Response:

We have done it.

Line 447: better: "It remains unknown whether the current findings can be extrapolated..."

Response:

We have done it.

Line 447: Please note the ODP drilling has recovered methane hydrates not just on the continental margin, but in trench sediments in the Peru Trench at > 5000m depth (Shipboard Scientific Party, 2003. Site 1230. In D'Hondt, S.L., Jørgensen, B.B., Miller, D.J., et al., Proc. ODP, Init. Repts., 201: College Station, TX (Ocean Drilling Program), 1–107. doi:10.2973/odp.proc.ir.201.111.2003)

Response:

We have revised it according to the reviewer's suggestion.

Referee #3 (Remarks to the Author):

I'm satisfied with the revisions made in this version of the manuscript. The authors have addressed the major concerns raised in the initial round of review, and the paper now presents a well-structured, compelling, and enjoyable account of hadal methane seeps and associated communities. It reads clearly and concisely, and represents an exciting contribution to deep-sea research. In my opinion, it is ready for publication pending a few very minor editorial adjustments and some general finalizing of the text, such as:

Response:

Thanks for the referee's comments.

- Line 54 – correct "research"

Response:

We have done it.

- Line 56 – remove “a” before “predominantly”

Response:

We have done it.

- Lines 102–105 – for clarity, consider adding commas after each faunal group or placing the taxonomic names in parentheses

Response:

We have done it.

- Line 111 – correct to “siboglinids”

Response:

We have done it.

- Line 116 – add a comma after “polychaetes”

Response:

We have done it.

- Line 162 – add a comma after “siboglinids”

Response:

We have done it.

- Line 163 – consider changing “east to” to “east of”

Response:

We have done it.

- Line 182 – the sentence could be refined

Response:

We have done it.

- Lines 200–205 – this sentence is a bit dense. Consider splitting it into two

Response:

We have done it.

- Line 268 – correct the spelling of “bivalves”

Response:

We have done it.

- Line 274 – suggest rewording as “the distribution ranges observed”

Response:

We have done it.

- Line 310 – remove the comma after “unknown”

Response:

We have done it.